Comprehensive immunogenetics / W.H. Hildemann, E.A. Clark, R.L. Raison.

Comprehensive Immunogenetics

W.H. Hildemann
E.A. Clark
R.L. Raison

BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD LONDON EDINBURGH BOSTON MELBOURNE

0-632-00788-5

Comprehensive Immunogenetics

William H. Hildemann, Ph.D.
University of California, Los Angeles

Edward A. Clark, Ph.D.
University of Washington, Seattle

and

Robert L. Raison, Ph.D.
University of Sydney, Australia

Thoroughly modern in perspective, this outstanding new text covers the entire field of immunogenetics. Emphasizing basic principles, problem solving, and applications, it examines topics now at the forefront of immunogenetic research:

— genetics of antibody molecules

— genetic control of immune responsiveness

— immunodeficiency and immunogenetic diseases

— phylogeny of immunocompetence

Comprehensive Immunogenetics is the ideal text for advanced undergraduate or graduate courses in the biomedical curriculum because it:

— is both comprehensive and contemporary

— assumes only elementary background in genetics, biochemistry, and immunology

— takes a sequential approach that has been successfully used in the classroom

— includes numerous illustrations integral to the presentation

(continued on back flap)

COMPREHENSIVE IMMUNOGENETICS

COMPREHENSIVE IMMUNOGENETICS

W. H. Hildemann
University of California, Los Angeles

E. A. Clark
University of Washington, Seattle

R. L. Raison
University of Sydney, Australia

BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD LONDON EDINBURGH BOSTON MELBOURNE

Blackwell Scientific Publications Ltd.
Osney Mead, Oxford OX2 OEL
8 John Street, London WC1N 2ES
9 Forrest Road, Edinburgh EH1 2QH
52 Beacon Street, Boston, Massachusetts 02108, USA
214 Berkeley Street, Carlton, Victoria 3053, Australia

First published in the United States of America in 1981
by Elsevier North Holland, Inc., 52 Vanderbilt Avenue, New York, NY 10017

ISBN 0-632-00788-5

Desk Editor Robert Glasgow
Design Edmée Froment
Design Editor Glen Burris
Art rendered by Vantage Art, Inc.
Production Manager Joanne Jay
Compositor Publishers Phototype, Inc.
Printer Haddon Craftsmen

Manufactured in the United States of America

To Dorothy, Yukiko, and Roslyn

CONTENTS

PREFACE

The first book entitled *Immunogenetics,* written solo by the senior author and completed in 1968, was also the first attempt to offer a comprehensive overview of this newly emerging field. By that time, the subjects of microbial antigens/serotype patterns, blood group alloantigen systems, and immunogenetics of tissue transplantation were already well explored. However, other topics now at the forefront of immunogenetic interest were still in early stages of study. These newer emphases are genetics of antibody molecules, genetic control of immune responsiveness, immunodeficiency and immunogenetic diseases, and phylogeny of immunocompetence. Although many excellent reviews of different aspects of immunogenetics have been written over the years, no single source of basic principles and their applications has been available for pedagogic or correlative purposes. The need for such a book has markedly increased with expanding emphasis in the interdisciplinary fields of tissue transplantation, genetic regulation of immune responsiveness and cancer.

The nine chapters of *Comprehensive Immunogenetics* now cover the entire field from the perspective of 1980. Indeed, this is the only book available that deals with the ramifications of immunogenetics as a whole. This book is intended to serve multiple needs. It is intended first as a text for advanced undergraduates and graduate students. For this purpose, only a very elementary background in genetics, biochemistry, and immunology is assumed by the authors. The introductory chapter accordingly outlines concepts and first principles—the scope of immunogenetics with emphasis on fundamentals, terminology, and basic methodology. This chapter ends with a consideration of types of genes affecting diverse immunologic characteristics. The stage is thereby set for the more specialized topics of subsequent chapters. The sequential approach adopted has evolved over many years of teaching immunogenetics to a broad range of students at the University of California, Los Angeles. *Com-*

prehensive Immunogenetics should also serve to enrich courses in genetics or immunology, including those offered for medical or professional students. Postdoctoral and faculty-level individuals in various biomedical sciences should find this book a frequently useful reference, suitable for self-instruction.

The first chapter is intended as a general foundation upon which later chapters depend in differing degrees. Chapter 2 deals with the genetics of antibody molecules, their production, structure, diversity, and their generation. Given this understanding, microbial antigens and serotype patterns are analyzed in Chapter 3 with emphasis on genetic polymorphisms and their significance in well-studied bacteria, protozoans, and viruses. At this juncture, the student should have a good basic grasp of the immunogenetics triad of immunogenes, antibodies, and antigens. Chapters 4 through 6 may be regarded as an integrated unit, dealing in turn with blood group alloantigen systems, immunogenetics of tissue transplantation, major immunogene complexes, and lymphocyte differentiation genes. These subjects provide an important background for understanding Chapter 7 on genetic control of immune responsiveness with its dependence on the major immunogene or histocompatibility complex. Chapter 8 then comes to grips with immunodeficiency and immunogenetic diseases. This takes the reader from the genetic basis of infectious disease resistance to immunogenetic correlates of cancer and aging. Finally, Chapter 9 on phylogeny of immunocompetence puts immunorecognition systems, allogeneic polymorphisms, and diversification of immunocyte functions in evolutionary perspective.

Since there are many interconnecting paths among the subjects considered in the separate chapters, attention is repeatedly called to basic interrelationships, especially where frontier questions are involved. This book then emphasizes principles and problem-solving. An entreé to the contemporary literature of immunogenetics is also provided in the text and in the annotated key references at the end of each chapter. These references, which are mostly recent reviews or definitive research articles, contain citations of pertinent earlier work the serious student may wish to pursue in greater detail. Given the new breadth of immunogenetics, an effort was made to achieve a stimulating compromise between the usual textbook and advanced monograph approaches. The illustrations are an integral part of the presentation. For the most part, each chapter may be read or used independently. Thus, instructors may choose to alter the sequence or otherwise select certain chapters to meet the needs of different groups of students.

We welcome the opportunity to acknowledge the lively stimulation of immediate colleagues, postdoctoral fellows and graduate students. For helpful suggestions or personal communications we thank Don Bailey, Nick Cohen, Carol Sibley, George Snell, Clyde Stormont and Paul Terasaki. At the same time, we apologize to investigators whose work may have been cited without pausing to assign credits. Carol Thiele skillfully converted our crude drawings

into the finished illustrations. For innumerable days of typing, proof-reading, and enthusiastic attention to other details, we salute Lois Bigger and Virginia Janczak.

Bill Hildemann
Ed Clark
Bob Raison

1

CONCEPTS AND FIRST PRINCIPLES

INTRODUCTION: THE SCOPE OF IMMUNOGENETICS

Immunogenetics was born about 1900 when Landsteiner identified ABO blood groups in humans while Ehrlich and Morganroth discovered individual-specific blood types in goats. This was the first use of immunological methodology to explore genetic variation, coincidentally at the time when rediscovery of Mendel's principles began to revolutionize biology. Some might contend that immunogenetics was really established in the mid-1920s with Bernstein's demonstration from analyses of family data of a three-allelic-gene basis for ABO blood types in man. However, the genetic basis of tissue transplantation incompatibility was already proved in 1916 as a result of experiments with inbred lines of mice by Little and Tyzzer. Numerous studies of blood types and of tissue transplant reactions during the 1920s established the early finding that normal cells of mammals carry individual-specific antigens under the control of Mendelian genes.

The scope of immunogenetics has progressively widened to include microbial antigens, the genetics of antibodies and other macromolecules, and the phylogeny of immune responsiveness. Immunogenetics may now be broadly defined to include studies in which the principles and techniques of both genetics and immunology are employed together. Studies of individual-specific and tissue-specific cellular antigens have become a major focus of immunogenetics, with important ramifications both in molecular biology and in medicine. Almost every facet of modern immunological research and its application involves important genetic considerations. Elucidation of host–parasite relationships depends upon understanding the inheritance of host immune-response capacities as well as antigenic characteristics of particular pathogens. Intraspecific differences are reflected in individual variations in chemical structure of cellular and soluble macromolecules. This intraspecific diversity is so great that, except for identical twins or equivalent highly inbred animals, ev-

ery individual within a species may be regarded as unique. Such uniqueness is best demonstrated across the whole spectrum of animal species by tissue transplantation. Given appropriate techniques, self-to-self transplants survive, whereas nonself exchanges reveal their lack of molecular identity by rejection. Incompatibility leading to eventual graft rejection is the universal rule within species of multicellular animals, both invertebrates and vertebrates. A normal, adult recipient reacts against the genetically and antigenically different tissue of the donor. This highly discriminating recognition of nonself applies to species ranging from corals to tunicates and fishes to mammals. The "uniqueness of the individual" and the "uniqueness of separate breeding populations" emerge as tenets of immunogenetics.

One of the most fundamental generalizations in biology is that genes specify and regulate the synthesis of macromolecules required for growth and development. In addition to nucleic acids containing the genetic code, the essential macromolecules that distinguish cells as well as individuals of all species are proteins and polysaccharides. The structural complexity and functional specificity of these molecules have only begun to be characterized. Animals dispose of nearly all foreign macromolecules that enter the body in a predictable manner. Some of these macromolecules are antigenic and may induce an immune response. All multicellular animals appear to recognize foreignness at the molecular level—whether introduced by a pathogenic microorganism or by intraspecies tissue graft. Invertebrates respond to such antigens with cell-mediated immunity associated with leukocyte-type cells, while vertebrates additionally respond with production of circulating antibodies.

The phylogenetic emergence of immunorecognition systems will be considered in detail in the last chapter. For introductory purposes, antigens may be defined as molecules that will induce an immune or antibody response when inoculated into an animal. Antigens will react specifically with the antibodies they have induced in vertebrates. Although antibodies are represented by diverse molecular classes in higher vertebrates, all are found in the globulin fractions of serum or plasma proteins (Figure 1–1). Antigen–antibody reactions are usually highly specific, and yet single antigens may evoke a multiplicity of antibodies of varying reactivities. A macromolecular "antigen," in the crude sense, is composed of various structural groups or sites, each capable of reacting with antibodies of corresponding stereospecificity. Small molecules, even ions associated with proteins or polysaccharides, may serve as antigenic determinants or haptens but will induce antibodies only when conjugated to a large molecule. In general, immunogenic molecules exceed 8,000 in molecular weight and are polyvalent with respect to determinant groups. The combining sites of individual antigens can be as large as six or seven sugar residues in polysaccharides and five or six amino acids in proteins. However, single sugars or amino acids within such sequences usually provide the predominant complementary configuration for specific antibodies (see Figures 3–2 and 4–1). Although nearly all antibodies have two identical combining sites per molecule or subunit, antibody molecules formed to a single antigen are not iden-

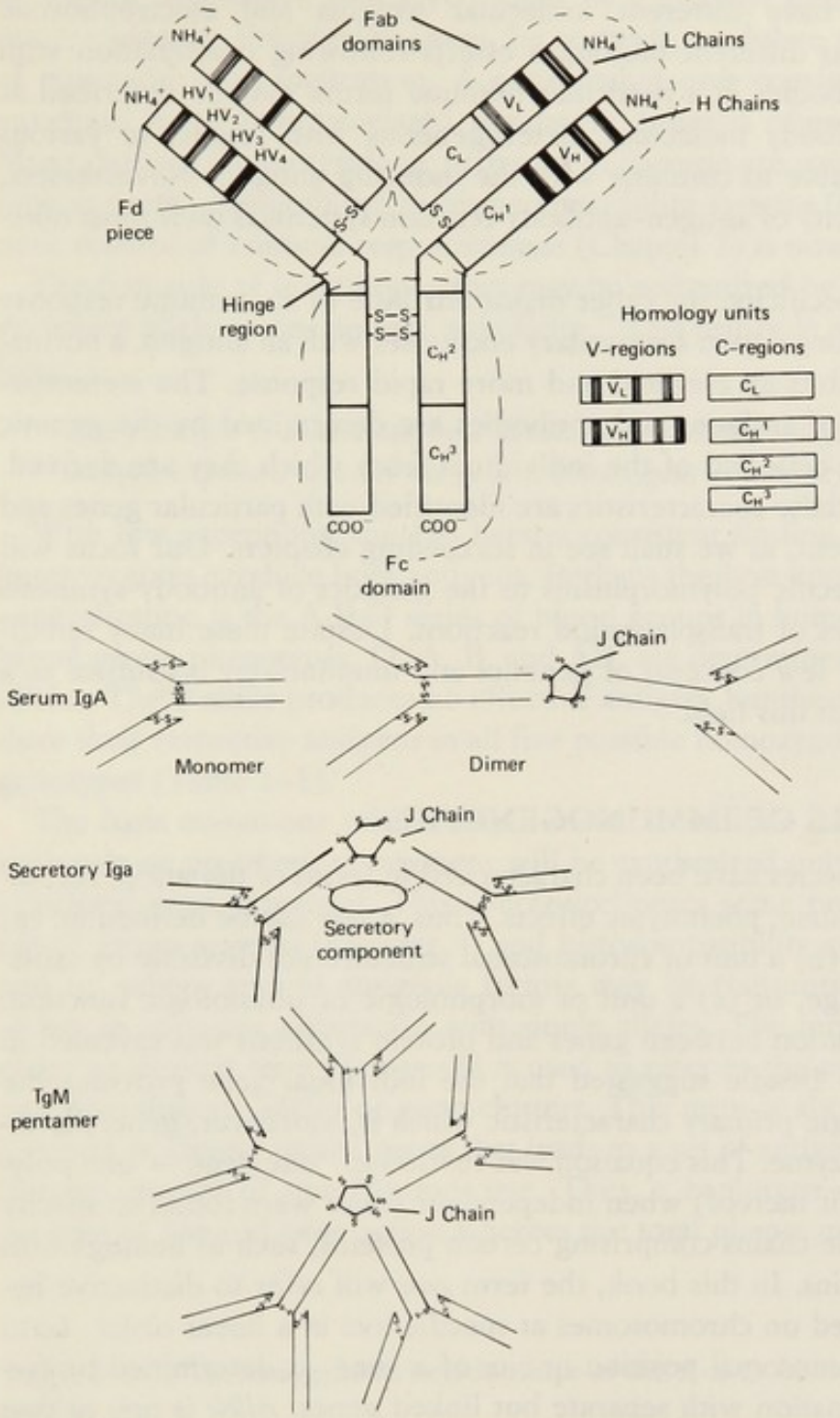


FIGURE 1–1. Schematic diagrams of mammalian antibody molecules of different immunoglobulin (Ig) classes. The essential molecule consists of four polypeptide chains, two identical heavy (H) chains of about 55,000 daltons and two identical light (L) chains of about 25,000 daltons, joined by interchain disulphide bonds (S-S). The antibody-combining site with complementary configuration for an antigenic determinant is located in V_H and V_L hypervariable (HV_1-HV_4) amino-terminal regions of each heavy–light chain pair. Immunoglobulin classes (e.g., IgM, IgA, IgG) are distinguished by heavy chain composition, polymerization involving joining or J chains, carbohydrate content, serum half-lives, and other properties. See Chapter 2 for genetics of antibody structure.

tical. They often have different molecular weights and electrophoretic mobilities as well as different biological effects following combination with antigen. Thus, antibodies in a particular immune serum may be described as populations of antibody molecules, heterogeneous with respect to various properties, but all able to combine with the inducing antigen. Nevertheless, the essential specificity of antigen–antibody reaction systems is their most noteworthy attribute.

Apart from its specificity, the other major attribute of an immune response is its selective memory. Upon a secondary encounter with an antigen, a normal animal usually exhibits an elevated and more rapid response. The stereospecific characteristics of antigens and antibodies are determined by the genetic constitutions of the cells and of the individuals from which they are derived. In many instances, these characteristics are identified with particular genes and stages of development, as we shall see in succeeding chapters. Our focus will range from intraspecific polymorphisms to the genetics of antibody synthesis and the complexities of transplantation reactions. Despite these many ramifications, a relatively few concepts of genetics and immunology do suffice as a basis for coping with this field.

FUNDAMENTALS OF IMMUNOGENETICS

Genes of diverse species have been characterized in terms of mutation, recombination, and, of course, phenotypic effects. Thus, a *gene* can be defined as: (a) a unit of mutation, (b) a unit of chromosomal structure not divisible by crossing over or breakage, or (c) a unit of morphologic or physiologic function. As the essential relation between genes and protein synthesis was revealed in the 1940s, George Beadle suggested that the individual gene provides the specificity for a single primary characteristic which is, moreover, generally associated with an enzyme. This equation was revised to "one gene → one polypeptide" (or subunit thereof) when independent genes were found to specify separate polypeptide chains comprising certain proteins, such as hemoglobins and immunoglobulins. In this book, the term *gene* will refer to distinctive hereditary units located on chromosomes at specific loci in a linear order. *Locus* designates the chromosomal position or site of a gene, as determined by frequency of recombination with separate but linked genes. *Allele* is one of two or more genes belonging to the same gene locus. Alleles then are regarded as functional alternatives which have not been separated by tests for recombination. A locus which has multiple alleles is said to be *polymorphic.* The term gene *complex* or *system* means a functional unit of multiple, closely linked genes. Why cells of different types, even from the same individual, express only some of the potentialities inherent in their *genome* (i.e., one complete set of genes or a haploid set of chromosomes) is the subject of the frontier realm of developmental regulation. Two classes of genes—*structural* and *regulatory*—may often be conveniently distinguished in immunogenetic studies. Structural genes are identified by their coding of amino acid sequence in a polypeptide

or specific attachment of a given sugar by a glycosyl transferase to a carrier macromolecule. Regulatory genes, by contrast, modulate the "on–off" status of structural gene expression. A coordinated unit consisting of a group of structural genes and associated regulatory genes is often termed an *operon.* Many, but not all, biosynthetic pathways in bacteria are consistent with the operon concept. Applicability to histocompatibility systems (Chapter 6) and genetic control of immune responsiveness (Chapter 7) is now emerging.

The first rule of immunogenetics may be epitomized by the aphorism "one dominant allele → one antigen specificity." This principle may be stated as follows:

> *Every antigen is determined by a dominant or codominant gene, i.e., the antigen is present whether the gene is homozygous or heterozygous.*

With few exceptions, this has been a consistent finding. Doubly dominant heterozygotes produce both antigens. Perhaps the best known example of this generalization is the A-B-O series of blood groups in humans. The four main blood group phenotypes, O, A, B, and AB, are determined by three multiple alleles. The *0* allele produces no effective antigen, but the *A* and *B* alleles produce their respective antigens in all five possible homozygous or heterozygous genotypes (Table 1–1).

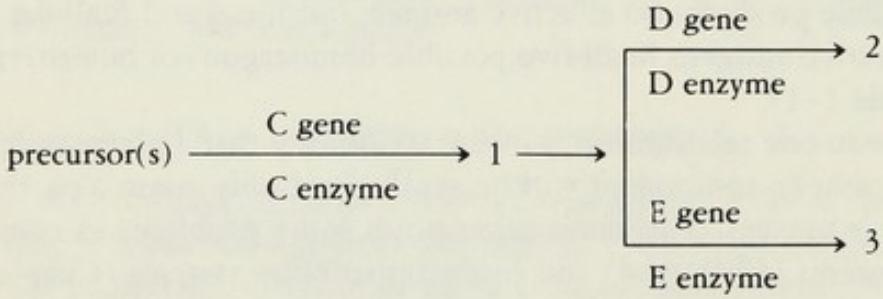
The basic one-to-one relationship is meant to indicate that a given antigenic molecule or prosthetic component will be synthesized only when a particular structural gene is present. This conception poses some problems in complex blood group systems (Chapter 4) and histocompatibility systems (Chapters 5 and 6), where several antigenic factors may be transmitted genetically as a group in apparent association with single alleles. The term *haplotype* (or the older equivalent term *phenogroup*) is used to refer to the product(s) of one of two homologous alleles or gene clusters. This term is also used to designate a cluster of closely linked genes that leads to a set of antigenic specificities under the control of one chromosome. Thus, a haplotype corresponds to the product of haploid gene action whereas the total phenotype is determined by

TABLE 1–1. Immunogenetic Relationships of the A-B-O Blood Group System in Man

Blood group phenotype	Possible genotypes	Reactions observed with antibodies		Blood contains	
		Anti-A	Anti-B	Erythrocyte antigens	Serum antibodies
AB	*A/B*	+	+	A,B	—
A	*A/A,A/0*	+	−	A	anti-B
B	*B/B,B/0*	−	+	B	anti-A
O	*0/0*	−	−	—	anti-A anti-B

the diploid genotype, or both alleles at each relevant genetic locus. The criteria of both genetics and immunology need to be satisfied before an antigen may be attributed to a given gene. At the genetic level, the problem is often to distinguish single genes from closely linked gene complexes. At the immunologic level, the problem is to distinguish monospecific reactions from multispecific or cross-reactions. Because even an antigenic determinant as small as a hydroxyl group on a sugar may evoke antibodies of differing specificity, as Landsteiner discovered long ago, the lack of one-to-one correspondence between a gene, its antigenic product, and evocable antibodies is hardly surprising. In Chapter 2 we shall come to grips with the potential multispecificity of antibodies as a function of their idiotypes or hypervariable regions involved in specific combining sites.

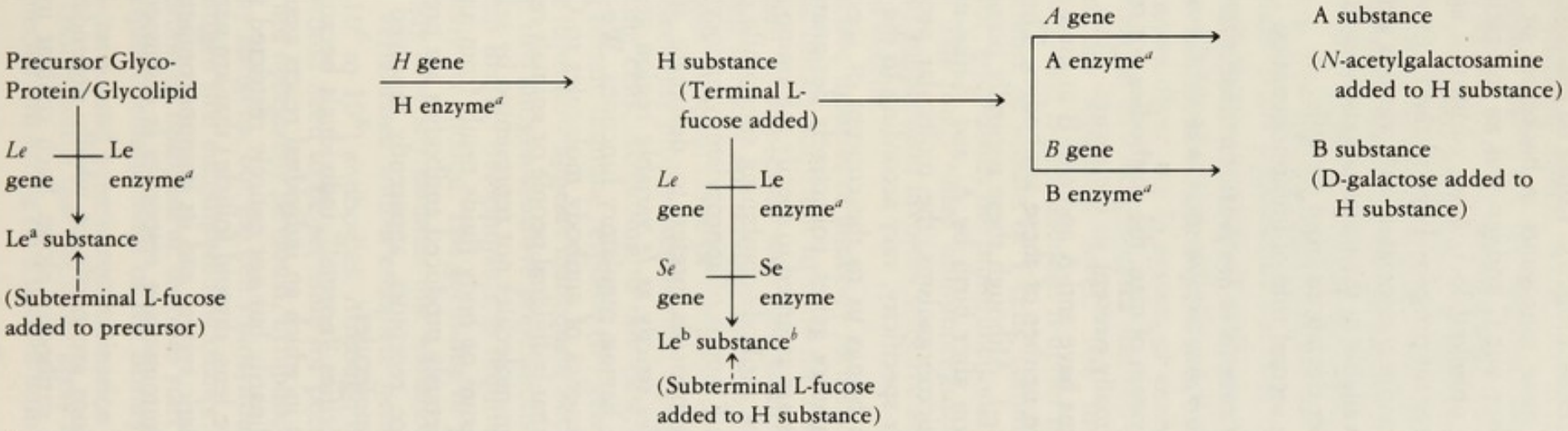
The relationship "one gene → one antigen" is not negated by the finding that more than one gene locus may affect an antigen or that a given locus may affect more than one antigen. As a hypothetical example, consider the following reaction sequence:



Product 2 might always appear to show a one-to-one dependence on the D gene even though one or more precursors dependent on other genes such as C were required earlier in the sequence. Moreover, antigenic products 2 and 3 could both be said to depend on the C gene as well as on the D and E genes, respectively. A notable example of such gene interaction involves the *Lewis, H,* and *Secretor* genes in man (Table 1–2). While the synthesis of the Le^a antigen requires only the *Le* gene, the presence of Le^b antigen on human erythrocytes and in secretions requires the action of *H, Le,* and *Se* genes. This and other apparent deviations from simple one-to-one relationships reflected in hybrid antigens or recessively determined antigens will be scrutinized later (Chapter 4).

In a model interaction system using two highly inbred or essentially homozygous but genetically disparate lines of mice, the interstrain cross AA × BB could yield F1 hybrids of the uniform constitution AB(C). In this symbolism, C represents an interaction or hybrid antigen dependent on the genes or gene products of A and B together. The existence of C could be demonstrated by immunizing another species such as a rat with F1 cells and then mixing the resulting antiserum with large numbers of cells from both parental strains to remove or "absorb out" antibodies to A and B. If complete absorption of anti-A and anti-B still left antibodies reactive with F1 cells (i.e., anti-C), presence

TABLE 1–2. Biosynthesis of A, B, H, Le[a], and Le[b] Antigens Detectable on Human Erythrocytes and in Secretions



[a]Each gene codes for a specific glycosyl transferase that adds a particular monosaccharide to a glycoprotein/glycolipid backbone.

[b]Combined action of dominant *H*, *Le*, and *Se* genes required for production of Lewis[b] substance on erythrocytes or in saliva.

of the additional nonparental antigen C would be established. Without further experiments involving gene segregants in backcross or F2 generations, we would not know whether our C antigen was an interaction product of two allelic genes or of two unlinked loci. Also, additional antiserums produced against the cells of segregant progeny beyond the F1 generation could reveal more than one hybrid antigen associated with various genotypes. This is one of several approaches available to the immunogeneticist to test for possible hybrid specificities or other deviations from the rule.

Now we come to the second rule of immunogenetics:

> *A normal individual possesses or can produce antibodies (or develops immunity) only against those antigens that he or she does not have.*

In the familiar A-B-O system of man, the antibodies not represented in the individual's cells are normally present in the serum. Thus, type O individuals lacking A and B antigens have anti-A and anti-B in their serum whereas type AB individuals produce neither of these antibodies. These relationships are summarized in Table 1–1. Although these naturally occurring antibodies appear to be induced soon after birth by A- and B-like molecules present in foods and intestinal microorganisms, the molecular properties of the antibodies, apart from their specificity, vary according to the genetic constitution of the individual. This brings us to the concept of specialized immune response or immunoregulatory genes, collectively designated *Ir* genes at present. Distinctive *Ir* genes are assumed to specify the amino acid sequence of the hypervariable regions of antibody-combining sites while other *Ir* genes regulate the selective "on–off" status of appropriate lymphocyte biosynthetic pathways. Some immunogeneticists prefer to designate structural genes for antibody-combining sites strictly as *Ig* variable region genes and restrict the term *Ir* genes to those having regulatory functions. We shall defer detailed consideration of the genetics of antibody molecules to Chapter 2. Here we simply make the point that individual people or animals react with an immune response only to foreign molecules not represented by self-constituents. Antibodies detectable in serum or body fluids result from antigenic stimulation. Any natural or spontaneously produced antibodies are present only in minute quantities as cell-surface receptors, apparently involved in initial immunorecognition of foreign molecules.

Our second rule specifies "normal" individuals because autoimmune diseases reflect exceptions in which an individual reacts against his own tissues. Autoantigenicity is ordinarily but not entirely restricted to those tissue components (e.g., brain, eye lens, thyroid follicle) that are naturally isolated from contact with the lymphatic system and its immunocompetent cells. The regulation of normal and autoimmune responses is discussed in more detail in Chapter 8.

Most immune reactions are partly or wholly dependent upon leukocytes rather than circulating antibodies alone. Such cellular immunity, as distinct

from serum antibody-mediated effects, is decisive in infections (especially virus) and tissue transplant rejections, but the mechanisms are poorly understood. In fact, the molecular basis of cell-mediated immunity remains a major unsolved problem. For present purposes, it is sufficient to note that immune reactions generally involve cooperative interaction of immune cells and humoral antibodies. The dual nature of the immune system is reflected in the separate functions of thymus-derived (T) lymphocytes and bone marrow-derived (B) lymphocytes. The B-cell populations are the source of secreted humoral antibodies, while T-cell populations underlie cell-mediated immunity and interact with B-cells in the initiation of antibody production. The distinctive functions of T- and B-lymphocytes underlie much of cellular immunology. Although much is known about the Ig system of antibodies derived from B-lymphocytes, the activation of T-lymphocytes and cell-mediated immunity remain mysterious at the molecular level. The integration or dynamic immunoregulation of T- and B-cell functions is under intensive study by many researchers. The presence of subsets of T-cells, commonly designated killer, helper, and suppressor, emphasizes the multifunctional and overriding importance of this system. The finely tuned immunoregulatory network of induction, modulation, and suppression involves selective activation of appropriate subsets of T-cells and B-cells.

Now popular selective theories of antibody formation assume that individuals possess a genetic library sufficient to code for a wide array of antibody-equivalent specificities. Lymphocytes with genetically determined antibody receptors on their cell surfaces are thought to be activated by encountering corresponding antigens. A small clone of lymphocytes activated by exposure to a given antigen selectively proliferates to yield a population of corresponding antibody-producing cells. According to this view, antigen signals the selective turning on of certain immune response (*Ir*) genes. The clonal selection theory in this simple form now appears inadequate because for many antigens interaction of at least two antigen-sensitive populations of T- and B-cells, each probably reactive with distinct determinants, is required for immunorecognition leading to antibody production. The germ-line or phylogenetic theory for generation of antibody diversity assumes that mutation and selection during evolution provided vertebrates with a complete genetic (DNA) library to make all required (10^6 to 10^8) antibodies. Although more than 1 percent of the DNA of a cell would need to be set aside for potential antibody production, selection need not necessarily be clonal. Lymphocytes could be potentially responsive to many antigens, but the cell's biosynthetic apparatus might be preempted by the antigen first encountered by chance.

Somatic diversification or ontogenetic theories start with a small library of germ-line genes. Required enlargement of the library is then thought to be achieved by hypermutation and genetic recombination during embryogenesis. The essence of this conception is that the large array of antibody specificities arises by a somatic mechanism acting on a limited number of germ-line genes.

The generation of antibody diversity poses formidable problems as we shall see in Chapter 2. A comprehensive theory of adaptive immune responsiveness has yet to be put forward. Such a theory should account for:

Self versus not-self recognition in both cell-mediated immunity and induction of antibody formation

Responsiveness to a very large number of distinct antigens or how antibody diversity is generated

Specificity of antigen–antibody interactions

Immune memory and qualitative differences between primary and secondary responses

Immunoglobulin antibodies or other immunorecognition molecules provide multicellular animals, all of astonishing molecular complexity, with the capacity to deal with an even greater array of foreign molecules. Our second rule simply avers the exquisite discrimination inherent in the normally adaptive immune response.

GENETICALLY DEFINED ANIMALS

Random breeding populations of any species are most often genetically heterogeneous. This is the real world of naturally occurring populations with which the immunogeneticist must ultimately come to grips. Indeed, for most species and animal taxa, wild-derived individuals or colonies may be the only material available for study. Genetic heterogeneity is undesirable in many experiments, however, because of wide variations in responsiveness. Moreover, polygenic effects will usually mask the effects of individual gene loci unless experimental and control animals are derived from highly inbred or otherwise selected homogeneous sources. Inherited characteristics of research animals can be controlled by various combinations of inbreeding and selection systems. Although many breeding systems are potentially available for special purposes, only the most commonly useful sources of animals are described here.

Breeding Systems and Inbred Strains

Inbreeding can take different forms, but the most commonly used is full-sibling, or brother–sister, mating involving breeding of individuals derived from the same parents. Because genetic heterogeneity is caused by segregation of alleles at heterozygous loci, the goal is to achieve homozygosity at nearly all loci. Consider the consequences of repeated brother–sister mating in terms of one locus with two codominant alleles, R^1 and R^2. Three genotypes may then occur: R^1R^1, R^1R^2, or R^2R^2. These genotypes can be paired in six combinations and four types of matings called incrosses, crosses, backcrosses, and intercrosses as shown in Table 1–3. If, for example, an intercross were made between

TABLE 1–3. Possible Types of Matings that May Occur in Terms of One Locus with Two Alleles Yielding Three Genotypes

Locus	Alleles	Genotypes	Mating types	
R	R^1	R^1/R^1	$R^1/R^1 \times R^1/R^1$	Incrosses:
	R^2	R^2/R^2	$R^2/R^2 \times R^2/R^2$	matings of like homozygotes
		R^1/R^2	$R^1/R^1 \times R^2/R^2$	Crosses: matings of unlike homozygotes
			$R^1/R^1 \times R^1/R^2$	Backcrosses:
			$R^2/R^2 \times R^1/R^2$	matings of homozygote and heterozygote
			$R^1/R^2 \times R^1/R^2$	Intercrosses: matings of heterozygotes

heterozygous littermates ($R^1R^2 \times R^1R^2$), all three types of progeny should occur in the ratio 1/4 R^1R^1:1/2 R^1R^2:1/4 R^2R^2. A subsequent brother–sister mating from this progeny would have a probability of $1/4 \times 1/4 = 1/16$ of being either $R^1R^1 \times R^1R^1$ or $R^2R^2 \times R^2R^2$. The combined probability of achieving homozygosity as either R^1R^1 or R^2R^2 is $2/16 = 1/8$. Once such a mating occurs either by chance or by selection of a desired genotype, the locus remains "fixed," i.e., homozygous in all future sib matings.

A very low probability exists for mutation to occur at a given locus (10^{-6} to 10^{-7}), and even such restored heterozygosity should be eliminated by continued brother–sister matings. Remember that the founders of an inbred line will usually be heterozygous at many different gene loci at the outset. Successive generations of full-sib matings gradually increase the probability of incrosses and thus the probability of homozygosity. This is because incrosses not only persist once established, but are increasingly produced by further incrosses, by backcrosses, and by intercrosses in the preceding generation. As shown in Figure 1–2, some twenty generations of brother–sister mating are required to achieve about 99 percent homozygosity. At this stage, a line is designated highly inbred, or simply *inbred.* Despite continued inbreeding, slight residual heterozygosity will theoretically be retained as a result of random mutation. The degree of homogeneity may be represented by the coefficient of inbreeding (F) as follows:

$$F = 1 - (1 - \Delta F)^n$$

where ΔF is the rate of inbreeding (equal to 0.19 for consecutive brother–sister matings) and n is the number of generations of inbreeding. Since the effect of inbreeding is cumulative, F increases asymptotically toward 100 percent. $F = 98.6$ percent after twenty generations of full-sib matings. Note that brother–sister inbreeding also reduces the chance of heterozygosity for sex-linked

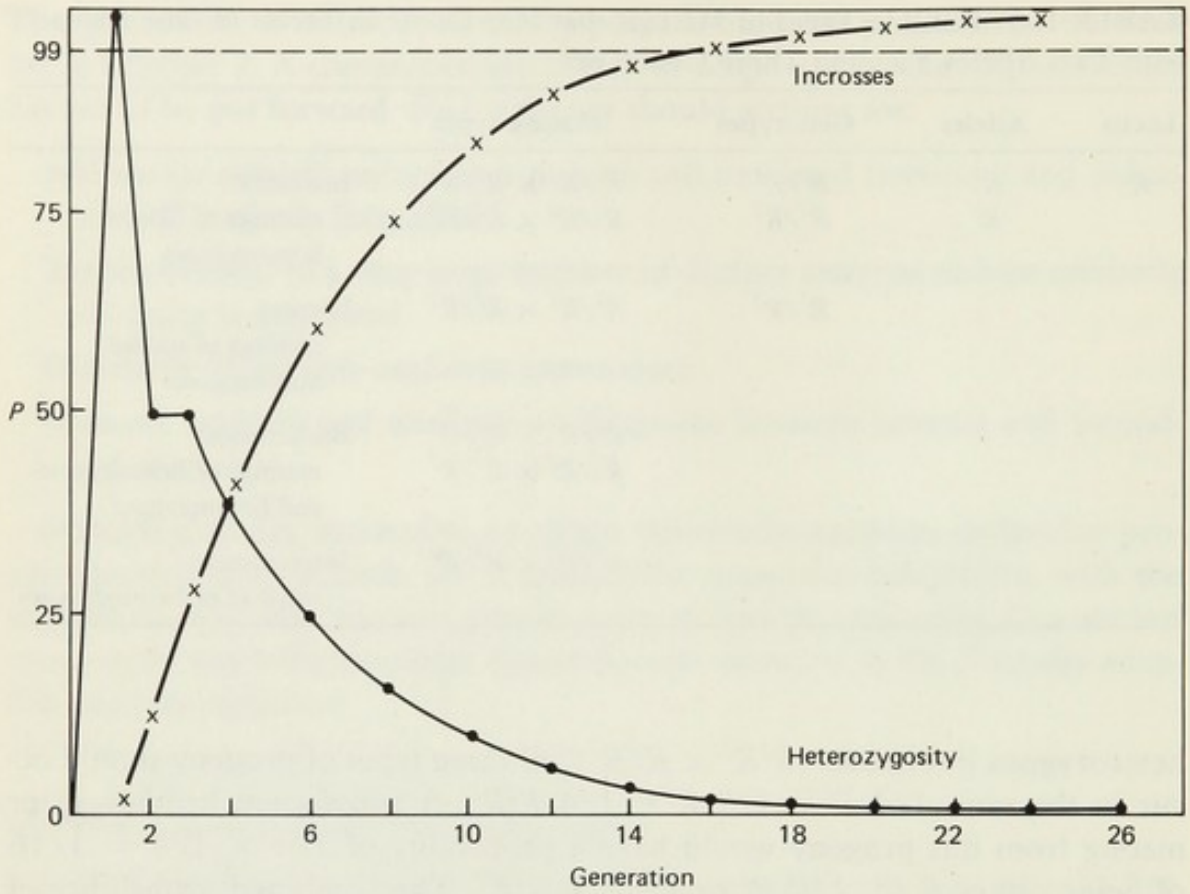


FIGURE 1–2. Probability (P) of incrosses or matings of like homozygotes and of heterozygosity in successive generations of brother–sister inbreeding. Note that homozygosity approaches but does not quite reach 100 percent after twenty generations.

loci in the homogametic sex, and in the heterogametic sex if there is crossing over between the sex chromosomes. A large number of distinctive inbred strains of mice and rats are available, while a less extensive array of inbred lines have been produced in guinea pigs, rabbits, hamsters, chickens, and a few species of teleost fishes.

Congenic Lines

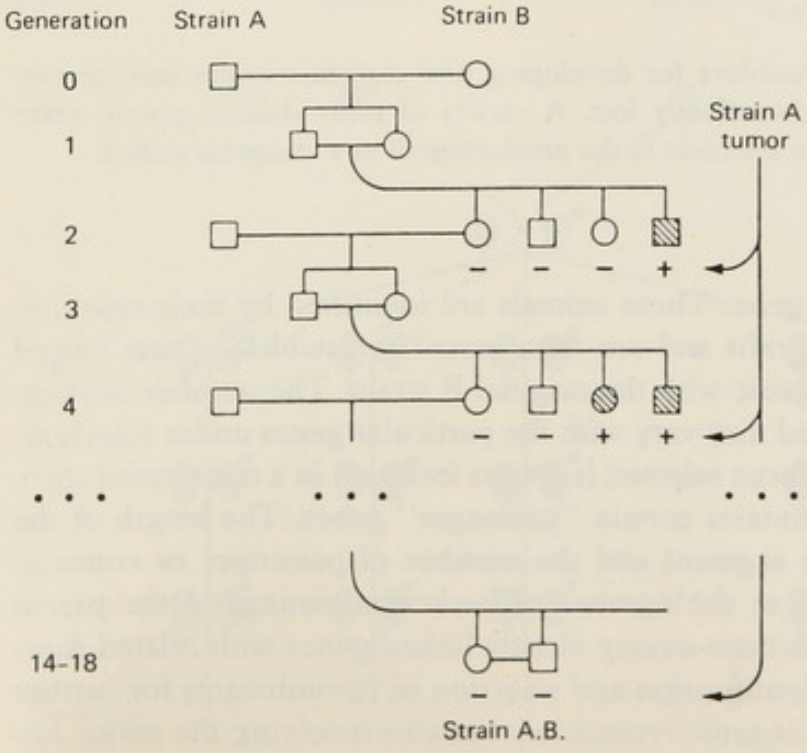
Two or more strains identical with an inbred line except for a difference at a single locus are *coisogenic* and can arise only by point mutation. Such strains differing for given cellular antigens or for genes affecting particular immune responses are rare because the desired mutants can be identified only by extensive and usually expensive screening. However, nearly coisogenic strains called *congenic strains* can be produced through appropriate crosses by introducing a chromosome segment containing a selected allele into an established inbred strain. These congenic lines approximating coisogenic strains can be produced either by the cross–intercross method of Snell (Figure 1–3) or by the cross–backcross–intercross system of mating employed by Bailey (Figure 1–4). In both procedures, the geneticist must be able to select for the desired trait in successive generations to assure that the differential gene is retained

in the new strain while all other genes become identical to those of the original inbred strain.

In the Snell system, strain A is any inbred strain while B is some other strain, inbred or not. Strains A and B are crossed and an F2 generation is raised. The F2s and subsequent even-numbered generations are inoculated with a transplantable tumor of A strain origin and specificity. The survivors of resistant animals, who have acquired a gene(s) for resistance from the B strain, are crossed to strain A in each odd-numbered generation to progressively increase the background of A genes. After fourteen or more generations, two tumor graft survivors are mated to yield a new congenic resistant strain A.B. At this stage grafts between A and A.B. should be rejected while grafts within either strain are accepted. Note that any selective trait such as coat color or high versus low antibody responsiveness to a particular antigen also might be used to produce new congenic lines. In Bailey's procedure (Figure 1–4), delaying selection in early backcross generations allowed histocompatibility (*H*) genes determining weak antigens to be isolated. The C gene carriers among the bc_5 progeny were identified by rejection of their skin grafts on B hosts. Such grafting was repeated in each subsequent backcross generation to select *H* genes of C-strain origin while otherwise increasing the background of B-strain genes. After ten to fourteen backcross generations, an intercross of bc progeny should yield offspring ($\cong 1/4$) which are homozy-

FIGURE 1–3. Diagram showing the cross–intercross method of producing congenic lines. Graft acceptance (+), graft rejection (−). Strain A.B should carry one histocompatibility gene derived from strain B which is different from the corresponding or allelic gene in strain A. Otherwise, strain A.B should be essentially isogenic with A.

Source: Modified from Snell, *J. Natl. Cancer Inst.* 21:844, 1958.



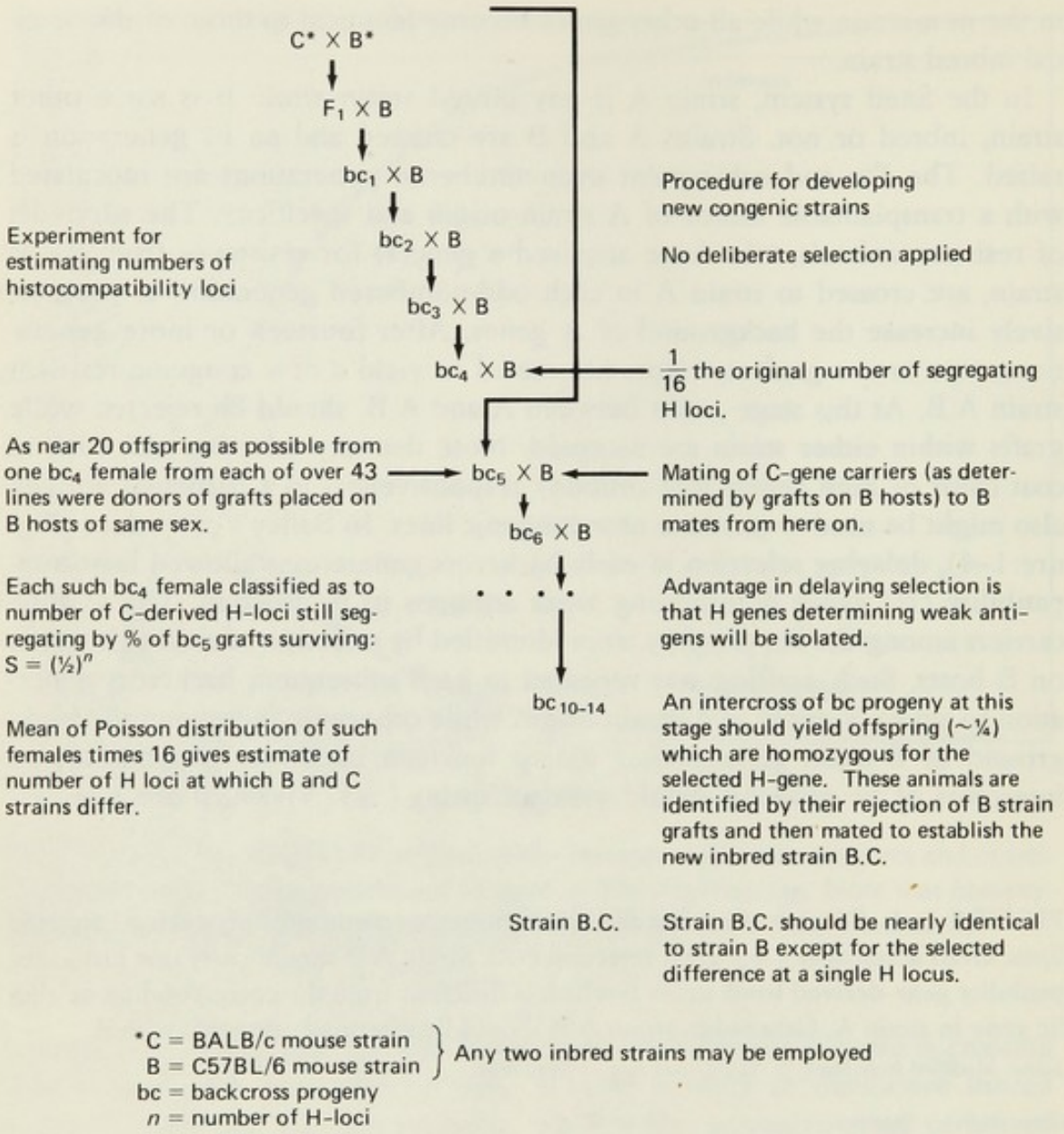


FIGURE 1–4. Bailey's procedure for developing new congenic strains and for estimating numbers of histocompatibility loci. A variety of other distinct genetic traits could be used for deliberate selection in the production of new congenic strains.

gous for the selected *H* gene. These animals are identified by their rejection of female B-strain skin grafts and are then mated to establish a new inbred strain B.C, which is congenic with the original B strain. The number of backcross generations required may vary with the particular genes under selection.

Bear in mind that the locus selected is always included in a transferred chromosome segment that contains certain "passenger" genes. The length of the introduced chromosome segment and the number of passenger or contaminant genes will decrease as the number of backcross matings to the parent strain is increased. Distinctions among closely linked genes with related functions may be made by identification and selection of recombinants for further study. Numerous such congenic recombinant strains involving the major his-

tocompatibility or immunogene complex (*H-2*) in mice now suggest that *H-2* is a "master gene cluster" or operon for immunorecognition and immunoregulation. The great advantage of congenic lines is that the functions of single gene loci and their multiple alleles can be evaluated on an otherwise identical genetic background. Congenic lines for various immunologically important traits are now available in mice, rats, and chickens. These lines have recently been exploited in characterization of transplantation incompatibilities (Chapters 5 and 6) and immunoregulatory genes (Chapter 7).

Recombinant-Inbred Strains

While inbreeding yields homozygous strains of animals of defined genotypes, segregation and recombination of known genes allow the researcher to map genes and to study their interactions or pleiotropic effects. *Recombinant-inbred* (RI) strains have been developed by Bailey and by Taylor to fix chance recombination of genes in a homozygous state in a group of strains derived from two unrelated but highly inbred progenitor strains. RI strains then are inbred lines independently derived from an F2 generation of a cross between two unrelated, inbred progenitor strains. The procedure for development of RI strains is summarized in Figure 1–5. If we consider only three independent loci distinguishing the parental strains (e.g., A_1A_1; B_1B_1; $C_1C_1 \times A_2A_2$; B_2B_2; C_2C_2), the F1 progeny will all be A_1A_2; B_1B_2; C_1C_2, but the F2 generation

FIGURE 1–5. Diagram illustrating development of recombinant-inbred (RI) strains for analyzing gene linkages and functions. Strain distribution patterns of diverse genes in RI strains may be established by comparison with established congenic strains.

Source: Bailey, *Transplantation* 11:325, 1971. © 1971 The Williams and Wilkins Co., Baltimore, MD.

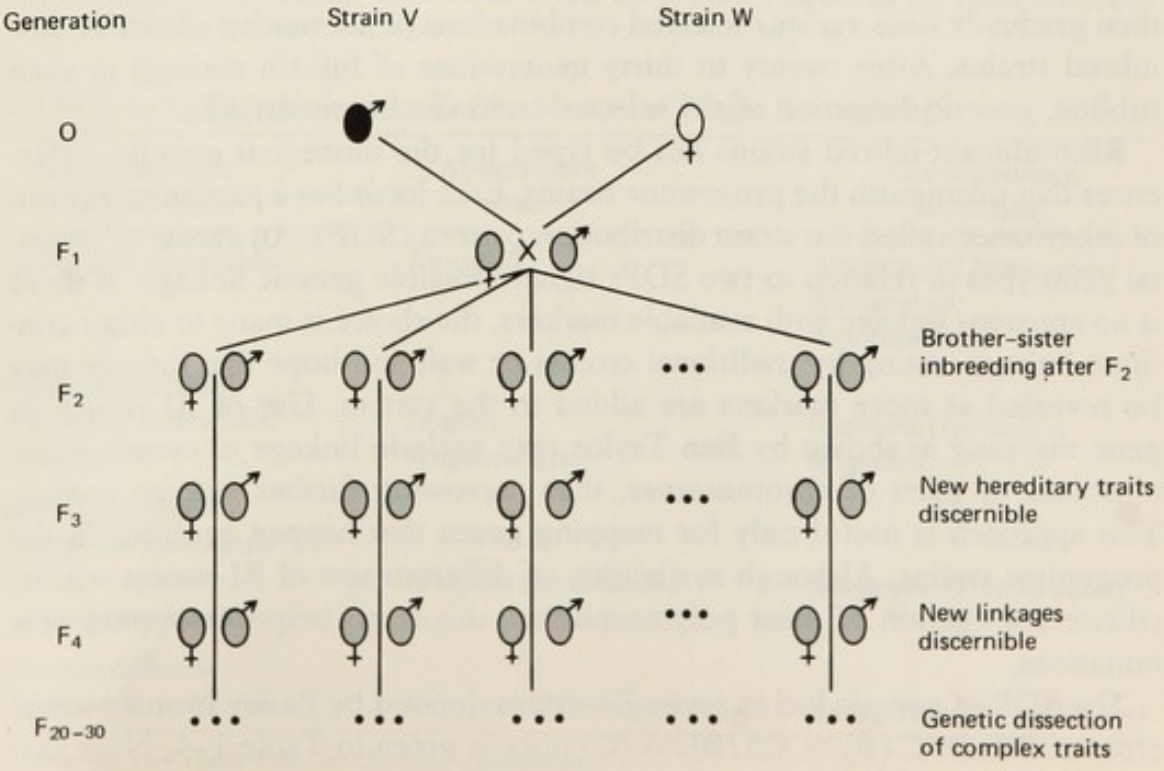


TABLE 1–4. Strain Distribution Patterns of Coat-Color Loci, Histocompatibility Loci, and Erythrocyte Alloantigen Loci in Seven RI Strains (D to K) Derived from a Cross of BALB/c × C57BL/6 Mice[a]

	Recombinant-inbred strains						
Locus[b]	CXBD	CXBE	CXBG	CXBH	CXBI	CXBJ	CXBK
a	C[c]	C	C	B	B	B	B
b	B[d]	B	C	C	C	C	B
c	B	B	C	B	C	B	B
H-1	B	B	C	B	C	B	B
H-2	C	B	B	C	B	B	B
H-8	C	C	B	B	C	C	C
H-18	C	C	C	C	C	B	C
H-19	B	C	B	C	B	C	B
H-21	B	C	C	C	C	C	B
H-22	B	C	B	C	C	C	B
Ea-4	C	B	B	C	B	B	B
Ea-6	C	B	C	B	B	B	B

[a] After Klein, *Biology of the Mouse Histocompatibility-2 Complex*, New York: Springer Verlag, 1975, p. 39.

[b] Symbols *a*, *b*, and *c* indicate coat-color loci, *H* symbols indicate histocompatibility loci, and *Ea* symbols designate erythrocyte alloantigen loci.

[c] C = BALB/c origin.

[d] B = C57BL/6 origin.

could yield sixty-four different genotypes ($2^3 = 8$ different gametes and $8^2 = 64$ gametic combinations). Such Mendelian segregation would, of course, be complicated by linkage and therefore nonindependent assortment of other genes of interest. Once the array of genes distinguishing the original parental strains have segregated in the F2 generation, strict brother–sister inbreeding then gradually fixes various selected combinations of alternative alleles in new inbred strains. After twenty to thirty generations of full-sib matings in each subline, genetic dissection of the selected traits can be undertaken.

Recombinant-inbred strains can be typed for the numerous genetic differences that distinguish the progenitor strains. Each locus has a particular pattern of inheritance called the strain distribution pattern (SDP). An excess of parental genotypes in relation to two SDPs signals possible genetic linkage. If there is no apparent linkage with available markers, the choice is made to either continue linkage testing by traditional crosses or wait and hope that linkage may be revealed as more markers are added to the system. Use of RI results in gene mapping as shown by Ben Taylor may exclude linkage to certain chromosomes or parts of chromosomes, thus narrowing further linkage testing. This approach is useful only for mapping genes that happen to differ in the progenitor strains. Although availability of different sets of RI strains will facilitate segregation of most polymorphisms, this is no help in mapping new mutations.

The SDP of genetic loci in seven RI strains derived by Bailey from an initial cross of BALB/C (B) × C57BL/6 (C) mice is given in Table 1–4. Note that

each strain shows a different pattern of alternative alleles (B or C origin) for the twelve loci listed. The segregating alleles from the original parent strains can be fixed in n lines in 2^n different patterns. Moreover, each segregant line is homozygous for one or the other allele at each segregating locus. Potential uses of RI strains include (a) *identification of gene loci:* the distinctive strain distribution patterns of RI genotypes can be used to identify newly isolated genes in new congenic lines; (b) *determination of gene functions:* correlation of particular phenotypic traits with given loci, alone or in combination, is feasible; (c) *analysis of traits dependent upon replicate observations:* traits such as resistance to particular infectious agents that often involve interactions among multiple loci (Chapter 8) may be correlated with SDPs of certain genes; (d) *detection of new gene linkages:* if a previously unknown gene exhibits the same strain distribution pattern of a known gene, the two may be linked. This should also be demonstrated by a conventional linkage test since identical strain distribution patterns could occur by chance with unlinked genes in a limited set of RI strains. Recombinant inbred lines have proved effective in the analysis of numerous traits including histocompatibility genotypes of congenic strains of mice (Chapter 6).

TERMINOLOGY OF IMMUNOGENETICS

Well-defined immunogenetic relationships have now become the rule in the evaluation of immunologic principles and pathways. In the field of comparative immunogenetics and especially tissue transplantation, precise terms are needed to indicate various donor–recipient relationships. There is now a reasonably consistent usage of terms, a happy state of affairs for the neophyte. First, consider the terms used to define sources of cells or tissue transplants as summarized in Table 1–5. Four roots or prefixes need to be remembered.

TABLE 1–5. Terminology to Define Sources of Tissue Transplants

Immunogenetic relationships	Appropriate noun[a]	Corresponding adjectives[a]
Self-to-self; compatible	Autograft	Autogeneic (Autologous) Autogenous Autochthonous
Between genetically identical individuals; compatible	Isograft Syngeneic graft	Isogeneic (Isologous) Syngeneic
Between genetically disparate individuals of same species; usually incompatible	Allograft (Homograft)	Allogeneic (Homologous)
Between different species; incompatible	Xenograft (Heterograft)	Xenogeneic (Heterologous)

[a]Old terms, now rarely used, are shown in parenthesis.

Auto- always defines a self-to-self, usually compatible relationship between cell sources. The generally equivalent relation between genetically identical individuals (i.e., monozygotic twins or animals of like sex from the same inbred strain) is denoted by *iso-* or *syn-*. The term syngeneic has become more popular than isogeneic, but the noun "syngraft" is not in use. *Allo-* always denotes genetically disparate individuals of the same species. The term "semiallogeneic" or "semisyngeneic" is often used to indicate a unidirectional disparity of inbred strain → F1 hybrid offspring, where the genotype of the parent is presumably not foreign to the F1 hybrid. This assumption can be invalid for sex-associated antigens as we shall see later. Finally, the term *xeno-* is restricted to relationships or disparities between different species.

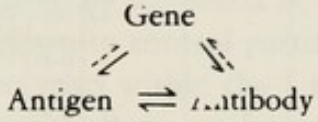
An etymologically consistent vocabulary is applied to types of antigens and antibodies, insofar as the terms *xeno-*, *allo-*, and *auto* are concerned (Table 1–6). However, several additional terms are employed. Certain potential antigens that are shared by widely disparate species ranging from microorganisms to mammals are called heterogenetic antigens. A notable example important in diagnostic microbiology is the Forssman antigen, a structural carbohydrate–lipoprotein complex of cell walls. Tissue-specific or organ-specific antigens identify specificities peculiar to a given tissue or organ, such as thyroglobulin

TABLE 1–6. Terminology for Types of Antigens and Antibodies

Immunogenetic designation			
Antigen	Antibody	Properties	Examples
Heterogenetic	Heterogenetic	Involve same or similar antigens shared by several or many species	Forssman antigen of diverse bacteria and vertebrates
Xenoantigens	Xenoantibodies	Involve species-specific antigens, characteristic of all individuals of a given species	Goat anti-human or Rabbit anti-mouse
Alloantigens	Alloantibodies	Involve individual-specific antigens, distinguishing individuals of different phenotype within a species	Human anti-HLA-A1 or Mouse anti-H-2.31
Organ-specific or Tissue-specific	Organ-specific or Tissue-specific	Involve antigens peculiar to a given tissue or organ	Thyroglobulin of thyroid gland or Myelin of central nervous system
Autoantigens	Autoantibodies	Involve self-antigens or antibodies to autogeneic molecules within an individual	Cardiolipin or Nuclear DNA

of the thyroid gland. Here we should also include "differentiation antigens," i.e., specificities characteristic of particular tissues at certain stages of development or maturation. Thus, lymphocyte-specific antigens designated Lyt 1, 2, and 3 in mice define distinctive subclasses of thymus-derived lymphocytes. There are also fetal antigens, often tissue-specific, that normally disappear in adult life.

In elementary terms, immunogenetics may be defined as the study of gene–antigen–antibody relationships. The "gene → antigen" component involves the elucidation of genetic fine structure and function in relation to structural determinants of macromolecular antigens (Chapters 3 to 6). "Gene → antibody" relationships may be decisive in regulating immune responses because given genes control specific antibody responses (Chapters 2 and 7). The manifestations and mechanisms of "antigen ⇄ antibody" reactions are, of course, central to general immunology as well as immunogenetics. The scope of immunogenetics may be illustrated with a triangle as follows:



Reciprocal arrows are meant to indicate all possible biosynthetic or regulatory interactions among specific genes, antigens, and antibodies. Relationships indicated by dashed arrows are little understood, but include the regulatory network of feedback signals that determine how and when immunocytes function.

TYPES OF GENES AFFECTING IMMUNOLOGIC CHARACTERISTICS

Many gene loci have now been identified with distinctive roles in immune responsiveness. These genes, governing a broad array of molecular properties, are scattered all over the chromosome map. Yet some with special interrelated functions occur in closely linked clusters, most notably the major histocompatibility or immunogene complex (*MHC* or *MIC*). The most numerous array of genes of immunogenetic interest have been identified in the laboratory mouse (*Mus musculus*). A partial linkage map of the mouse showing the distribution of some ninety genes localized to twenty of the twenty-one chromosomes is represented in Figure 1–6. Many other loci affecting immunologic characteristics (i.e., immunogenes) have been identified by genetic analysis of experimental progenies, but remain to be mapped. Comparison of mouse and human linkage maps reveals many similarities; many murine immunogenes thus are likely to have human counterparts.

For introductory purposes we may divide immunogenes into six broad categories. First is the diverse category of alloantigen-determining loci indicated by asterisks in Figure 1–6. Individual-specific or strain-specific markers in this grouping are demonstrable by serologic tests with monospecific antibodies.

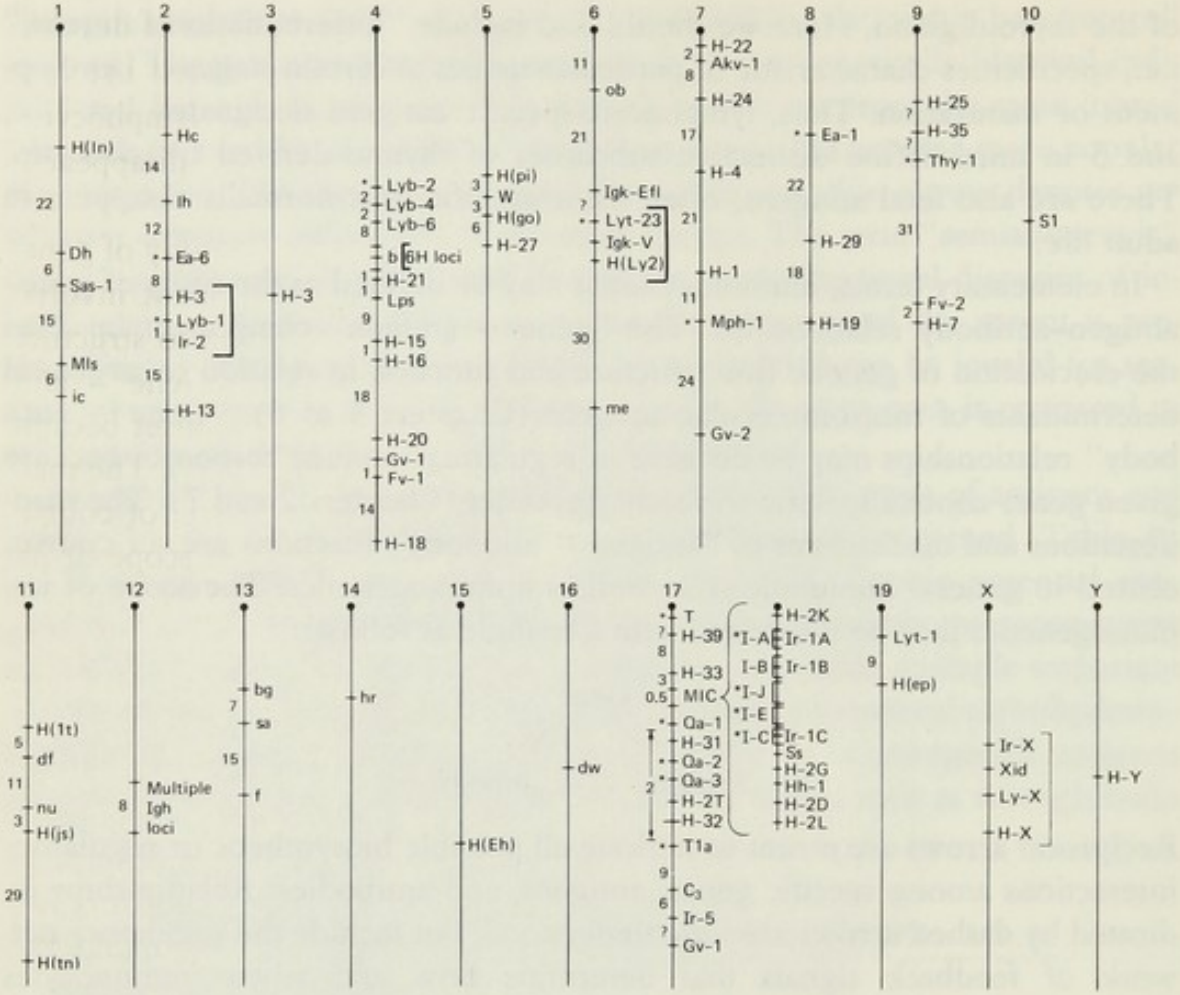


FIGURE 1–6. Partial linkage map of the mouse identifying loci of immunogenetic interest. Many other loci affecting immunologic characteristics have been identified but remain to be mapped. Alloantigen-determining loci, including tissue-specific or differentiation antigens demonstrable serologically with monospecific antibodies, are indicated by asterisks. Histocompatibility loci with products known to be capable of provoking allograft rejection are designated by an *H* followed by a number (e.g., *H-1* to *H-39*) or a provisional letter symbol (e.g., *H (ep)*). Immune response or immunoregulatory loci known to control particular antibody responses are designated by the prefix *Ir*. Genes specifying immunoglobulin structure are given the prefix *Ig*. Numerous genes affecting pigmentation, hair growth, and other visible traits (e.g., *ic*, *W*, *ob*, *me*, *Sl*, *nu*, *bg*, *sa*, *hr*, *dw*) also influence development of the immune system. These immunogenes also affect susceptibility to certain infectious diseases or leukemia. Still other murine genes control virus expression (e.g., *Fv-1* and *2*, *Gv-1* and *2*, *Akv-1*). Brackets enclosing linked genes on chromosomes 2, 4, 6, and X indicate that sequential order of genes is unknown. See text for further details.

Certain alloantigens are distinctive markers for the cell membranes of erythrocytes (e.g., Eal to Ea6) or leukocytes (e.g., Mph-1, Thy-1). Others appear to be quite tissue-specific, including differentiation antigens limited to certain cell types at selective stages of maturation. Thus, Tla and Lyt antigens are found only on thymus-derived lymphocytes, while Lyb antigens occur principally on antibody-producing B-lymphocytes. In addition, there are allotypes

characteristic of immunoglobulin polypeptides and other serum proteins. For example, the closely linked *Ig-1* to *Ig-6* genes recently mapped to chromosome number 12 in mice specify immunoglobulin heavy chain (*Igh*) differences (Chapter 2).

A second major category is defined by the histocompatibility (*H*) loci specifying cellular alloantigens known to be capable of provoking allograft rejection. At least forty *H* loci, most represented by multiple alleles, have been detected in mice, but many have yet to be definitively mapped. Allogeneic disparities at given loci between donor and recipient strains are often termed strong, moderate, or weak, depending on the intensity of the reactions or the duration of allograft survival. The strength of a histocompatibility barrier depends more on the interallelic combination and tissue source of the test graft than the *H* locus as such (Chapters 5 and 6). However, the *MIC* now appears to represent a unique continuum of closely linked genes essentially involved in immunorecognition and immunoregulation.

Our third category, designated by the prefix *Ir,* consists of immune response or immunoregulatory loci known to control particular antibody responses. Although at least several *Ir* loci map within the I region (i.e., *Ir-1A, Ir-1B,* and *Ir-1C*) of the *MIC* in mice, many others are known to be genetically independent. Known *Ir* genes are mainly immunoregulatory, influencing T- or B-lymphocyte activity in specific responses to defined antigens (Chapter 7). For example, different alleles of these loci often regulate high versus low responsiveness, or the capacity to switch over from early IgM to later IgG antibody production. Numerous structural genes for variable portions of immunoglobulin heavy chains (*Igh*) are clustered on chromosome 12, whereas variable region loci for light chains map elsewhere (e.g., *Igk-V* on chromosome 6; see Chapter 2). The designation *immune response genes* is often used in a generic sense to include both structural genes governing antibody specificity and regulatory genes controlling immune pathways as well as high versus low responsiveness. Three gene loci (*C3, Ss,* and *Hc*) specifying three (C3, C4, and C5, respectively) of some twenty known serum complement components have now been mapped, with the *Ss* for component C4 residing within the *MIC.* This multiple source of nonspecific immune amplification collectively designated *complement* remains an enigma.

The fourth group of immunogenes are associated with primary immunodeficiencies. Here we include nude or *nu,* a recessive gene on chromosome 11 yielding hypothymic mice with a profound deficiency in the T-lymphocyte functions of cell-mediated immunity. In contrast, spleenless mice with restricted antibody-producing capacity result from the presence of the dominant hemimelia gene *Dh* on chromosome number 1. Two independent recessive dwarf mutants, *df* and *dw,* are attributable to defective development of the anterior pituitary gland. Absence of the pituitary hormones precludes normal development of the immune system, although precise causes and effects, including thymic hormone regulation, are obscure. Genes in this category obviously affect major pathways involved in nearly all immune responses.

A fifth group is comprised of numerous genes identified secondarily with immunodeficiencies. Included here are genes affecting pigmentation, hair growth, obesity, and other visible traits: e.g., *ic* (ichthyosis), *W* (dominant spotting), *ob* (obesity), *me* (motheaten), *Sl* (steel), *bg* (beige), *sa* (satin), *hr* (hairless), *f* (flexed tail). These genes also influence susceptibility to certain infectious diseases or to leukemia, and thereby qualify as immune response genes (Chapter 8).

Finally, other multiple independent murine genes (*Fv-1* and *-2; Gv-1* and *-2*) control proliferation or expression of given viruses such as Friend and Gross leukemia viruses, possibly by influencing lymphocyte and macrophage functions. Expression of the G_{IX}^{+} thymocyte phenotype in mice requires the presence of positive alleles at both *Gv-1* and *Gv-2* loci. Because of quasi linkage, it is not clear whether *Gv-1* is located on chromosome 4 or chromosome 17. (Quasilinkage describes nonindependent assortment of two genes that cannot be truly linked because they are situated on different chromosomes.) Although genetic resistance has a polygenic basis in these and other virus infections (e.g., polyomavirus and mammary tumor virus), effective control by single dominant genes (e.g., Group B togaviruses or ectromelia) or by a recessive gene (hepatitis virus) has been reported. In this broad category, inherited susceptibility as a function of host genotype assumes a uniform pathogen genotype and a reasonably uniform environment.

This, then, is an introductory overview of the concepts and scope of immunogenetics circa 1980. The next seven chapters deal with each major topic in depth. The biological relevance of immunogenetics for medicine emerges in Chapters 2 through 8; the last chapter presents a phylogenetic perspective.

KEY REFERENCES

Books

Altman, P. L. and Katz, D. D. (eds.). *Inbred and Genetically Defined Strains of Laboratory Animals. Part 1. Mouse and Rat.* Bethesda: Federation of American Societies for Experimental Biology, 1979. A biological handbook containing detailed tabular summaries of immunogenes and their phenotypic manifestations.

Benacerraf, B. (ed.). *Immunogenetics and Immunodeficiency.* Baltimore: University Park Press, 1975. A multiauthored volume dealing with the genetics of immunoglobulins, major histocompatibility complexes, and genetic control of immune responses.

Fudenberg, H. H., Pink, J. R. L., Wang, A. C., and Douglas, S. D. *Basic Immunogenetics,* 2d ed. New York: Oxford University Press, 1978. Emphasizes genetics of immunoglobulin molecules and human blood groups.

Hildemann, W. H. *Immunogenetics.* San Francisco: Holden-Day, 1970. The original monograph covering the whole field from the perspective of the 1960s.

Hood, L. E., Weissman, I. L., and Wood, W. B. *Immunology.* Menlo Park: Benjamin/Cummings, 1978. A concise, well-illustrated textbook of immunology.

Landsteiner, K. *The Specificity of Serological Reactions.* Rev. Ed. New York: Dover Publications, 1962. A classic monograph, still worth perusing, by an outstanding early pioneer.

Strickberger, M. W. *Genetics,* 2d ed. New York: Macmillan, 1976. A comprehensive introductory text in genetics.

Articles

Bailey, D. W. Recombinant-inbred strains. An aid to finding identity, linkage, and function of histocompatibility and other genes. *Transplantation* 11:325–327, 1971.

Borysenko, M. Phylogeny of Immunity: An Overview. *Immunogenetics* 3:305–326, 1976. A brief review of major findings in comparative immunogenetics.

Giblett, E. R. Genetic polymorphisms in human blood. *Annu. Rev. Genet.* 11: 13–28, 1977. A brief review of red cell allotypes, white cell allotypes, plasma protein allotypes, and their disease associations in man.

Green, E. L. Breeding systems. In E. L. Green (ed.). *Biology of the Laboratory Mouse.* New York: McGraw-Hill, 1966. Pages 11–22. Systems of random breeding and of inbreeding and selection to produce genetically defined animals are described.

Haldane, J. B. S. The genetics of cancer. *Nature* 132: 265–267, 1933. This was the first presentation of the "one autosomal dominant gene → one antigen" theory, extending the classic concepts of C. C. Little (see also *Science* 40: 904–906, 1914).

Hood, L., Campbell, J. H., and Elgin, S. C. R. Organization, expression, and evolution of antibody genes and other multigene families. *Annu. Rev. Genet.* 9: 305–353, 1975. Analyzes genetic bases of antibody diversity in a broad perspective.

Irwin, M. R. The beginnings of immunogenetics. *Immunogenetics* 3: 1–13, 1976. Brief historical review of the marriage of genetics and immunology by a pioneer.

Little, C. C. and Tyzzer, E. E. Further studies on inheritance of susceptibility to a transplantable tumor of Japanese waltzing mice. *J. Med. Res.* 33: 393–425, 1916. A classic early study in the field of transplantation immunogenetics.

Snell, G. D. Histogenetic methods. In G. D. Snell, J. Dausset, and S. Nathenson. *Histocompatibility.* New York: Academic Press, 1976. Pages 11–21. Production and analysis of congenic resistant strains are described.

Taylor, B. A. Recombinant inbred strains: Use in gene mapping. In H. C. Morse (ed.). *Origins of Inbred Mice.* New York: Academic Press, 1978.

Wachtel, S. S., Thaler, H. T., and Boyse, E. A. A second system of alloantigens expressed selectively on epidermal cells of the mouse. *Immunogenetics* 5: 17–23, 1977. A consideration of tissue-specific antigens and differentiation antigens in the context of cell-surface immunogenetics.

Weigert, M. and Riblet, R. Genetic Control of Antibody Variable Regions. *Cold Spring Harb. Symp. Quant. Biol.* 41: 837–846, 1977. Summarizes evidence concerning organization of immunoglobulin genes and germ-line versus somatic generation of antibody diversity.

2

GENETICS OF ANTIBODY MOLECULES

THE PRODUCTION OF ANTIBODIES

Antibodies are molecular mediators produced by plasma cells that are the differentiated end product of stimulation and proliferation of B-lymphocytes. The ability of the immune system to produce antibodies capable of specifically reacting with virtually all possible antigens suggests unusual complexity of structure and function in these molecules. Both the structural characteristics and the production of antibodies are under genetic control; the former being governed by complex multigene families and the latter by immune response or immunoregulatory (*Ir*) genes to be discussed in Chapter 7.

Cellular Pathways

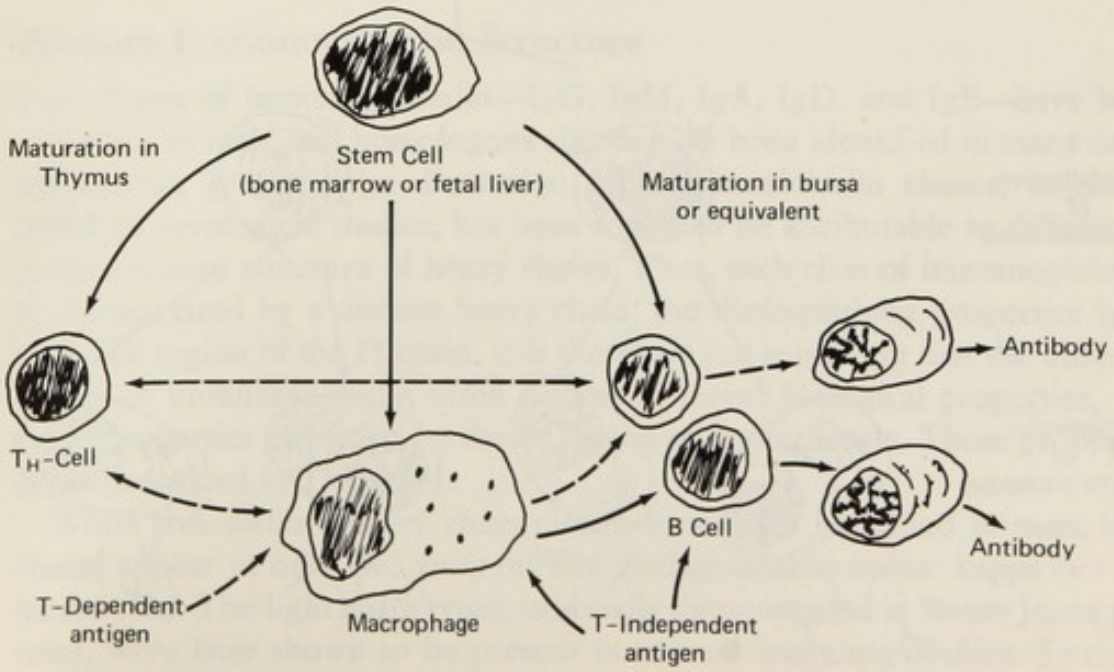
The pathways of cellular involvement in antibody production are outlined in Figure 2–1. Depending on the nature of the antigen, initial cellular stimulation may occur at either the T- or B-lymphocyte level. Many polymeric antigens such as pneumococcal polysaccharides, polymerized bacterial flagellin, and hapten conjugated Ficoll are capable of eliciting antibody in the absence of functional T-lymphocytes and are therefore said to be T-independent. However, most immunogens, particularly the common protein antigens, require the presence of functional T- and B-lymphocytes in order to elicit a humoral response. The mechanisms of such cellular cooperation are still not fully understood, but experiments using hapten–carrier systems (i.e., a small molecule coupled to a "carrier" macromolecule) indicate that the T-cell reactivity is carrier-specific and that T-cells may recognize larger "carrier determinants" in all T-cell-dependent antigens. The helper T-cell role in such responses is seen as one of pattern recognition and antigen focusing to enable the presentation of the antigen to the B-cell in a suitably stimulating form. The collaboration of T- and B-lymphocytes in humoral immunity is restricted by genes of the major histocompatibility or immunogene complex (*MHC* or *MIC*). Thus, recognition

or display of specific MIC products on the cell surface is usually essential for T/B collaboration. Moreover, the subpopulation of T-lymphocytes involved in this process, the T-helper cells, depend on regulatory *Ir* genes residing in the *I* region of the *MIC* in mice.

Macrophages (i.e., histiocytes and monocytes) also play an important, but as yet unclear, role in T- and B-cell collaboration as shown by the fact that in vitro systems devoid of macrophages are incapable of yielding antibody-forming cells. Soluble factors, produced by T-cells in response to antigen stimulation, also appear to participate in T/B collaboration. It has been proposed that an antigen-specific factor released from the T-cell may fix to macrophages and enable them to bind high concentrations of antigen for presentation to the B-cell. Furthermore, in vitro B-cell activation by soluble factors released from macrophages has been demonstrated. Macrophages also secrete products inhibitory to B-cell clonal expansion through mitogen stimulation, and it therefore appears probable that macrophages exercise a regulatory role in T/B collaboration and the consequent clonal proliferation of B-cells.

In view of the fact that both T- and B-cells are able to interact with antigen, it is obvious that both cell types possess antigen-binding receptors. On B-cells, this receptor has been identified as membrane bound immunoglobulin of the same specificity that will ultimately be secreted by the stimulated cell. The T-

FIGURE 2–1. Cellular involvement in antibody production. The two lymphocyte lines, B and T, are derived by different processing pathways from multipotent hemopoietic stem cells in the bone marrow. Macrophages also differentiate from stem cells. Certain antigens, those that are T-independent, are able to act directly on B-cells, causing plasma cell formation and synthesis of specific antibody. T-dependent antigens require collaboration of T-cell subsets and B-lymphocytes, and macrophages as well, in order to elicit antibody production by the B-cell.



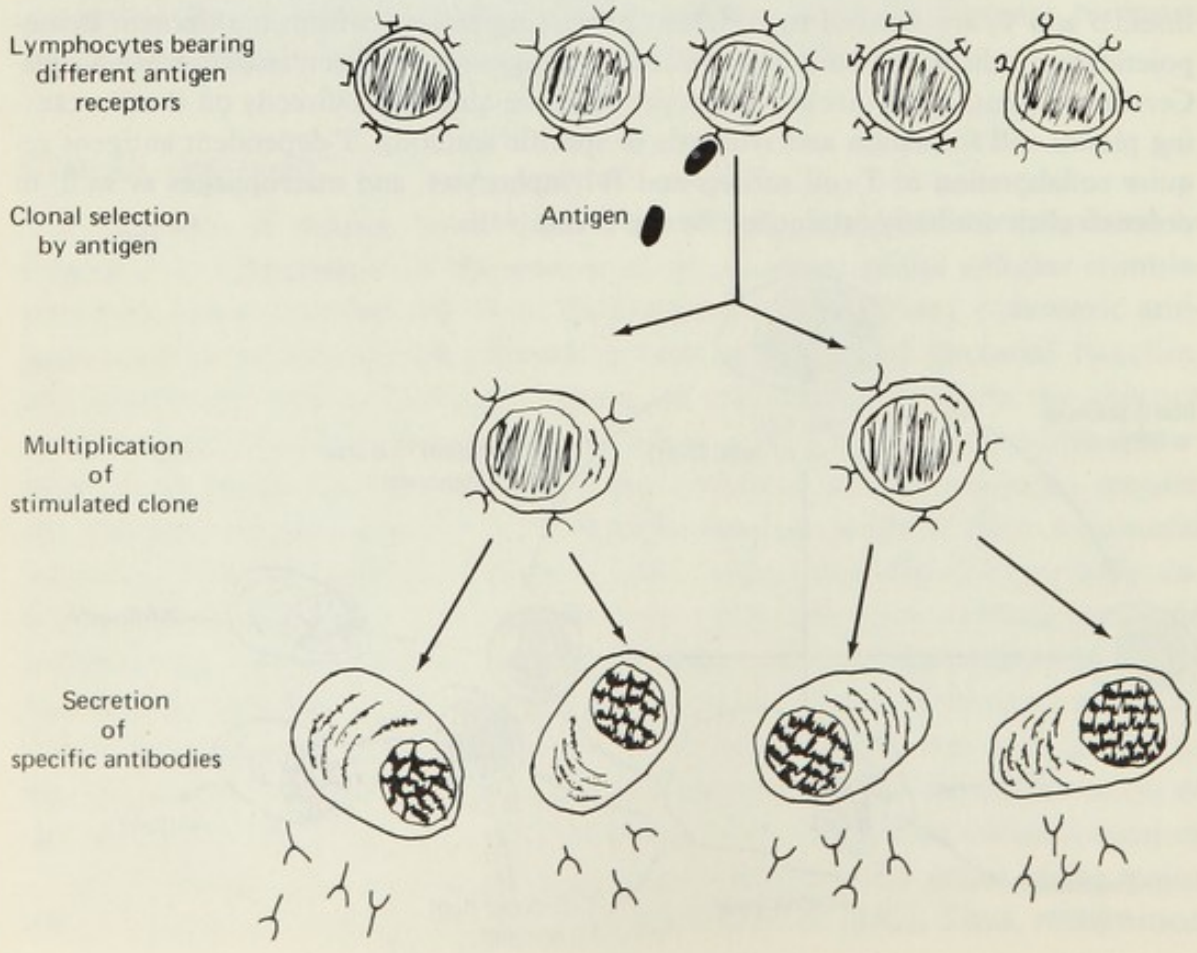
cell receptor remains a point of conjecture with products of the *MIC* and/or a modified immunoglobulin being the major contenders.

Antibody is secreted by a differentiated B-lymphocyte, the plasma cell, or antibody-forming cell (AFC). A single AFC is committed to producing antibody of a single antigen-binding specificity, although an AFC clone may produce antibodies of different classes during the progression of the immune response.

Clonal Selection

The term clonal selection was introduced by Burnet to describe the process of expansion and differentiation of the immunocompetent small lymphocyte (B-cell) to the formation of the antibody forming cell. As shown in Figure 2–2, this process is driven by stimulation of those particular immunocompetent cells in the total population that bear receptors specific for the antigen introduced. These cells, representing only a small number of the total population,

FIGURE 2–2. Clonal selection model. Antigen sensitive lymphocytes each express a pair of *V* region genes for V_H and V_L in the form of an antigen receptor on the cell surface. Antigen interacts with the appropriate cell by combining specifically with the membrane-bound receptor, thus stimulating the cell to undergo rapid division leading to a clone of daughter cells synthesizing and secreting the specific antibody.



are stimulated to undergo rapid cellular division and differentiation and thus produce a clone of antibody-forming cells. Thus, the initially small number of responding cells is greatly amplified. Finally, some of the differentiated cells become long-lived memory cells that are capable of rapid differentiation to antibody-forming cells upon second contact with the antigen.

ANTIBODY STRUCTURE

The Multichain Structure of Immunoglobulins

The pioneering studies of Porter on rabbit γ globulins and Edelman on a human myeloma protein demonstrated the multichain structure of the immunoglobulin G molecule and led to an upsurge of endeavor in the field of molecular immunology. The results of enzymic digestion using pepsin and papain and reduction of disulphide bonds under varying conditions led Porter to propose that rabbit IgG consisted of four polypeptide chains, two heavy chains (H) each of 50,000 daltons and two light chains (L) each of 20,000 daltons, held together by disulphide bonds (Figure 2–3). The products of enzyme cleavage and of reduction of disulphide bonds are also illustrated in Figure 2–3. The Fab fragment obtained by papain digestion of the immunoglobulin molecule retains the antigen-binding specificity of the intact molecule. However, as this fragment is monovalent it is unable to precipitate or agglutinate antigen. The Fc fragment, so named for its ability to form crystals (a property indicating the homogeneity of its primary structure), is important in conferring distinctive biological properties on each molecular class of immunoglobulin molecules. These properties include the ability of the intact molecule to fix complement or to cross the placenta.

Primary Immunoglobulin Structure

Five classes of immunoglobulin—IgG, IgM, IgA, IgD, and IgE—have been described in man, and homologous classes have been identified in many other mammalian species. The distinction of immunoglobulin classes, originally based on serological studies, has been found to be attributable to differences in the primary structure of heavy chains. Thus, each class of immunoglobulin is characterized by a distinct heavy chain, the distinguishing properties lying in the Fc region of the H chain. It is therefore not surprising that the different classes of immunoglobulin often possess different biological properties, i.e., those properties governed by the Fc region of the molecule. These properties are summarized in Table 2–1.

While five distinct heavy chain classes have been identified in man, light chains appear in only two antigenically distinguishable forms: kappa (κ) and lambda (λ). The light chain types, originally demonstrated in Bence Jones proteins, were later shown to be present in normal immunoglobulins. So-called

Bence Jones proteins are L-chain monomers and dimers abundantly present in the urine of individual mammals with multiple myeloma, a cancer of B-cells in which a clone of plasma cells secretes a homogeneous immunoglobulin called myeloma protein. This protein, distinctive in each afflicted individual, may comprise 95 percent of the serum immunoglobulin. The antigenic difference of κ and λ are reflected in variations in amino acid sequence of the carboxyl terminal half of the light chain molecule. Two light chain types, analogous to human κ and λ, have been identified in the mouse and the rabbit.

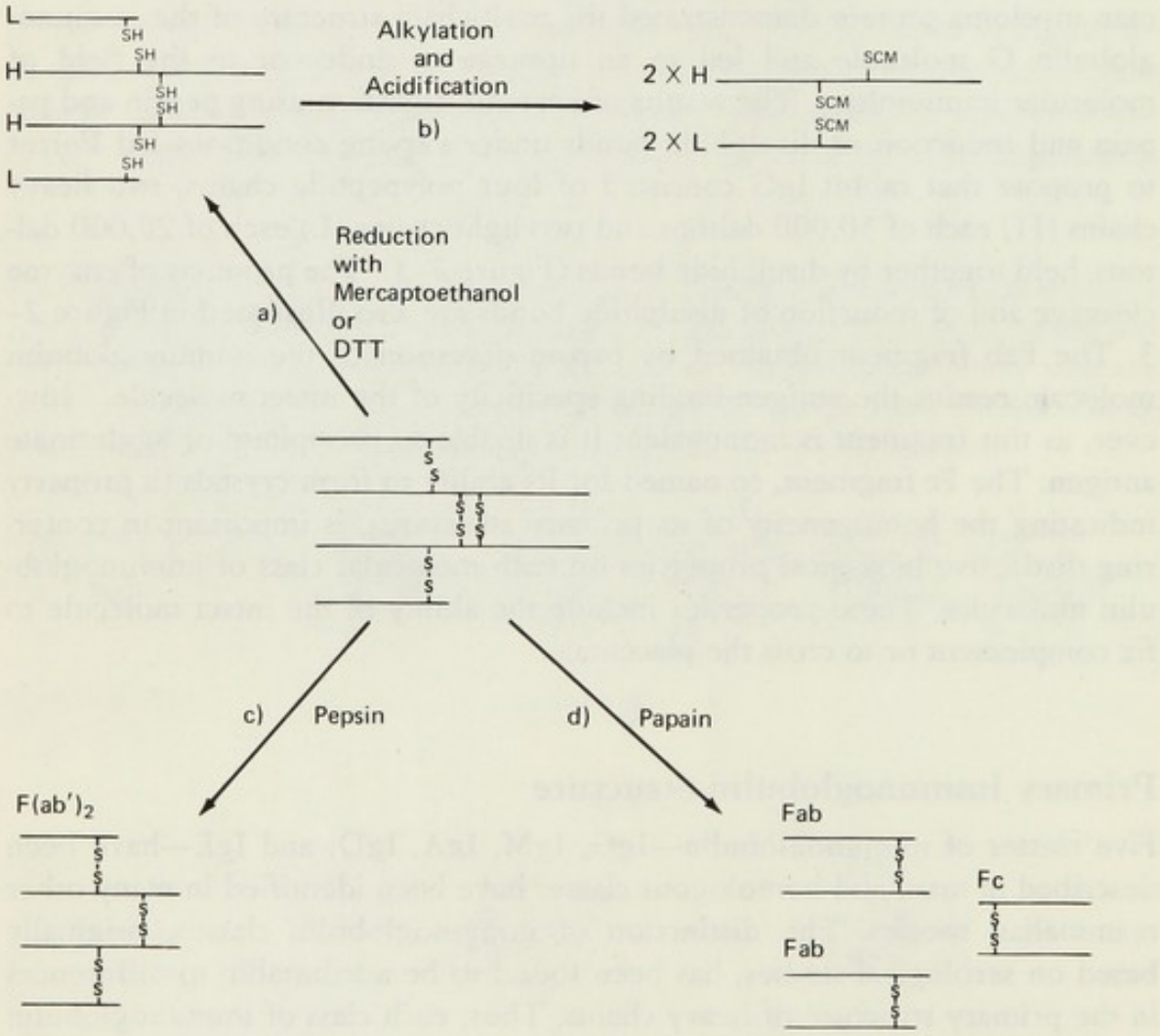


FIGURE 2–3. Degradation of IgG. (a) Reduction of IgG in neutral buffers using 0.1M 2-mercaptoethanol or 0.02M dithiothreitol results in cleavage of all interchain disulphide bonds. (b) Alkylation of reduced IgG with iodoacetamide introduces carboxymethyl groups onto the free sulfhydryl groups, thus preventing reformation of disulphide bonds. Under acid conditions the carboxymethylated polypeptide chains can be separated by gel filtration. (c) Digestion with pepsin yields a divalent $F(ab')_2$ fragment and a small fragment representing the remainder of the H chains. (d) Papain digestion results in formation of two univalent Fab fragments and the Fc fragment consisting of the COOH terminal halves of the H chains joined by disulphide bonds.

TABLE 2–1. Distinctive Features of Major Molecular Classes of Human Immunoglobulins

Immunoglobulin class	Sedimentation constant (S)	Number of four-polypeptide chain subunits	Heavy chain MW	Mean serum concentration mg/ml	Complement fixation pathways		Placental transfer	Fix to mast cells and basophils
					Classical	Alternate		
IgG	7	1	51,000–60,000	12	+[a]	–	+	–
IgM	19	5	65,000	1.5	+	–	–	–
IgA	7	1	52,000–56,000	3.5	–	+	–	–
sIgA	11	2	52,000–56,000	0.05	–	+	–	–
IgD	7	1	70,000	0.03	–	–	–	–
IgE	8	1	72,500	0.00005	–	–	–	+

[a]Human IgG_4 does not fix complement via the classical pathway, but the remaining IgG_1, IgG_2, and IgG_3 subclasses apparently do.

Amino acid sequence analyses of light and heavy chains revealed the existence of regions of constant and variable sequence within these chains. Thus, sequence comparison of human κ chains showed the amino terminal half (107 residues) to be highly variable while the carboxyl half (107 residues) was constant in sequence except for single amino acid interchanges at positions 153 or 191 reflected in the Km or InV allotypes (see p. 39). Constant and variable regions of amino acid sequence were similarly identified in H chains. The variable region of the H chain extends 110 to 115 residues from the amino terminus, the remainder of the chain being constant in sequence within members of the same heavy chain class. The constant region of the heavy chain can be further divided into three regions of homology or domains. The heavy chain constant domains, each extending 101 to 107 residues, are homologous in sequence to one another as well as to similar domains in the light chain. Both the variable regions and the constant homology regions contain an intrachain disulphide bond, giving these domains a characteristic loop structure (Figure 2–4). The differences in the classes of heavy chain, which have been ascribed

FIGURE 2–4. Diagrammatic representation of the structure of human IgG showing the loops formed by the folding of the polypeptide chains within the intrachain disulphide bonds. The homology regions, or domains, are designated as: VL, VH—homology units of variable regions of heavy (H) and light (L) chains—CL, CH_1, CH_2, CH_3—homology units of the constant regions of L and H chains.

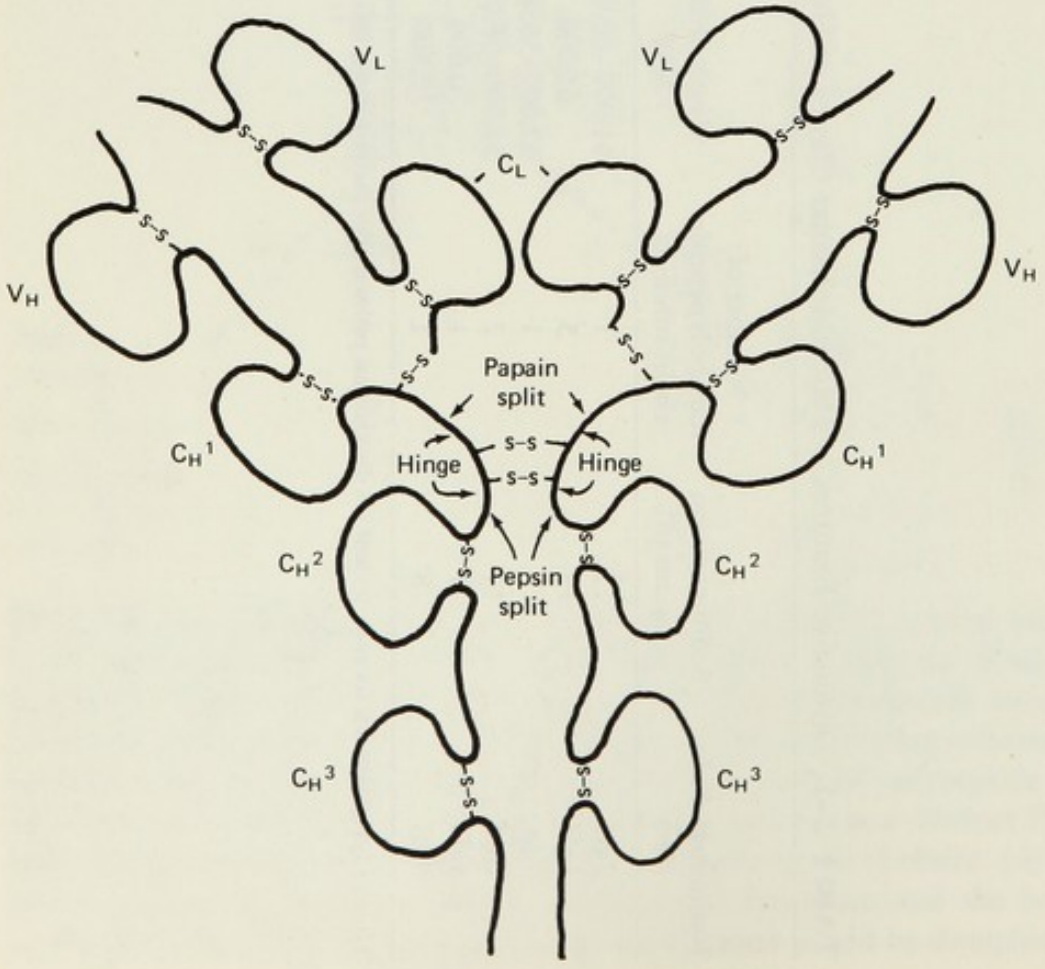
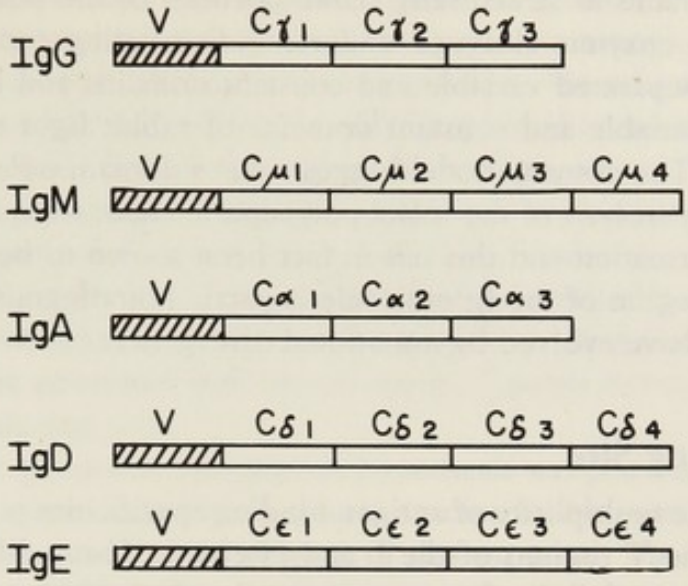


TABLE 2–2. Heavy Chain Homology Regions of the Five Major Classes of Mammalian Immunoglobulins



to the Fc portion of the molecule, are reflected in different numbers of domains making up the constant region of the heavy chains (Table 2–2).

Even within the variable regions, some sequence homologies can be seen when comparing these regions within a given polypeptide chain type. On the basis of these homologies, the variable regions of human κ L chains can be assigned to one of three subgroups: $V\kappa_I$, $V\kappa_{II}$, or $V\kappa_{III}$. Human λ chains are subdivided into four subgroups ($V\lambda_I \rightarrow V\lambda_{IV}$), and the variable regions of human heavy chains may belong to one of four proposed subgroups ($VH_I \rightarrow VH_{IV}$). A survey of a large number of variable region sequences from human light chains revealed three areas within the V region that exhibit hypervariability of sequence. These hypervariable regions of the light chain are: L1, residues 23 to 36; L2, residues 52 to 58; and L3, residues 91 to 99. Corresponding hypervariable regions have been found in the heavy chain from residues 31 to 36 (H1), 49 to 66 (H2), and 99 to 104 (H3). A fourth hypervariable region, He, extending from residues 81 to 85, has recently been described in human heavy chains. The implications of these hypervariable regions in determining binding specificity of the antibody are discussed later in this chapter.

The Domain Model of Immunoglobulin Structure

The demonstration of homology regions within the light and heavy chains of the immunoglobulin molecule formed the basis of the domain hypothesis proposed by Edelman. In this model, Edelman proposed that each domain, formed by a separate V or C homology region, has a discrete function and is stabilized by an intrachain disulphide bond which holds the homology region

in a loop formation (see Figure 2–4). X-ray diffraction studies have revealed the folding pattern of a homology region to be consistent with this model. The existence of domains as structurally stable portions of the polypeptide chains is supported by enzymic cleavage under nondissociating conditions of light chains to yield separated variable and constant domains and by specific cleavage between variable and constant domains of rabbit light chains by dilute acid hydrolysis. The domain model suggests that a domain isolated by selective proteolysis or hydrolysis of the intact polypeptide chain would retain its main structural conformation and this has in fact been shown to be the case. Note that the hinge region of the Ig molecule is partly homologous to the CH domains and may have evolved by mutational divergence.

The Combining Site

The origin of the multiplicity of antigen-binding specificities possessed by antibodies lies in the V regions of the L and H chains. X-ray diffraction studies on several immunoglobulin fragments have shown that the combining site is formed by bringing together the hypervariable regions of VL and VH. Affinity labeling studies of hapten-binding myeloma proteins have identified residues in VL and VH regions as being in close contact with bound hapten. These residues all lie within the previously described hypervariable regions. Obviously, the primary structure of the V regions outside the hypervariable sequences must influence the nature of the combining site through the effect exerted on the tertiary structure of the chain and in particular the folding and spatial positioning of the hypervariable regions. The nonhypervariable residues of the V regions, known as the framework portions of the chain, are remarkably invariant, and x-ray crystallography of human and mouse V domains has shown striking homologies in structure in the framework portions of VH and VL regions. Thus, the framework of the V domains tends to stabilize the architecture, thereby allowing the hypervariable regions to interact to form the combining site. The fine architecture, or antigen-binding specificity, of the combining site is then determined by the sequences of the hypervariable regions. Although a single antibody molecule has a unique three-dimensional structure, it can combine not only with the inducing antigenic determinants, but with similar structures that thereby become cross-reacting antigens. In this sense, individual antibodies can be regarded as potentially multispecific.

Two Genes, One Polypeptide Chain?

The existence of both variable and constant regions within immunoglobulin in L and H chains is an apparent paradox; however, the immunoglobulin molecule has dual functions of specific antigen binding and broad biological activities. What mechanism may operate that allows for the genetic coding of such diverse areas within a single molecule? The most plausible explanation is that the individual polypeptide chains of the immunoglobulin molecule (L

and H) are each encoded by two gene families, one for the variable region of the molecule and the other for the constant region. Thus, the single polypeptide chain arises from two structural genes or clusters of genes through some process of joining at an early stage leading to immunoglobulin synthesis.

The existence of multiple human Vκ regions (i.e., Vκ subgroups) associated with a single constant region (Cκ) is readily explained by the existence of separate *V* and *C* region genes. Hence, multiple *V* region genes exist, any of which can be expressed in association with the *C* region gene. Moreover, a given *V* region gene can join to more than one *C* region gene as in instances where human V_H subgroups have been found in association with C regions showing different immunoglobulin class specificity. In essence, a single V_H gene can become associated with two or more C_H genes during differentiation of antibody-producing cells.

While the two gene–one polypeptide hypothesis was postulated on the basis of indirect experimental evidence, recent studies by Hozumi and Tonegawa have supplied concrete data in support of this proposal. Their approach was to cleave cellular DNA with a restriction enzyme and then hybridize the resulting DNA fragments with an mRNA specific for either a complete κ chain or only the C region of the chain. This technique showed that the *V*κ and *C*κ genes in embryonic cells are separated on the chromosome, while in the fully differentiated plasma cell, the two genes are recombined to form a more contiguous stretch of DNA (Figure 2–5). Several models have been proposed to explain the mechanism by which *V* and *C* region genes separated in the embryo may be joined during differentiation of B-lymphocytes. In effect, a given *V* gene is inserted in the chromosome adjacent to a *J* or joining gene contiguous with the *C* gene. Widely separated *C* and *V* genes may thus be brought together when the DNA between particular *V* and *J* genes loops out or is otherwise deleted. Rearrangement of *V*, *J*, and *C* genes by DNA recombination during differentiation of B-lymphocytes is illustrated in Figure 2–6.

However, the rearrangement of *V* and *C* genes at the DNA level does not result in a continuous DNA sequence encoding the entire immunoglobulin chain. An intron or intervening sequence, identified around the junction of the V and C regions, prevents direct transcription and translation of the DNA to form the intact polypeptide chain. The final removal of this intervening sequence appears to occur in the nuclear RNA material. A 27S species of nuclear RNA from MOPC 21 cells has been shown to contain the intron seen in the DNA while a 13S nuclear RNA species isolated from the same cells lacks the intervening sequence and therefore encodes the final continuous polypeptide chain. Thus, the final joining of V and C coding sequences or exons occurs at the nuclear RNA level, possibly by some looping out process to remove the intervening nucleotide sequence followed by ligation of the V and C RNA sequences. The V region of mouse K chains is evidently encoded by a multiple cluster of *V* genes specifying the first ninety-nine amino acids and a V-C joining or *J* gene encoding the region from residues 100 to 112. The J region then is a splicing DNA segment adjacent to the V region that joins

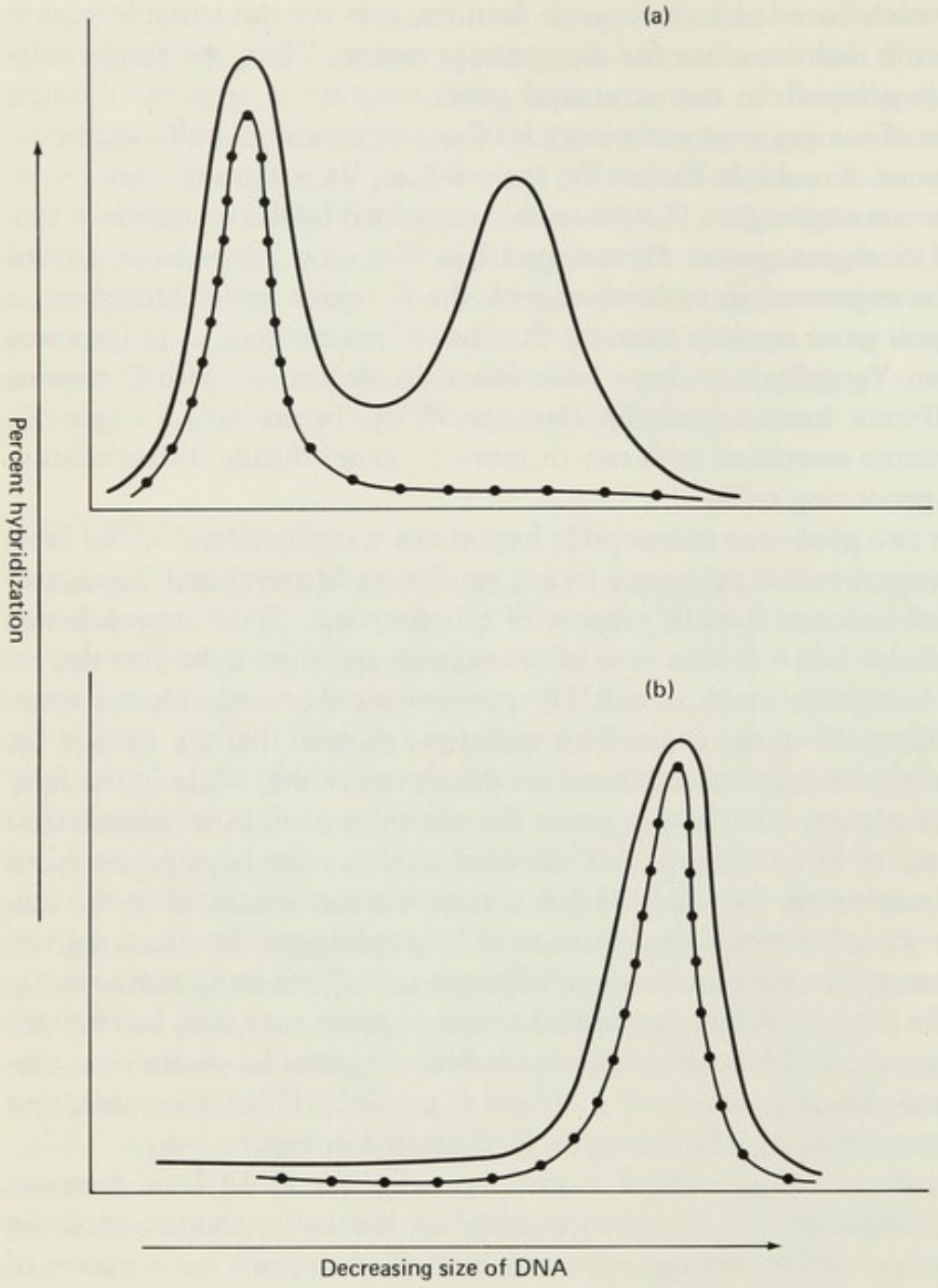


FIGURE 2–5. Hybridization of mRNA probes with fragments of DNA from mouse embryo cells (a) or mouse tumor cells MOPC 321 (b). Whole DNA was digested with the restriction enzyme BAM HI to yield DNA fragments that were separated by electrophoresis in an agarose gel. The fragments were then tested for their ability to hybridize with mRNA for whole MOPC 21 K chain (———) or mRNA for the C region of MOPC 21 (–●–●–●–). The results show that embryo DNA contained two components, one hybridizing with the C region probe and another, smaller fragment, hybridizing with *V* gene sequences. However, the tumor DNA (representing DNA from a differentiated cell) contained only a single fragment that hybridized both V and C region probes. This fragment was smaller than either of the fragments observed in embryo DNA.

Source: Adapted from Hozumi and Tonegawa, *Proc. Natl. Acad. Sci. USA* 73:3628, 1976.

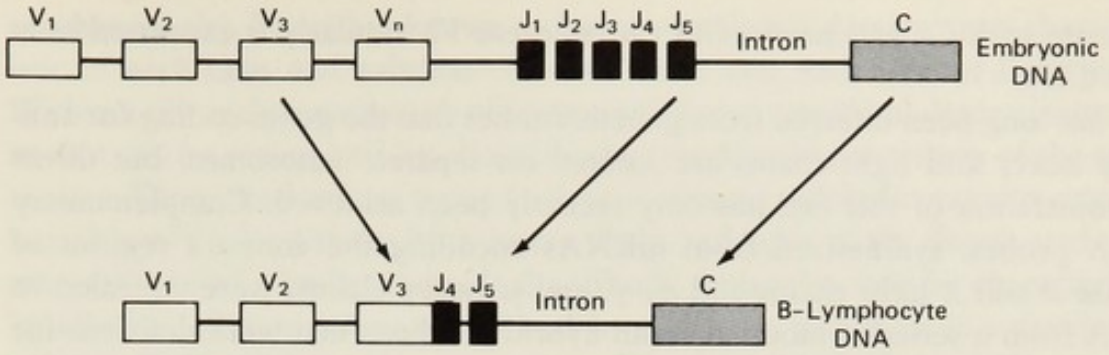


FIGURE 2–6. Arrangement of *V*, *J*, and *C* genes in germ-line or embryonic DNA and in B-lymphocyte DNA after joining of *V* and *J* by recombination. In this example, V_3 becomes joined to J_4 with deletion of the intervening segment from V_n through J_3. The intron or intervening sequence DNA between the *C* and *J* regions presumably remains intact. This recombined DNA is then active in immunoglobulin synthesis in B-lymphocytes.

Source: Adapted from Seidman et al., *Nature* 280:370–375, 1979. © 1979 Macmillan Journals Limited.

and perhaps activates *V* gene segments. Its existence has been established by sequence analysis at the DNA and polypeptide levels. Thus, in the mouse κ system, the polypeptide chain is encoded by at least three disparate DNA segments: one for the major portion of the V region, one for the J region, and one for the constant region.

Further, in the mouse K system nucleotide sequences have been identified for numerous Vκ regions and at least five J regions. Somatic recombination joins one of the available *V* region genes with a *J* segment that is located close to the *C* region gene (Figure 2–6). An intervening sequence between the joined *V-J* segments and the *C* region gene is deleted during processing of the mRNA to yield a functional continuous nucleotide sequence encoding the entire K chain. The implications of V-J joining in the generation of antibody diversity are discussed later in this chapter. In essence, existence of multiple *J* region genes allows for creation of diversity by assortment of *V* and *J* genes.

The Structural Genes of Immunoglobulins

The preceding discussion gives us some idea of the complexity of the genes encoding the immunoglobulin structure. In mammals, at least three autosomal, multigene families coding for antibodies must exist, κ, λ, and H. A *multigene family* is defined as a group of nucleotide sequences or genes that exhibit four properties: multiplicity, close linkage, sequence homology, and similar or overlapping phenotypic functions. Although immunochemical and genetic data indicate that κ and λ constitute distinct gene families, the H chains, despite the existence of H chain classes, are encoded within a single multigene family. The three unlinked multigene families are arranged as shown in Table 2–3. The κ and λ gene families are seen as separate because *V*κ genes only

associate with Cκ and never with *C*λ, whereas *V*λ similarly is expressed only with *C*λ.

It has long been inferred from genetic studies that the genes coding for antibody heavy and light chains are located on separate autosomes, but direct demonstration of this fact has only recently been achieved. Complementary DNA probes, synthesized from mRNAs encoding the constant regions of mouse κ and λ light chains and α, γ, and μ heavy chains, were annealed to DNA from a series of mouse–human hybrid cell lines that were deficient for various mouse chromosomes. This elegant method clearly demonstrated the separate autosomal locations of *C*κ, C_λ, and C_H genes as well as further indicating that the class-specific *C*α, *C*γ and *C*μ heavy chain genes were located on the same autosome. Thus, the three classic immunoglobulin gene families are located on different non-sex-linked chromosomes in the mouse. Nuclear transcripts of mouse heavy chain immunoglobulin genes contain only the expressed class of C region sequences (i.e., Cα, Cγ, or Cμ). The switch from one class of heavy chain to another during B-cell ontogeny apparently depends on some type of DNA rearrangement similar to that found with *V* and *C* light chain genes.

The discovery of genetic markers, that is, heritable structural characteristics, of immunoglobulins has greatly aided the interpretation of structural data and provided a rationale for theories of antibody diversity. Three levels of genetic markers have been defined in immunoglobulins. Each molecular class of immunoglobulins (e.g., M, G, A, and their subclasses) possesses distinctive structural characteristics recognized by antiserum prepared against Ig chains in

TABLE 2–3. Multigene Families of Human Immunoglobulins

Gene family	*V* gene members	*C* gene members
κ	$V\kappa_I$, $V\kappa_{II}$, $V\kappa_{III}$	C_k
λ	$V\lambda_I$, $V\lambda_{II}$, $V\lambda_{III}$, $V\lambda_{IV}$	$C\lambda OZ^+$[a], $C\lambda OZ^-$ $C\lambda Kern^+$, $C\lambda Kern^-$ $C\lambda Mz^+$, $C\lambda Mz^-$ $C\lambda Ch^+$, $C\lambda Ch^-$ $C\lambda Mcg^+$, $C\gamma Mcg^-$
H	VH_I, VH_{II}, VH_{III}, VH_{IV}	$C\gamma_1$ $C\gamma_2$ $C\gamma_3$ $C\gamma_4$ $C\alpha_1$ $C\alpha_2$ $C\mu_1$ $C\mu_2$ $C\delta$ $C\epsilon$

[a]The C region of the λ chain is characterized by ten isotypes reflecting one to three amino acid substitutions. These variants are nonallelic and therefore each isotype represents the product of a distinct structural gene.

another species. These are known as *isotypes*—antigenic determinants characteristic of each class and subclass of heavy chain and each type of light chain. Within a given class or isotype of immunoglobulin, structural determinants exist that can be recognized by alloantibodies produced in members of the same species. These markers are termed *allotypes*—immunoglobulin variants or polymorphisms distinguishing both heavy chain and light chain classes at the intraspecific level. Finally, individual antibody molecules may be characterized by idiotypic determinants, recognized by antiserum produced in a previously naive individual of the same genotype. In other words, the anti-idiotype reactivity is directed against the antigen-binding specificity of antibody molecules. The immunizing antibodies should be otherwise identical to those of a naive syngeneic recipient. In essence then, *idiotypes* are antigenic determinants of antibodies that distinguish one V domain from all other V domains. Idiotypic antibodies should be specific for the unique determinants of the variable regions of given antibodies. Such determinants may reside both inside and outside actual antigen-binding sites.

Human Ig Allotypes

Let us consider in sequence the Ig allotypes of humans, mice, and rabbits. We focus on alloantigenic determinants of immunoglobulins which are detected serologically by antiserum produced in genetically different members of the same species. The expression of allotypic markers on immunoglobulins occurs in a codominant Mendelian pattern referable to single, autosomal gene loci, each possessing two or more alleles. As we shall see, allotypic amino acid changes are mostly localized to C regions and therefore facilitate the genetic mapping of *C* genes.

Polymorphic forms of human immunoglobulins were first observed by Grubb and coworkers in 1956 while examining serums from patients with rheumatoid arthritis. They found that Rh+ erythrocytes coated with nonagglutinating Rh antibodies could be agglutinated by some, but not all, rheumatoid serums depending on the source of the anti-Rh. Using this as an indicator system, two allotypes were initially discerned in human immunoglobulins. Normal human serums capable of inhibiting this hemagglutination by antiglobulin were designated Gm(a+), and noninhibiting serums were designated Gm(a−). Genetic studies showed that Gm(a+) was inherited as a dominant Mendelian trait that was not sex linked. The Gm(a) determinant, now designated G1m(1), is present only on the IgG1 subclass and has been localized to the CH 3 domain of the heavy chain. Gm antibodies have been induced by direct immunization of Gm-negative individuals and by maternal–fetal incompatibility. Monospecific typing serums have been procured from rabbits following immunization with isolated human myeloma proteins or fragments thereof and subsequent absorption with normal human serum. Either whole serum, an isolated immunoglobulin, or a polypeptide subunit may be used as a source of blocking or inhibiting antigen in the hemagglutination–inhibition assay. Since the initial recognition of the Gm allotypes, some twen-

ty-four Gm determinants have been identified on the heavy chains of IgG1 and the other three subclasses of human IgG. Five sets of allotypic markers are now recognized on human immunoglobulins. The closely linked *Gm, Am,* and *Mm* systems govern allotypes on heavy chains of IgG, IgA, and IgM, respectively. These *Gm* loci are structural genes for amino acids of the constant regions of gamma heavy chains; the alternative amino acids and their positions have been identified with certain Gm antigenic determinants as indicated in Table 2–4. The independently inherited *Km* or *Inv* system specifies three Cκ region variants differing by one or two amino acids in kappa-type L chains. The most recently discovered *Hv(1)* system controls a variable-region marker found on mu, gamma, and alpha heavy chains (Table 2–4).

The expression of the Gm determinants is complex and a single heavy chain often possesses more than one Gm determinant, all located in the CH region. If we assume that one gene encodes the entire CH region, it then follows that a single gene can control more than one Gm specificity. This is supported by the frequent finding of associated Gm determinants inherited as if encoded by a single gene. For example, IgG1 molecules bearing Gm determinants 1 and 17 or 1, 2, and 17 occur frequently in Caucasian populations and are encoded by allelic genes $Gm^{1,17}$ and $Gm^{1,2,17}$ as shown by family studies. These could be regarded as examples of one gene yielding one complex or multideterminant antigen, but the *Gm* system may well represent a multigenic complex or

TABLE 2–4. Allotype Markers Associated with Human Immunoglobulin Chains

Chain	Allotype antigen[a]	Amino acid residue or function	Residue position
Gamma 1	G1m(1)	Asp, Leu	356, 358
	G1m(1−)	Glu, Met	356, 358
	G1m(4)	Arg	214
	G1m(4−)	Lys	214
Gamma 2	G2m(23)	?	?
Gamma 3	G3m(5)	Phe, Phe	296, 436
	G3m(21)	Tyr, Tyr	296, 436
Mu	Mm(1)	?	?
Alpha 2	A2m(2)	Disulphide bridge between H and L chains	131
	A2m(1)	No H-L chain bridge	131
Gamma, Mu, and Alpha	Hv(1)	Variable region marker	?
Kappa	Km(1)	Val, Leu	153, 191
	Km(1,2)	Ala, Leu	153, 191
	Km(3)	Ala, Val	153, 191

[a]The *Gm, Mm,* and *Am* loci are structural genes for constant regions of different heavy chains and are in the same linkage group, whereas the *Km* (or *Inv*) locus for kappa light chains and the *Hv* locus for variable regions of several heavy chains are each genetically independent and probably on separate chromosomes.

cluster of genes rather than single, alternative alleles. In any event, occurrence of multiple antigenic specificities in *different* amino acid positions of the same Ig chains has been found for both Gm and Km specificities. Numerous combinations of Gm determinants, presumably controlled by allelic genes, have been recognized for human IgGs. In an individual homozygous for the $Gm^{1,17}$ gene, all IgG1 molecules will bear the 1 and 17 determinants. However, in a heterozygous individual (e.g., $Gm^{1,17}/Gm^{1,2,17}$) a single IgG1 molecule is encoded by only one of the two alleles and possesses either the 1 and 17 combination or the 1,2,17 determinants. Similarly, the myeloma protein of IgG1 subclass is an individual who is heterozygous G1m(1)/G1m(4) will exhibit either G1m(1) or G1m(4), but not both. This phenomenon is termed molecular *allelic exclusion*—only one of the two alternative allotypic markers is expressed in each mature B-lymphocyte. Allelic exclusion in heterozygous Ig-synthesizing B-cells is usually attributed to the turning on or off of alternative homologous genes or chromosomes by chance. Otherwise, activated B-cells could become functionally hemizygous for *Ig* genes by the rearrangement of *C* and *V* genes inherent in the generation of antibody molecules. *Allotype suppression* resulting from allelic exclusion can be produced experimentally in mice or rabbits by injection of heterozygous neonates with specific antiallotype serum. This outcome, by whatever mechanism, must be understood in terms of specific gene activation or differential gene expression.

A single allotype antigen, Mm(1), has been demonstrated on Mu(μ) chains of human IgM, but alternative alleles for Mm(1 –) phenotypes have yet to be detected. The Mm^1 allele segregates as a dominant trait associated with a single autosomal locus. Human IgA may be grouped into two subclasses, IgA1 and IgA2, and allotypy has been demonstrated only in the IgA2 subclass. Two alleles, $A2m^1$ and $A2m^2$, have been identified with this heavy chain variation associated with amino acid position 131. IgA2 molecules bearing the A2M(1) determinant are unique in that the L and H chains are not joined by disulphide bonds; rather, the L chains are disulphide linked.

While allotypic markers have not been identified on human λ chains, the Km (formerly Inv) determinants have been demonstrated on κ chains. Three determinants, Km(1), (2), and (3) have been identified and ascribed to amino acid sequence changes at positions 153 and 191 of the chain. Km(2) is always associated with Km(1), and this is explained by the fact that the Km(1) determinant is defined by leucine at position 191, while the Km(2) determinant requires the combination of leucine at 191 and alanine at 153. Km(3) is distinguished by valine at position 191 (see Table 2–4). Each of the three Km allotypes is distinguished from the other two by a single amino substitution.

A new allotypic determinant, Hv(1), has recently been identified on human IgG, IgM, and IgA using a hemagglutination–inhibition assay system. The allotype has been localized to the variable region of the immunoglobulin heavy chains and thus represents the first known V region allotype of human immunoglobulins. Inheritance studies of the alleles, $Hv(1)^+$ and $Hv(1)^-$, support a Mendelian mode of transmission, but quantitative differences in expression of $Hv(1)^+$ suggest the involvement of multiple loci or regulatory genes. The

TABLE 2–5. Allotypic Specificities of Heavy Chains of Murine Immunoglobulins*

Immunoglobulin	Locus	Allele	Specificities	Prototype strains
IgG2a	*Igh-1*	*Igh-1*^{a}	1.1, 1.2, 1.6, 1.7, 1.8, 1.10, 1.12	BALB/cJ; BC-8; SJA
		Igh-1^b	1.4,1.7	C57BL/10J; BALB.Ig^b
		Igh-1^c	1.2, 1.3, 1.7	DBA/2J; BALB.Ig^c
		Igh-1^d	1.1, 1.2, 1.5, 1.7, 1.12	AKR/J; BALB.Ig^d
		Igh-1^e	1.1, 1.2, 1.5, 1.6, 1.7, 1.8, 1.12	A/J; C57BL/6.Ig^e; B10.D2b.Ig^e
		Igh-1^f	1.1, 1.2, 1.8, 1.11	CE/J; BALB.Ig^f
		Igh-1^g	.1.2, 1.3	RIII/J; BALB.Ig^g
		Igh-1^h	1.1, 1.2, 1.6, 1.7, 1.10, 1.12	SEA/Gn
IgA	*Igh-2*	*Igh-2*^{a,h}	2.2, 2.3, 2.4	BALB/cJ; SEA/Gn
		Igh-2^b		C57BL/10J
		Igh-2^{c,g}	2.1	DBA/2J; RIII/J
		Igh-2^{d,e}	2.3	AKR/J; A/J
		Igh-2^f	2.4	CE/J
IgG2b	*Igh-3*	*Igh-3*^{a,c,h}	3.1, 3.2, 3.4, 3.7, 3.8	BALB/cJ; DBA/2J; SEA/Gn

(continued)

*Adapted from Nisonoff, Hopper, and Spring (1975) and Herzenberg et al. (1976). The *Igh* prefix in lieu of *Ig* has recently been suggested to indicate the heavy chain (h) relationship of these loci (see Green, 1979).

**See Goding, J.W., Scott, D.W., and Layton, J.E. *Immunol. Rev.* 37:152–186, 1977.

†Fast and slow Fc refers to the electrophoretic mobility of the Fc fragments at pH 8.2.

current World Health Organization nomenclature and molecular basis for human immunoglobulin allotypes is summarized in Table 2–4. Allotype markers for IgG4, IgA1, IgD, and IgE heavy chains remain to be identified.

Mouse Ig Allotypes

Allotypy in mouse immunoglobulins was originally demonstrated for IgG1, IgG2 (a and b), and IgA and has recently been extended to include IgM, IgD, and kappa light chains. The majority of mouse immunoglobulin allotypes have been localized to the H chain of the molecule and in particular the Fc portion

TABLE 2–5. *(continued)*

Immunoglobulin	Locus	Allele	Specificities	Prototype strains
		Igh-3^{b}	3.4, 3.7, 3.8, 3.9	C57BL/10J
		Igh-3^{d}	3.1, 3.3, 3.7, 3.8	AKR/J
		Igh-3^{e}	3.1, 3.3, 3.7	A/J
		Igh-3^{f}	3.1, 3.2, 3.3, 3.4	CE/J
		Igh-3^{g}	3.1, 3.2, 3.4	RIII/J
IgG1	*Igh-4*	*Igh-4*^{a,c,d,e,f,g,b}	Fast Fc[†]	BALB/cJ; DBA/2J; AKR/J; A/J; CE/J; RIII/J; SEA/Gn
		Igh-4^{b}	Slow Fc	C57BL/10J
IgD**	*Igh-5*	*Igh-5*^{a}	5.1	CBA; BALB/c, C3H; SJA, NZB; A/J, C57BL/6.Ig^{e}
		Igh-5^{b}	5.2	C57BL/6; SJL; BALB/c.Ig^{b}; C3h.SW.Ig^{b}
IgM**	*Igh-6*	*Igh-6*^{a}	6.1, 6.2	CBA; BALB/c, C3H; SJA
		Igh-6^{b}	6.2, 6.4	C57BL/6; SJL, BALB/c.Ig^{b}; C3H.SW.Ig^{b}
		Igh-6^{e}	6.3, 6.4	NZB; A/J; C57BL/6.Ig^{e}

of that chain. However, immunization of guinea pigs with mouse Fab from IgG has revealed two specificities apparently located in the Fd or N-terminal half of the H chain. Kappa L chain allotypes have recently been identified in mice associated with strain-specific peptides occurring in the V region; normal mouse kappa chains of certain strains can be differentiated by peptide mapping. The occurrence of myeloma proteins in association with highly inbred strains has greatly contributed to the study of allotypy in mouse immunoglobulins. Antiserum recognizing allotypic specificities is prepared by immunizing one strain of mouse with immunoglobulin, usually a homogeneous myeloma protein, from another inbred strain. Otherwise, potent precipitating antibodies, detectable by double diffusion of antigen and antibody on agar gel, can be produced by other means such as alloimmunization with antibacterial antibodies bound to bacterial cells. Absorption of multispecific antiserums in double diffusion in agar tests can be conveniently achieved by prefilling the antibody wells with different allogeneic serums. This procedure permits the

absorbing serum to permeate the surrounding agar and provide a zone of excess antigen.

A set of multiple allotypic specificities controlled by a single allele is often defined as an allotype, although the same antigenic specificities recur in different combinations as products of different alleles or haplotypes (Table 2–5). Thus, immunoglobulins of differing allotype may carry one or several distinguishing allotypic specificities. In this context the allotypic specificity is a single alloantigenic determinant. The six allotype loci designated *Igh-1* through *Igh-6* all appear to be structural genes for C regions of heavy chains; close linkage of these loci is strongly suggested by the absence of recombinant types in numerous crosses, as illustrated in Table 2–6. Indeed, this cluster of heavy chain genes has recently been mapped to chromosome 12 and given the generic symbol *Igh*. Table 2–5 summarizes the properties of the mouse heavy chain allotypes, the specificities that make up the allotypes, and the prototype inbred strains whose immunoglobulins carry the specificities. Twelve antigenic determinants are now recognized as products of eight alleles of the *Igh-1* locus. These allotypic determinants or specificities probably represent one or more amino acid substitutions in the constant portion of IgG2a heavy chains, but the residue positions affected are unknown. Polymorphism is also characteristic of the *Igh-2*, *Igh-3*, and *Igh-4* loci; the apparent complexity appears proportional to the extent of investigative effort thus far. The *Igh-4* allelism is based on differences in electrophoretic mobilities of the Fc fragment of IgG1 molecules rather than on the usual serologic methods. The *Ig* prefix is appropriately used for all allotype loci (genes) in mice. This is unfortunately not the case in other species.

TABLE 2–6. Linkage of *Ig-1* (*Igh-1*) and *Ig-2* (*Igh-2*)

Cross	Progeny	Number tested
(C3H X DBA/2)F1, X C57BL	Parental Types	
	$\frac{Ig\text{-}1^a \quad Ig\text{-}2^a}{Ig\text{-}1^b \quad -}$	78
$\frac{Ig\text{-}1^a\ Ig\text{-}2^a}{Ig\text{-}1^c\ Ig\text{-}2^c} \times \frac{Ig\text{-}1^b -}{Ig\text{-}1^b -}$	$\frac{Ig\text{-}1^c \quad Ig\text{-}2^c}{Ig\text{-}1^b \quad -}$	71
	Recombinant Types	
	$\frac{Ig\text{-}1^a \quad Ig\text{-}2^c}{Ig\text{-}1^b \quad -}$	0
	$\frac{Ig\text{-}1^c \quad Ig\text{-}2^a}{Ig\text{-}1^b \quad -}$	0
	Total Tested	149

From Herzenberg, *Cold Spring Harb. Symp. Quant. Biol.*, 29:460, 1964.

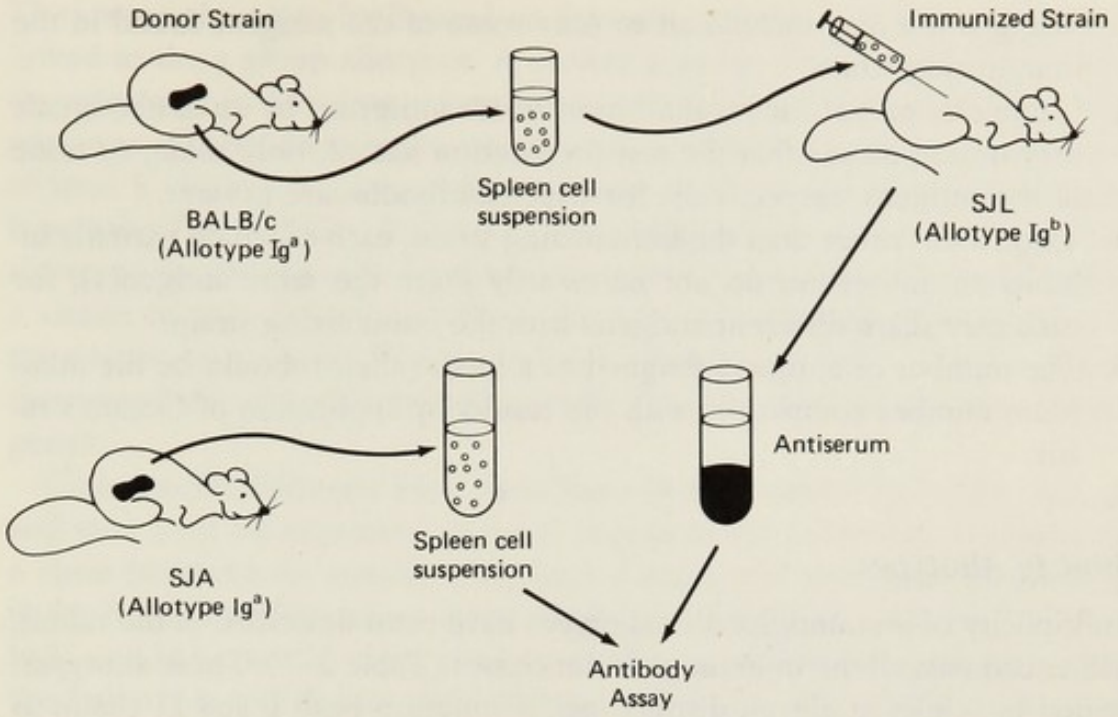


FIGURE 2–7. Regimen used to identify Igh-5 and Igh-6 allotypes on murine IgD and IgM. The donor strain (BALB/c) and immunized strain (SJL) differ at *H-2* loci and in immunoglobulin allotype so that antibodies to H-2 as well as allotype will be produced. To avoid reactions due to anti-H-2, the antiserum is tested in a strain (SJA) with the same *H-2* haplotype as the immunized strain, but the same allotype as the donor strain.

The allotypes Igh-5 and Igh-6 have recently been identified on IgD and IgM molecules, respectively, using alloantiserums made against spleen cells. To avoid interference from antibody to H-2 antigens and other nonimmunoglobulin cell surface determinants, each alloantiserum was tested against spleen cells from mice congenic with the immunized strain, but bearing the allotype of the immunizing donor (Table 2–5 and Figure 2–7). Examination of several congenic strains indicates that *Igh-5* and *Igh-6* loci do in fact belong to the previously identified linkage group governing mouse heavy chain specificities. Finally, an *Igk-V* allotype locus governing peptide sequences in the variable region of kappa light chains has been mapped to chromosome 6, closely linked to the *Lyt 2,3* loci (see Figure 1–6). The *Igk-V* alleles exhibit a classic Mendelian pattern of segregation in crosses yielding allotypes that are distinguishable by peptide mapping.

The general rules followed in the analysis of the Ig antigens are potentially appropriate to analogous studies. They may be cited as follows:

1. The immunizing strain should possess *all* the antigens to which the antiserum reacts, and the recipient strain should have *none* of the alloantigens corresponding to antibodies produced by it.
2. An antiserum produced in a given donor–recipient combination will not necessarily detect all of the antigens characteristic of the donor strain.
3. The antigens detected by an antiserum in a strain other than the immu-

nizing strain may include all or only some of the antigens found in the immunizing strain.

4. Complete, partial, or no inhibition of an antiserum by an alloantigenic preparation means that the test preparation has all, only some, or none of the antigens, respectively, for which antibodies are present.
5. Two strains other than the immunizing strain, each of which partially inhibits an antiserum, do not necessarily share the same antigen(s), for each may share different antigens with the immunizing strain.
6. The number of antigens assigned to a locus (allele) should be the minimum number compatible with the results by application of Occam's razor.

Rabbit Ig Allotypes

A multiplicity of immunoglobulin allotypes have been described in the rabbit, which encompass all the immunoglobulin classes (Table 2–7). These allotypes, specified by alleles at eleven distinct loci, distinguish both L and H chains as well as V or C regions within those chains. Typical codominant inheritance of these alloantigens has been observed. The present nomenclature is something of a geneticist's nightmare, with small letters for dominant genes and nonsequential numbering of alleles.

The original specificities described are controlled by the *a* and *b* allotypic loci. The *a* locus has three allelic alternatives corresponding to the allotypes a1, a2, and a3 which are located in the Fd region of the H chain. The group a allotypes are associated with the N-terminal regions of γ, μ, α, and ϵ chains and are thus described as genetic markers of the VH region. The group b allotypic determinants originally described were designated b4, b5, and b6.

TABLE 2–7. Allotypes of Rabbit Immunoglobulins

Allotypic locus	Alleles and antigenic products[a]	Location of allotypic determinants
b	*b4, b5, b6, b9*	κL
c	*c7, c21*	λL
a	*a1, a2, a3*	VH
x	*x32*	VH
y	*y33*	VH
d	*d11, d12*	Cγ
e	*e14, e15*	Cγ
f	*f71, f72, f73*	Cα1
g	*g74, g75*	Cα2
ms	*ms1, ms2, ms3, ms4, ms5, ms6*	Cμ
n	*n81, n82*	Cμ

[a]A one-to-one correspondence between individual alleles and their distinctive antigenic products is assumed, contrary to the situation found for mouse and human Ig allotypes.

They were shown to be located on immunoglobulin L chains and not to be linked to the a group allotypes. A further b group determinant, b9, was later demonstrated in breeding experiments with rabbits of known *b4*, *b5*, and *b6* genotype. Oudin described a population of rabbit L chains (5 to 20 percent of total L chains) that lacked the b group allotypes and he designated these b negative (b−) chains. A second L chain allotypic locus, the *c* locus, has been shown to be present only on the b negative L chains, which are analogous to λ chains of man and mouse. Thus, the *b* locus is expressed on κ chains and the *c* locus on λ chains. The two *c* locus allotypes, *c7* and *c21*, segregate as if they are allelic, although breeding studies suggest they may be closely linked genes.

Class specific allotypes have been described for rabbit IgG, IgA, and IgM and thus must be expressed in the C region of the respective H chains. The λ chain possesses the specific allogroups d and e, and they have been localized in the Fc region of the chain. Rabbit IgA has been divided into two subclasses, IgA_1 and IgA_2, on the basis of susceptibility to papain digestion. IgA_1 carries the f allotypic specificities with alleles *f71*, *f71*, and *f73*, and IgA_2 the g allogroup with alleles *g74* and *g75*. Six allotypic determinants, ms1 to ms6, have been confirmed as specific for IgM. Two separately identified determinants, n81 and n82, have also been found on IgM but not on IgG or IgA. However, insufficient data is available to conclusively demonstrate that n81 and n82 are not two of the ms allotypes previously described. More recently, two new allotypes designated x32 and y33 have been demonstrated in the V regions of λ, μ, and α chains. Breeding studies indicate that these markers are nonallelic and associated with loci closely linked to the *a* locus.

The differences in the allotypic specificities discussed above have been demonstrated primarily by serological techniques. However, the validity of allotypes as genetic markers of structural genes of immunoglobulins requires evidence of structural differences among immunoglobulins of different allotypes. Correlation of primary structural difference with allotype variation has been shown for the d and e allotypes. The alleles *d11* and *d12* reflect an amino acid interchange, methionine for threonine, in the hinge region of the γ chain, while *e14* and *e15* correlate with threonine or alanine at position 309.

The positioning of the a allotypes in the VH region was indicated by the presence of these specificities on all classes of rabbit immunoglobulin. Confirmation of the a allotypes as VH markers comes from the demonstration of recombination between the *a* locus specificities and the d and e allotypes of the Cγ region and ultimately from amino acid sequence determinations on pooled IgG and IgA variable regions of known a allotype. The b allotype specificities of the κ chain are referable to amino acid sequence variations in the constant region, particularly in the C terminal hexapeptide. Some sequence correlations have been found in the V region, but do not hold for all chains of the same b allotype. This apparent correlation may rather indicate a linkage of selective combination of VL and CL regions as has been demonstrated for VH and CH regions.

Recently, anomalies in expression of certain allotypic determinants have been observed. These include the finding of pluriallelic rabbits, apparently expressing more than two alleles at either the *a* or *b* locus. Latent allotypes have also been detected, arising to significant levels during hyperimmunization but not normally detected in preimmune serum of the same animal. Similar examples of unexpected expression of allotypes have been reported in the mouse, in rats, and in man. Actually, the supposed one-to-one correspondence between alleles and antigenic specificities in rabbits is atypical. One suspects that additional alloantigens or specificities common to different alleles have been overlooked. Moreover, closely linked loci, such as *a, x,* and *y* affecting the same VH regions, could exhibit complementation or otherwise interact in an integrated biosynthetic pathway. One possible explanation for unexpected rabbit allotypes may be that the structural genes depend on one or more regulatory genes. Occurrence of unexpected allotypes may then result from a disturbance of the regulating system, thereby permitting expression of normally suppressed genes. It has been suggested that some rabbits carry latent genes which may only be expressed in a transitory fashion, while in the majority of rabbits the genes behave in a normal Mendelian manner. Detailed analysis at the DNA level of the immunoglobulin structural genes of single antibody-forming cells should eventually provide new insights.

The remarkable phenomenon of *allotype suppression* in heterozygous rabbits is worthy of special mention. Heterozygous b4/b5 offspring of b4/b4 mothers immunized with paternal b5 antigen show suppression of production of immunoglobulins with the b5 allotype concurrent with a compensatory increase in immunoglobulins of b4 allotype. Such offspring then have normal levels of IgG. Injection of newborn b4/b5 rabbits with anti-b5 serum can also produce b5 allotype suppression. Passive or transplacental allotype suppression persists at least up to sixty weeks of age or throughout life, whereas the antibodies causing the suppression remain detectable for only six weeks. Similarly, a1a3 offspring of a1a1 mothers producing anti-a3, or offspring of the same genotype from a3a3 mothers producing anti-a1, exhibit only low levels of the respective paternal allotypes. The phenotypic expression of the *b-5* allele derived from the paternal genome was unaffected in these same a1 a3 b4 b5 offspring. This alteration of *a* locus allotypes persisted for at least twenty-three months. Thus allotype suppression may occur separately in relation to either heavy or light chains in doubly heterozygous rabbits, although normal IgG levels are maintained. Presumably some central or feedback control of synthesis of IgG is triggered by allotype-specific antibodies early in life, and continued suppression is not dependent upon indefinite persistence of the anti-allotype molecules. Allotype suppression may be a form of directed *allelic exclusion,* since hybrid allelic molecules are not naturally occurring in heterozygous rabbits.

The allelic exclusion characteristic of all Ig-producing cells may be equated with *haplotype exclusion* as demonstrated for mouse IgM and IgD allotypes and receptors. This means that on any given B-cell, these markers come from the

same chromosome and share the same variable region. Presumably, individual lymphocytes become committed to one *Igh* gene complex early in development.

Idiotypes

The antigenic determinants, present on a specific immunoglobulin, that are capable of eliciting an antibody response in the same inbred strain from which the immunoglobulin was originally isolated are known as idiotypes. Oudin originally defined idiotypic determinants as "those antigenic determinants in a population of antibody molecules which are not observed in other immunoglobulins of the donor animal nor in antibody directed to the same antigen from other animals of that species." This definition has been modified to include cross-reacting idiotypes in members of the same species, as shall be discussed below. Considerable effort has been expended in the study of idiotypes and in establishing their validity as genetic markers of immunoglobulin V regions, as suggested by their relationship to antigen-binding specificity.

Inheritance of idiotypic determinants, first demonstrated in rabbit antibodies to streptococcal carbohydrate, has also been observed in mouse anti-streptococcal carbohydrate antibodies and antibodies to phosphorylcholine. The A5A idiotype, present on a murine monoclonal antibody to group A streptococcal carbohydrate, occurs in 90 percent of all A/J strain mice immunized with group A streptococci, but is not expressed in BALB/c mice. However, (A/J $\times$ BALB/c)F_1 hybrids express the A5A idiotype in a high proportion of individuals, indicating that the idiotype is encoded by a gene present in A/J but absent in BALB/c. In contrast, the idiotype T15, first recognized on TEPC 15, a phosphorylcholine (PC)-binding myeloma protein occurring in BALB/c mice, is regularly expressed in the induced anti-PC molecules of many mouse strains. Thus, the germ-line genes encoding idiotypic determinants may be confined to a given strain or may occur extensively throughout different strains of the same species.

Several types of idiotypic determinant are distinguishable. Binding-site idiotypic determinants are dependent on the integrity of the antigen-binding site of the immunoglobulin molecule for expression. Thus, the expression of binding-site idiotypes is blocked if the binding site is occupied as, for example, when it is affinity labeled with a reactive hapten. Nonsite associated determinants are not blocked by ligands occupying the combining site. A given antibody molecule may possess both binding-site and nonsite associated idiotypic determinants; the dinitrophenyl-binding mouse myeloma protein, M315, is an example of such a molecule. Further studies with this myeloma protein have shown that the nonsite idiotype is not present on isolated L or H chains of M315. Thus, the expression of both binding-site associated and nonsite associated idiotypes may require the presence of a specific H-L chain combination. The necessity, therefore, for the coordinate expression of two unlinked genes

to give rise to these idiotypes may explain why in some antibody systems it has been impossible to demonstrate simple inheritance patterns for idiotypic determinants.

Of recent interest has been the discovery of chain-specific idiotypes. These idiotypic determinants may be localized to either the VL or VH regions of the antibody molecule. The chain-specific idiotypes may prove to be extremely useful genetic markers of the V regions of immunoglobulins as they do not require the complexity of coordinate inheritance and expression of two unlinked genes as is the case with the idiotypes dependent on both VL and VH regions. In addition to individual idiotypes, unique to a specific antibody molecule, cross-reactive idiotypes (CRI) have been observed in several mouse systems including the anti-PC system discussed earlier. This cross-reactivity represents a sharing of a V region antigenic determinant among functionally related immunoglobulins. In fact, an individual antibody may possess both unique and cross-reactive idiotypes. CRI are idiotypes shared by antibodies of similar specificity in many animals of the same inbred strain, whereas unique or private idiotypes are found in only a single animal of that strain. CRI have been extensively studied in antibodies to p-azophenylarsonate (Ars) in A/J mice. Amino acid sequence analysis of ten H chains from anti-(Ars) antibodies bearing the CRI show complete homology of the framework residues (up to residue 30), which strongly suggests the existence of a germ-line gene for at least this section of the H chains.

From several lines of evidence then, the genes coding for idiotypic determinants are believed to be *V* region genes. In mice and rabbits, these genes are linked to the *CH* region genes as shown by their association with CH allotypes. One such series of V_H genes in mice is now known to fall within the *Igh* cluster on chromosome 12 (Figure 1–6). Genetic studies suggest that the *V* genes encoding different idiotypes are usually nonallelic but may be closely linked on the chromosome. This even appears to hold for two *V* genes which encode the same antigen-binding specificity. The idiotypes A5A and S117 are associated with mouse antibodies to group A streptococcal carbohydrate and, although they may segregate as if allelic in certain mouse strains, their concurrence in one recombinant strain points to nonhomologous positions of these two idiotype genes on the chromosome.

Idiotypes may play an important role in the regulation of the immune response. Immunosuppression of idiotype can be achieved by administration of anti-idiotype antibodies which in turn may lead to production of idiotype-specific suppressor T-cells. Either anti-idiotype Ig or suppressor T-cells could then act on idiotypic determinants of B-cell surface immunoglobulin. The regulatory function of idiotypes is inherent in network theories of immunoregulation (Figure 2–8). Jerne proposes that no one idiotype (antigen-binding site) is present in sufficient concentration in the absence of antigenic stimulation to induce autoidiotypic antibodies. The initial paucity of given idiotype molecules would follow from the requirement for a large repertoire of 10^6 to 10^8

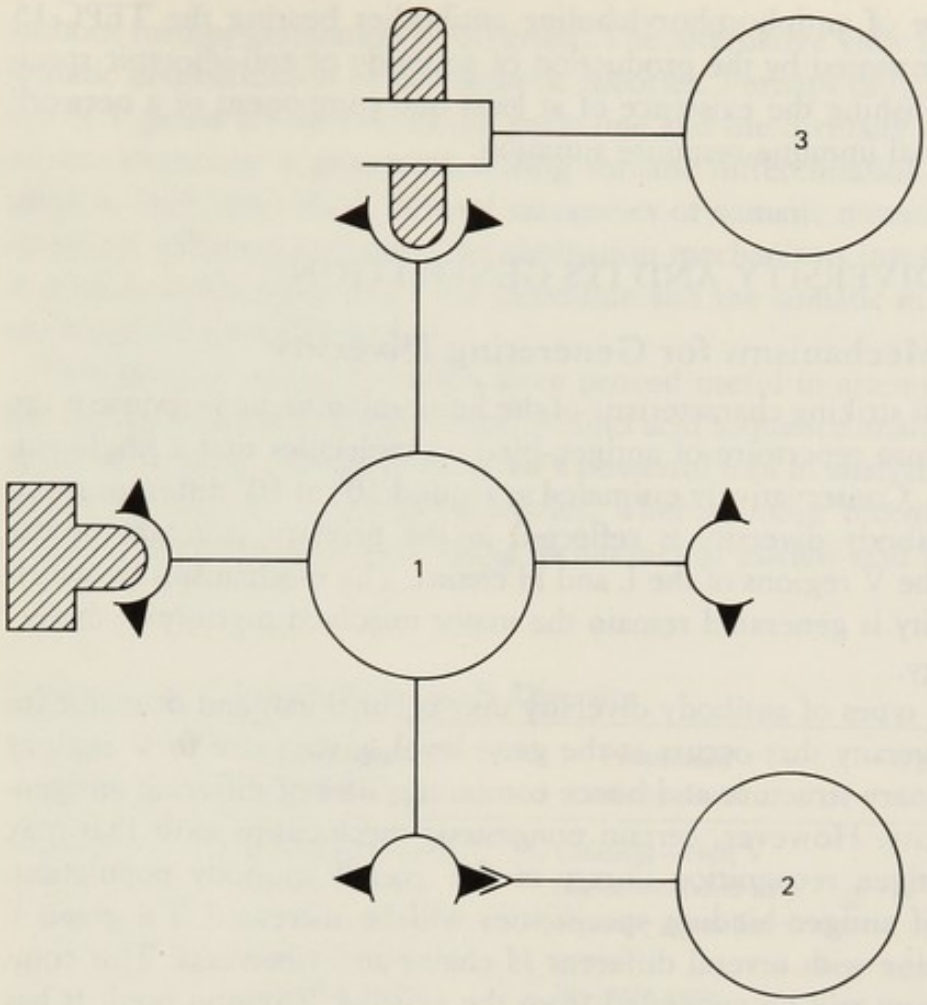


FIGURE 2–8. Regulation of the immune response via a network of idiotypes and anti-idiotypes. Cell type 1—antigen reactive set with receptors bearing idiotype ▲. Cell type 2—cells bearing antibodies or receptors that will bind idiotype ▲, i.e., anti-idiotype set of cells. Cell type 3—cells bearing antibodies or receptors with determinants that cross-react with the antigen (•) and are thus recognized by cell set 1. Both T- and B-cells are assumed to participate in this regulatory network, regulation being achieved through the balance of the suppressive and stimulatory effects exerted upon the antigen binding set (1) of lymphocytes.

Source: From Raff, M. *Nature* 265:205–207, 1977. © 1977 Macmillan Journals Limited.

antigen-binding sites to be selectively available in any one animal. However, during the course of an immune response the concentration of specific idiotypes of the induced antibodies will increase and stimulate an anti-idiotype response. It is this anti-idiotypic antibody (either circulating or cell membrane associated) that is supposed to specifically regulate given immune responses through its action on idiotype-bearing lymphocytes. Many recent findings have given credence to the network theory. Idiotypic determinants have been shown to be present on T- and B-lymphocytes and anti-idiotypic antibodies can act to suppress or stimulate the cells through these determinants. Production

in BALB/c mice of antiphosphorylcholine antibodies bearing the TEPC-15 idiotype is accompanied by the production of antibody of anti-idiotypic specificity, thus establishing the existence of at least one component of a network system in a normal immune response situation.

ANTIBODY DIVERSITY AND ITS GENERATION

Sources and Mechanisms for Generating Diversity

Perhaps the most striking characteristic of the humoral immune response is the obviously immense repertoire of antigen-binding molecules that a single animal may possess. Conservatively estimated at around 10^6 to 10^7 different specificities, this antibody diversity is reflected in the primary structure of the molecule, i.e., the V regions of the L and H chains. The mechanisms by which antibody diversity is generated remain the major unsolved mystery of molecular immunology.

Two possible types of antibody diversity may occur. First, and of major interest, is the diversity that occurs at the gene level, giving rise to V regions of different primary structure and hence combining sites of differing antigen-binding specificity. However, certain nongenetic mechanisms exist that may increase the antigen recognition library of the overall antibody population. The diversity of antigen-binding specificities will be increased if a given L chain can combine with several different H chains and vice versa. This combinatorial diversity will be generated from the existing *V* region pool. It has also become clear that a given antigen-binding site may exhibit multispecificity and hence be capable of binding more than a single antigenic species. The combined effect of these nongenetic generators of diversity is to reduce the number of *V* region genes required to code for an estimated 10^6 different antibody combining sites.

However, the main source of antibody diversity lies in the *V* region genes as indicated by amino acid sequence studies of antibody V regions. Two general theories exist to explain how this genetic diversity arises. Germ-line or phylogenetic theories hold that all the genes required to code for the antigen-binding repertoire of antibody molecules are inherited in the zygote or germline of the organism and that needed diversity has been generated during evolutionary development by mutation and selection. The large number of germ-line genes required for synthesis of the vast array of antigen-binding molecules could have arisen by gene duplication and diversification during phylogeny. This would imply that antibody diversification is an increasing process and that early evolved vertebrate species, by now extinct, would have possessed a less diversified repertoire of immune response genes, including variable region genes, than contemporary species. The evolutionary origins of antibody diversity will be discussed more fully in Chapter 9. Germ-line theories further hold that the *V* region genes, inherited in the zygote, are passed on to individual lymphocytes during somatic development of the organism

without further generation of diversity. The alternative view is represented in somatic diversification or ontogenetic theories. Perhaps only a minimal number of *V* genes are carried in the germ-line and the diversity expressed in the mature organism is generated during somatic differentiation and thus arises anew in each individual. Several categories of somatic mutation theory exist based on mutation and gene recombination mechanisms invoked to generate *V* gene heterogeneity. Both the germ-line and the somatic mutation theories are summarized in Table 2–8.

Two lines of experimentation have proved useful in attempting to resolve the question of antibody diversity. Amino acid sequence analysis of immunoglobulin variable regions has proved a powerful tool in analyzing the diversity and relatedness of antibody molecules, whereas more recently, direct gene counting has been attempted using techniques of nucleic acid hybridization.

TABLE 2–8. Theories of Antibody Diversity

Basic theory	Generation of diversity	Postulated mechanisms	Proposed number of genes
Germ-line or Phylogenetic	Antibody genes are encoded in the germ-line (i.e., zygote) of the organism and diversity is generated during evolutionary development.	1. Classical—each V region encoded by a separate germ-line gene. 2. Multiple gene interactions—each hypervariable and each framework region encoded by a separate germ-line gene and any framework gene may combine with any 3 (or 4) hypervariable genes to encode the intact V region. An episomal mechanism for insertion of hypervariable region sequences has been suggested.	1,000s Difficult to estimate due to uncertainty on limitation of combinations of the 4 or 5 gene groups.
Somatic or Ontogenetic	A limited number of *V* region genes are encoded in the germ-line and these are diversified in somatic cells.	1. Successive point mutations, perhaps involving some hypermutation process, followed by antigen driven selection. 2. Recombination followed by antigen driven selection.	< 1000 but varies with V system being examined.

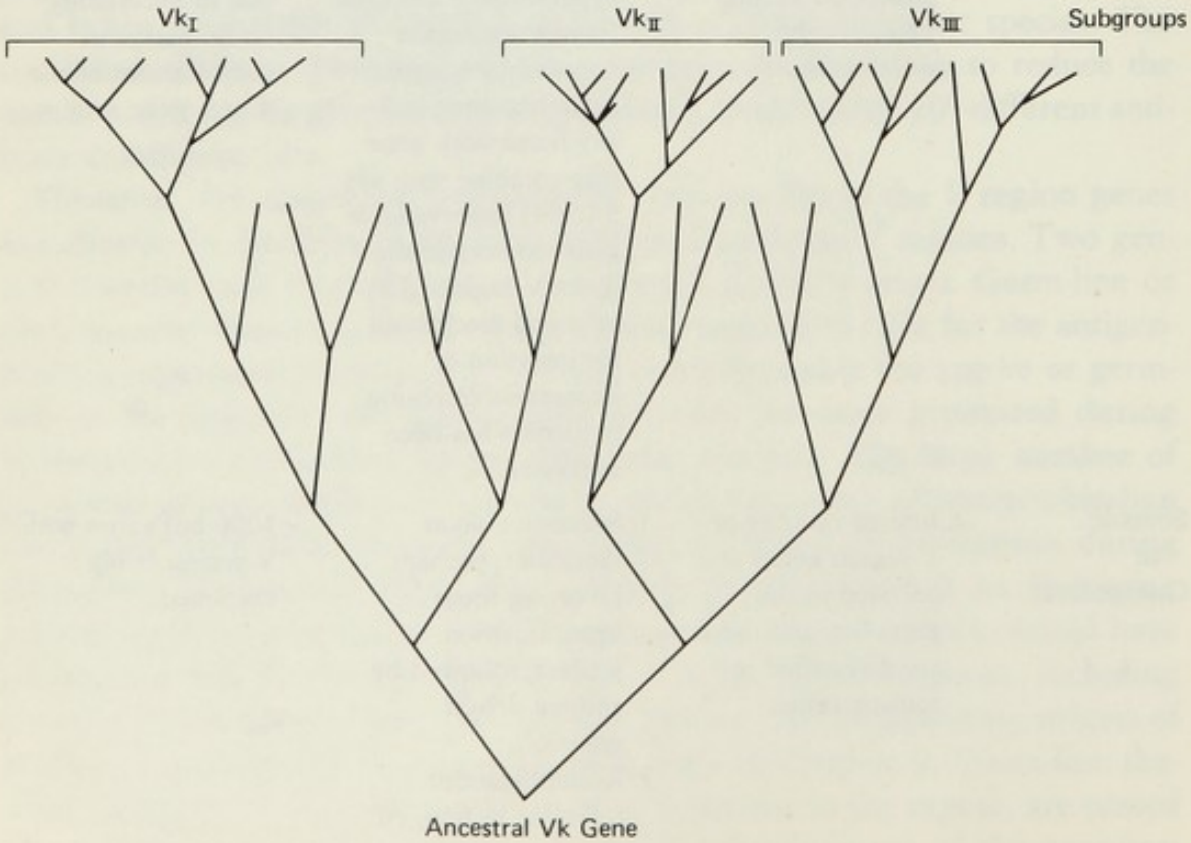
V Region Sequences

Sufficient amino acid sequence data have accumulated to establish the existence of Vκ, Vλ, and VH subgroups in human, mouse, and rabbit immunoglobulins. When comparing sequences within a given family of V regions (e.g., human Vκ), it is apparent that the diversity of sequence between representatives of each subgroup is large. Much less sequence diversity is observed within members of a given subgroup.

Recent analysis of extensive sequence data on the first twenty-three amino acids from sixty-one BALB/c and NZB κ chains has led to an expansion of the subdivisions within the mouse Vκ regions. Defining a set of Vκ regions

FIGURE 2–9. Hypothetical genealogic tree for human Vκ regions. The genealogic tree represents a hypothetical construction of the genetic events leading from an ancestral *V* region gene (*V*κ) to the genes coding for the present-day Vκ sequences. Each branching point in the scheme represents a gene duplication resulting in two daughter genes which diverge from each other via mutation or deletion. These genetic events are represented by the lines between branching points, with the lengths of the lines indicating the number of point mutations taking place. The tree is constructed by taking contemporary Vκ sequences and working backwards to arrive at a common ancestral gene.

Source: From Hood et al., *Cold Spr. Harb. Symp. Quant. Biol.* 41:817, 1976. © 1976 Cold Spring Harbor Laboratory.



possessing extensive sequence homology over their first twenty-three amino acids as a group, the BALB/c Vκ regions fall into twenty-six groups and NZB Vκ regions into fourteen groups. As in the case of the previously defined subgroups, each mouse Vκ group must be encoded by at least one germ-line gene if extensive parallel somatic mutation is excluded. Furthermore, analyses of twenty-two region sequences belonging to the Vκ 21 group suggest that this group is encoded by at least six germ-line genes.

Thus, the minimum germ-line gene assignment is one per subgroup, if diversity within a subgroup is assumed to arise by somatic mutation. The assignment of a germ-line gene for each subgroup is reinforced by examining the genealogy of V region sequences. The construction of genealogic trees is an attempt to show how present-day V region sequences may have arisen from a natural sequence of mutation and selection of germ-line genes during evolution. The genealogic tree shown in Figure 2–9 depicts the minimum number of mutations necessary for the present-day observed human Vκ and Vλ sequences to have evolved from a common ancestral sequence. The division of L chain sequences into κ and λ type and, further, into subgroups within each type can be clearly seen.

Using this type of analysis, one can begin to assess the minimal level at which germ-line genes must encode the observed sequence variability. One may surely exclude, on the grounds of the number of required coincident or parallel mutational events, placing the germ-line gene level at any point preceding the branching point for each subgroup. However, attempting to raise the germ-line gene level to include individual sequences within each subgroup is highly dependent on the system being analyzed. Genealogic analysis of the diversity within the Vκ region of BALB/c myeloma proteins suggests that the sequence diversity observed lies largely at the germ-line level; however, the similarity observed in the sequences of eighteen mouse Vλ regions suggests the possibility of a single germ-line gene encoding this family. Therefore, although genealogic analysis is helpful in assessing the minimum number of germ-line genes, it does not exclude a role of somatic mutation in generating V region diversity.

Nucleic Acid Studies

Direct quantitation of the number of *V* region genes has been attempted by hybridization analysis. In this method, complementary DNA (cDNA) probes first isolated by annealing to a myeloma κ messenger RNA (mRNA) are then hybridized with mRNAs from other related or unrelated L chain mRNAs. The degree of protection from nuclease attack of the labeled cDNA in the DNA–RNA hybrid is used as a measure of hybridization. The greater the protection by the hybrid of nuclease attack on the cDNA, the greater the extent of hybridization or complementarity of the cDNA and mRNA species. Rehybridization of the purified cDNA to the original mRNA template serves as a

control that should form an almost completely nuclease-resistant duplex. Initially these methods yielded inconclusive results because of the use of cDNA probes that did not code for a significant portion of the V region. However, the use of well-defined probes for Vκ indicates that the Vκ 21 group of mouse light chains is encoded by four to six germ-line genes. As the Vκ group contains more than twenty different sequences, it is apparent that the germ-line gene complement does not encode all the diversity of the group. It may thus be inferred that the greater diversity of the Vκ 21 group is generated by somatic mutation from four to six germ-line genes. If Vκ 21 is representative of all the Vκ groups, and one takes five as the average number of germ-line genes encoding each group, then the entire Vκ system may arise from 250 germ-line genes since there are estimated to be fifty groups in the family.

Complete nucleotide sequences of cloned germ-line (embryonic) DNA and a somatically recombined active gene coding for MOPC-41 κ chain indicate that, apart from the recombinational event that joins the MOPC-41 *V*κ gene with *J* segment, the germ-line gene remains unaltered in sequence. Thus, no somatic mutation events occur that may further contribute to antibody diversity. It would therefore appear that diversity in the κ system is encoded in the germ-line and expanded by different combinations of *V* and *J* genes during somatic development.

The mouse λ system represents a much more restricted V region family, only seven V region sequences being identified out of eighteen individual chains examined. Hybridization studies in this system using cDNA probes extending well into the *V* λ region indicate that only one copy of the *V* λ gene may be required and that the seven identified sequences may be produced as a result of somatic mutation of this single gene. However, it should be noted that the mouse Vλ system is apparently unique in the degree of restriction observed and, in fact, it is possible that it may simply be analogous to a subgroup as seen in Vκ sequences. Furthermore, the λ chain occurs in less than 5 percent of normal mouse immunoglobulin, which may again reflect a special case.

From the preceding discussion it is apparent that at least some antibody diversity must be generated during somatic differentiation. Comparison of the sequences of the genes from a number of related *V* regions revealed extensive sequence homology in the areas flanking the *V* genes themselves. This has formed the basis of a proposed intergenic recombination mechanism to explain how diversity may be generated from a fixed number of germ-line genes. The extensive sequence homology adjacent to the *V* region sequences would allow for extensive recombination between nonallelic variable region genes. The flanking sequence homologies appear to be restricted to the set of genes constituting a group, thus allowing for recombination only within that group. In immunoglobulin precursors there is an *N*-terminal extra peptide segment (18 to 29 residues long) exhibiting sequence variability similar to that found in hypervariable regions of L chains. The DNA segment coding for the extra piece may be involved in the regulation of *Ig* gene transcription. In any event, the *V* region gene complex is larger than previously realized.

Genetic Combinatorial Diversity and Recombination

The adaptation to permanent growth in tissue culture of a mouse plasmacytoma (MOPC 21) allowed the direct assessment of the occurrence of spontaneous mutants during repeated cloning of this line. In a sample of 7,000 subclones, five mutant clones were obtained based on variation in the isoelectric focusing pattern of the antibody produced compared to that of the parental cell line. Of the four mutant antibodies examined, all showed genetic changes in the C_H regions. No mutations involving V regions were observed. Although the occurrence of somatic mutation was elegantly demonstrated, the results do not directly implicate somatic mutation as a generator of V region diversity.

Several mechanisms contribute to the generation of antibody diversity. In the mouse κ system, a significant portion of the variability is carried in the germ-line that includes at least 250 *V*κ genes. The remaining diversity seen in the Vκ amino acid sequences may be generated in either of two ways: (1) the arrangement of major *V* region genes with different *J* region genes, a process that may be termed genetic combinatorial diversity; (2) recombination events occurring within members of a given group during evolutionary and somatic development. This working model of generation of antibody diversity brings together the once opposing theories of germ-line and somatic mutations, but still leaves uncertain the approximate number of *V* genes encoded in the germ-line. Recent developments in the field of nucleic acid chemistry may soon provide more definitive answers. The concept of separately functional *V* genes, *C* genes, and *J* genes is somewhat misleading. Functional *Ig* gene complexes are rather synthesized by joining two types of DNA segments, one specifying the V region and the other specifying the C region. Viewed in another way, activation of a particular *V* gene for transcription may require its splicing to an appropriate *J* and *C* gene. In essence, active immunoglobulin heavy and light chain genes are generated through somatic rearrangements in DNA. The immune system utilizes two combinatorial mechanisms in generating diverse antibodies: combinatorial association of heavy chains and light chains, and combinatorial translocation of *V* genes with different *C* genes.

KEY REFERENCES

Books

Glynn, L. E., and Steward, N. W. (eds.). *Immunochemistry: An Advanced Textbook.* New York: John Wiley & Sons, 1977. Contains comprehensive chapters on immunoglobulin structure, genetics, and diversity.

Hood, L. E., Weissman, I. L., and Wood, W. B. *Immunology.* Menlo Park: Benjamin-/Cummings, 1978. A concise, well-illustrated textbook of immunology.

Nisonoff, A., Hopper, J. E., and Spring, S. B. *The Antibody Molecule.* New York: Academic Press, 1975. Pages 346–406 contain an excellent treatment of allotypes of rabbit, man, and mouse immunoglobulins.

Putnam, F. W. (ed.). *The Plasma Proteins.* vol. III. 2d ed. New York: Academic Press, 1977. The chapters by Putnam on structure, function, and genetics of immunoglobulins are especially informative.

Articles

Immunoglobulin Structure

Davies, D. R., and Padlan, E. A. Three dimensional structure of immunoglobulins. *Annu. Rev. Biochem.* 44:639–667, 1975. A review of crystallographic studies of human immunoglobulins and immunoglobulin fragments confirming the domain hypothesis of immunoglobulin structure.

Edelman, G. M. Antibody structure and molecular immunology. *Ann. N.Y. Acad. Sci.* 190:5–25, 1971. A contemporary discussion of the structure of human IgG and elaboration of the domain hypothesis of immunoglobulin tertiary structure.

Edelman, G. M., et al. The covalent structure of an entire γG immunoglobulin molecule. *Proc. Natl. Acad. Sci. USA* 63:78–85, 1969. This paper reports the first complete amino acid sequence of a human IgG myeloma protein including the location of all disulphide bonds.

Porter, R. R. The structure of gamma-globulin and antibodies. In Gellhorn, A. and Herschberg, E. (eds.). *Basic Problems of Neoplastic Disease.* New York: Columbia University Press, 1962. Pages 177–194. Summarizes Porter's earlier experiments characterizing enzyme cleavage fragments of normal immunoglobulin.

Wu, T. T., and Kabat, E. A. An analysis of the sequences of the variable regions of Bence-Jones proteins and myeloma light chains and their implications for antibody complementarity. *J. Exp. Med.* 132: 211–250, 1970. Variability analysis of amino acid sequences of human L chains indicates the existence of three regions of hypervariable sequence in the VL region.

Antibody Structural Genes

Binz, H., and Wigzell, H. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. *J. Exp. Med.* 142: 197–211, 1975. Antigen-binding receptors on T-cells share idiotypic determinants with IgG antibodies with the same antigen-binding specificity.

Eichmann, K. Idiotype expression and the inheritance of mouse antibody clones. *J. Exp. Med.* 137: 603–621, 1973. Expression of the idiotypes of two monoclonal antibodies to streptococcal carbohydrates varies in frequency within the A/J strain. Both idiotypes are strain-specific and are transmitted to F1 hybrids. Evidence for C region allotype linkage with the idiotypic determinants was obtained.

Green, M. C. Genetic nomenclature for the immunoglobulin loci of the mouse. *Immunogenetics* 8:89–97, 1979. In the newly adopted nomenclature, the heavy(h)-, kappa(k)-, and lambda(l)-chain constant subregion loci are designated *Igh-1, Igh-2, Igk-2,* etc. Variable subregion loci are designated, for example, *Igh-Dex, Igh-Pc,* and *Igk-Ef1;* the symbol to the right of the hyphen identifies the corresponding antigen.

Herzenberg, L. A., et al. Surface markers and functional relationships of cells involved in murine B-lymphocyte differentiation. *Cold Spr. Harb. Symp. Quant. Biol.* 41:33–45, 1976. The murine allotypes Igh-5 and Igh-6 are present on IgD and IgM, respectively. These allotypes, present on B-cell surface immunoglobulin, provide an excellent tool for tracing the ontogeny of murine B-cells.

Hozumi, N., and Tonegawa, S. Evidence for somatic rearrangement of immunoglobulin genes coding for variable and constant regions. *Proc. Natl. Acad. Sci. USA* 73:3628–3632, 1976. DNA from Balb/c mouse embryo, or from MOPC-321 plasmacytoma, was digested with restriction enzymes and the resulting fragments hybridized with mRNA probes for whole κ chain or the Cκ region only. The results

indicate that *Vκ* and *Cκ* genes are separated in embryonic DNA but form a contiguous stretch in the DNA of the fully differentiated cell.

Margolies, M. N., Cannon, L. E., Kindt, T. J., and Fraser, B. The structural basis of rabbit VH allotypes: Serologic studies on al H chains with defined amino acid sequence. *J. Immunol.* 119:287–294, 1977. Amino acid sequence analysis of VH regions of three homogeneous antipneumococcal antibodies showed a correlation between sequence in framework regions and the group a allotypic specificity.

Seidman, J. G., Max, E. E., and Leder, P. A *K*-immunoglobulin gene is formed by site-specific recombination without further somatic mutation. *Nature* 280:370–375, 1979. Direct evidence at the nucleotide sequence level is presented for somatic recombination of germ-line genes leading to the joining of a *V* region gene with that of the *J* segment. No point mutational events were detected when comparing germ-line sequence with active somatic sequence.

Strosberg, A. D. Multiple expression of rabbit allotypes: The tip of the iceberg? *Immunogenetics* 4: 499–513, 1977. A review article examining recent anomalies in the expression of rabbit allotypes including such phenomena as pluriallelism and transitory and latent allotypes.

Tonegawa, S., Maxam, A. M., Tizard, R., Bernard, O., and Gilbert, W. Sequence of a mouse germ-line gene for a variable region of an immunoglobulin light chain. *Proc. Natl. Acad. Sci. USA* 75:1485–1489, 1978. The nucleotide sequence of a gene encoding the V region of a mouse immunoglobulin confirms that the *V* gene is separated from the *C* gene.

Wang, A. C., Mathur, S., Pandy, J., Siegal, F. P., Middaugh, C. R., and Sitman, G. W. Hv(1), a variable region genetic marker of human immunoglobulin heavy chains. *Science* 200: 327–329, 1978. Hv(1) represents the first description of a V region allotype in human immunoglobulins as it has been identified, using a hemagglutination–inhibition assay, on human IgG, IgM, and IgA.

Yarmush, M. L., and Kindt, T. J. Isolation and characterization of IgG molecules expressing latent group b allotypes from pedigreed b^4b^4 rabbits. *J. Exp. Med.* 148: 522–533, 1978. Latent group b allotypes were detected in IgG from rabbits of homozygous *b4,b4* genotype. The latent allotypes were shown to reside on a population of molecules not bearing the b4 marker.

Antibody Diversity

Capra, J. D., and Kindt, T. J. Antibody diversity: Can more than one gene encode each variable region? *Immunogenetics* 1:417–427, 1975. Episomal insertion is proposed as a mechanism of generating diversity in immunoglobulin V regions. Three gene groups are proposed to encode a single immunoglobulin polypeptide chain; one encoding the hypervariable regions, a second the V region framework residues, and a third the C region.

Hood, L., et al. The structure and genetics of mouse immunoglobulins: An analysis of NZB myeloma proteins and sets of BALB/c myeloma proteins binding particular haptens. *Cold Spr. Harb. Symp. Quant. Biol.* 41:817–836, 1976. An analysis of the theories of antibody diversity in the light of sequence data available for NZB murine myeloma proteins and hapten binding BALB/c myeloma proteins.

Milstein, C., et al. Somatic cell genetics of antibody-secreting cells: Studies of clonal diversification and analysis by cell fusion. *Cold Spr. Harb. Symp. Quant. Biol.* 41: 793–803, 1976. An experimental system for assessing somatic mutation events in mouse immunoglobulin genes is described. The potentials of somatic cell fusion as a tool for investigating antibody diversity are elaborated.

Seidman, J. G., Leder, A., Nau, M., Norman, B., and Leder, P. Antibody diversity: The structure of cloned immunoglobulin genes suggests a mechanism for generating new sequences. *Science* 202: 11–17, 1978. Variable region genes are segregated into small

families of genes of closely related sequence (subgroups). The areas of extensive gene homology shared by members of a subgroup are seen as targets for intragenic recombination leading to extension of the *V* gene repertoire.

Valbuena, O., Marcu, K. B., Weigert, M., and Peery, R. P. Multiplicity of germ line genes specifying a group of related mouse κ chains with implications for the generation of immunoglobulin diversity. *Nature* 276:780–784, 1978. Saturation hybridization analysis of the Vκ 21 group of murine light chains indicates only four to six germ-line genes specifying these sequences. This infers the requirement for a non-germ-line mechanism of diversity generation.

Weigert, M., Gatmaitan, L, Loh, E., Schilling, J., and Hood, L. Rearrangement of genetic information may produce immunoglobulin diversity. *Nature* 276:785–790, 1978. Amino acid sequence analysis of twenty-two Vκ regions from NZB mice demonstrates that the κ gene is divided into three segments, *V, C,* and the *J* or joining segment. Different combinations of *V* and *J* genes contribute to antibody diversity.

Weigert, M., and Riblet, R. Genetic control of antibody variable regions. *Cold Spr. Harb. Symp. Quant. Biol.* 41:837–846, 1976. A comprehensive examination of germline and somatic generation theories, particularly related to data obtained from the Vλ system.

3

MICROBIAL ANTIGENS AND SEROTYPE PATTERNS

There are many theoretical and practical reasons for continuing interest in the antigenic specificities of microorganisms. In the realm of infection and disease, survival of pathogenic microorganisms depends on their ability to circumvent or override the host response to their foreignness or antigenicity. The identification of pathogenic bacteria or viruses and the preparation of vaccines often require the characterization of both species- and strain-specific antigens. Subtle but important differences in cellular or viral constituents may often be discerned only by appropriate antigen–antibody reactions. Living microorganisms, like cells in general, contain many specific antigens, some of which are effective and others poor inducers of antibody production. However, it is noteworthy that the virulence of a pathogenic organism is usually referable to a certain class of antigen. The capsular polysaccharides of the pneumococci, for example, are type-specific as well as essential for the virulence of the organism. In this species, polymers of basic sugar units are combined in various sequences to give nearly 100 distinct serologic types. The virulence-determining antigens of microorganisms include polysaccharides, proteins, carbohydrate–protein conjugates and polysaccharide–lipid–protein complexes; the last are found in many Gram-negative bacteria located in the intestinal tract.

In diagnostic microbiology and epidemiology, serologic typing is often the principal tool for identification of etiologic agents. Spontaneous mutations in antigenicity are known to yield new strains of bacteria and viruses, which then possess a selective advantage to the extent that potential hosts lack preexisting immunity to the antigens of the new strains.

Immunogenetic characterization of microorganisms poses special problems because many novel patterns of sexual and asexual reproduction substantially complicate genetic analyses. Many fungi and protozoa, as well as bacteria and viruses, are haploid or contain only one complete set of genes. When zygotes or sexual products are formed, they soon undergo division to yield haploid forms again, including recombinants. Under these circumstances, and also in

asexually dividing cells, mutations will be expressed immediately and exposed to environmental selection.

In many species of fungi and protozoa, sexual reproduction occurs only between individuals of different mating types. Such compatibility patterns, which we shall consider in some detail, are heritable and involve antigenic surface constituents that may be characterized as serotypes. Comparable sexuality occurs in many bacterial species in the form of a conjugation mechanism in which genetic recombination is effected by the one-way transfer of genes from donor to recipient bacteria. Donor bacteria in conjugation possess an episomal sex factor which is readily transmitted along with part of the donor bacterial chromosome to recipient cells lacking the factor. However, the donor bacteria generally transfer only part of their genome to recipients with the consequent formation of incomplete zygotes.

It is now clear that episomes are infectious genetic determinants present in addition to the normal cellular genome. Episomes are thought to include all elements capable of independent replication and transfer that show genetic interaction with the genomes of microbial host species. They may have alternative cytoplasmic and chromosomally integrated states. Three types of episomes or "conjugons" involved in direct cell-to-cell, sexual exchange have so far been described: the *F* (fertility) factors, the *Cf* (colicinogenic) factors, and the *RTF* (resistance transfer) factors. In genetic transfers involving transduction, bacteriophages of low virulence act as episomal carriers of one gene or a few closely linked genes. This parasexual phenomenon is common in Gram-negative bacillary species. Elevated mutability in bacteria may be caused by mutator genes and by interaction between chromosomal genes and episomes. Mutator genes are known in *Salmonella typhimurium, Escherichia coli,* and *Neisseria meningitidis.*

Finally, there is the process of transformation, probably uncommon in nature, in which single donor genes in the form of unencumbered deoxyribonucleic acid may induce homologous hereditary changes upon assimilation by recipient bacteria. The essential peculiarity of sexual reproduction of all kinds in bacteria is the unequal genetic contribution of the two parents. It is sufficient for present purposes to realize that the genetic determination of antigenic macromolecules in microorganisms often involves unusual events beyond the scope of Mendelian inheritance as applied to higher animals.

Since our purpose is to emphasize principles rather than details of many species of microorganisms, only certain illustrative studies will be considered in depth. Extensively studied antigenic determinants and serotype patterns in bacteria will be considered first, with particular attention to the cell wall polysaccharide and flagellar protein antigens of *Salmonella* species. We then proceed to serotype systems in both free-living and parasitic protozoa. Ciliary antigen polymorphism in *Paramecium* and *Tetrahymena* is evaluated in the light of complex mating types and unusual nucleo-cytoplasmic interactions. This sets the stage for trying to understand antigenic variation in parasitic trypanosomes

and in malaria. Lastly, we come to grips with virus-associated antigens, especially the now well-studied systems involving interactions with known murine genes.

ANTIGENIC DETERMINANTS AND SEROTYPE PATTERNS IN BACTERIA

Antigenic variation in Enterobacteriaceae, especially *Salmonella* and *Escherichia,* has long been studied intensively to facilitate diagnosis of infectious disease. With the discovery of a conjugational system of genetic recombination and of phage-mediated transduction, genetic analyses of the molecular basis of virulence and pathogenicity have been forthcoming. Large numbers of distinctive serotypes, often reflecting subtle differences in the structure of cell surface lipopolysaccharides and proteins, may be identified among otherwise similar bacteria by use of appropriately absorbed antiserums. A consideration of serotype patterns in the enterobacterial group of intestinal pathogens is instructive from both genetic and immunologic standpoints. More than 700 strains or "species" have now been classified in the genus *Salmonella* on the basis of differences in serotypes. Two major genetic systems of serotypes, designated O for the somatic or cell wall polysaccharides and H for the flagellar protein antigens, are found in *Salmonella* species. Nearly eighty serotypes, dubiously classified as if they were separate species, are distinguishable on the basis of O antigens alone. Despite the great diversity of antigenic polysaccharides, a simplified classification is possible because many clones or serotypes exhibit strong cross-reactions and thus must possess similar antigenic determinants. The finding of major common determinants has led to the grouping of serotypes together in classes. However, cross-reactions between O antigens of different species such as *Salmonella* and *Klebsiella,* and *E. coli* and *Shigella,* occasionally complicate matters.

Characteristics and Genetic Control of O and H Antigens in *Salmonella*

First we consider serologic distinctions based on defined antigen–antibody reactions, and then we examine the structural and genetic bases. The Kauffman–White (K–W) scheme of serologic classification of *Salmonellae* (Table 3–1) designates major groups, usually including multiple serotypes, by capital letters (e.g., B, C3, D, E4). Over 95 percent of strains isolated from natural sources fall into one of five groups, A through E. Each group shares a common somatic antigen. Individual O determinants, as defined by exhaustive cross-reaction and cross-absorption tests with antibodies obtained by immunizing rabbits, are distinguished by number. Where two or more somatic antigens are present, one determines the group to which the strain is assigned. Thus, group B consists of those serotypes carrying O antigen 4, and group D serotypes

TABLE 3–1. Illustration of Kauffman–White Scheme of Serological Classification of Salmonellae

Group	Serotype or strain	O Antigens[a]	H Antigens	
			Phase 1[c]	Phase 2
A	*S. paratyphi A*	1, *2*, 12	a	—
B	*S. abortus-equi*	*4*, 12	—	e, n, x
	S. paratyphi B	1, *4*, 5, 12	b	1, 2
	S. stanley	*4*, 5, 12	d	1, 2
	S. bredeney	1, *4*, 12, 27	1, v	1, 7
C1	*S. paratyphi C*	*6*, 7, Vi	c	1, 5
	S. montevideo	*6*, 7	g, m, s	—
C2	*S. newport*	*6*, 8	e, h	1, 2
C3	*S. kentucky*	(8)[b], 20	i	Z_6
D	*S. sendai*	1, *9*, 12	a	1, 5
	S. typhi	*9*, 12, Vi	d	—
	S. enteritidis	1, *9*, 12	g, m	—
	S. panama	1, *9*, 12	1, v	1, 5
E1	*S. london*	*3*, 10	1, v	1, 6
E2	*S. newington*	*3*, 15	e, h	1, 6
E3	*S. minneapolis*	(*3*), (15), 34	e, h	1, 6
E4	*S. senftenberg*	1, *3*, 19	g, s, t	—

[a]The group-specific or private specificities are italicized below.

[b]Parenthesis is used to indicate that this serotype posseses only part of the somatic antigen designated within bracket.

[c]These antigens have already illustrated the limitations of an alphabetical notation by exceeding twenty-six in number.

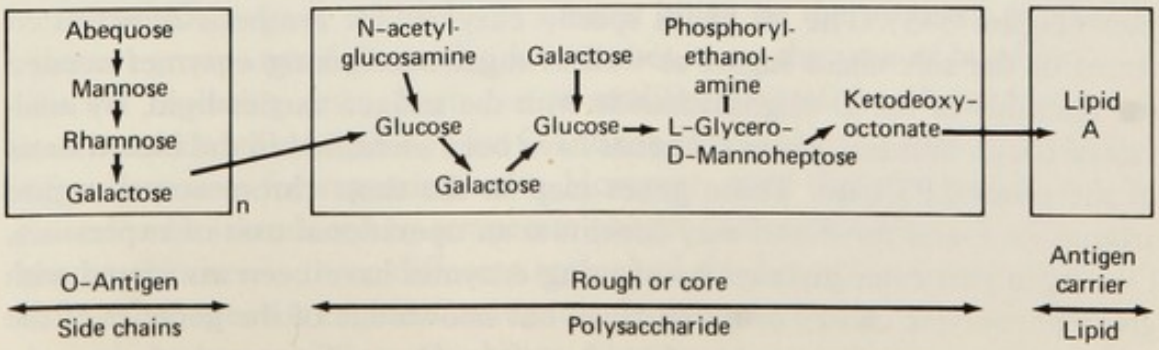
share O antigen 9. Several groups are divided into subgroups whose members each possess a second antigen in common. Groups A, B, and D_1 have almost identical factors 12 in common; all are also sensitive to phages P22 and P27, and a common antigen appears in the three groups after conversion by these phages. Such modifications of lipopolysaccharide (LPS) specificity have often been found to be dependent on bacteriophages, a phenomenon called *antigenic conversion.*

The H or flagellar protein antigens may exist in two alternative forms, designated phase 1 and phase 2. The determinants of the specific phase 1 are either peculiar to a given serotype or are shared by only a few other serotypes. However, the group phase 2 components are shared by many other O types (Table 3–1). A culture of a given diphasic strain may consist entirely of bacilli in either the specific phase or the group phase, or may include representatives of both phases. However, the two sets of flagellar antigens do not exist as mixtures in the same, individual bacteria. A clone usually remains in one phase for many generations but retains the capability of transforming into the alter-

native phase. Certain strains such as *S. paratyphi A* and *S. abortus-equi* are monophasic, that is, apparently capable of exhibiting only one set of flagellar antigens. Note in the K–W scheme that H antigenic complexes of both phases 1 and 2 are found within the major O groups. Unfortunately, designating H antigens by the combined use of small letters and Arabic numerals is potentially confusing. Many of the antigenic specificities identified by a single numeral or letter in the K–W scheme are themselves complex, consisting of several distinctive determinants. Thus, the antigenic constitution of most serotypes is more complex than is suggested by the notations in Table 3–1. Because of "minor" somatic or flagellar antigens, cross-agglutination may occur between serotypes which appear to have no common antigens in terms of K–W entries. Only "major" antigens of differential diagnostic importance have been regularly recorded.

Apart from epidemiologic identification of salmonellosis, the biochemical attributes and gene control of the antigenic macromolecules of *Salmonella* are of fundamental interest. The determinants of specificity in the O antigens reside entirely in the polysaccharide moiety of the cell wall lipopolysaccharides. The whole macromolecule consists of three units: outer nontoxic O-antigen side chains, a rough or core polysaccharide, and an antigen carrier lipid (lipid A) as illustrated in Figure 3–1. This lipopolysaccharide (LPS) is highly immunogenic and is an integral component of the protein–lipid–polysaccharide endotoxin which is responsible for the fever, diarrhea, edema, and internal hemorrhage produced by injection of heat-killed bacteria. The core of LPS, endotoxin without O chains, has a similar structure in all *Salmonella,* whatever their O antigenic character. All O-antigen specificities of a given serotype are located in a single polysaccharide molecule and the constituent determinants are referable to certain monosaccharide or oligosaccharide groupings. Purified oligosaccharides of low molecular weight have been characterized for their haptenic specificity by inhibition tests with rabbit antibodies directed against intact lipopolysaccharides of different clones. This hapten inhibition is the methodology of choice for identification of these antigenic determinants. The

FIGURE 3–1. Schematic structure of *Salmonella* lipopolysaccharide. A glucosyl residue of a branched pentasaccharide joined to a heptosyl disaccharide serves as the core attachment for the O antigen side chains. Glycosidic bonds are represented by arrows.



test sugar which possesses the highest affinity for the antibodies of a monospecific serum is termed the *immunodominant* sugar mainly responsible for a designated O specificity.

Only the wild-type strains possessing the entire antigenic side chains are pathogenic. Yet loss of the side chains or even part of the core molecule appears to have little effect on the ability of mutant strains to multiply in vitro. Presumably the complete lipopolysaccharide with its "smooth" O-polymer inhibits phagocytosis and thereby facilitates host invasion. The capsular polysaccharides of the pneumococci are also known to confer resistance to phagocytic digestion. No mutant *Salmonella* are known to lack the lipid-backbone portions of the lipopolysaccharide, which suggests that these components are indispensable.

Salmonella lipopolysaccharides may contain as many as eight different sugars. Because all of the major antigens of the A through E groups, with the exception of C_1, contain galactose, mannose, and rhamnose, the overall specificity must depend on the linkage and sequence of these sugars. However, nearly half of the many O antigens also contain 3, 6-dideoxyhexoses (e.g., tyvelose, abequose, colitose, paratose) that have been shown to serve as strong immunogenic determinants. In essence, diverse monosaccharides with multifarious linkages provide a large array of molecular alternatives. Immunodominant sugars responsible for the specificity of some O antigens are shown schematically in Figure 3–2. These sugars need not be terminal structures. The individual O specificities of a *Salmonella* serotype, all carried by one and the same macromolecule, are not separable as small molecules by chemical or serological fractionation. However, serotype distinctions may be assigned to single sugars—for example, the presence of paratose in antigen 2 versus abequose in antigen 4 versus tyvelose in antigen 9. Various blood group antigens in man are also known to derive their specificity from oligosaccharide units (Chapter 4).

Investigation of "rough" mutants (i.e., those without O antigen side chains) in *S. typhimurium* revealed some to have defects attributable to *rfa* genes affecting only the core portion of LPS, whereas a quite separate group of *rfb* genes governed synthesis of the O-specific portion. The most striking feature on the genetic side is that each major O specificity segregates in conjugational or transductional crosses as a unit character. All the clustered *rfb* genes for synthesis of the O repeating unit characteristic of a given O group are located in the *Salmonella* chromosome close to the *his* (histidine biosynthesis) gene complex (Figure 3–3). The *rfb* genes specify enzymes for synthesis of activated forms of the side chain sugars as well as sugar-transferring enzymes needed for assembly of the O oligosaccharide with the antigen carrier lipid. By analysis of rough mutants, seven *rfa* genes have been identified in the biosynthesis of the outer LPS core. These genes map in the short chromosomal region around *cys E* and *pyr E* and may constitute an operational unit of expression. Defects in particular glycosyl-transferring enzymes have been associated with given phenotypic classes of *rfa* mutants, but knowledge of the genetics of the LPS core is much less complete than that of the O-specific part.

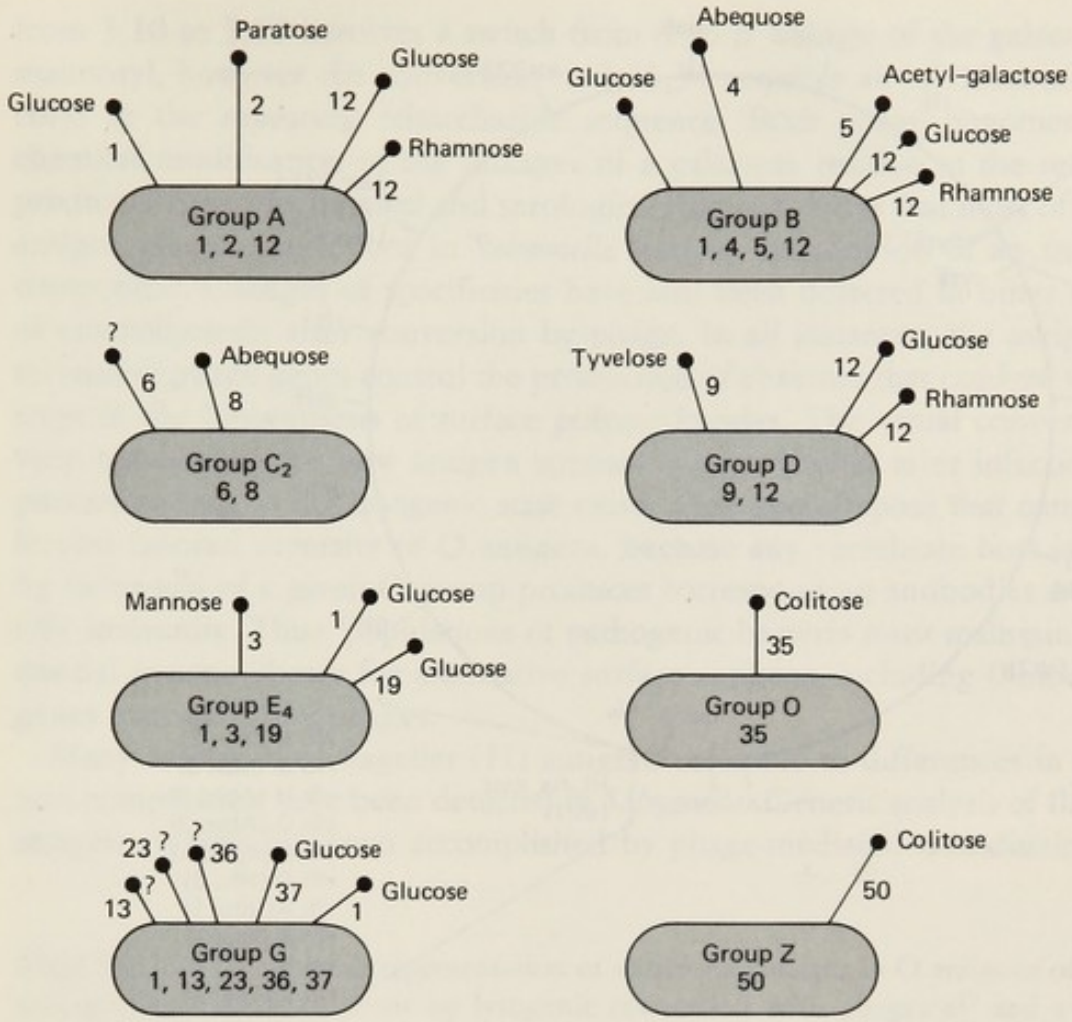


FIGURE 3–2. Schematic representation of sugars responsible for the specificity of some O antigens of *Salmonella* as determined by serological inhibition studies. The group-specific O determinants of the Kauffman–White scheme are shown within the soma (e.g., 1, 2, 12 in Group A). The "immunodominant" sugars, terminal or otherwise, which possess the highest affinity for the corresponding antibodies are shown at the ends of lines projecting from the soma. Note that O antigens containing the same sugars may still belong to different serogroups, e.g., antigens 4 and 8, or 35 and 50, contain terminal abequose and colitose, respectively, but each numbered determinant is serologically distinct.

Certain O antigens in the K–W scheme hinge on the presence or absence of acetyl groups or of glucosyl side branches or on the type of bond between adjacent repeating units. Such minor modifications of the O polysaccharide have been identified with *oaf* (for O antigen factors) loci or with *rfc* loci yielding polymerases for repeating units. However, most *O*-modifying genes have proved to be introduced by temperate phages and map at preferred prophage attachment sites in three different regions of the bacterial chromosome (Figure 3–3). Genes for certain O-antigenic determinants appear to be carried exclusively by lysogenic bacteriophages. Changes in serotype from 3,10 to 3,15,

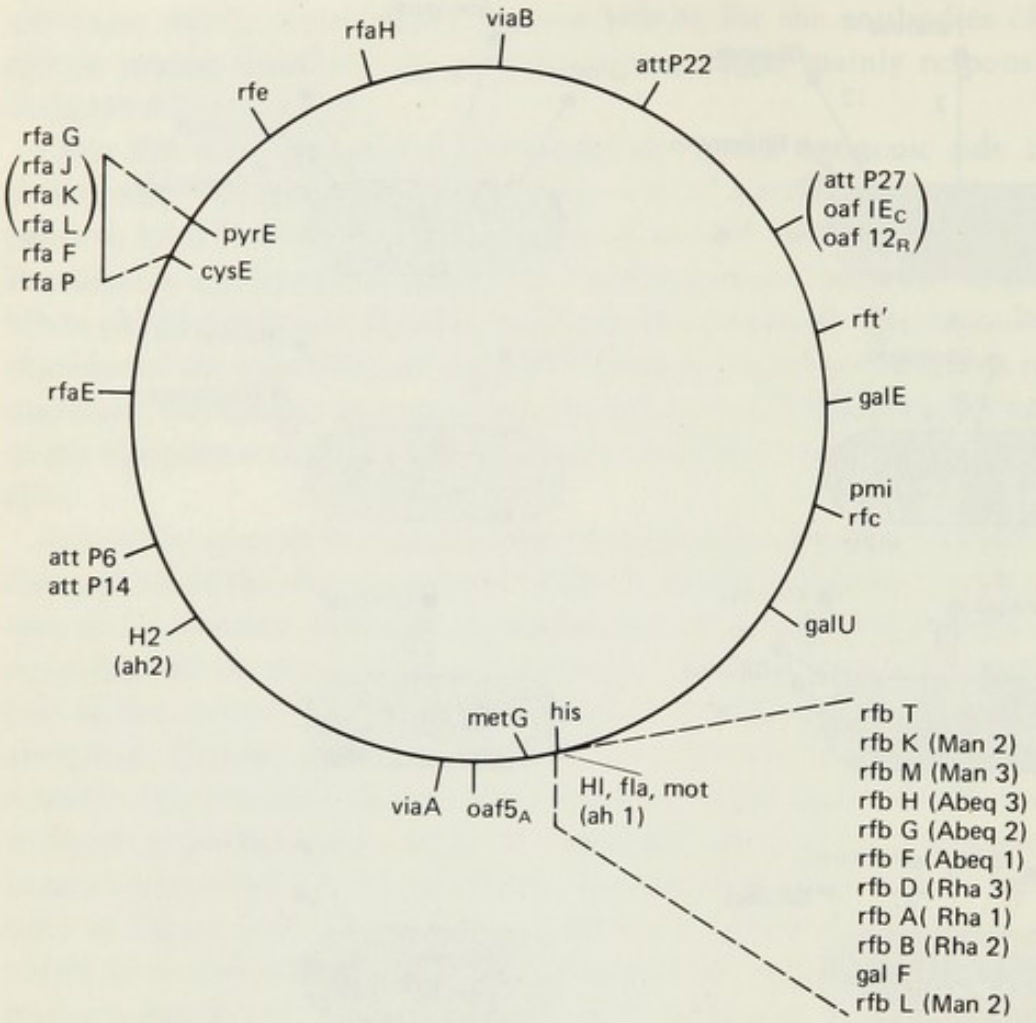


FIGURE 3–3. Immunogenetic linkage map of *Salmonella* with genes known to affect polysaccharide or flagellar protein synthesis indicated outside the circular chromosome. Loci are listed in known relative order except where shown in brackets. Gene symbols: *rf* = LPS biosynthesis; *rfa* for core, *rfb* for O repeating unit, *rfc* for polymerization of repeating units, *rfe* undefined, *rft'* for T1 side chain synthesis. The functions of biosynthetic enzymes determined by *rfb* genes are indicated in parentheses, e.g., (Man 3) for the third enzyme in the GDP-mannose pathway. Otherwise, *att* = prophage attachment site for phages 6, 14, 22 and 27: *cysE* = cystine biosynthesis; *gal* = enzyme for galactose metabolism; *H1* and *H2* = flagellar phase proteins with respective activator (*ah1* and *ah2*) genes: *his* = histidine biosynthesis; *met* = methionine biosynthesis; *oaf* = O antigen factors determined by modification of repeating unit, e.g., *oaf* ^{1E}C governs factor 1 in Group E; *pmi* = phosphomannoisomerase; *pyr E* = pyrimidine biosynthesis; *via* = Vi antigen synthesis.

Source: Modified from Stockert and Mäkelä, *Microbial Toxins*, vol. IV. New York: Academic Press, 1971. p. 390.

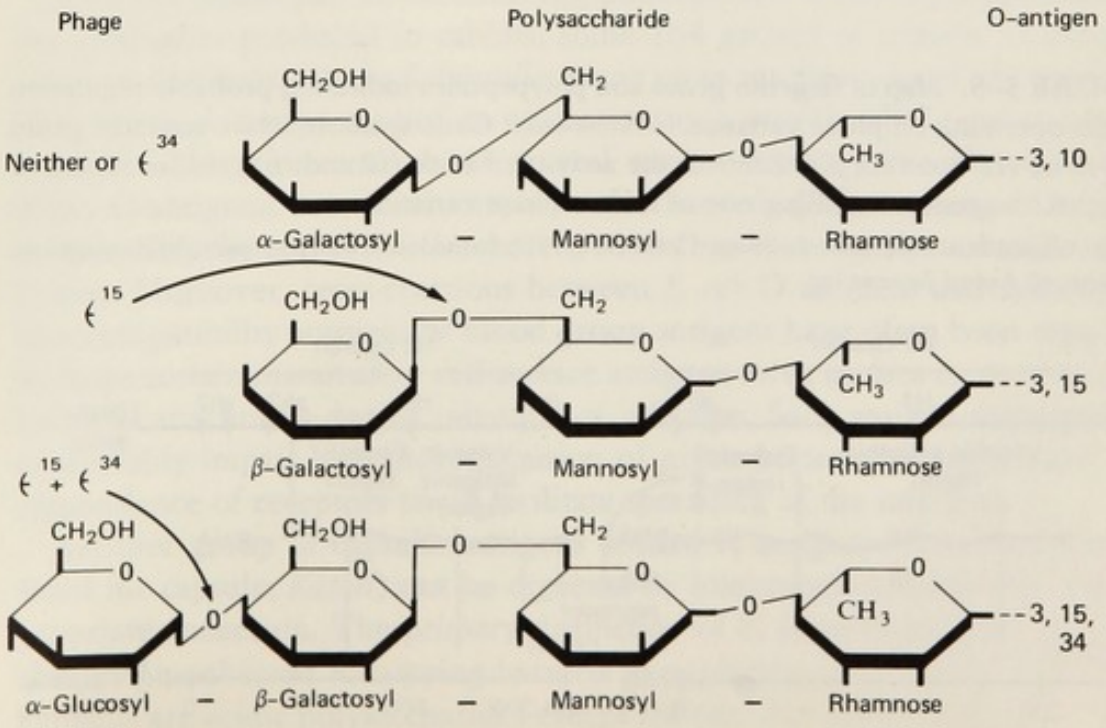
and finally to 3,15,34 produced by antigenic conversion in the E group of *Salmonella* are illustrated in Figure 3–4. When O serotype 3,10 is infected with phage E^{15}, a new serotype soon appears which is either 3,15 or 3,15,34, depending on whether the original strain carried E^{34} prophage prior to infection with E^{15}. If both phages are present, the conversion is to 3,15,34. The change

from 3,10 to 3,15 involves a switch from α to β linkage of the galactosyl to mannosyl, however the conversion to 3,15,34 requires an addition of α-glucosyl to the repeating trisaccharide sequence. Both phage genomes cause chemical modification of the linkages of a galactose residue in the sequence predicted from biochemical and serological findings. Note that most of the O-antigen phage conversions in *Salmonella* lead to the addition of an antigenic component. Changes of specificities have also been detected in other species of enterobacteria after conversion by phage. In all instances, the antigen-determining phage genes control the production of enzymes that catalyze specific steps in the biosynthesis of surface polysaccharides. The actual conversion is very rapid, since the new antigen appears within minutes after infection and persists as long as the lysogenic state exists. One may suppose that natural selection favored diversity of O antigens, because any vertebrate host infected by *Salmonella* of a given O group produces corresponding antibodies and specific immunity. Thus, populations of pathogenic bacteria must maintain a substantial genetic library for alternative surface antigens, including *O*-modifying genes introduced by phages.

Many serotypes of flagellar (H) antigens referable to differences in amino acid composition have been detected in *Salmonella.* Genetic analysis of flagellar antigen systems has been accomplished by phage-mediated transduction and

FIGURE 3–4. Schematic representation of structural changes in O antigens of *Salmonella* group E brought about by lysogenic conversion with phages ϵ^{15} and ϵ^{34}. The phages provoke the appearance or depression of bacterial enzymes, which produce the new sugar linkages shown by the arrows. Phages may lead to replacement of one antigen by another or to the addition of one or more new antigenic specificities.

Source: Modified from Robbins and Uchida, *Federation Proc.* 21:704, 1962.

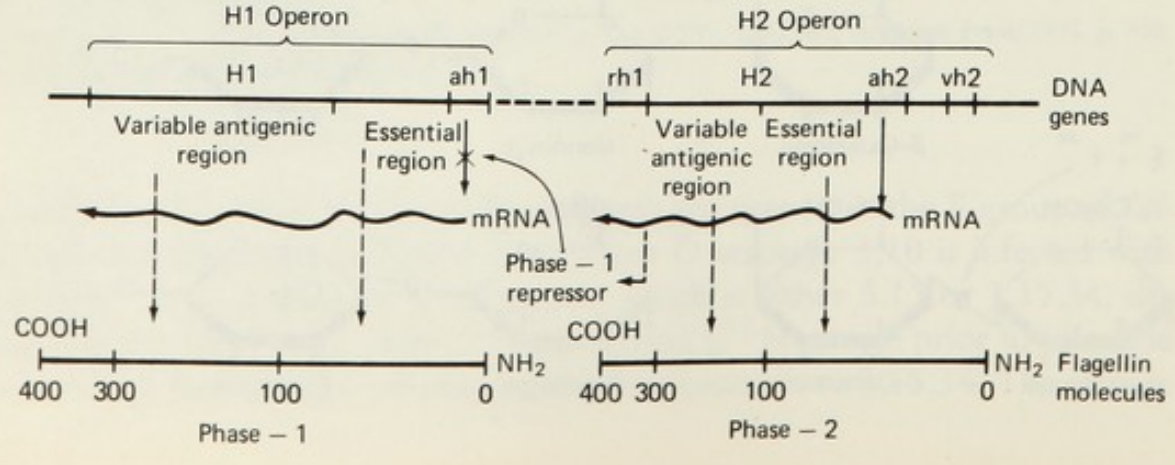


by sexual conjugation. Transduction between different diphasic serotypes indicates that the specificities of alternative phases 1 and 2 (Table 3–1) are mediated by "independent" loci designated *H1* and *H2*, respectively. In other words, these bacterial chromosomal loci are sufficiently separated (Figure 3–3) so simultaneous transduction by a single phage does not occur. Transductional experiments between different serotypes have revealed the existence of *H1* and *H2* operons, each with multiple alleles. The H serotype of each phase includes multiple antigenic determinants reflecting differences in amino acid sequence of the flagellin molecules primarily responsible for the characteristic antigen type. The extensive polymorphism of flagellin suggests the existence of evolutionarily variable and conservative regions in the molecule. Genetic analysis of the *H1* complex revealed nearly a quarter of the cistron from the terminus adjacent to *ah1* responsible for nonflagellate phenotype (Figure 3–5). This conservative or "essential region" may correspond to the portion of polypeptide chain essential for the specific conformation of flagellin. The "variable antigenic region" determines the sites of specific antigens and may correspond to exposed surface components of the flagellin molecule. Although antigenic specificities reside in the central region of flagellin polypeptide, the sites of amino acid substitution responsible for antigen mutations remain to be identified. One may note that flagellins with unrelated antigens such as (b) and (g) differ by as many as thirty-nine amino acid substitutions, whereas antigenically similar flagellins, e.g., (g,m), (g,m,s), and (g,s,t), differ by only a few amino acids from a total of nearly 380 (see Table 3–1).

The regulatory system determining phase variation in *Salmonella* probably operates as diagrammed in Figure 3–5. When H2 is active producing phase-2 flagellin, the synthesis of phase 1 flagellin by H1 is repressed. Activator genes, *ah1* and *ah2* are responsible for turning on the adjoining *H* gene. A repressor gene, *rh1*, was found to constitute an operon with *H2* and *ah2*. The

FIGURE 3–5. Map of flagellin genes and polypeptides indicating probable regulation system operating on phase variation in *Salmonella*. Gene symbols: *ah* = activator genes for *H1* or *H2* operons; *ah2* controls the activity of both *H2* and *rh1*; *rh1* = repressor gene; *vh2* = gene controlling rate of shift in phase variation.

Source: Adapted from Iino, *Annu. Rev. Genet.* 11:161–182, 1977. Reproduced, with permission, by *Annual Review of Genetics*, © Annual Reviews Inc.



phase-1 repressor product of *rh1* blocks the transcription of the *ah1-H1* operon, and this appears to account for alternative synthesis of phase-specific mRNA. An initial locus (*vh2*) in the *H2* operon controls the rate of shift in phase variation. Other regulatory genes involved in flagellar functions are *fla,* governing flagellar formation, and *mot,* controlling locomotive action. The largest cluster of flagella genes, including *H1, fla,* and *mot,* resides near the *rfb-his* operons (Figure 3–3).

In addition to H and O antigens, some *Salmonella* strains possess a polysaccharide somatic antigen often associated with virulence known as Vi antigen. Although only one major category of Vi antigen has been detected by agglutination, precipitation, and protection experiments, subtle differences in this antigen can be recognized through bacteriophages. Differing components of Vi antigen evidently serve as receptors to which specific phages are adsorbed. Nearly eighty distinctive Vi serotypes governed by several *via* loci of the typhoid bacillus have now been identified on the basis of their susceptibility to lysis by specific phages. From a basic standpoint, it should be apparent that the number of antigenic or serotypic differences detectable in a species or genus is limited only by the resourcefulness and persistence of the investigator.

Serology and Genetics of O and K Antigens of *Escherichia coli*

The *E. coli* group or species consists of many serofermentative types known as *serovars* that may be classified by ability to ferment certain sugars, but are most readily differentiated on the basis of antigenic surface structures. Because *Escherichia* strains occur both as normal inhabitants of the intestinal tract and as intestinal or extraintestinal pathogens, genetic mechanisms underlying their variable pathogenicity in diverse mammals, including man, have recently aroused wide interest.

With the techniques of bacterial agglutination and immunoprecipitation using antibodies produced in rabbits, some 164 groups of somatic O antigens are now detectable. As in *Salmonella,* many cross-reactions occur among single O antigens, so assignment of an unknown strain to a certain O group usually requires absorption analysis of several strains with specific O antiserums. Many O-antigenic cross-reactions have also been discerned among *E. coli, Salmonella, Shigella,* and *Klebsiella,* thereby revealing shared polysaccharide specificities. Moreover, cross-reactions between *E. coli* O antigens and mammalian histocompatibility antigens or blood group antigens have often been reported. Perhaps certain mammalian cell-surface antigens serve as sites or receptors for bacterial attachment during initiation of infection. Such antigen sharing could conceivably impair immunorecognition of given bacterial serotypes and correspondence of receptors could facilitate spreading of the infection.

Another group of capsular antigens termed K antigens (from the German word for capsule, *Kapsel*) can be detected by immunoelectrophoresis with appropriate antiserum. The primary distinction of K from O antigens is the inability of nonheated K-carrying bacteria to agglutinate in O antiserum. All K antigens are acidic polysaccharides except for two that are proteins. The acidic

polysaccharide K antigens contain unusual sugars such as N-acetylneuraminic acid or N-acetylmannosaminuronic acid. Immunodominant sugars may be branch substituents such as galactose in the K27 antigen, or a repeating oligosaccharide of a polysaccharide main chain as with the K30 and K42 antigens. Other K and O antigens appear to be "conformational determinants" depending on specific spatial arrangements, such as helix or coil formation in a polysaccharide.

Distinct groups of the K antigens are specified by two quite separate gene loci on the *E. coli* chromosome. One locus termed *kps A* (*k* for K antigen, *ps* for polysaccharide, and *A* because it was the first locus described) controls the synthesis of K1, K4, K10, and K54 antigens and is close to *ser A.* The other *kps B,* near the *his* operon, specifies ten known K antigens and is closely linked to the *rfb* gene cluster that determines the O-specific polysaccharides. The pertinent linkage map of loci affecting the antigenic properties of *E. coli* as derived mainly from conjugation experiments is shown in Figure 3–6. Some kps B antigens require an additional *rfc* locus for complete expression. This *rfc* locus may yield a K-specific polymerase with a function similar to the rfc enzyme of *Salmonella* responsible for polymerization of O-specific oligosaccharide repeating units.

A few K antigens are found in protein surface structures of *E. coli* known as fimbria or pili which appear to function as adhesive organs. The determinants of the K88 and K99 protein antigens are located on transferable plasmids and thus are doubly distinct from the other two groupings of K antigens. Another capsular polysaccharide antigen known as M antigen surrounds the bacterial cell as a thick slime layer, but is not a type-specific K antigen because the same specificity is found in many Enterobacteriaceae. Mutations in two regulator genes designated *lon* and *cap S* can derepress synthesis of M antigen and thereby allow its overproduction. Another gene located near *his,* termed *non-9,* inhibits M antigen capsule formation.

The close similarity of the genetic maps of the related *Escherichia* and *Salmonella* genera has been confirmed by both conjugational crosses and transduction experiments. Indeed, many genes derived from a strain of one genus function normally when transferred to bacteria of the other genus. However, genes controlling flagellar antigen(s) in the two genera exhibit striking differences. Most *Salmonella* species possess two flagellar serotypes displaying diphasic variation, i.e., exclusive expression of either the *H1* allele or the *H2* allele in a given bacterium. In contrast, *E. coli* strains yield only one flagellar antigen with no phase variation. The flagellin-determining locus of *E. coli,* now termed *hag,* corresponds to *H1* in *Salmonella.*

The attractive idea that host–parasite interactions in infectious diseases provide continual natural selection for cell-surface and immunogenetic polymorphisms was first put forward by J. B. S. Haldane in 1949. According to this view, the *raison d'etre* for highly discriminating immunosurveillance by metazoan animals is defense against infectious agents. Conversely, microorganisms could counteract adaptive immune responsiveness by continual production of new protective surface coats. Indeed, the enormous diversity of antigenic pat-

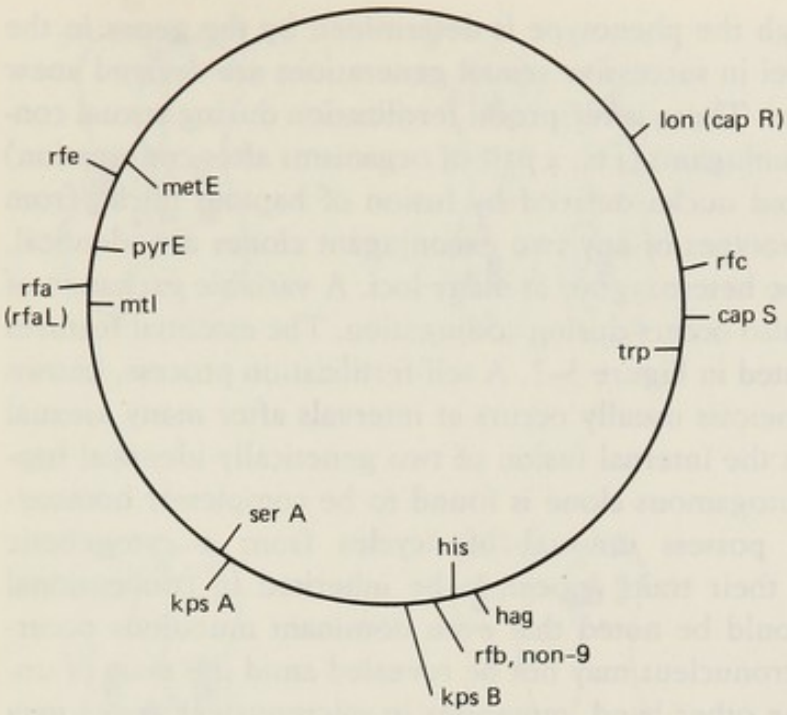


FIGURE 3–6. Immunogenetic linkage map of *Escherichia coli*, with genes known to affect polysaccharide or flagellar protein synthesis indicated outside the circular chromosome. Loci are listed in probable relative order, although exact positions may be inaccurate. Gene symbols: *rf* = LPS biosynthesis; *rfa* = core; *rfb* = O repeating unit; *rfc* = polymerization of O repeating units; *rfe* = undefined; *kps A* and *kps B* = polysaccharide K antigen biosynthesis; *non-9* = block in M antigen capsule formation; *lon* (previously *cap R*) and *cap S* = regulator genes for M antigen synthesis; *hag* = flagellin-specifying locus; *his* = histidine biosynthesis; met E = methionine biosynthesis; *mtl* = mannitol biosynthesis; pyr E = pyrimidine biosynthesis; ser A = serine biosynthesis; trp = tryptophan biosynthesis.

Source: Adapted from Ørskov et al., *Bacteriol. Rev.* 41:691, 1977. © 1977 American Society for Microbiology.

terns found in populations of enteropathogenic bacteria and certain viruses is consistent with this view. Yet nonpathogenic, common yeasts may also exhibit a great variety of surface antigens depending mainly on oligosaccharide structures similar to those characteristic of microbial pathogens. Obviously, such polymorphism is of value to many microorganisms also in nonparasitic environments.

SEROTYPE SYSTEMS IN FREE-LIVING AND PARASITIC PROTOZOA

Ciliary Antigen Polymorphism in *Paramecium* and *Tetrahymena*

Unlike most microorganisms, the ciliates are diploid or polyploid at the somatic level. During periods of fission (i.e., asexual reproduction) in the most extensively studied species, *Paramecium aurelia*, individuals possess two diploid micronuclei and a single macronucleus containing forty or more diploid sets

of chromosomes. Although the phenotype is determined by the genes in the macronucleus, macronuclei in successive sexual generations are derived anew from germinal micronuclei. There is reciprocal fertilization during sexual conjugation which yields exconjugants (i.e., a pair of organisms after conjugation) containing identical diploid nuclei derived by fusion of haploid nuclei from each mate. Thus, the genotypes of any two exconjugant clones are identical, although such cells may be heterozygous at many loci. A variable exchange of cytoplasmic constituents also occurs during conjugation. The essential features of conjugation are illustrated in Figure 3–7. A self-fertilization process, known as autogamy, involving meiosis usually occurs at intervals after many asexual generations. Autogamy is the internal fusion of two genetically identical haploid nuclei and each exautogamous clone is found to be completely homozygous. Although ciliates possess unusual life cycles from a cytogenetic standpoint, nearly all of their traits appear to be inherited in conventional Mendelian fashion. It should be noted that even dominant mutations occurring in the polyploid macronucleus may not be revealed amid the mass of unchanged genomes. On the other hand, mutations in micronuclear genes may be detected after one or two sexual generations.

Perhaps most remarkably, the breeding relations in ciliates involve systems of mating types or multiple sexes. Different stocks or clones, i.e., progenies of single individuals, may be assigned to groups such that all stocks of the same group interbreed freely while different groups of stocks are incompatible and do not mate successfully. Each variety or syngen is represented by diverse clones and contains one or usually two mating types that will agglutinate and conjugate when mixed. Thus, conjugation requires contact between two individuals of complementary mating types, but the mode of recognition of complementarity is not known. In recent studies, the terms *clone* and *syngen* have been used in preference to *stock* and *variety,* respectively. The differentiation of mating type systems shows remarkable similarities to the serotype systems of ciliary antigens, which may now be considered in some detail.

The extensive polymorphism of ciliary antigens in *P. aurelia* and *Tetrahymena pyriformis* provides scope for increased understanding of gene–antigen relationships as well as nuclear–cytoplasmic interactions. Each individual of any one clone has the macronuclear genetic potential for the expression of a series of alternative ciliary antigens or serotypes. Ciliates of each serotype may be immobilized by dilute homologous antiserum, but not by antiserum against other serotypes. The antiserum is usually prepared by repeated immunization of a rabbit. The serotype specificity resides in a single, water soluble protein localized primarily in the cilia and body wall. Molecular weight determinations of immobilization antigens from several different serotypes indicate they are proteins of 310,000 to 320,000 daltons. After appropriate isolation, immobilization antigens may also be compared by immunodiffusion tests in agar gels.

Although multiple alleles at many loci are associated with diverse immobilization antigens in *P. aurelia,* each antigen is usually referable to a single gene or cistron. Normally only one of these antigens is detectably present at any

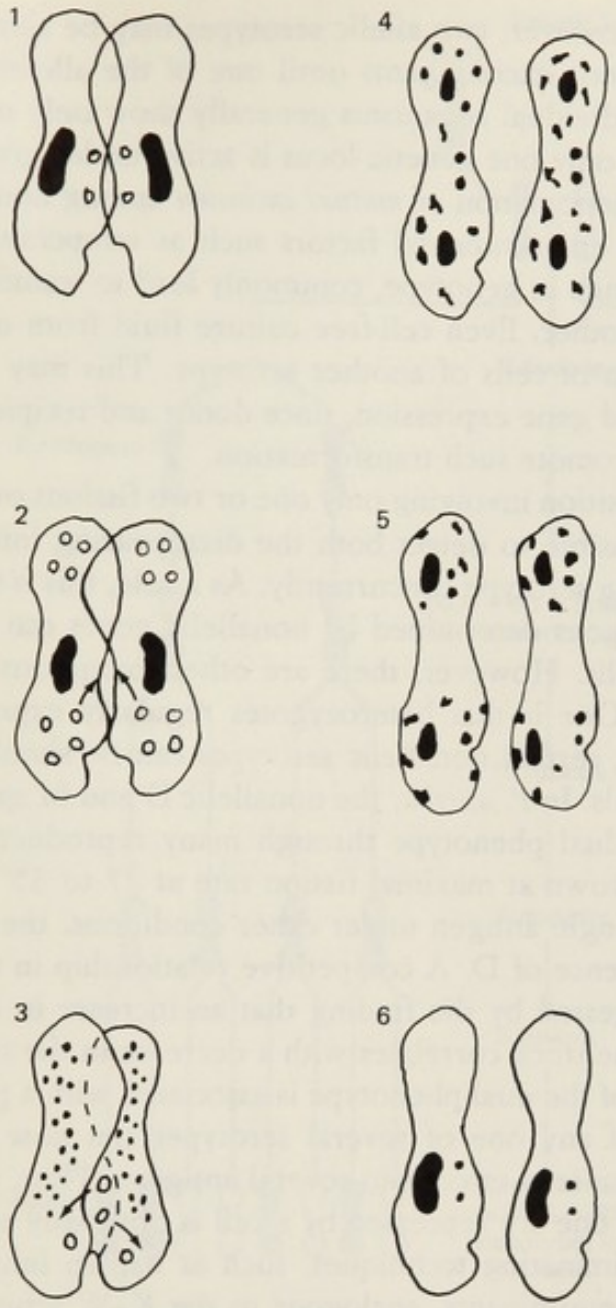


FIGURE 3–7. Essential features of sexual conjugation in ciliates of compatible mating types: (1) Fusion of two parental organisms of opposite mating type, each with one macronucleus and two diploid micronuclei. (2) Production of eight haploid nuclei from the germinal micronuclei of each conjugant. Seven of these nuclei disappear and the remaining nucleus in each conjugant passes into the paroral cone. (3) The nuclei in the paroral cones divide mitotically, forming "male" and "female" gamete nuclei. The female gamete nuclei enter the cytoplasm of the parental animals and fusion of male and female gamete nuclei from opposite mates occurs, followed by two mitotic nuclear divisions. The old macronuclei disintegrate. (4) Two products of each fusion nucleus differentiate into new macronuclei, while the other two become new micronuclei. Old macronuclear fragments gradually disappear. (5–6) Asexual fission yields typical organisms with two micronuclei and one macronucleus.

one time in a single organism. However, two allelic serotypes may be simultaneously expressed in heterozygous exconjugants until one of the alleles is eliminated by autogamy. Since individual organisms generally show only one serotype at a time, it follows that only one genetic locus is active under given conditions. This is the unusual phenomenon of *mutual exclusion* among nonallelic ciliary antigens. Changes in environmental factors such as temperature and growth rate, without any change in genotype, commonly lead to transformations from one serotype to another. Even cell-free culture fluid from one serotype can cause transformation of cells of another serotype. This may be regarded as an example of induced gene expression, since donor and recipient cells of identical genotypes can promote such transformation.

During the short period of transition involving only one or two fissions over some twenty-four hours, it is possible to detect both the disappearing initial serotype and the newly developing serotype concurrently. As a rule, this is the only time that two different antigens determined by nonallelic genes can be found concomitantly in single cells. However, there are other exceptions to the mutual exclusion principle. One is that heterozygotes regularly express both allelic serotypes. Moreover, certain nonallelic serotypes can be simultaneously expressed in single animals. In *P. aurelia,* the nonallelic D and M specificities can be maintained as a dual phenotype through many reproductive cycles when the organisms are grown at maximal fission rate at 27 to 35° C. Where serotype D occurs as a single antigen under other conditions, the M antigen is found only in the presence of D. A competitive relationship in the biosynthesis of D and M is suggested by the finding that an increase in the quantity of one antigen in any one stock correlates with a decrease in the other. Moreover, the manifestation of the dual phenotype is associated with a particular M allele. Many clones of any one of several serotypes can now be shown by antibody immobilization tests to contain several antigens. Thus, the assumption that all serotypes but one are repressed by a cell is obviously subject to qualification. More discriminating techniques, such as hapten inhibition, might reveal a mosaic of determinants, analogous to the K–W scheme of *Salmonella,* rather than just a single ciliary antigen characteristic of each serotype. The multispecificity of particular antiserums and possible cross-reactions between chemically similar antigens should be considered in this connection. Complicating exceptions notwithstanding, the novel phenomena of *mutual exclusion* and *serotype transformation* of ciliary antigens emerge as operational principles. Transformation of serotype may be succinctly defined as the reversible induction of a new ciliary antigen type and the complete disappearance of the previous type resulting from switches in gene activity. Analogous induction of *antigenic modulation* in certain mammalian cells is described in later chapters.

An example of the inheritance of allelic serotypes in *P. aurelia* from the work of Beale at Edinburgh University is shown in Figure 3–8. Each of two clones designated 60 and 90 in syngen 1 may exhibit three serotypes: type S at low temperatures, type G at intermediate temperatures, and type D at high

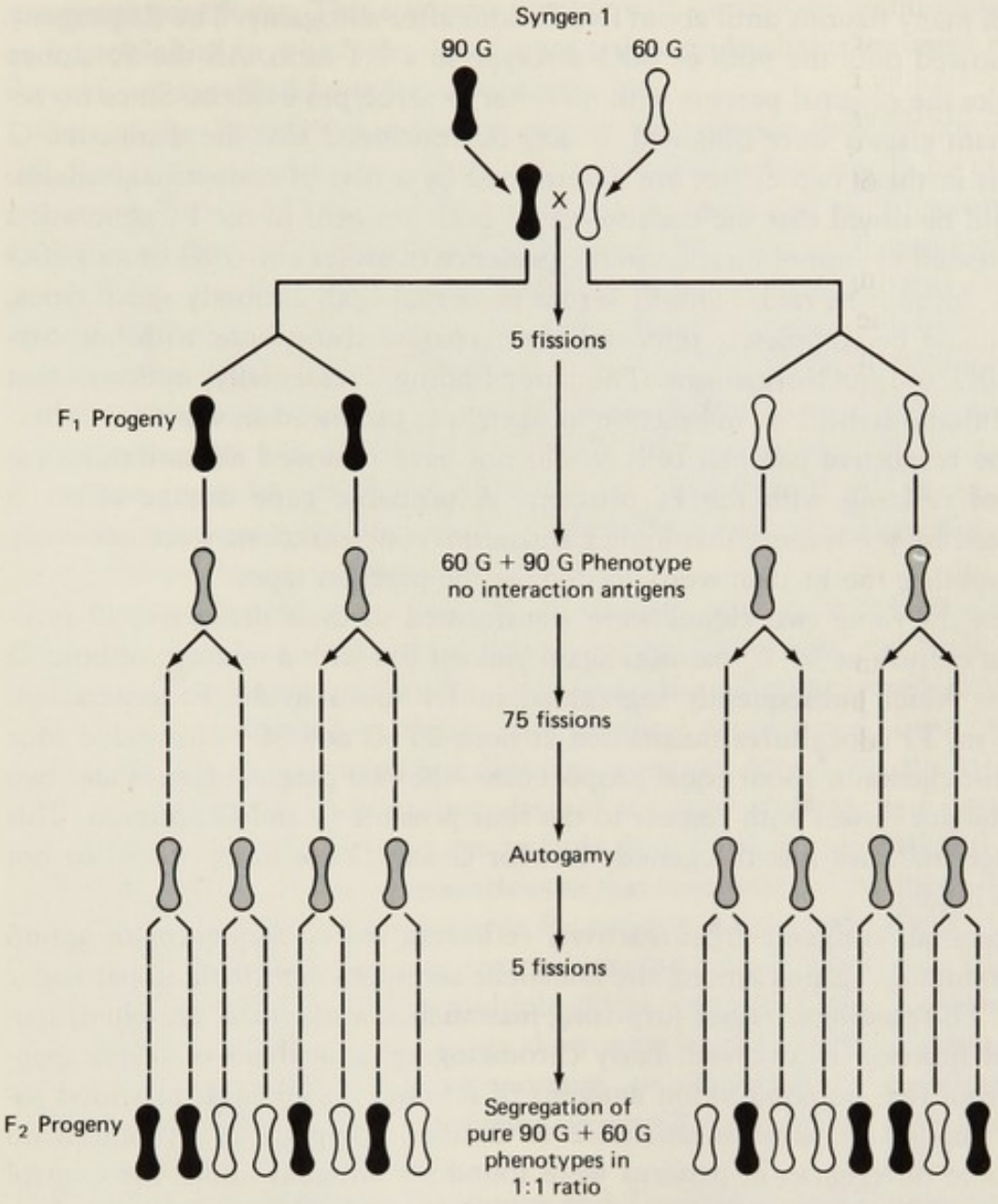


FIGURE 3–8. Segregation of allelic antigenic types 90G and 60G in F1 and F2 progeny.

Source: Modified from Beale, 1954; also see Beale, 1974.

temperatures. The G and D antigens were found to be clone-specific, as evidenced by immobilization reactions in appropriate antiserums. At a constant temperature of about 25° C, conjugation between 90G and 60G yielded F1 hybrids with both G serotypes expressed simultaneously. This codominant expression in the hybrid phenotype was detectable only after about five fissions. Prior to this stage, the hybrid clones showed only the serotype of the parent from which the cytoplasm was derived. Evidently, the proteins resulting from the new F1 macronucleus replace the preexisting ciliary protein gradually as required during sequential cell divisions. The dual hybrid phenotype persisted

through many fissions until about five fissions after autogamy. The F2 progeny then showed only the 90G or 60G serotype in a 1:1 ratio. All the F2 clones were like the original parents with no other G serotypes evident. Since no recombinant classes were obtained, it may be concluded that the distinctive G antigens in these two clones are determined by a pair of codominant alleles. It should be noted that the coexistence of both antigens in the F1 generation was revealed by immobilization in the presence of either anti-90G or anti-60G serums. Moreover, rabbit anti-F1 serum possessed both antibody specificities, which could be completely removed after separate absorptions with homozygous 90G and 60G organisms. The latter finding, incidentally, indicates that no additional hybrid or interaction antigen was produced in the F1s. Otherwise the respective parental cells would not have removed all antibodies capable of reacting with the F1 progeny. A probable gene dosage effect is suggested by the finding that higher antiserum concentrations were necessary to immobilize the F1 than were needed for the parental types.

When the same two clones were transformed to their distinctive D serotypes in culture at 30° C, matings again yielded F1s with a mixture of both D antigens which subsequently segregated in 1:1 ratios in the F2 generation. However, F2 subcultures maintained at both 25° C and 30° C revealed four antigenic classes in about equal proportions—the two parental classes and two recombinant classes with respect to the four possible G and D antigens. This evidence indicates that the genetic loci for G and D are independent or not closely linked.

In general, antigenic cross-reactivity reflecting shared sequences of amino acids is notably lacking among the nonallelic serotypes detectable in particular clones. This finding is rather surprising inasmuch as a structural protein of specialized function is involved. Early chromatographic analyses of tryptic peptides from five immobilization antigens of *P. aurelia,* syngen 4, provided so-called fingerprint patterns amenable to detailed comparisons. Although no detectable differences in patterns were found for antigens under the control of these alleles, the nonallelic antigens exhibited quite different fingerprints. However, no two of nine allelic D types were chemically identical in a later study, although five were indistinguishable by immunological criteria. Nonallelic serotypes bear surface proteins differing in amino acids in many peptides, whereas allelic subtypes exhibit much less variation. A close correlation has been shown between similarity of peptide maps and degree of serological cross-reactivity. Structurally, purified antigenic protein molecules consist of multiple polypeptides joined by disulphide bonds. The process of serotype transformation evidently requires the synthesis of polypeptides with different amino acid sequences to replace the protein characteristic of the previous ciliary antigen. However, allelic genes determine structurally similar proteins that share numerous antigenic determinants. The allelic D antigens of syngen 1, for example, are partially cross-reactive with specific antiserums and chromatographically separable as well. The genetic evidence of a one allele → one ciliary protein relationship suggests that an entire protein is specified by one

structural gene locus. This contrasts notably with vertebrate immunoglobulins or hemoglobins in which the constituent polypeptide chains, or even regions thereof, are specified by independent gene loci.

Beyond the Mendelian modes of genetic transmission thus far considered, ciliary antigen phenotypes appear to be controlled at least partly by cytoplasmic components. As noted earlier, the dual expression of allelic serotypes in F1 progeny requires several fissions following conjugation. Immediately after conjugation, the serotype of the parental cytoplasm is retained. When matings involve different nonallelic serotypes, the original parental serotypes usually persist in the two conjugant clones. However, under conditions of massive cytoplasmic exchange during conjugation between clones of different serotype, the exconjugants usually yield clones of identical serotype, with the serotype expressed depending on the conditions of the cross. Such clones also show the same mating type; again, the type expressed is determined by the environmental conditions during conjugation.

In essence, therefore, the traits of the progeny tend to be indistinguishable when cytoplasmic mixing occurs during mating. Otherwise, the traits remain distinct and segregate predictably. It is clear that different cytoplasmic states can become established in ciliates with identical genotypes. Crosses between strains of identical genotype but different serotypes reveal that the cytoplasm plays a decisive role in the maintenance of serotypic phenotypes. Considering the life cycle as a whole, there can be little doubt that the primary flow of genetic information is from micronucleus to macronucleus to cytoplasm. The system of genetic control of serotypes in *P. caudatum* is essentially identical to that found in *P. aurelia.* Differences among homologous serotypes from crosses of various stocks are governed by multiple alleles, whereas crosses involving nonhomologous or nonallelic serotypes show cytoplasmic control of gene expression. The extent to which autonomous or semiautonomous cytoplasmic episomes may influence differentiation or contribute hereditary information in ciliates is still largely unknown.

The serotype systems in *Paramecium* and *Tetrahymena* both show many similarities and also significant differences. In both species, single clones can differentiate or can be transformed into a variety of serotypes, albeit a particular cell normally expresses only one antigen at a time. However, in *Tetrahymena* the mutual exclusion of distinctive serotypes in heterozygotes generally occurs with allelic genes as well as nonallelic genes. Both interlocus and interallelic exclusion are also found in *Paramecium primaurelia;* in heterozygotes displaying allelic exclusion, the parental antigen phenotypically excluded is absent from both the cell surface and the cytoplasm. All heterozygous *Tetrahymena* combinations, though derived from homozygous parents, yield sublines showing considerable variability in phenotype. Although these sublines eventually express one allele or the other, a minority of intermediate phenotypes showing both antigens in different degrees may persist through many fissions. The particular allelic combination established in a heterozygote determines the direction of allelic repression. The interallelic exclusion of the most studied *H* locus

antigens in *Tetrahymena* appears to be strictly under nuclear control. Cytoplasmic transmission of serotype from one generation to the next has not been found. The results of various F1 × F1 and F1 × parental clone crosses show that the phenotypes of the heterozygotes do not influence the phenotypes of the next generation. In other words, the micronuclear genotype is not modified when a heterozygote undergoes serotype transformation.

The distribution of mating types and serotypes among progeny of a contrasting pair indicates that differences in both traits in cells of identical genotype are associated with macronuclear differentiation. Despite initial variability, all clones produce pure phenotypes in both systems at the same rate, calculated by Nanney to be 0.0113/cell division. Nevertheless, the *H* serotype and mating type systems are attributable to independent genetic loci. No positive correlation is found between the serotypes and mating types of mature cultures involving genotypes that allow both forms of differentiation. Similar but separate mechanisms must regulate the phenotypic expression of the two systems. Recent studies reveal about forty-five macronuclear gene copies in each cell of *T. pyriformis,* syngen 1. Segregating genetic units in the macronucleus are in effect haploid and are randomly distributed during fission. Thus, interallelic repression need not be postulated to account for phenotypic assortment in heterozygous clones. For nearly every locus examined, heterozygotes assort irreversibly into sublines that express either alternate macronuclear allele but remain heterozygous for the micronuclei.

Five serotype systems have been identified in *T. pyriformis,* syngen 1. Their phenotypic expression hinges on certain regulatory genes, selective agents, and environmental temperature: the L serotype occurs below 20° C, H serotypes (Ha, Hc, Hd, He) predominate at 20 to 35° C, T serotypes (Ta, Tb, Tc) are favored at 36 to 40° C, I serotypes are expressed at 25° C in the presence of dilute anti-H serum, and St serotypes appear at 25° C in the presence of dilute NaC1. The He and Tb antigens were found to be proteins of 29,000 and 23,000 daltons, respectively. As in *Paramecium,* serological specificity is referable to differences in the primary sequence of amino acids in these proteins. There is a sharp discontinuity between the expression of H and T serotypes as determined by immobilization tests with specific rabbit antiserums; cells reactive with both antiserums were found only in cultures maintained at 36 to 37° C. R. B. Phillips detected the distinctive serotypes of both parents in T heterozygotes initially, but cellular multiplication of clones subsequently yielded only one of the two types in nearly equal proportions. Such T serotype differentiation occurred within about thirty fissions after conjugation at 25° C, even in the previous absence of T antigen expression. The kinetics of stabilization were similar to those found for H serotypes. At least two loci, *R-1* and *T,* are responsible for normal expression of T serotypes, but three loci, *R-1, R-3,* and *H,* are involved in the production of H serotypes. Mutant alleles $R\text{-}1^r$ and $R\text{-}3^r$ can modify the normal expression of L, H, and T antigens. Specifically, Doerder has shown that in cells homozygous for $R\text{-}1^r$, no L, H, or T antigens are detectable when clones are grown at temperatures appropriate

for their expression. However, cells homozygous for *R-3*^{r} can synthesize T antigens but not H antigens. In both of these homozygotes, a previously unidentified antigen, r, is manifest whenever the normal serotype is absent; *R-1* and *R-3* then function as regulatory gene loci. The structural loci for ciliary serotypes in *Tetrahymena* and *Paramecium* typically involve codominant alleles, and their respective alternative expression is analogous to allotypic immunoglobulins (Chapter 2) produced by heterozygous mammalian lymphoid cells.

Variation in Surface Antigens of Trypanosomes

The protozoan parasites *Trypanosoma congolense, T. vivax,* and *T. brucei* cause extensive disease in livestock in large areas of central Africa identified with the habitat of the tsetze fly (*Glossina* sp.) vector. In many instances trypanosomiasis results from mixed infections of all three species. *Trypanosoma gambiense* and *T. rhodesiense* can produce in man either nonfatal chronic disease or sleeping sickness which leads to death within three months. Establishment of chronic infections in mammals favors transmission of the parasite to the intermediate host, thereby completing the life cycle. Immunization has yet to prove successful because of the capacity of the parasites to evade the host immune response by repeatedly changing the antigenic structure of the surface coat. The unusual features of this antigenic polymorphism and its genetic control are now under intensive investigation. Before we cope with sources or mechanisms of antigenic variation, the life cycle and known host responses to trypanosome infections require consideration.

Tsetze flies ingest parasites while taking a blood meal from a mammalian host. Inside the gut of the fly, the trypanosomes lose their surface glycoprotein coat, divide, and migrate to the fly's salivary glands. Here developmental changes lead to appearance of a new surface coat and the parasite becomes infective to another mammal. The parasites are usually not evident in mammalian blood during the first two to eight days but later appear in great numbers. Most of the trypanosomes transmitted by the tsetze fly have an antigenic type different from that of the population ingested by the fly. The trypanosomes reproduce by fission in a logarithmic manner. Neither conjugation nor other evidence of sexual mating has been detected under any conditions, although the negative evidence is open to doubt. Host antibodies against antigens comprising the surface coat become abundant within a week after appearance of the hemoflagellates. Such antibody production in mice can reduce the parasitemia from 10^9 per ml to undetectable levels. Within three to five days thereafter, however, the number of circulating parasites will return to the previous high level, and this "relapse population" will express a new surface coat antigen. Serologic testing with specific antiserums reveals that each succeeding population of trypanosomes carries an entirely different antigen characteristic of the surface glycoprotein. In other words, each trypanosome is capable of expressing a spectrum of surface antigens, but normally expresses only one at a time. This oft-repeated finding of *antigenic modulation* is strongly suggestive

of the serotype transformation and mutual exclusion of ciliary antigens in *Paramecium* and *Tetrahymena.* Moreover, the antigenic determinant of each new trypanosome serotype does not cross-react with any antiserum previously produced by the host in response to earlier serotypes. A host reacts to each new relapse population by producing variant-specific antibodies that eventually eliminate the new serotype from the bloodstream. In other words, each new wave of parasites is recognized by the immune system of the host as being antigenically distinct. A final or late antiserum may contain several specificities of antibodies that exhibit some cross-reactivity toward parasite populations from other hosts, as shown by neutralization of infectivity or agglutination. By morphologic criteria, successive relapse populations are not new species or subspecies. Indeed, experiments are now initiated with cloned strains by injecting a single trypanosome into lethally irradiated (900R) mice. According to one view, the number of different antigenic coats a trypanosome strain can express in a given host may be limited only by the life span of the diseased host. Although some twenty-two antigenic variants have been obtained from a single strain, one would predict on immunogenetic grounds a finite number of variants that could arise from a given clone in a single host. Thus far, no more than seven distinct variants have been identified before the death of a single infected mouse.

The surface coat can be removed from trypanosomes with retention of viability by exposing the parasites to nonideal culture conditions or by trypsin digestion. The coat consists of the variant, immunogenic glycoprotein, and a lipid fraction. Each glycoprotein is comprised of a single polypeptide chain with a molecular weight of about 65,000 and contains around 600 amino acid and 20 monosaccharide residues. Preliminary structural studies of variant polypeptides indicate large differences in the amino acid sequences dispersed over more than half of the polypeptide chain. Each clone of *T. brucei* has yielded a characteristic predominant glycoprotein which induced clone-specific immunity to trypanosome infection in mice. In essence then, these variant-specific antigens demonstrable by antibody precipitation, agglutination, or immunoelectron microscopy also elicit protective immunity. Antibodies to each population become detectable in the serum soon after the disappearance of the variant population, that is, after the system is no longer in antigen excess. Most studies reveal only one serologically definable antigenic determinant per clone at a given time, but the possibility of coexistence of different clones derived from a single clone or of more than a single variant-specific antigen on the cell surface remains tenable. An immunofluorescence technique modified by Doyle and coworkers to allow identification of variant antigens carried by individual living blood trypanosomes in vitro repeatedly revealed coexistence of two or more successive antigenic types in individual mice. The common or species-specific antigens of trypanosomes are internal and probably have structural/enzymatic functions with no impact on protective immunity. The additional presence of tightly bound hostlike protein antigens in trypanosome cell surfaces suggests antigenic mimicry, but the parasite versus host origin of these proteins is not known.

The most striking feature of antigenic variation in African trypanosomes is the predictable sequence of variant serotypes that ensues following mammalian infection. Thus all naive hosts infected by a tsetze fly bite will develop a parasite population expressing serotype A or antigen A. This "basic antigen" is always the first one to appear in a new infection, regardless of mode of transmission. After clearance of this population and appearance of host anti-A, all or nearly all trypanosomes in the ensuing relapse population will express antigen B. The cycle repeats in less than a week with the emergence of a C variant, then D, E, and so on. If the E serotype is transferred to a naive host, the sequence is not continued but begins all over with serotype A. If a naive mouse or rabbit is passively immunized against the first four variants in the series, the arising serotype after infection will express the fifth variant. Thus, the appearance of new serotypes can hardly be attributed to random mutation in a gene coding for a single antigen.

The genome of an individual trypanosome apparently codes for numerous serotypic variations in a glycoprotein with certain constant characteristics. Quite possibly, several gene loci or a bacterial-type operon are involved. Perhaps each clone's variant antigen or corresponding gene is the suppressor for the serotype which follows it in the normal infection sequence. However, variant populations comprising a "strain" may have different fission rates, some serotypes being more virulent than others. Differential multiplication of intrastrain variants could conceivably account for sequential appearance of predominant types early in infection. This complication can be controlled by inoculating animals of the same inbred strain, age, and sex with single trypanosomes. Apart from the apparent order in the appearance of new serotypes, underlying genetic hypotheses should be able to account for (a) the competence of trypanosomes to display a previously expressed antigen, (b) the ability of different populations to express similar antigens in the course of cyclic transmission, and (c) the molecular heterogeneity of the variant-specific glycoproteins. Although host antibodies appear to select against the predominant variant(s) at any given time, it is not clear whether the overall immune response (including macrophages, T-cells, and B-cells) is strictly selective, inductive, or both. In simplified terms, both selection of mutants or variant clones and induced phenotypic changes in clones by surface antigen–humoral antibody reactions have been suggested. Sequential antigenic variation occurs only in parasites multiplying in normal mice, not in lethally irradiated, totally immunosuppressed mice. There is a conspicuous paucity of information concerning the influence of host genotype, especially immune response (*Ir*) genes and histocompatibility systems, in this connection.

The sequential variation in protein coat specificities is probably precoded in the trypanosome genome. Serotype expression and transformation may then be triggered by specific as well as seemingly nonspecific factors. Both have been shown to operate in *Paramecium* and *Tetrahymena* without the added complication of a two host–one parasite relationship. Data concerning the extent of antigenic variation and adaptive immunity in the invertebrate host could reveal the sources of genetic triggering. Somatic diversification of germ-line

genes obviously has inherent restrictions in this serotype system. Apparent lack of sexual reproduction in trypanosomes precludes direct analysis of gene–antigen correlations.

Antigenic Variation in Malaria

Malaria, still the worst blight of the tropics, each year strikes 150 million people and in Africa alone kills more than 1 million children. Malarial parasites, transmitted to humans by the female *Anopheles* mosquito, multiply in the liver and then invade erythrocytes, producing fever, chills, and weakness. About one-fourth of the adult population of Africa suffers recurrent bouts of the disease, and an estimated 2 billion people reside in endemic areas. Widespread resurgence of malaria has occurred in recent years because mosquitoes have developed resistance to insecticides such as DDT and the parasites have evolved resistance to chloroquine, the standard drug for prevention and treatment of the disease.

Serotype variation analogous to that in trypanosomes has been conclusively shown in chronic erythrocytic malaria caused by at least six species of *Plasmodium.* Such variation occurs with high frequency in chronic *P. knowlesi* infections in monkeys. Parasites isolated at seven-day intervals were found to possess different serotypes. Each strain appears to carry a distinct genetic repertoire yielding new variants during infections of many months' duration. Variant-specific antibodies are demonstrable by agglutination of infected red cells or by opsonization correlated with in vivo protection. The molecular nature of the intraspecific surface antigens is unknown. Whether the antigenic variation appears in a sequential or predictably ordered manner during the course of mammalian and mosquito vector infections is also unknown. Since genetic recombination has been demonstrated among strains of malarial parasites, both sexual and asexual sources of serotype variation may operate.

Although onset of protective immunity is characterized by a sudden drop in numbers of blood parasites, antigenic variation continues during subclinical phases. Serotype transformations can also proceed in the absence of protection or effective parasite destruction. However, most experiments suggest that phenotypic change is induced in these protozoans by the presence of specific nonlethal antibody. According to this view, low affinity antibodies are variation-inducing, while high affinity antibodies tend to be parasiticidal. "Antigenic drift" of influenza virus mutants by immunoselection has similarly been attributed to low affinity antibodies. K. N. Brown has suggested a mechanism of selective gene activation and repression in response to environmental pressures, including combination of extracellular malarial merozoites (forms released after red cell rupture) with specific antibody. The antigenic change itself may not take place until nuclear division at schizogony (asexual reproduction by multiple division) a supposition in accord with the finding that serotype changes in *Paramecium* are correlated with mitosis. As with trypanosomes, the most probable working hypothesis is that serotype genes at

one or more loci preexist in a polymorphic array in the nuclear DNA of malarial organisms. If this is valid, then the regulatory network or feedback signals that induce selective gene expression obviously invite identification.

Although a regulatory role for serum antibodies in serotype expression appears certain, actual immunity to malaria clearly hinges on T-lymphocytes. Neonatally thymectomized rats fail to develop effective immunity, and T-cell suspensions free of antibody-secreting B-cells can adoptively transform protective immunity. Perhaps helper T-cells recognize common determinants on variant-specific molecules and thereby play a crucial role in the overall immune response. More information is again needed concerning effects of host immune response genes in inbred and congenic animals. This whole field is ripe for investigation.

VIRUS ASSOCIATED ANTIGENS

We may begin with the solid generalization that viruses, like other microorganisms, can be nicely identified serologically on the basis of group-, species-, and strain-specific antigens. Because many copies of the viral chromosome are usually present in any one animal cell, genetic exchange may occur among numerous chromosomes in the production of virus progeny. This exchange of chromosomal genes in a host cytoplasm may generate polymorphism within restrictions imposed mainly by mutation and selection of viable recombinants. Virus species or strains that achieve latency or replication synchronized with host cell division, rather than cause cell death, are of course more likely to survive as successful parasites. Much work on cell-surface antigens associated with the presence of viruses or viral genes has been motivated by the search for specificities distinctive for malignant cells. Almost all virus-induced tumors in experimental animals show perpetuation of viral genes as a heritable factor in the tumor cells. These genes usually elaborate virus-specific antigens even when no infectious virus is produced. With oncogenic viruses, it is now established that induced tumors acquire unusual cellular alloantigens distinct from viral antigens. Some of these surface markers are not strictly tumor-specific, but may be reexpressed fetal antigens or differentiation antigens associated with certain stages or pathways of cellular differentiation. Other "neoantigens" are presumably determined by the virus genome rather than that of the host, because they are virus-specific regardless of the strain or even the species of the host. Such tumor-associated antigens have been found in all classes of virus-induced solid tumors and leukemias. These antigens are demonstrable by both tissue transplantation reactions and serological tests. Tumors induced by different viruses have different antigens, but tumors induced by closely related viruses (especially RNA viruses) may display some antigens in common. Techniques employing specific serum antibodies have proved most powerful in sorting out the array of gene products expressed on the surface of tumor cells. Let us consider some of the highlights of these ongoing studies.

Antigens Evoked by DNA Tumor Viruses

Solid tumors are regularly inducible in newborn rodents following inoculation of oncogenic DNA viruses known as papovaviruses (SV_{40} and polyoma) and certain serotypes of adenoviruses. These different viruses have similar effects in mediating tumorigenesis, malignant transformation of animal cells in culture, and production of distinctive antigens. Although these viruses are widespread in diverse environments, there are few indications that they are responsible for naturally occurring cancer in animals or humans. Since the late 1960s, however, at least three herpesviruses, a family of complex DNA viruses, have been implicated in the causation of cancer. These are Epstein-Barr virus (EBV) associated with Burkitt lymphoma and nasopharyngeal carcinoma in certain populations, and herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2). These viruses are familiar human pathogens. EBV commonly causes infectious mononucleosis or "kissing disease" in young people, HSV-1 provokes the "cold sores" of the oral cavity, and HSV-2 usually infects urogenital areas following venereal transmission. Both HSV-1 and HSV-2 may persist in people for long periods of time and produce recurrent symptoms. The herpes simplex viruses transform cells in culture and their distinctive antigens are present in corresponding tumor cells. Fulfillment of Koch's postulates to prove that any of these suspected oncogenic viruses actually causes cancer in man has not been achieved. Thus indirect evidence of virus-associated products in malignant cells and in individuals with cancer has attracted intensive investigation. Four groups or categories of antigens have emerged in conjunction with oncogenic DNA viruses as follows:

1. *Nonvirion antigens.* These are viral gene products coded by the viral DNA, but are not themselves part of the viral particles. Malignant transformation of cells by adenoviruses and papovaviruses is regularly associated with expression of these antigens. In host animals, serum antibodies against nonvirion antigens are readily detectable. In patients with advanced carcinomas of the oral and urogenital regions, antibodies against nonvirion antigens of HSV-1 and HSV-2, respectively, are present. Moreover, lip and cervical carcinomas contain HSV nonvirion antigens not found in normal tissue or unrelated tumor. The possibility remains, of course, that these viruses are merely opportunists that are biosynthetically active in malignant cells but tend to remain latent in normal cells.
2. *T (tumor) antigens.* These early antigens appearing in transformed cells are mainly virus-specific protein(s) found only in the nucleus in the case of papovaviruses and EBV virus, but in both nucleus and cytoplasm of adenovirus-infected cells. T antigen is virus-specific, since cells transformed by polyoma virus react only with antiserum from animals bearing tumors induced by this virus and not with serum from animals with tumors induced by SV_{40}, and vice versa. T antigens do not appear to be

part of the viral capsid, because antivirion antibody does not react with tumor or transformed cells and anti-T serum does not neutralize the infectivity of the corresponding virus. T antibodies are detectable by either immunofluorescence or complement fixation. Although T antigens are produced in all infected cells, they are not essential for tumorigenesis; adenoviruses which are nononcogenic can still induce T antibodies in hamsters. Synthesis of T antigens appears to depend on the transcription and translation of some viral genes but not on the expression of the entire viral genome. Indeed, T antigen synthesis is not dependent on the replication of viral DNA, because appearance of the antigen is not blocked by inhibitors of DNA synthesis such as cytosine arabinoside or 5-fluorodeoxyuridine. In essence, T antigens could be virus-coded proteins, derepressed cell proteins, or proteins derived by interaction of viral and cellular proteins; their function is unknown.

3. *Tumor-associated transplantation antigens (TATA).* Animals immunized with polyoma virus or SV_{40} became resistant to subsequent challenge with transplantable tumors produced by the corresponding virus. In other words, new TATA found in DNA virus-induced tumors are detectable by the resistance of adult syngeneic animals to challenge with viable tumor cells consequent to previous infection with the homologous virus. This acquired immunity following infection is attributable to the appearance of transformed antigenic cells. Tumor rejection is mediated by sensitized immune lymphocytes (T-cells) and is highly specific. There is no cross-reactivity between cells transformed in vitro with SV_{40} or polyoma virus or between tumor cells induced by these two viruses. The same transplantation antigen is evidently present in cells of different species transformed by the same virus. Thus, tumor rejection is demonstrable within a species previously immunized with tumor cells produced by the same virus but in different host strains or species. In other words, the antigens induced by a given virus are very similar or identical. The only definitive assay for transplantation antigen is the tumor rejection test. Virus-specific tumor transplantation immunity may be elicited without the concomitant appearance of antibodies to viral or tumor antigens. The "one virus genome → one transplantation antigen" relationship appears to be remarkably specific in that different strains of polyoma virus produce different transplantation antigens. Although the transplantation antigens are virus-specific, it is again not known whether the antigen is coded by the virus, or is a derepressed host or altered-self protein.
4. *Other surface (S) antigens.* Certain antigens not exposed on normal cells can be detected on the surface of virus-transformed cells by immunofluorescence, cytotoxic antibodies, mixed hemagglutination tests, and colony inhibition tests. S antigen can often be demonstrated on normal cells after mild treatment with trypsin or chymotrypsin. Thus, such antigens are not coded by virus; their chemical identity is unknown. The S antigens are not species-specific because infection with a particular virus will

expose cross-reacting antigens on the surfaces of cells of different species. Viral transformation has been observed to produce alterations in cell surface glycoproteins as well as gross changes in surface glycolipids. Evidently each different virus exposes or alters a particular preexisting cell-surface molecule that is potentially antigenic. S antigens may or may not function as effective TATA.

All the evidence taken together supports the assumption that the nonvirion antigens, T antigens, TATA, and viral antigens are distinctive. Given the diversity of antigens expressed or induced by DNA viruses, the reader may well wonder how any potential tumor cells escape destruction by the immune system. Most animals and people do, in fact, cope effectively, despite the ubiquitous presence of adenoviruses, papovaviruses, and herpesviruses. Those who are or become susceptible presumably have a deficient repertoire of *Ir* gene functions in their macrophages, T-lymphocytes, or B-lymphocytes. Although induction of immunologic tolerance might be invoked to account for the susceptibility of perinatal animals to oncogenesis by DNA viruses in contrast to the resistance of adults, there is evidence against this possibility. Newborn mice inoculated with polyoma virus not only develop viral antibody in conjunction with tumors, but may become resistant to later transplants of polyoma tumor cells from isogenic animals. Moreover, a markedly diminished incidence of tumors consequent upon neonatal administration of SV_{40} virus or adenovirus type 12 can be achieved by subsequent inoculation of more virus or of irradiated tumor cells during the period preceding oncogenesis. These experiments also suggest that appropriate immunization can prevent the progressive proliferation of incipient tumor cells. Evidently the immunogenicity of the initial tumor cells is insufficient to cause their rejection.

Systems Connected to RNA Tumor Viruses

A variety of oncogenic RNA viruses (also called oncornaviruses) are known to evoke production of both transplantation antigens and soluble antigens in tumor cells distinct from those of intact virus. The group of murine leukemia viruses (MuLV), named Gross, Friend, Moloney and Rauscher after their discoverers, contain two outer surface glycoproteins responsible for their C-type virus-specific antigenicity as well as four internal proteins. The products of the four genes of RNA tumor viruses involved in murine oncogenic transformations and their functions are summarized in Figure 3–9. The largest internal protein gives rise to the group-specific antigenicity unique to the murine viruses. The MuLV are broadly classified into two serological subgroups, the Gross (G)-AKR subgroup and the FMR (Friend–Moloney–Rauscher) subgroup, according to the specificities of antigens found on the leukemic cells as detected by cytotoxicity and immunofluorescence tests. Naturally occurring leukemias all result in production of cell-associated and soluble antigens of the G-AKR group, whereas FMR antigens are found only in experimentally in-

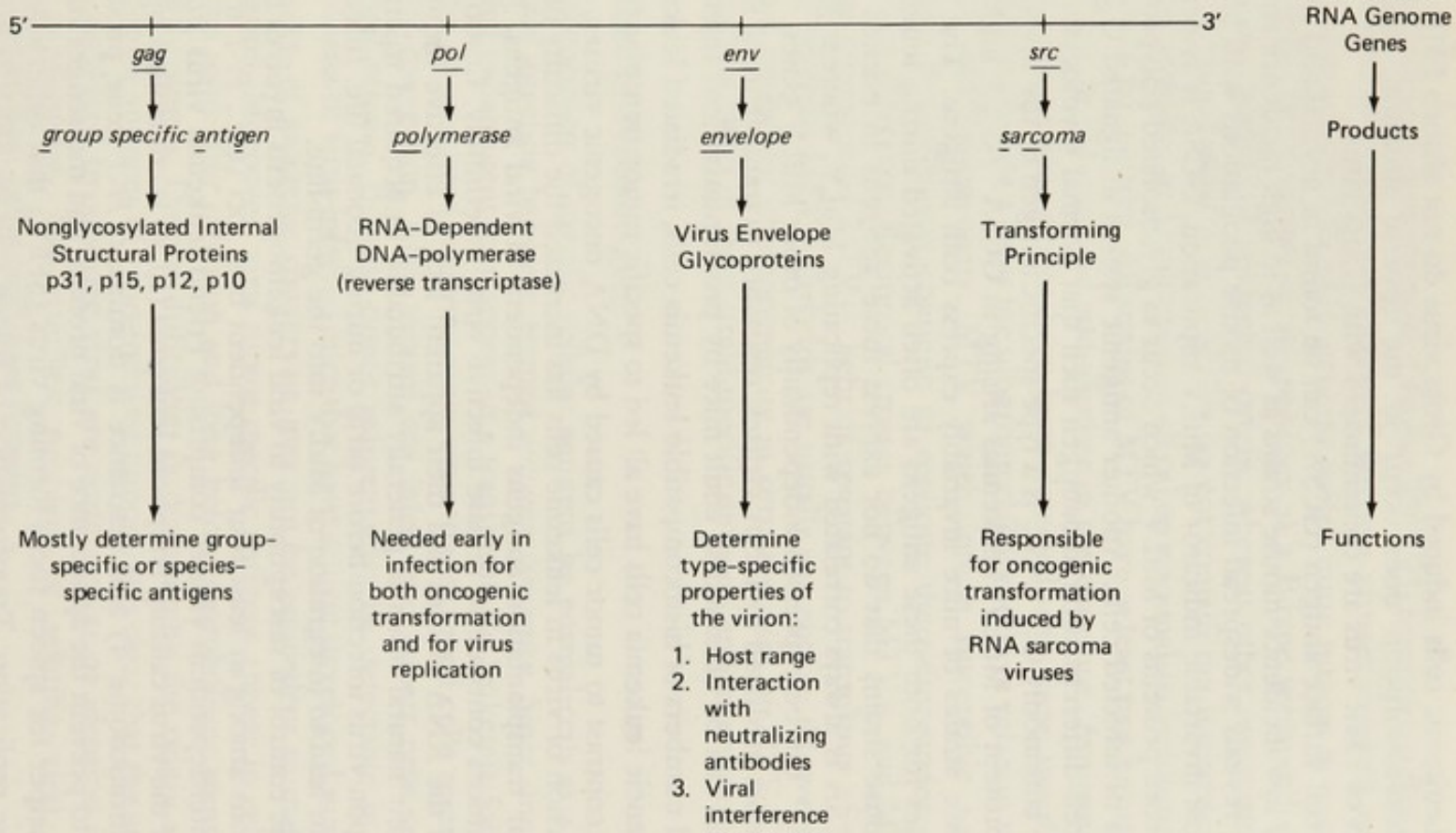


FIGURE 3–9. Genes of RNA tumor viruses involved in murine oncogenic transformations.

Source: Based on Vogt and Hu, *Annu. Rev. Genet.* 11:207–216, 1977. Reproduced, with permission, by *Annual Review of Genetics*, © Annual Reviews, Inc.

fected mice. Leukemic cells induced by Gross virus do not absorb FMR antibody, but cross-absorption does occur in the reverse situation, possibly because stocks of FMR virus are contaminated with Gross virus.

The Gross cell surface antigen (GCSA) can be found in normal and malignant tissues of low incidence mouse strains as well as in high incidence strains. GCSA typing reveals widespread infection of mouse populations with MuLV and is an almost invariable indicator of MuLV replication. GCSA is associated with internal core proteins of MuLV which occur as glycosolated polyproteins on the surface of infected cells. Another antigenic system designated G_{IX} appears as a T-cell differentiation alloantigen such that normal thymocytes and leukemias are commonly G_{IX}^+. G_{IX} is a type-specific antigen of the major envelope glycoprotein of MuLV. Leukemias arising in $GCSA^+/G_{IX}^+$ high leukemia incidence strains of mice invariably express both antigens. The viral structural genes for both these antigens are often activated during leukemogenesis in mouse strains that do not express these antigens in normal life. However, GCSA is always correlated with replicating MuLV, whereas genes coding for G_{IX}^+ can be expressed independently of other MuLV genes.

As with tumors mediated by DNA viruses, resistance to transplants of RNA virus-leukemias can be obtained in adult mice by preimmunization. Injection of virus, small numbers of histocompatible leukemia cells, irradiated leukemia cells, or allogeneic leukemia cells have all led to specific tumor transplantation immunity. In contrast to tumor cells caused by DNA oncogenic viruses, continued production of virus in leukemic cells has increased the difficulty of detecting cellular transplantation antigens independent of viral antigens. Most RNA virus tumors continue to release infective virus indefinitely. Continued production of the RNA viruses and their apparent lack of effective immunogenicity in their natural hosts is generally attributable to acquired tolerance consequent upon virus infection before birth or during neonatal life. Infection of embryos can lead to integration of MuLV into the germ-line.

Host genetic control of susceptibility to viral leukemogenesis involves complex interactions among at least four independent host loci (e.g., *Fv-1* to *Fv-4*) and two interdependent viruses comprising Friend leukemia virus stocks. Replication of the virus causing lymphoid leukemia (LLV) is controlled by the *Fv-1* locus (chromosome 4) and resistance is dominant. *Fv-1* gene products may act early to prevent the appearance of viral proteins and messenger RNA. LLV acts as helper for spleen focus-forming virus (SFFV) that otherwise exhibits defective replication. Transformation of spleen cells by SFFV is in turn controlled by the *Fv-2* locus (chromosome 9), and resistance is recessive. *Fv-3* may determine whether the infected host is immunodepressed by Friend virus, while *Fv-4* affects a broader range of MuLV strains of differing host range than *Fv-1* does. Other murine loci known to affect resistance to viral leukemogenesis include *S1, W, Gv-1, Gv-2, Tla,* and *H-2* (see Chapter 8). The *H-2* complex influences not only the threshold dose of virus sufficient for emergence of malignant disease, but also the likelihood of recovery from the disease once it occurs. Obviously, the outcome of viral–host interactions depends at least as much on the genotype of the host as on the strain of virus.

The mouse mammary tumor viruses (MTV), comprising five or six strains, are similar to murine leukemia and sarcoma viruses in being enveloped particles with a 60 to 70S RNA genome which cause tumors in susceptible mice. However, MTV differs in morphology as a B-type virus and causes tumors (e.g., carcinomas) of epidermal origin, whereas other oncoviruses transform cells of mesodermal origin. MTVs do not contain the interspecific or group-specific antigen (gs 3) common to all other mammalian leukemia and sarcoma viruses so far isolated. All strains of MTV have at least two group-specific antigens, one in the nucleoid and another in the envelope. Antigenic differences among the various strains have been detected, and these specificities should ultimately provide a basis for subgroup classification in relation to varying virulence and oncogenicity. MTV which infects susceptible newborn mice through the milk may continue to replicate thereafter under conditions of complete tolerance. Reciprocal crosses between MTV+ and MTV− inbred mouse strains yield MTV− hybrids resistant to transplants of mammary tumors, whereas MTV+ hybrids are usually susceptible. However, mammary tumors may show immunogenicity in certain strains of MTV+ mice, which suggests an effect of virus strain-specific antigens and incomplete tolerance.

Sources of Neoantigens

The acquisition of specific cellular antigens by normal cells as a consequence of virus infection has been termed *antigenic conversion.* Since one or more additional neoantigens may appear in a transplantable leukemia following infection by an unrelated leukemogenic virus, transformed cells remain subject to further conversion. Also, viruses can infect and impart new antigenicity to nonviral tumors. Because tumor antigens may be detectable in normal cells and plasma of virus-infected mice that have not yet developed tumors, the phenomenon of antigenic conversion may not be equivalent to malignant transformation. Moreover, viral tumor-specific antigens appear in cells inoculated in vitro soon after infection and long before morphologic transformation of the cell cultures. Despite difficulties in determining the origin and nature of the diverse antigens of virus-induced tumors, it is clear that the antigens of tumors produced by any one virus are quite similar or identical in their serological reactivity. The complex problem of relating viral products to transformed host cell specificities remains.

Because most or all of the genome of the RNA tumor viruses is present in the malignant cells, it may be argued that continued functioning of the viral genome is necessary to maintain the malignant change. With the DNA tumor viruses, the viral genome appears essential for the initiation of the malignant change and not for its perpetuation, insofar as infective virus usually fails to persist. The question remains open whether the TATA are virus-coded proteins or altered host-cell surface components. The latter alternative gains support from the finding that antigens of solid tumors induced by a given chemical carcinogen are generally distinct for each tumor. This diversity of tumor antigens associated with a given chemical cancerogen, like methylcholan-

threne in genetically defined mice, is usually taken as a strong argument against a viral etiology of these tumors. The number of distinctive antigens demonstrable in chemically-induced tumors appears limited in a given host genotype. These antigens could arise by mutation of histocompatibility genes or derepression of normal cellular genes for embryonic "differentiation" molecules (Chapter 6). The rationale here is that tumors and embryos may share antigenic properties not present in the normal adult. Indeed, malignant tumors in adult animals frequently contain high concentrations of molecules, such as alpha fetoprotein, found only in fetal tissues. A carcinoembryonic antigen can be detected immunochemically at high concentration in the serum of patients with gastrointestinal malignancy. Among both virally and chemically triggered neoplasms, relatively strong or weak immunogenicities may be found. Curiously, stronger antigenicity is often associated with shorter latent periods for oncogenesis.

Atypical appearance or disappearance of cell-surface antigens exhibiting restricted expression on normal host cells has complicated the problem of distinguishing causes and effects in tumorigenesis. The most striking phenomenon in this connection is that of *antigenic modulation* exemplified by the *Tla* system of thymus leukemia antigens in mice (see Chapter 6). In brief, Tla antigen is found on both thymocytes and leukemia cells of certain mouse strains (e.g., A strain), but is strictly leukemia-specific in other strains (e.g., C57BL). The Tla^+ phenotype in normal mice is inherited as a Mendelian dominant trait with the responsible locus *Tla* closely linked to the *H-2* complex on chromosome 17. The assumption that all mice possess the structural gene for Tla, which is not normally expressed in Tla^- strains, is consistent with the anomalous appearance of Tla^+ leukemias in Tla^- strains. The *Tla* locus may also be viewed as a regulatory gene that is activated or derepressed as a consequence of leukemogenesis resulting in the appearance of TL antigen of several allelic variants on leukemia cells.

Antigenic modulation is demonstrated as an alteration in the phenotypic expression of Tla antigens in the presence of Tla antibodies. Tla^+ cells undergo phenotypic suppression of Tla after exposure to Tla antibodies. When leukemia cells are transplanted to syngeneic mice containing Tla antibody they remain Tla^-, but after passage to nonimmune mice they revert to Tla^+ and regain high sensitivity to the cytotoxic action of Tla antibodies. Passive transfer of Tla antibody can also induce antigenic modulation of either normal thymocytes or leukemic cells of Tla^+ mice both in vivo and in vitro. All Tla specificities probably reside on a single molecule, since antibodies to an individual specificity of the *Tla* complex cause modulation of other Tla antigens. Finally, different Tla^+ cell types modulate at different rates with leukemia cells changing more rapidly (e.g., within one hour) than normal thymocytes. Antigenic modulation provides an effective mechanism for Tla^+ leukemia cells to escape immune elimination. *Tla* activation by MuLV may occur during the preleukemic phase of x-ray leukemogenesis in view of the finding that MuLV is often detectable during this period. Two distinct pathways of T-cell

TABLE 3–2. Categories of Serologically Demonstrable Surface Antigens on Mouse Leukemia Cells

Conventional alloantigens	H-2D, H-2K
Differentiation alloantigens	Lyt-1,2,3,4, Thy-1, TL.1,2,3
MuLV-structural antigens	MuLV-gp70, p30, p15
MuLV-related antigens	GCSA, G_{IX}, X.1, $G_{(RADA1)}$, $G_{(ERLD)}$
Transformation-specific MuLV antigens	None defined
MTV-related antigens	ML
Derepression antigens	TL.1,2,4 (in TLa^- strains)
	GCSA, G_{IX}, $G_{(RADA1)}$, X.1 (in strains not expressing MuLV-related antigens in normal tissue)
Individually unique antigens	None defined
Species antigens	MSLA
Embryonic and fetal antigens	None defined

After Old and Stockert, *Annu. Rev. Genet.* 11:155, 1977. Reproduced, with permission, from *Annual Review of Genetics*, © 1977 Annual Reviews Inc.

leukemogenesis may exist, but the relation between the Tla class and MuLV class of mouse leukemias remains to be clarified. Numerous categories of surface antigens can now be recognized on mouse leukemia cells (Table 3–2). Some not considered in this chapter are described later. Note that no transformation-specific MuLV antigens, individually unique antigens, or embryonic/fetal antigens have been defined in mouse leukemias despite intensive study.

The mechanism of antigenic modulation, like that of serotype transformation in protozoans, appears to involve feedback regulation of normal gene expression, perhaps including conformational changes in preexisting determinants. By contrast, antigenic conversion involves acquisition of "new" cell-surface determinants referable to abnormal genes, often of viral origin.

KEY REFERENCES

Books

Kabat, E. A. *Structural Concepts in Immunology and Immunochemistry.* New York: Holt, Rinehart and Winston, 1968. Summarizes classic evidence concerning microbial polysaccharides and oligosaccharides as antigenic determinants.

Strickberger, M. W. *Genetics,* 2d ed. New York: Macmillan 1976. A well-illustrated, reasonably up-to-date coverage of microbial genetics is included in several chapters.

Articles

Bacteria and Fungi

Ballou, C. E., and Raschke, W. C. Polymorphism of the somatic antigen of yeast. *Science* 184:127–134, 1974. Nonpathogenic yeasts exhibit a diversity of polysaccharide cell-surface antigens analogous to animal and bacterial antigens.

Enomoto, M., and Stocker, B. A. D. Integration, at *HAG* or elsewhere, of *H2* (Phase-2 flagellin) genes transduced from *Salmonella* to *Escherichia coli. Genetics* 81:595–614,

1975. Confirms close similarity of genetic maps of the two genera, but reveals striking differences in the genes controlling flagellar antigens.

Haldane, J. B. S. Disease and evolution. In *Symposium on Ecological and Genetic Factors in Animal Speciation,* Supplemento A "La Ricerca Scientifica," Anno 19, 1949. Pages 68–76. A classic paper suggesting that infectious diseases provide continual natural selection for cell-surface and immunogenetic polymorphisms in both hosts and pathogens.

Hirata, A. A., McIntire, F. C., Terasaki, P. I., and Mittal, K. K. Cross reactions between human transplantation antigens and bacterial polysaccharides. *Transplantation* 15:441–445, 1973. Lipopolysaccharides from several species of *Salmonella* and several strains of *E. coli* were found to combine specifically with antibodies to various human HLA antigens.

Iino, T. Genetics of structure and function of bacterial flagella. *Annu. Rev. Genet.* 11:161–182, 1977. Summarizes analysis of flagellin genes and polypeptides yielding flagellar antigens. Interaction of H1 and H2 operons is evaluated in terms of regulatory system operating on phase variation in *Salmonella.*

Jann, K., and Westphal, O. Microbial polysaccharides. In Sela, M. (ed.). *The Antigens,* vol. III. New York: Academic Press, 1975. Pages 1–125.

Lüderitz, O., Jann, K., and Wheat, R. Somatic and capsular antigens of Gram-negative bacteria. In Florkin, M., and Stotz, E. H. (eds.). *Comprehensive Biochemistry,* vol. 26A. Amsterdam: Elsevier 1968. Pages 105–228. An extensive review of the somatic and capsular antigens in diverse bacterial species.

Ørskov, I., Ørskov, F., Jann, B., and Jann, K. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli. Bacteriol. Rev.* 41: 667–710, 1977. A recent review with emphasis on the genetic polymorphisms of capsular polysaccharide antigens.

Robbins, P. W., and Wright, A. Biosynthesis of O-antigens. In Weinbaum, G., Kadis, S., and Ajl, S.J. (eds.). *Microbial Toxins,* vol. IV. New York: Academic Press, 1971. Pages 351–368. A nice resumé of variations in *Salmonella* 0 antigen structures with attention to antigenic conversion by bacteriophages.

Schmidt, G. Genetical studies on the lipopolysaccharide structure of *Escherichia coli* K12. *J. Gen. Microbiol.* 77: 151–160, 1973.

Stockert, B. A. D., and Mäkelä, P. H. Genetic aspects of biosynthesis and structure of *Salmonella* lipopolysaccharide. In Weinbaum, G., Kadis, S., and Ajl, S. J. (eds.). *Microbial Toxins,* vol. IV. New York: Academic Press, 1971. Pages 369–438. An extensive review of *Salmonella* immunogenetics with emphasis on gene mapping functions.

Protozoans

Beale, G. H. Genetics of antigenic variation in *Paramecium:* A model system. In *Parasites in the Immunized Host: Mechanisms of Survival.* Ciba Found. Symp. No. 25, 1974. Pages 21–33. Argues cogently for switches in serotype gene activity as the essential mechanism for antigenic variation.

Brown, K. N. Antigenic variation and immunity to malaria. In *Parasites in the Immunized Host: Mechanisms of Survival.* Ciba Found. Symp. No. 25, 1974. Pages 35–51. Intraspecies diversity of plasmodial antigens reflects changes in phenotype during persistent infections in response to host production of corresponding antibodies. For more up-to-date review by same author, see Chap. 1, in *Immunity to Blood Parasites of Animals and Man.* New York: Plenum Press, 1977. Pages 5–25.

Capdeville, Y. Intergenic and interallelic exclusion in *Paramecium primaurelia:* Immunological comparisons between allelic and non-allelic surface antigens. *Immunogenetics* 9:77–95, 1979. Surface antigens coded by different loci *G* and *D* show no immunologic cross-reaction either in vivo or by immunodiffusion, whereas surface antigens of a given allelic series, even those displaying no cross-reactivity in vivo, reveal

common antigenic determinants by immunodiffusion. Allelic exclusion is associated with total absence of the corresponding antigenic parental polypeptide.

Cross, G. A. M. Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitology* 71:393–417, 1975. Each variant-specific glycoprotein consisted of a single polypeptide containing about 600 amino acid and 20 monosaccharide residues.

Doerder, F. P. Regulatory serotype mutations in *Tetrahymena pyriformis*, syngen 1. *Genetics* 74:81–106, 1973. Demonstrates that at least two regulatory gene loci govern the normal expression of several serotype systems.

Doyle, J. J. Antigenic variation in the salivarian trypanosomes. In *Immunity to Blood Parasites of Animals and Man*. New York: Plenum Press, 1977. Chap. 3, pages 31–63. Considers possible mechanisms of antigenic variation in terms of alternative genetic hypotheses.

Finger, I., Heller, C., and Von Allmen, C. Specific cell-induced transformation of *Paramecium* surface antigens. *Differentiation* 11: 51–57, 1978. A very recent analysis of antigenic transformation as a function of gene regulation.

Grass, F. G. Genetics of the *St* serotype system in *Tetrahymena pyriformis*, syngen 1. *Genetics* 70: 521–536, 1972. A serotype system revealed under conditions of salt stress is analyzed in relation to other serotype loci.

Juergensmeyer, E. B. Serotype expression and transformation in *Tetrahymena pyriformis*. *J. Protozool.* 16: 344–352, 1969. Describes the L and I serotype systems. See also Doerder and Grass references cited above.

Margolin, P. An exception to mutual exclusion of the ciliary antigens in *Paramecium aurelia*. *Genetics* 41: 685–699, 1956. See also Finger et al., Clonal variation in Paramecium. I. Persistent unstable clones. *Genetics* 72: 17–33, 1972. Clones of any one of several serotypes can express several antigens simultaneously in single animals, apparent exceptions to the rule that different serotypes found in homozygous lines are mutually exclusive.

Orias, E., and Flacks, M. Macronuclear genetics of *Tetrahymena*. I. Random distribution of macronuclear gene copies on *T. pyriformis*, syngen 1. *Genetics* 79: 187–206, 1975. Establishes that macronucleus is approximately 45-ploid, with many functional copies of each gene, and suggests that segregating genetic units in the macronucleus are in effect haploid.

Phillips, R. B. Inheritance of immobilization antigens in syngen 7 of *Tetrahymena pyriformis:* Evidence for a regulatory gene. *Genetics* 67: 391–398, 1971. A penetrating study of gene–environmental interactions in serotype regulation.

Preer, J. R. Genetics of protozoa. In Chen, T. T. (ed.). *Research in Protozoology*, vol. 3. New York: Pergamon Press, 1968. Pages 129–278. This extensive review remains valuable to serious students of protozoan immunogenetics.

Sommerville, J. Serotype expression in *Paramecium*. *Adv. Microb. Physiol.* 4: 131–178, 1970. Shows that genetic differences in serotype specificity are referable to differences in the primary sequence of amino acids in the antigenic molecule.

Vickerman, K. Antigenic variation in African trypanosomes. In *Parasites in the Immunized Host: Mechanisms of Survival*. Ciba Found. Symp. No. 25, 1974. Pages 53–80. Each trypanosome is genetically capable of expressing a whole range of surface antigens, but usually expresses only one at a time in an ordered sequence as a mechanism of survival in immunized hosts.

Viruses

Baltimore, D., Huang, A. S., and Fox, C. F. (eds.). *Animal Virology*. New York: Academic Press, 1976. Presents diverse, recent studies of viral genetics, tumor viruses, and tumorigenesis.

Boyse, E. A. The G_{1x} system in relation to C-type viruses and heredity. *Immunol. Rev.*

33: 125–145, 1977. A penetrating analysis of viral gene–host interactions in relation to tumorigenesis and disease.

Kurth, R., Fenyö, E. M., Klein, E., and Essex, M. Cell-surface antigens induced by RNA tumor viruses. *Nature* 279: 197–201, 1979. Review of antigens coded by avian, murine, and feline oncornaviruses.

Möller, G. (ed.). Tumor associated embryonic antigens. *Transplantation Rev.* 20: 3–129, 1974. Contains several articles focusing on alpha-fetoprotein, carcinoembryonic antigen and other differentiation antigens.

Old, L. J., and Stockert, E. Immunogenetics of cell surface antigens of mouse leukemia. *Annu. Rev. Genet.* 11: 127–160, 1977. A far-ranging review of cell-surface antigens related to MuLV viruses, the TL system, antigenic modulation, and techniques of analysis.

Steeves, R., and Lilly, F. Interactions between host and viral genomes in mouse leukemia. *Annu. Rev. Genet.* 11: 277–296, 1977. Explores the complexities of susceptibility versus resistance to leukemias in terms of type C viruses and host genes.

Tooze, J. (ed.). *The Molecular Biology of Tumour Viruses.* New York: Cold Spring Harbor Laboratory, 1973. An admirably comprehensive coverage of the classic work in this field.

Vogt, P. K., and Hu, S. S. F. The genetic structure of RNA tumor viruses. *Annu. Rev. Genet.* 11: 203–238, 1977. Describes genes of oncoviruses, their products, functions, and mapping.

4

BLOOD GROUP ALLOANTIGEN SYSTEMS

IMMUNOHEMATOLOGY

Blood Group Methodology

Discovery of the ABO blood types in man by a combination of serologic and family studies marked the beginnings of immunogenetics. Indeed, the first principles—(1) each dominant allele yields one antigen specificity and (2) nonself antigens normally evoke corresponding antibodies—were originally deduced from analyses of blood groups in mammals as outlined in Chapter 1. Serological tests have long provided the central methodology to identify antigenic characteristics. Antibody reagents dependably reveal inherited differences among individuals as well as species. Immune serums and, to a lesser extent, serums from normal individuals have been very successfully employed in identifying polymorphic erythrocyte and leukocyte antigens. Antibodies easiest to discern are those that give a visible reaction with the antigen in ordinary mediums.

The most straightforward test is agglutination or clumping together of saline-diluted erythrocyte suspensions, a method sufficient for detection of such antigens as AB or MN in man. Antibodies incapable of producing agglutination in a saline diluent are called "incomplete antibodies." Alloantibodies evoked by Rh blood groups are often of this type. Colloidal substances such as polyvinylpyrrolidone or high molecular weight dextran must then be used as diluents to achieve visible agglutination. Otherwise, red cells pretreated with enzymes (e.g., trypsin or papain) can be made agglutinable by incomplete antibodies in saline. Since no serologic method is universally applicable, an investigator must adapt techniques to suit given antigen–antibody systems. Antiglobulin tests have proved particularly useful for the detection of incomplete antibodies. In essence, red cells coated with nonagglutinating antibodies can be linked together into visible aggregates by addition of antibodies to immunoglobulin (e.g., rabbit anti-human globulin) that form intercellular bridges.

Existence of a spectrum of antibodies of similar specificity is revealed by occurrence of at least four categories of alloagglutinins (i.e., alloantibodies): (1) those demonstrable in saline solutions, (2) those detectable only when colloids are used, (3) those detectable only by the antiglobulin test, and (4) those detectable both in colloidal diluents and by the antiglobulin test. Microcytotoxic tests involving killing or immune lysis of target cells in the presence of serum complement are applicable to both erythrocytes and nucleated cells, and are usually preferable with the latter.

Even seed extracts from plants are a source of lectins or antibodylike molecules which may exhibit a high degree of serological specificity. Thus, *Dolichos biflorus* provides a lectin which agglutinates cells carrying the human blood group substance A, and this agglutinin reacts so much more strongly with subtype A_1 than with A_2 that it is nearly specific for A_1. Although the specificity of serological reactions is usually quite sharp, it is rarely absolute. Antigens of very similar chemical structure, as noted with certain serotypes in *Salmonella,* may cross-react to a large extent with the same antibodies. Conversely, it should be remembered that every antiserum, even though produced in an inbred animal by immunization with a highly purified antigen, will contain a heterogeneous population of antibodies in terms of combining site specificities (idiotypes) as well as other molecular properties. Under these circumstances, appropriate absorption of a high titer antiserum may yield a subset of antibody molecules with precise specificity.

Antibody Reagents and Absorption Analyses

In all antigenic typing, it is desirable that positive reactions be referable to antibodies of a single specificity. To derive antibody reagents specific for a single antigen, antiserums containing multiple antibody specificities must be absorbed with cells or solutes lacking the particular antigen in order to remove unwanted antibodies. In other words, an antiserum containing antibodies against two erythrocyte antigens (e.g., A and B) could be made specific for antigen B following absorption with cells containing antigen A obtained from an individual lacking B. The anti-B reagent produced could then be used to detect the presence of antigen B on the cells of other individuals. Such antiserums are defined as monospecific or unit reagents if each reactive cell type absorbs all antibody capable of reacting with any other positive cell, while negative cells remove none of the relevant antibody. Thus, in this example, cells from diverse individuals with the B antigen should absorb out all antibody from the anti-B serum capable of reacting with the cells of *any* other individual of the species. If this does not hold true, as frequently happens, an additional antibody specificity is thereby revealed. Another monospecific reagent may then be prepared to detect the newly discovered antigen.

Antiserums usually contain antibodies of multiple specificities directed against diverse antigenic determinants of the cells or even purified proteins or polysaccharides used in immunization. Monospecific reactions are best detect-

ed by cross-absorption analysis using cells or other antigenic preparations obtained from numerous individuals or sources reflecting different genetic constitutions. In a strict sense, one may contend that no antiserum raised by conventional-immunization is truly "monospecific." With the exception of monoclonal antibodies produced by hybrid cell lines (Chapter 6), even a highly specific antiserum will contain antibodies of different properties. Although the methodology of antibody absorption will effectively separate populations and subpopulations of antibodies, the antibodies remaining even after repeated absorptions may never be entirely specific for only one antigenic determinant. This reservation notwithstanding, the term *monospecific* is used in reference to antiserums which appear to be reactive operationally with only single antigenic specificities. The detailed consideration of absorption analyses to follow may require careful, repeated study by the novice.

An absorption analysis leading to the recognition of two molecular specificities is illustrated in Table 4–1. Note that the zeros (no reaction) along the diagonal dotted line are essential controls showing all antibodies for the respective absorbing cells were removed during the absorptions. The column under individual 1 indicates that absorption of this antiserum by these cells removed all antibodies capable of reacting with further number 1 test cells, but left behind antibodies for antigens on the cells of individuals 2, 4, 5, and 6. If the antibodies capable of reacting with the individual antigens in this panel were present in high concentration, several successive absorptions with cells from given individuals might be required to remove all antibodies capable of giving positive reactions with the absorbing cells. When xenogeneic antibodies are induced in a foreign species (e.g., rabbit anti-rat cell), many are directed toward species-specific antigens. Under these conditions, the unabsorbed immune serum invariably reacts with the cells of all individuals of the donor species. In the example of Table 4–1, which is often found in intraspecies or alloimmune systems, cells of individual 3 failed to react with the unabsorbed serum; this indicates that all the antigens which individual 3 had in common with the donor were also shared by the recipient who produced the antibodies. It also follows (see column under individual 3) that number 3 cells will not absorb out the antibodies capable of reacting with cells from the re-

TABLE 4–1. Absorption Analysis Revealing Two Antigenic Specificities

Test cells from individual	Antigenic symbols assigned	Unabsorbed	Antiserum absorbed with cells of individual					
			1	2	3	4	5	6
1	A	+	0	+	+	0	+	0
2	B	+	+	0	+	0	0	0
3	–	0	0	0	0	0	0	0
4	AB	+	+	+	+	0	+	0
5	B	+	+	0	+	0	0	0
6	AB	+	+	+	+	0	+	0

maining individuals in the panel. The behavior of each cell source in both the absorption columns and test rows, relative to all other cell sources tested, must be consistent with the antigen symbols designated. A complete cross-absorption analysis of the type illustrated provides a two-dimensional confirmation of the validity of the individual tests.

One may assign symbols to the individual antigens (or antibody subpopulations) detected in Table 4–1 in a systematic way as follows: Beginning with the unabsorbed serum, read down the columns and assign a different letter symbol to the "antigen" detected (positive reactions) in each column. This will be a preliminary or initial assignment of factors to account for the observed reactions.

Individuals	Initial assignment of antigen symbols
1	A — C D — E —
2	A B — D — — —
3	— — — — — — —
4	A B C D — E —
5	A B — D — — —
6	A B C D — E —

Note the identical configuration of the A and D columns and the C and E columns; the pairs of letter symbols describe the same pattern of reactions. Deletion of unnecessary symbols may be achieved by reading across and then down the columns of reactions and striking out any symbol that is not required to account for the differences among the individuals tested.

Individuals	Symbols retained after deletions	Final assignment of symbols
1	— C	A
2	B —	B
3	— —	—
4	B C	AB
5	B —	B
6	B C	AB

The initial columns of symbols A and D are not required because the *differences* among the six individuals are already satisfied by the B and C columns. On the assumption that we are dealing with a previously unknown system, the letters A and B could be used in the final assignment of symbols; the alphabetical sequence would then be continued as new antigens were discovered. If the two antigens assigned are sufficient to account for the individual differences observed, it should follow that absorption of this antiserum with the combined cells of individuals 1 = (A), and 2 = (B) should remove all antibodies capable of reacting with cells of the remaining individuals. Further-

more, one may note that individual 2 reacts with antibodies not absorbed by 1, whereas individual 1 reacts with antibodies not absorbed by 2. These antibodies could be called anti-A and anti-B, thus explaining the whole pattern in these terms. The symbols may stand for stereochemical or fine structural features of either the antigens or their complementary (reciprocal) antibodies. The precise meaning of such symbols often engenders considerable speculation and controversy among immunogeneticists.

The reader may check his understanding of the above analysis by assigning symbols of his own to characterize the pattern of observed reactions. In the final assignment of symbols, the so-called principle of parsimony or Occam's razor should be applied: The least number of symbols that satisfies the observed reactions is to be chosen.

Using the results of an absorption analysis, one may then prepare monospecific typing reagents by appropriate absorptions. In our example, absorption of the antiserum with cells of individual 1 should remove all anti-A and yield an anti-B reagent which could subsequently be employed to identify the B antigen in any other individual. Supposed monospecific serums on further absorption analysis with antigens of additional individuals will often reveal previously undetected antibody specificities. In other words, absorptions with antigenic preparations from other individuals might reveal additional subpopulations of antibodies, even in what appeared to be monospecific reagents in early tests. In such complex situations, true monospecific reagents are obtained only after further absorptions with antigenic preparations of different types. Absorption analyses of other immune serums would probably reveal additional antigenic types. However, given a standardized and reproducible technique, a battery of monospecific serums that would characterize the antigenic constitution of individuals tested at random can eventually be accumulated. The simple serologic situation where antigen from any positive individuals will absorb the antibodies for all other positive individuals usually reflects a single gene difference. In progeny tests, positive individuals should be either heterozygous or homozygous for an allele producing the antigen, but negative individuals should be homozygous for a different allele at the same gene locus.

The possible results of matings involving one dominant and one recessive or inactive allele are given in Table 4–2. The distribution of the antigen in the progenies is that expected of Mendelian traits. If one or both parents are homozygous dominant, only progeny possessing the antigen should occur. Negative parents should behave as if they were homozygous recessive and produce only progeny lacking the antigen. Although the zygosity of positive parents may be unknown, it can often be deduced after testing of the progeny. Note that negativity in this instance refers only to a given antigen.

In the Table 4–1 example, four phenotypes were found: one possessing only A, one possessing only B, one possessing both A and B, and one possessing neither. Genetically, this result probably reflects either one gene locus with three alleles (*A, B,* and *neither*) or two independent loci with two alleles each (*A, non-A,* and *B, non-B*). Allelism versus independence of the genes produc-

TABLE 4–2. Expected Proportions of Positive (+) and Negative (−) Individuals in Progenies Involving a Single Antigen Produced by One of Two Alleles

Type of mating	Segregation in progeny (proportion)	
	Positive	Negative
+/+ × +/+	1	0
+/+ × +/−	1	0
+/+ × −/−	1	0
+/− × +/−	3	1
+/− × −/−	1	1
−/− × −/−	0	1

ing antigens A and B can be differentiated by antigenic characterization of progeny from matings between parents possessing both A and B and those possessing neither (AB × _). If A and B are determined by alleles, only A and B type progeny should occur, and in essentially equal numbers. If independent genes are involved, then AB progeny will occur and the other possible three types as well, depending on the genotype of the AB parent.

A similar distinction between allelic and nonallelic genes can be made from matings between AB parents: allelic genes could not result in progeny negative for both antigen determinants if there were no alleles able to code for both determinants. The possibility that such alleles exist cannot be excluded. However, extensive progeny testing may be required to distinguish alleles from closely linked genes.

HUMAN BLOOD GROUPS

The ABO and Lewis Systems

Immunogenetic relationships inherent in the familiar ABO system were summarized in the discussion of first principles in the introductory chapter. The three allelic genes, *A, B,* and *O* yield six genotypes *AA, AO, BB, BO, AB,* and *OO,* but only four phenotypes, A, B, AB, and O, based on the routine availability of only anti-A and anti-B for typing (Table 1–1). Accordingly, the mating of O to AB should never result in either O or AB offspring. The *ABO* locus, incidentally, is near the tip of the long arm of chromosome 9, where it is closely linked to the AK_1 locus for the polymorphic red cell enzyme adenylate kinase. The alloantibodies against A and B antigens are mostly of the IgM class, which function as complete agglutinins, but antibodies may also reside in the IgG and IgA classes. Some five subgroups of A and three of B are characterized by weak expression of these antigens on erythrocytes, except for the A_1 and A_2 subgroups. Among other indications of qualitative distinction between A_1 and A_2, many A_2 and A_2B individuals contain specific anti-A_1 in

their serums. With the additional A_1 versus A_2 distinction, the reader may verify a larger array of six phenotypes and ten genotypes in the *ABO* system. It is not possible to distinguish AA from AO or BB from BO phenotypes except by family studies, since an anti-O capable of reacting with any distinctive product of the *O* gene in single dose has not been found. The *O* gene is evidently inactive and exerts no effect on the *H* gene →H substance pathway, the precursor of all blood group antigens in this system (cf. Table 1–2). Individuals of type A, B, or AB all retain some H substance as a result of incomplete biosynthetic conversion. Only rare *hh* individuals (Bombay phenotype) lack H, A, and B antigens entirely. To understand this, we must hasten to consider the sequence and products of gene action.

A revealing pattern of relationships between gene action, chemical structure, and immunologic specificity has been determined for the *ABO* and closely related *Lewis* blood group systems in man. Attempts to determine antigenic structure by direct extraction of erythrocyte components have not proved fruitful until quite recently because the desired determinants constitute only a small portion of the erythrocyte and are bound to lipoproteins of the cell membrane in a manner which makes purification quite difficult. However, blood groups A, B, H, Le^a, and Le^b are present not only on red cells but also in tissue fluids and secretions in water-soluble form. These water-soluble determinants are identified and assayed by their capacity to react specifically with given blood group antibodies so that agglutination fails to occur upon subsequent addition of the corresponding erythrocytes. Because the activity of most enzymes is inhibited by their products, enzymatic inhibition methods have also been used to support and confirm the results of serologic inhibition tests. Molecules that specifically inhibit hemagglutination also occur in various plants and bacteria. Such heterogenetic antigens common to different species have provided another source of blood group substances for chemical studies. The most concentrated sources of blood group alloantigens in normal secretions are gastric juice and saliva.

Purified A, B, H, and Lewis substances from secretions are glycoproteins containing about 85 percent carbohydrate and 15 percent amino acids. Their average molecular weights from different individuals range from 3×10^5 to 1×10^6. There is apparently some molecular heterogeneity in purified preparations even from a single individual and a single type of secretion. Although the detailed structure of these macromolecules remains to be determined, their properties are consistent with the assumption that they consist of many short oligosaccharide chains covalently bound to a peptide backbone. Integrity of the entire macromolecule appears essential for maximal serological reactivity, even though inhibition methods indicate that specificity is associated with the oligosaccharide units. The A, B, and H membrane antigens are complex and highly branched glycosphingolipids (i.e., antigenic lipids containing a ceramide moiety), but unlike their soluble counterparts, all of the terminal galactoses are linked $\beta 1, 4$ to *N*-acetylglucosamine.

Although A and B antigens are usually found on the secretions as well as

on the red cells, about 20 percent of persons with A or B on their erythrocytes do not have these antigens in their secretions. This characteristic was found to be controlled by a locus designated Secretor, such that a dominant allele *Se* results in secretion whereas the recessive *se* leads to nonsecretion. Unlike the *A* and *B* alleles, the *O* allele does not yield a specific product. Secretions from *OO, Se* individuals will neutralize only those antibodies in anti-O reagents that are also neutralized by A, B, or AB secretions. Since AB individuals cannot have an *O* gene, the neutralizing antigen associated with type O was named H. With the finding of rare individuals who lack H as a recessive trait (*hh*), H reactivity was assignable to a separate gene locus. The Le^a antigen of the *Lewis* system was found to be a product of the *Le* gene, whereas the absence of this antigen accompanies the recessive *lele* genotype. The Lewis B specificity of this system is *not* dependent upon an additional allele but occurs as an interaction product of the *H* and *Le* genes (Table 4–3). Individuals of the rare "Bombay" phenotypes (*hh*) lack A, B, and H antigens both on their cells and in their secretions. However, the Le^a antigen is still normally synthesized in the absence of both *H* and *Se* genes. A meaningful understanding of these genetic relationships hinges on immunochemical identification of the antigenic determinants involved.

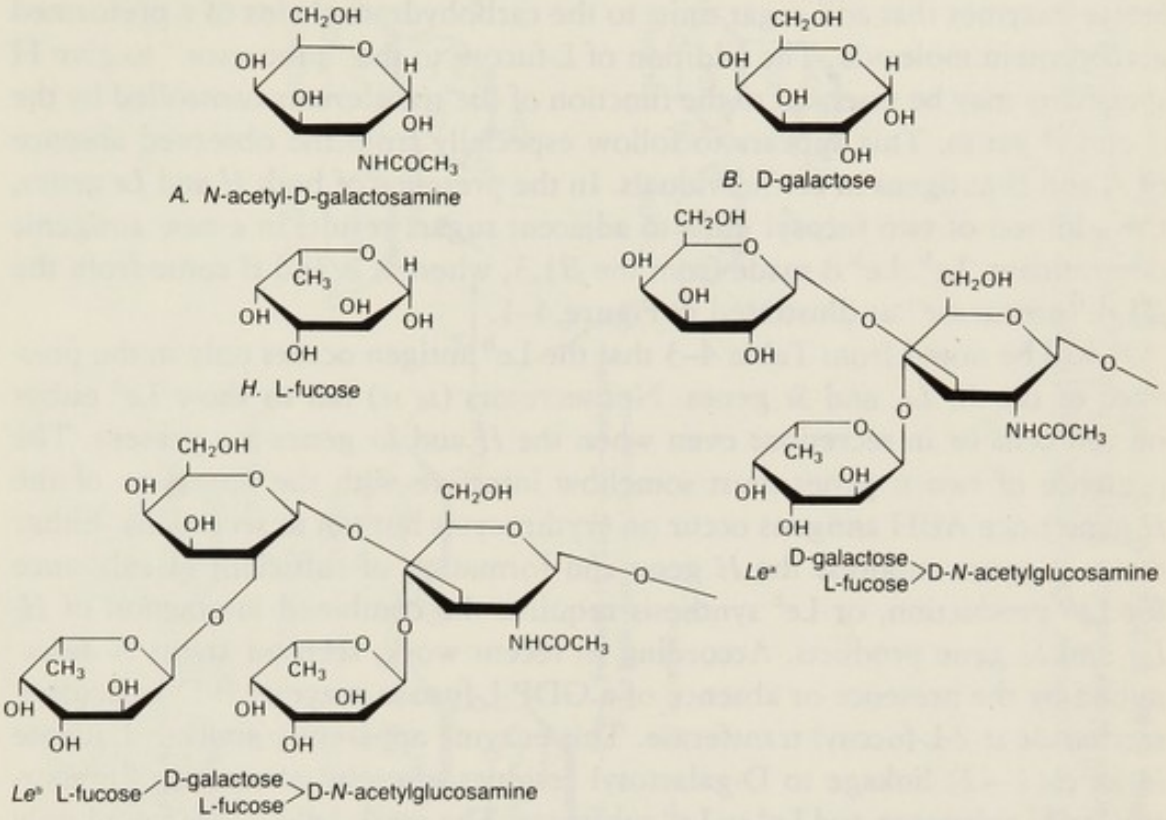
The different serological properties of A, B, H, and Le^a substances are not attributable to qualitative differences in composition. Each is composed of the same fifteen amino acids and each contains five sugars: a methyl pentose, L-fucose; a hexose, D-galactose; two amino sugars, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine; and *N*-acetylneuraminic acid (sialic acid). However, purified A preparations show higher *N*-acetylgalactosamine values, Le^a preparations usually contain less fucose, and removal of *N*-acetylneuraminic acid does not lead to loss of serological activity. After years of painstaking investigation, especially by Watkins and Morgan in Britain and by Kabat in the

TABLE 4–3. Six Blood Group Combinations Distinguishable on the Basis of *H, A, B, Lewis, and Secretor* Gene Activities[a]

	Antigens on erythrocytes							Antigens in secretions							
Gene combinations	H	A	B	Le^a	Le^b	Le^c	Le^d	H	A	B	Le^a	Le^b	Le^c	Le^d	
H–, ABO, Le–, Se–	++	++	++	–	++	–	–	++	++	++	+	++	–	–	
H–, ABO, Le–, se se	++	++	++	++	–	–	–	–	–	–	++	–	–	–	
H–, ABO, le le, Se–	++	++	++	–	–	–	++	++	++	++	–	–	–	++	
H–, ABO, le le, se se	++	++	++	–	–	++	–	–	–	–	–	–	++	–	
hh, ABO, Le–, Se–, or *se se*	–	–	–	++	–	–	–	–	–	–	++	–	–	–	Rare Bombay
hh, ABO, le le, Se–, or *se se*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

[a] *H–, Se–,* and *Le–* indicate people either homozygous or heterozygous at these loci; each person has any two of the *ABO* genes. Entries: ++, strong specific reactivity; +, weak reactivity; –, no reactivity. H activity is stronger in OO individuals than in those of other A or B genotypes, but is missing entirely in hh (Bombay) phenotypes.

United States, those blood group specificities have been identified with particular sugars and their linkages at the nonreducing ends of the carbohydrate chains. The determinant monosaccharide constituents of A, B, and H, and the oligosaccharide determinants of Le^a and Le^b are as follows:



The involvement of L-fucose in the H, Le^a, and Le^b antigens indicated that the linkage of the terminal sugar to the second sugar, or both the structure of the sugars and the nature of their linkage, was responsible for the observed serological specificity. Only oligosaccharides containing two fucose residues attached to adjacent sugars as shown were active inhibitors of Le^b hemagglutination. Thus, the Lewis antigens appear to derive their specificity from the branching as well as the nature and sequence of sugar units. The difference between A and B, on the other hand, is reflected only in the occurrence of an $-NHCOCH_3$ versus an $-OH$ group on carbon 2 of a terminal galactose residue. Precipitation experiments with monospecific antiserums reveal these several antigens to be present on the same macromolecule. When purified soluble antigen from an AB person is precipitated with anti-B serum, both A and B activities are precipitated. Similarly, both H and Le^a antigens are found in macromolecules with A, B, or AB activity. Moreover, enzymatic release of a single sugar unit may unmask a different determinant, indicating that the antigens were originally part of the same carbohydrate chain. The chains in H and Le^a substances are identical to those of A and B after removal of the terminal sugar. The products of gene action described are summarized in Figure 4–1.

Since A, B, H, and Le^a substances cross-react with horse antibody to type

XIV pneumococcal polysaccharide even after enzymatic or acid degradation to the extent that specific activity is lost, the type XIV or a similar specificity appears to be the "precursor" oligosaccharide upon which the blood group genes act. The combined immunogenetic and biochemical evidence points to the conclusion that the *A, B, H,* and *Le* genes control specific glycosyl transferase enzymes that add sugar units to the carbohydrate chains of a preformed glycoprotein molecule. The addition of L-fucose to the "precursor" to give H specificity may be essential to the function of the transferases controlled by the *A* and *B* genes. This appears to follow especially from the observed absence of A and B antigens in *hh* individuals. In the presence of both *H* and *Le* genes, the addition of two fucosyl units to adjacent sugars results in a new antigenic determinant, Le^b. Le^b is made from the β1,3, whereas A and B come from the β1,4 "precursor" as illustrated in Figure 4–1.

It will be noted from Table 4–3 that the Le^b antigen occurs only in the presence of the *H, Le,* and *Se* genes. Nonsecretors (*se se*) fail to show Le^b either on red cells or in secretions even when the *H* and *Le* genes are present. The presence of two *se* genes must somehow interfere with the operation of the *H* gene since ABH antigens occur on erythrocytes but not in secretions. Either the *se se* genes suppress the *H* gene and formation of sufficient H substance for Le^b production, or Le^b synthesis requires the combined interaction of *H, Le,* and *Se* gene products. According to recent work, secretor status is determined by the presence or absence of a GDP-L-fucose enzyme: B-D-galactosyl-saccharide α-2-L-fucosyl transferase. This enzyme apparently attaches L-fucose in an α-(1→2) linkage to D-galactosyl residues, thereby converting "precursor" to H substance and Le^a to Le^b substance. The weak Le^a activity found only in the secretions of persons with Le^b activity obviously suggests that most potential Le^a substance is utilized in the formation of Le^b. Moreover, the strongest Le^a activity is found in nonsecretors with or without the *H* gene, indicating that the Le enzyme is not competing for substrate. Le^a antigen is present but not bound to red cells at birth. Erythrocytes from infants with both *Le* and *Se* genes are first Le(a+b−), then Le(a+b+), and finally Le(a−b+) after one or more years. Thus, Le antigens are not intrinsic to red cells but become absorbed from the plasma during maturation.

Two additional Lewis antigens, designated Le^c and Le^d have recently been found in Le(a−b−) individuals of *le le* genotype. Le^c appears on the red cells and in the saliva of nonsecretors, while Le^d alternatively occurs in secretors. Thus, the *le* allele can no longer be considered a recessive amorph, but rather the source of a fucosyl transferase that places a fucose in the α1,3 position on *N*-acetylglucosamine, giving rise to Le^c in nonsecretors or Le^d in secretors in whom the *H* gene is also active (Figure 4–2). Le^a and Le^b by contrast, display an α1,4 linkage of the subterminal fucose and a β1,3 linkage of the terminal galactose (Figure 4–1). In essence then, Le(a−b−c+) individuals are *le le, se se,* whereas Le(a−b−d+) people are *le le, Se.* The perceptive reader will again note that particular linkages of sugars, rather than the mere presence or absence of given sugars, are often decisive determinants of antigenic specificity.

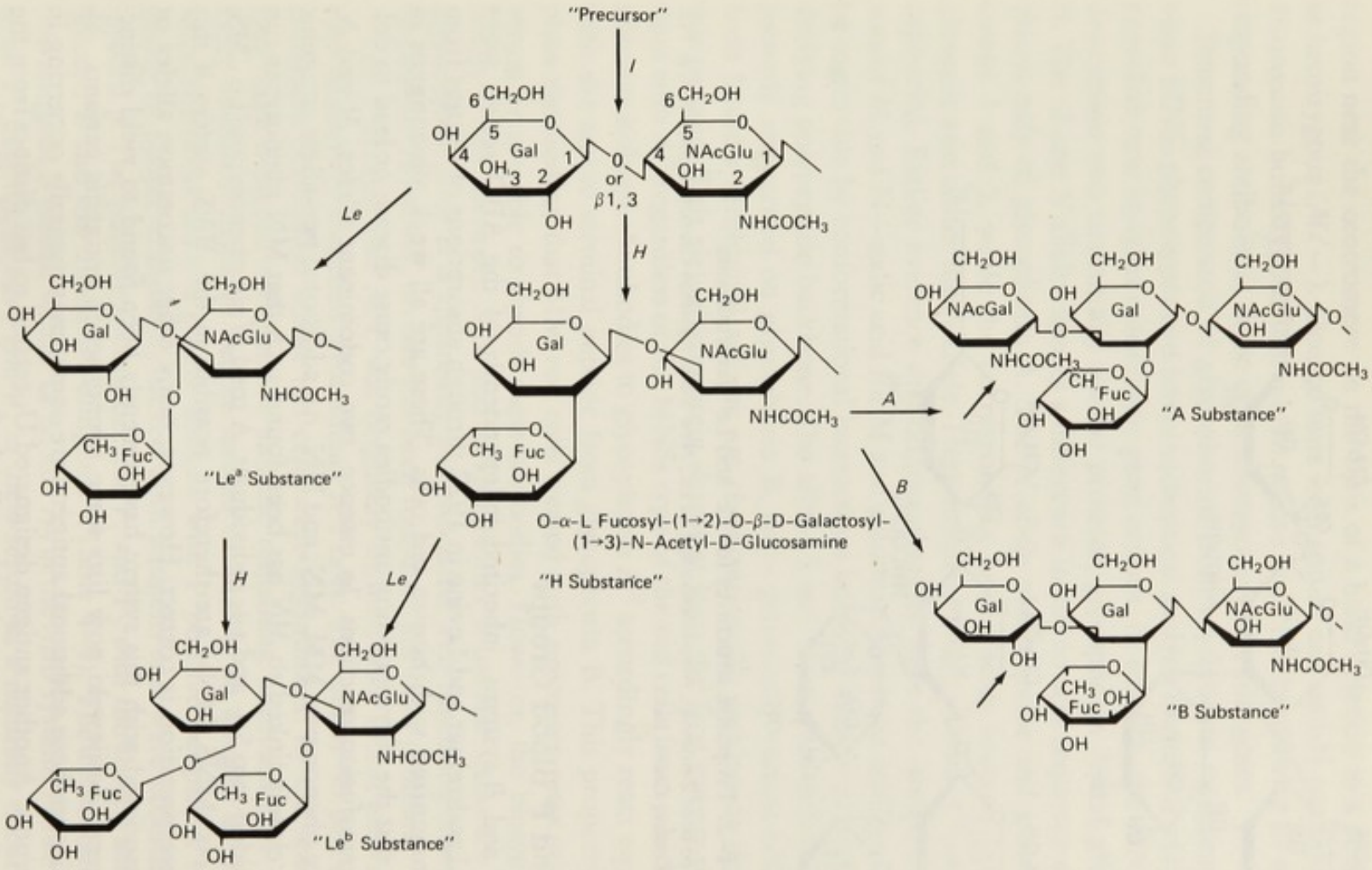


FIGURE 4–1. Sequential sugar additions to precursor glycoprotein by glycosyl transferases controlled by the *I*, *H*, *Le*, *A*, and *B* blood group genes. Precursor occurs in two forms with β1,3 and β1,4 linkages. Le^a and Le^b are made from the β1,3, whereas A and B as well as Le^c and Le^d are derived from the β1,4 precursor.

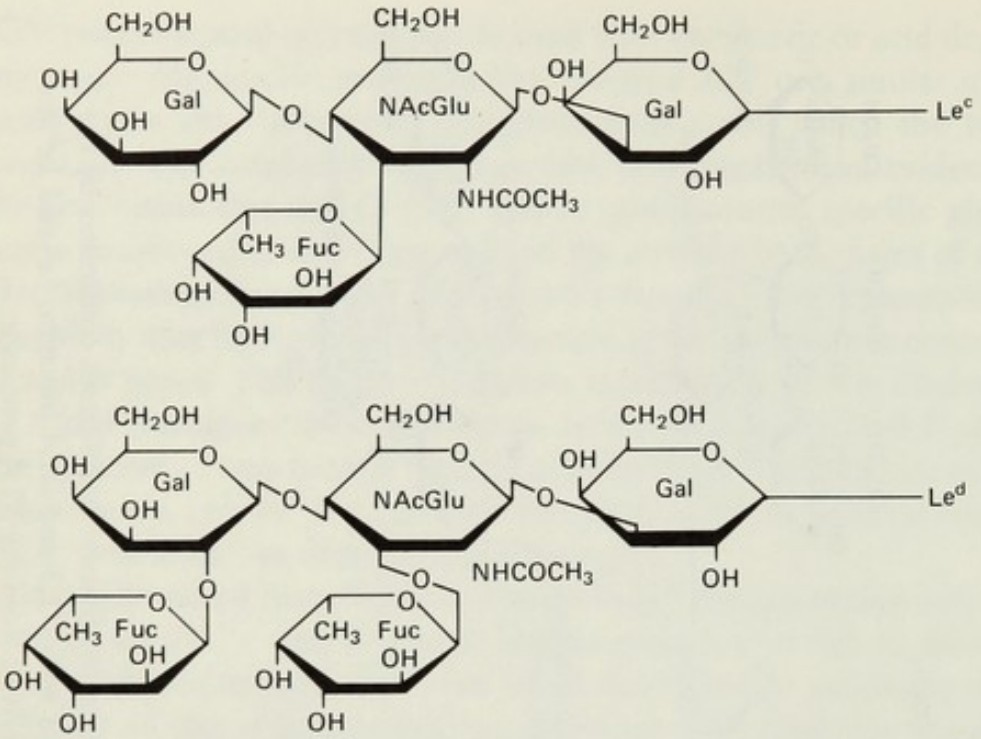


FIGURE 4–2. Proposed structures for Le^c and Le^d determinants.

Source: Adapted from Zmijewski, *Immunohematology*, 3d ed., New York: Appleton-Century-Crofts, 1978, p. 112. © Appleton-Century-Crofts, Inc.

MNSs and P Blood Groups

The *MN* and *P* systems, inherited independently of the ABO series, were found by Landsteiner and Levine in 1927 through absorptions of serums from rabbits immunized with human red cells. These are all weak alloantigens in the sense that the corresponding antibodies rarely cause disease or lead to deleterious transfusion reactions. In essence, two codominant alleles, *M* and *N*, yield three phenotypes: MM, MN, and NN. An additional two-allele antigenic system *Ss* closely linked to *MN* has been found such that MN phenotypes can be subdivided into S+ and s+ individuals. A total of six phenotypes (i.e., MS, Ms, NS, Ns, MNS, MNs) are therefore possible in the *MNSs* system if this genetic interpretation is correct. However, many other uncommon alleles or subloci associated with this system have recently been found to yield distinctive antigens. Zmijewski now lists some twenty-seven antigenic variants. Alloantibodies to these additional antigens are sometimes naturally occurring in human serums. Another antigen designated U, related to but distinct from the S and s antigens, was detected with antibody from the serum of a black woman who suffered a fatal hemolytic transfusion reaction. The U antigen occurs in all whites, but is absent from about 5 percent of blacks, mainly those who also lack the S and s antigens. The immunogenetic complexity of the *MNSs* system now appears formidable and can no longer be described in terms of a few alternative alleles independently yielding corresponding antigens. This genetic region has been tentatively assigned to the long arm of chromosome 2 in a

region near the centromere on the basis of a band deletion in a person typed as hemizygous ($M/-$). Dosage tests with anti-M and anti-N can reproducibly distinguish homozygous individuals whose red cells bind twice as much corresponding antibody as those of heterozygotes or hemizygotes.

Structural comparison of glycophorins from erythrocyte membranes of diverse MNSs phenotypes and immunochemical analysis of genetic variants have provided new insights concerning gene actions. In essence, the *MNSs* system determines two structurally similar proteins, glycophorin A and glycophorin B. The *M* and *N* alleles specify a difference in amino acid sequence at two positions only of glycophorin A. The *M* allele places serine and glycine in positions 1 and 5, while the corresponding amino acids become leucine and glutamic acid under the *N* allele. Heterozygotes produce both A^M and A^N glycophorins. Earlier evidence that oligosaccharides were the immunodeterminants of M and N—sialic acid for M and galactose plus sialic acid for N—could be explicable by conformational interactions between these sugars and the underlying polypeptide backbone. The allelic S and s antigenic determinants apparently are carried on glycophorin B, since genetically variant cells lacking both S and s serological activity are characteristically lacking or deficient in this protein. This sialoglycoprotein shares at least the first twenty-three residues with A^N regardless of the MNSs type of the red cells from which the protein was isolated. Antibodies to glycophorin A^N accordingly react equally well with the amino terminal peptide from glycophorin B. This property may explain the finding that MM red cells treated with proteases react with anti-N serum or are able to absorb these antibodies. However, the biosynthetic relation, if any, between MN and Ss specificities is unclear. Absence of either glycophorin A or B is apparently not associated with functional abnormalities or disease. However, no one has been found to lack both proteins.

The *P* system, like *MN,* was originally defined with rabbit anti-human serum selectively absorbed with human red cells. The nearly 80 percent of the American population whose red cells were agglutinated by the absorbed serum were designated P positive and the remaining nonreactors, P negative. Later certain P negative people instead of lacking an antigen of the system were found to share a potent antigen with P positive people. A third rare group of people completely lacked both antigens and were accordingly designated p. The positive groups became P_1 and P_2 with the shared antigen designated P and the distinctive antigen P_1(Table 4–4). The relationship of the two antigens is similar to that of A_1 and A_2 in the *ABO* system. However, nearly all P_2 individuals have anti-P_1 in their serum as a naturally occurring antibody. The rare *pp* recessives have both anti-P_1 and anti-P in their serums. Unusual variants termed P^k found among Finns normally produce anti-P, but family studies show that P^k is not an allele at the *P* locus, since P^k positives may also have P_1 and P_2 genes, which are inherited by their children.

A glycosphingolipid with P_1 activity from human erythrocyte stroma has the structure of paragloboside with a terminal D-galactose added. The terminal trisaccharide [Gal(α,1→4) Gal (β,1→4) Glc NAc(β,1→3)] of the P_1 deter-

TABLE 4–4. The P Blood Group System of Man

Phenotype	Antigens on red cells	Possible genotypes	Approximate frequency (%)	Antibodies in normal serum
P_1	P_1, P	P_1P_1	30	
		P_1P_2	50	none
		P_1p	~0.1	
P_2	P	P_2P_2	19.5	anti-P_1
		P_2p	~0.1	
p	none	pp	~0.1	anti-P_1 anti-P

minant is identical in both glycoprotein and glycolipid sources of the antigen. From hemagglutination-inhibition data, the P_2 antigen is equivalent to normal human globoside [i.e., Gal N Ac(β,1→3) Gal(α,1→4) Gal(β,1→4) D-glucose *N*-acylshingosine], whereas P^k antigen is ceramide trihexoside [i.e., Gal(α,1→4) Gal(β,1→4) D-glucose *N*-acylshingosine]. Cells of p phenotype have no detectable globoside or ceramide trihexoside (CTH). Presumably the genes of the *P* system specify galactosyl transferases governing biosyntheses as follows:

1. Paragloboside $\xrightarrow[\alpha\text{-galactosyl transferase}]{P_1\ \text{Gene}}$ P_1 antigen

2. Ceramide trihexoside (CTH) $\xrightarrow[\beta\text{-N-acetylgalactosaminyl transferase}]{P_2\ \text{Gene}}$ P antigen (globoside)

The P^k antigen in the form of CTH may be viewed as the precursor of the P antigen whenever the P_2 gene is present and synthesizing its acetylgalactosaminyl transferase. The P_2 gene product is apparently a β-Gal N Ac transferase that uses trihexosylceramide as substrate, while the P_1 gene product is an α-Gal transferase with paragloboside as substrate. Thus one should question whether these genes are truly allelic.

Rh Blood Groups

In 1940 Landsteiner and Wiener reported the discovery of the *Rhesus* (*Rh*) system which has turned out to be an important cause of maternal–fetal incompatibility in man. They detected the *Rh* system by injecting blood from a rhesus monkey into rabbits; after the induced antibodies for previously known human antigens had been absorbed, the immune rabbit serums still agglutinated the red cells of about 85 percent of the people tested. Since these people possessed a cellular antigen in common with the rhesus monkey, the factor was

named Rh. Although such Rh positivity is inherited as a dominant Mendelian trait, use of many different Rh antiserums has revealed the existence of eight common or "standard" *Rh* alleles, each leading to the expression of multiple antigenic specificities. Most of these antiserums have been obtained from humans who have been immunized by Rh antigens from other humans, either as a consequence of blood transfusions, the passage of cells from a fetus into a mother, or experimental inoculation of Rh negative volunteers. Rh alloantibodies are not naturally occurring but appear following prolonged immunization, and are mostly in the IgG class.

The phenomenology of the *Rh* system has become so complex at the serological level of antigen–antibody reactions that three nomenclatures reflecting different theories of inheritance have evolved. The contrasting Wiener and Fisher-Race nomenclatures designate genotypes and phenotypes of the Rh blood groups on the basis of different conceptions of the interrelation between genes, antigens, and antibodies. The Wiener school interprets the evidence on the classic assumption of a single locus with many alleles. These are given the letters *R* and *r* with distinguishing superscripts like R^o and r'. Individual antigenic determinants are called Rh, rh, and hr with superscripts or subscripts, and the corresponding antibodies are designated anti-Rh, anti-rh, anti-hr. The blood group substance or macromolecule as such is regarded as the complex antigen or immunogen ("agglutinogen" in the older literature), whereas the constituent antigenic determinants ("blood factors") are individually defined in terms of agglutination reactions with known antiserums. The molecular meaning of the letter symbols assigned to complementary antigens and antibodies is still a source of controversy. We shall return to this issue in several connections. In the Wiener scheme, a complex antigen or "agglutinogen" is defined by two or more determinants or "blood factors." The following diagram summarizes the sequence of relationships invoked:

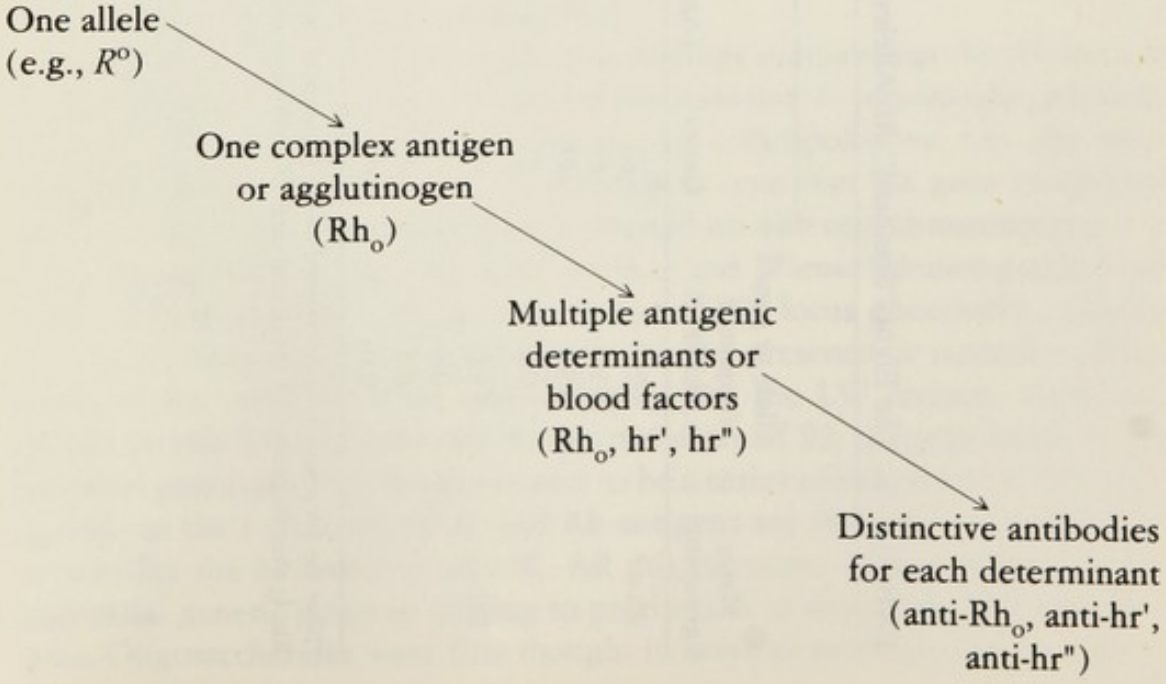
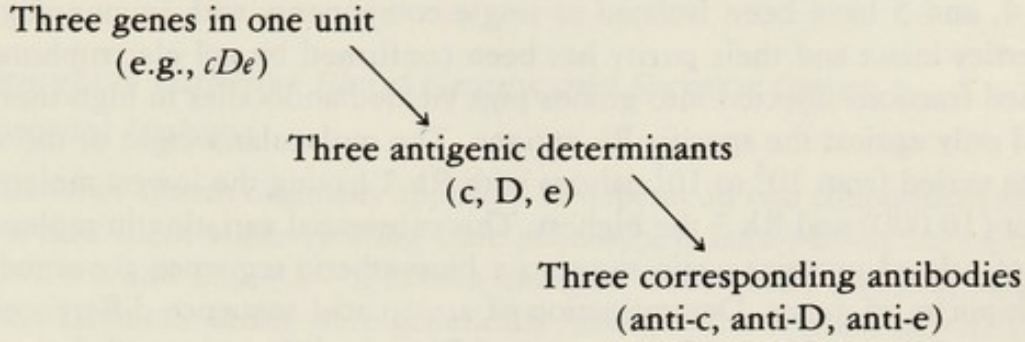


TABLE 4–5. The Eight "Standard" *Rh* Alleles and Their Antigenic Products Compared by Three Nomenclatures

		Wiener			Fisher-Race		Rosenfield et al.	
Major grouping	Allele or gene frequency in whites (%)	Genes	Antigen complex (Agglutinogens)	Antigenic determinants	Genes	Corresponding antigens	Genes	Antigenic structure of products
Rh+[a]	2.7	R^0	Rh_0	Rh_0, hr′, and hr″	*cDe*	c, D, and e	$R^{1,4,5}$	Rh: 1,4,5
	41.0	R^1	Rh_1	Rh_0, rh′, and hr″	*CDe*	C, D, and e	$R^{1,2,5}$	Rh: 1,2,5
	15.0	R^2	Rh_2	Rh_0, rh″, and hr′	*cDE*	c, D, and E	$R^{1,3,4}$	Rh: 1,3,4
	0.2	R^z	Rh_z	Rh_0, rh′, and rh″	*CDE*	C, D, and E	$R^{1,2,3}$	Rh: 1,2,3
Rh−	38.0	*r*	rh	hr′ and hr″	*cde*	c, d, and e	$R^{4,5}$	Rh: 4,5
	0.6	*r′*	rh′	rh′ and hr″	*Cde*	C, d, and e	$R^{2,5}$	Rh: 2,5
	0.5	*r″*	rh″	rh″ and hr′	*cdE*	c, d, and E	$R^{3,4}$	Rh: 3,4
	0.01	r^y	rh_y	rh′ and rh″	*CdE*	C, d, and E	$R^{2,3}$	Rh: 2,3

[a]Note the Rh positivity is determined by the presence of one or two genes (alleles) leading to the expression of the equivalent antigens or blood factors designated Rh_0, D, or Rh: 1; over 90 percent of the clinical cases of erythroblastosis are attributable to the production of antibodies against this antigen.

Fisher, Race, and Sanger, on the other hand, assume a complex *Rh* locus comprised of a series of very closely, if not absolutely, linked genes. Each of these genes is supposed to have two or more alleles, e.g., *C* and *c*, *D* and *d*, *E* and *e*, with correspondingly designated antigens and antibodies in a strictly one-to-one relationship (cf. Table 4–5).



The nomenclature of Rosenfield adopts a straightforward numerical correspondence between gene and antigen symbols. Thus the *Rh* allele designated $R^{1.4.5}$ yields an antigenic product symbolized as Rh 1,4,5. Given the increasing complexity of possible test reactions in this and similar systems (e.g., *HLA* and *Gm* in man; *H-2* in mice; *Rt-1* in rats) a numerical nomenclature is much to be preferred. This usage also has the virtue of neutrality concerning the possible molecular connotations of gene versus antigen symbols. Indeed, the definition of a gene locus at the DNA level is now complicated by evidence of long "spacer" or regulatory sequences separating structural DNA sequences specifying a single molecule. One may note from Table 4–5 that the four "recessive" genes leading to Rh negativity (i.e., absence of Rh_O, D, or 1) nevertheless yield antigenic products which also occur in the presence of the dominant alleles. The existence of hypothetical antigen d in the Fisher-Race scheme remains doubtful. Some thirty-five Rh antigens are now known and more variants will probably be identified.

Combined serological and cytogenetic findings indicate that the *Rh* locus is on the distal portion of the short arm of chromosome 1. Incidentally, the Duffy blood group system, of much interest to anthropologists, has also been mapped to chromosome 1. It is interesting to note that the gene complexes for the *ABO*, *MNSs*, and *Rh* loci are all located on different chromosomes. Another antigen named LW (after Landsteiner and Wiener) demonstrable with rabbit anti-rhesus serum but governed by an *LW* locus genetically independent of *Rh*, was found to be correlated with the presence or normal manifestation of Rh antigens. Thus, Rh null cells lack the LW antigen. However, certain people lacking LW have the normal array of Rh antigens representing different genotypes. There now appear to be a series of subgroups of LW comparable to the subgroups of A, and Rh antigens are thought to serve as precursors for the biosynthesis of LW. All this of course implies existence of a sequential genetic pathway leading to production of the various Rh-type antigens. Oligosaccharides were first thought to serve as serological determinants

of Rh specificity. Specific inhibition of Rh 1 antibody by compounds containing neuraminic acid has been repeatedly reported. One study identified Rh 1 specificity with a human ganglioside composed of D-galactose, D-glucose, *N*-acetylgalactosamine and *N*-acetylneuraminic acid. However, more recent work with Rh antigens purified from human erythrocyte membranes by isoelectric focusing associates specificity with a family of related proteins. Rh 1, 2, 3, 4, and 5 have been isolated as single components with immunological properties intact and their purity has been confirmed by gel electrophoresis. Purified fractions injected into guinea pigs yielded antibodies in high titer directed only against the specific Rh antigen. The molecular weight of the subgroups varied from 10^3 to 10^5 daltons with Rh 1 having the lowest molecular weight (10,000) and Rh 3 the highest. This substantial variation in molecular sizes of related proteins again suggests a biosynthetic sequence governed by a multiplicity of genes. Determination of amino acid sequence differences in these proteins should reveal the extent of *Rh* gene differences and their evolution. Involvement of the *Rh* system in disease is considered later in connection with other blood group systems.

Other Human Alloantigen Systems

The *ABO, Lewis, MNSs, P,* and *Rh* systems will have been considered in detail by us because of their biomedical and basic immunogenetic interest. Some fourteen well-defined blood-group systems have been identified in man (Ta-

TABLE 4–6. Well-Defined Blood Group Systems of Man in Decreasing Order of Antigenic Polymorphism

System	Gene or antigen complexes involved	Total number antigenic specificities
Rh	Rh 1 – Rh 35; LW*	~38
MNSs	M, N, S, s; 8M variants plus rare antigens Cl^a, He, Hil, Hu, Mi^a, Mur, Mt^a, N_2, Ri^a, S_2, Sj, St^a, Sul, T^m, U, V^r, V^w	~29
Lutheran	Lu1– Lu20	~20
Kell	K1 – K19	19
ABO	H*, h†, A_1–A_5, B_1–B_3, O†	9
Duffy	$Fy1$ – $Fy5$	5
I	I^F, I^D, I^T, i_1, i_2	5
Lewis	Le^a, Le^b, Le^c, Le^d	4
P	P, P_1, p†, p^k*	3–4
Kidd	Jk^a, Jk^b, Jk†	2
Diego	Di^a, Di^b	2
Cartright	Yt^a, Yt^b	2
Vel	Vel_1, Vel_2	2
Xg	Xg^a, Xg†	1

*Genetically independent locus governing an antigenic product associated with the biosynthetic pathway of the system.

†A silent or null allele with no antigenic product.

ble 4–6). These are arranged in decreasing order of apparent antigenic complexity or polymorphism so the reader will be conscious of the broad range from some thirty-eight specificities identified with the Rh system to the sex-linked system *Xg* characterized by only a single antigen. Although discussion of all these blood groups is beyond the scope of this chapter, several do illustrate important principles worthy of attention.

Polymorphic Lutheran Blood Groups and Secretor Genes: Autosomal Linkage

The *Lutheran* system originally appeared to depend on two codominant alleles plus a rare silent allele yielding four phenotypes: Lu(a+b−), Lu(a+b+), Lu(a−b+), and Lu(a−b−). It then emerged from family pedigree analysis that the Lutheran alleles were genetically linked to the secretor genes. Thus, parents of genotypes Lu^a, Lu^b; *Se se* × Lu^bLu^b; *se se* yielded only two types of children: Lu^bLu^b; Se se and Lu^aLu^b; se se. In other words, Lu(a+) children irrespective of sex were all nonsecretors in this situation, an outcome attributable to autosomal linkage. The rare Lu(a−b−) phenotype now appears to depend on a dominant suppressor gene rather than a recessive null allele with no antigenic product. This was deduced from matings of the type Lu(a−b−) × Lu(a−b+) that yielded numerous children, all Lu(a−b−). The dominant inhibitor gene *In*(*Lu*) occupies a locus separate from *Lu* on the basis of observed crossing-over. The far more common and normal recessive allele for noninhibition or nonsuppression is called *in*(*Lu*). This same suppressor locus also influences the expression of *P* and *I* system antigens. At least twenty Lu antigens have now been identified, although all have not been shown to be controlled exclusively by alleles of the *Lutheran* system. Thus, a supposedly simple three-allele–two-antigen system of ten years ago has, in fact, revealed substantial complexity as a result of the zeal of numerous blood group workers.

The Kell System

Here again we begin with a two-antigen system, K and k, of special interest because either antigen can provoke a potent immune response in transfused patients or pregnant women who lack the antigen. Since the K antigen is present in only about 9 percent of all individuals (*KK* or *Kk*), some 91 percent of the population is vulnerable to alloimmunization. Both K and k are strong immunogens. Many variant antigens, now totaling nineteen, have been found in recent years to belong to or to be associated with the *Kell* system. The growing complexity of the *Kell* system parallels that just described for the *Lutheran* system. A rare Kell null phenotype has been described as attributable to a recessive k° allele. The K15 or K_x antigen appears to be a precursor for the synthesis of other K antigens. K15 is absent in patients with X-linked chronic granulomatous disease. According to one scheme, presence of a dominant X-linked gene (X^1k) leads to production of K15 antigen in cells of both ery-

throid and myeloid series. The recessive X^2k allele in XY males yields no K15 on either red cells or granulocytes and such granulocytes exhibit defective bactericidal phagocytosis. In any event, a biosynthetic pathway involving multiple gene loci governs production of Kell antigens even though multiple alleles at a single autosomal *Kell* locus account for many of the specificities detectable. However, neither the precursors nor the end products have as yet been biochemically defined.

The Ii System

As a cellular alloantigen system, five classes of Ii determinants exhibit unusual characteristics of fundamental interest. First, the Ii antigens are identified by a set of IgM autoantibodies that function as cold hemagglutinins, i.e., they react preferentially at 4° C. The I antigens are expressed on the red cells of most adults (> 99.9 percent of Caucasians), while the i antigens are readily detected on red cells of rare adults lacking I and on umbilical cord blood cells. The anti-I and anti-i are often found in patients with autoimmune acquired hemolytic anemia following certain infections such as those caused by *Mycoplasma pneumoniae* and Epstein-Barr virus (EBV). Indeed, anti-i regularly appears in conjunction with infectious mononucleosis which is caused by EBV. I-negative reactors can be divided into two categories: i_1 characteristic of whites; and i_2 characteristic of blacks and only occasionally found among whites. The two main components of the I antigen are designated I^F(fetal) and I^D(developed), because I^D is gradually expressed after birth whereas I^F occurs on both fetal and adult red cells. The i condition can no longer be regarded as the absence of I, because fetal and newborn cells have been shown to react with specific anti-I as well as anti-i. To further complicate the story, an additional antigenic variant I^T appears to be a transitional molecule between i and I. In essence, normal development of the *Ii* system entails quantitative loss of i antigen concomitant with the gain in I antigen leading to expression of the I^D determinant at maturity. Practically no individual of i phenotype is completely devoid of the I^D antigen, as shown by capacity to absorb anti-I activity.

The apparent biosynthetic pathway is as follows:

$$i \rightarrow I^F \rightarrow I^T \rightarrow I^D \dashrightarrow H \begin{array}{l} \nearrow A,B \\ \searrow Lewis \end{array}$$

Yet this pathway now emerges as merely part of a more complex sequence leading to appearance of I, H, A, B, and P_1 in erythrocyte membrane glycolipids. The poly(glycosyl)ceramides (PGC) with these several blood-group activities are branched glycosphingolipids with twenty to sixty sugar residues per mole of ceramide. The group I activity of PGC is associated with Gal $\beta \rightarrow 4$ Glc N Ac structures. The *I* gene locus then may code for a transferase responsible for branching of inner galactopyranosyl residues of glycolipids with *N*-

acetylglucosamine. The detection of seemingly compound antigens IH, IA, or IB is now understood on the basis of their proximity on the same macromolecule (PGC) rather than as a result of interaction between *I* and *ABO* genes. The Lewis $^{a,b,c,\ \&\ d}$ determinants of red cells are acquired by a quite different mechanism involving absorption of glycolipids from blood plasma. In terms of sequential gene action, the *I* gene acts early, followed by *ABH* genes, and then *Le* genes. In other words, Ii determinants are expressed on the precursors of the ABH and Lewis blood group antigens.

The Sex-Linked Blood Group System

The finding of an antigen Xg^a governed by a gene locus on the X chromosome has provided a quite useful marker for studies of sex-linked disease, anthropology, and phylogeny. This is the simplest known alloantigen system with only two phenotypes Xg(a+) and Xg(a−) attributable to a dominant Xg^a allele and a silent *Xg* allele, respectively. From the mating of an Xg(a+) man and an Xg(a−) woman, all sons will be Xg(a−) and all daughters Xg(a+). Among Northern Europeans, the phenotype frequencies are about 88 percent Xg(a+) for females and 66 percent Xg(a+) for males. In other ethnic groups the frequency ranges from a high of 85 percent in New Guineans to a low of 38 percent in Taiwanese. The Xg^a antigen has been found in gibbons and is indistinguishable from that in man on the basis of antibody absorption studies. However, numerous other species of primates, dogs, and mice evidently lack this antigen.

The Lyon hypothesis of random inactivation of one of the X chromosomes in each female somatic cell during embryogenesis is generally accepted, but curiously, heterozygous Xg^a/Xg females do not exhibit corresponding red cell mosaicism. Various X-linked genes have now been mapped in relation to the *Xg* gene locus. A gene for ocular albinism and genes contributing to ichthyosis, retinoschisis, and angiokeratoma have all been mapped close to *Xg*.

Alleles and Population Structure

Most macromolecules of similar structure and function show antigenic polymorphism traceable to multiple alleles or complex loci. Why such molecular diversity persists is obscure in most instances. The far-ranging implications of the uniqueness of individuals is worth pondering. Long series of multiple alleles distinguishing individuals or subpopulations are commonly found within species ranging from protozoa to man. It should be noted that allelic genes, in general, cause only slight differences in their end products. Substantial differences would presumably destroy the functional integrity of most allelic products. Thus, the human blood group alleles *A* and *B* appear to differ only in that *A* mediates the addition of an *N*-acetylgalactosamine and *B*, a D-galactose, to the same precursor glycoprotein. Allelism may be discernible from serologic data alone. When tests reveal that two antigens always occur in

individuals alone or together and no individuals lacking both antigens are found, it is highly probable that the determinative genes are alleles. However, appropriate mating tests are usually essential to confirm such findings.

To understand the genetic structure of different populations, one needs to determine the frequencies of known alleles. The following section may prove difficult initially to the reader who has never encountered the concepts of population genetics. The reader may also want to consult additional reading concerning genes in populations.

If an allele A has a frequency p in a population and its only alternative B has a frequency q, then $p + q = 1$, and the distribution of genotypes in a population breeding at random and in the absence of selection should be $p^2AA + 2pqAB + q^2BB$. In other words, when gametes with chromosomes carrying this gene locus combine in pairs at random, the probability that both will have A is $p \times p = p^2$, and the probability that both will possess B is q^2. The AB heterozygotes then should have a frequency of $pq + pq = 2pq$. This $p^2 + 2pq + q^2$ distribution is equivalent to the binomial $(p + q)^2$. As long as the allele frequencies p and q remain essentially constant, a random-mating population of three genotypes in the proportions $p^2:2pq:q^2$ may be assumed to be in equilibrium, as is usually the case. The formula $p^2AA + 2pqAB + q^2BB$ and its extensions are known as the Hardy-Weinberg law. The stability and predictability of this relationship depend on: (a) random mating in a large population with all possible genotypes equally fertile, (b) sexes evenly distributed among genotypes in a large population, and (c) individuals compared at similar developmental stages. The last consideration is to avoid being misled by age-dependent antigens.

Genetic drift may often alter the gene frequency equilibrium of a small population, especially when one allele is present in low frequency. Such drift is defined as a random departure from the original gene frequency and is caused by limited interbreeding in a small population. The unusual blood group frequencies found in smaller Eskimo and North American Indian communities are perhaps best explained in terms of genetic drift. Differential mutation or selection favoring one genotype or phenotype may modify allele frequencies in successive generations. Changes in isolated, small populations caused by migrations, as is typical of today's mobile society, also alter the gene frequency significantly. Differences in cultural or behavioral traits, however, often tend to restrict gene exchange between ethnic groups.

Direct demonstration of changes in allele frequencies is achieved only by comparison of successive generations. Bentley Glass studied blood groups on a small community of Dunkers in Pennsylvania. This religious sect was derived from twenty-seven families who came from the German Rhineland to North America in the early 1700s. Their ABO frequencies now differ significantly from those typical of Rhineland Germans as well as from other Americans. The frequency of type A among Dunkers is about 60 percent, as compared with 40 to 45 percent in the other two populations. The B allele is nearly absent among Dunkers. Type O is less common in Dunkers than in Germans or Americans. Although there is no difference in the ABO frequen-

cies of three current generations of Dunkers, there is a substantial difference in their MN blood groups. The older Dunkers have an *M* allele frequency of 55 percent, which is similar to that of German and American populations. The middle-aged group contains 68 percent of *M* alleles whereas the frequency in the youngest group has risen to 73 percent. Since the difference between the oldest and youngest groups is highly significant, the shift in allele frequency among Dunkers can probably be attributed to genetic drift.

Because population immunogenetics often requires gene frequency analysis, one should understand the application of the Hardy-Weinberg principle, at least, to simpler systems like human ABO. Let p, q, and r represent the frequencies of the three major allelic genes *A*, *B*, and *O*, respectively, so $p + q + r = 1$, or 100 percent. By expansion of the trinomial $(p + q + r)^2$, the frequencies of the four phenotypes can be expressed as follows:

Phenotypes	Inclusive genotypes	Corresponding phenotype frequencies
O	*OO*	r^2
A	*AA* + *AO*	$p^2 + 2pr$
B	*BB* + *BO*	$q^2 + 2qr$
AB	*AB*	$2pq$

If $\overline{O}$, $\overline{A}$, and $\overline{AB}$ symbolize the phenotype frequencies in a population, the gene frequencies may be calculated as follows:
Since

$$r^2 = \overline{O}, r = \sqrt{\overline{O}}, \tag{1}$$

$$\overline{O} + \overline{A} = p^2 + 2pr + r^2 = (p + r)^2.$$

Therefore,

$$p + r = \sqrt{\overline{O} + \overline{A}}.$$

So

$$p = \sqrt{\overline{O} + \overline{A}} - \sqrt{\overline{O}}. \tag{2a}$$

Similarly,

$$q = \sqrt{\overline{O} + \overline{B}} - \sqrt{\overline{O}}. \tag{3a}$$

Since $p + q + r = 1$, $p + r = 1 - q$ and $q + r = 1 - p$; therefore $1 - q = \sqrt{\overline{O} + \overline{A}}$, and $1 - p = \sqrt{\overline{O} + \overline{B}}$.

$$p = 1 - \sqrt{\overline{O} + \overline{B}}, \tag{2b}$$

$$q = 1 - \sqrt{\overline{O} + \overline{A}}. \tag{3b}$$

Finally,

$$\sqrt{\overline{O} + \overline{A}} + \sqrt{\overline{O} + \overline{B}} - \sqrt{\overline{O}} = 1. \tag{4}$$

In Caucasian American populations, blood group frequencies are approximately 45 percent O, 42 percent A, 10 percent B, and 3 percent AB. By substituting these values in the formulas above, one can determine the respective gene frequencies (1, 2, and 3) and test the validity of the theory of multiple alleles in this system (formula 4).

Maternal–Fetal Incompatibility and Natural Selection

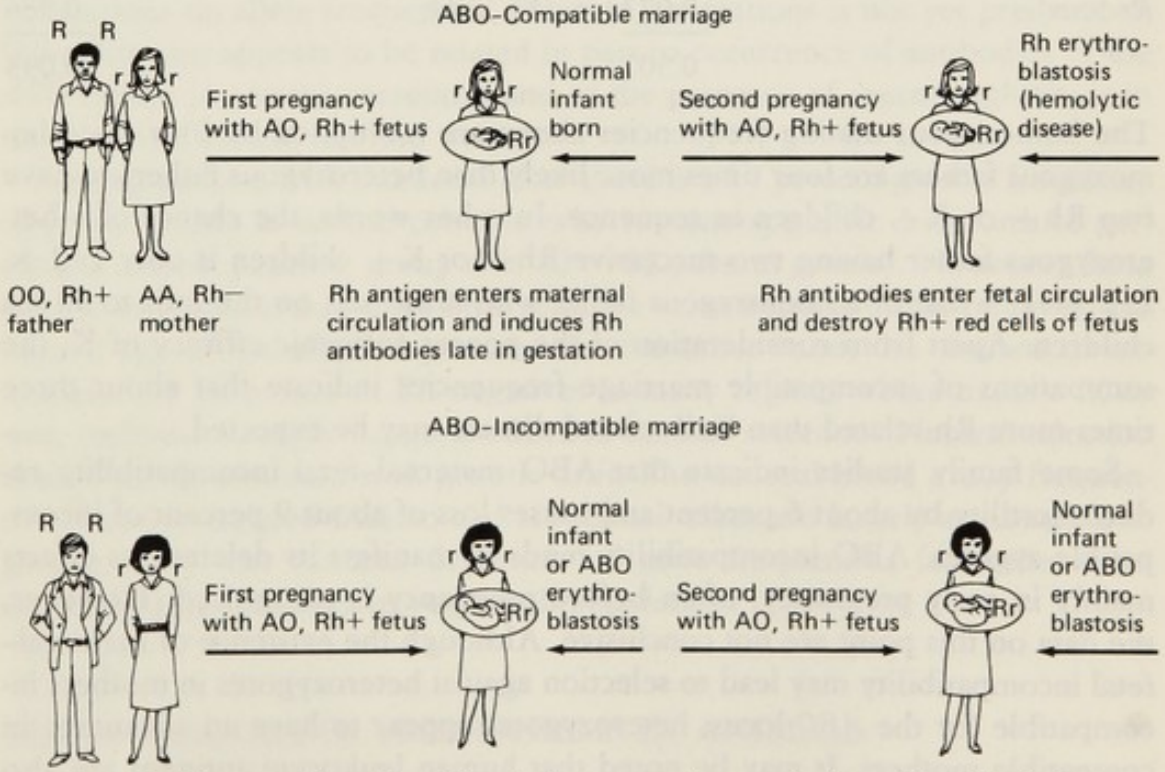
The observed differences in frequencies of alleles (and phenotypes) for various blood groups suggest that natural selection plays an important role in the establishment and maintenance of alleles in populations. The ABO and Rh blood groups in man reveal several levels at which selection may operate. The Rh antigens are the most important cause of maternal–fetal incompatibility detected clinically. Rh+ cells derived from the fetus often induce antibodies, mostly of IgG type, in Rh− women. These antibodies, particularly in second or later pregnancies, readily cross the placenta in sufficient concentration to produce fetal death or severe hemolytic disease in newborns. Rh-negative women have the highest stillbirth rate. In North America until recently, about one birth in every 200 to 300 showed disease attributable to Rh incompatibility. Incompatibility in the ABO system alone may have similar though less deleterious consequences in terms of injury to A, B, or AB newborns by the respective antibodies derived from the mother. Infants inheriting genes which determine weaker expressions of A or B do not usually show clinical evidence of disease. However, early embryonic deaths may also be caused by ABO incompatibility. Studies in European and Japanese populations indicate a smaller proportion of A children from marriages of O mothers and A fathers than in the reciprocal situation, suggesting that A antibodies from O mothers lead to the early death of A embryos. ABO erythroblastosis (i.e., presence of erythroblasts in lieu of mature erythrocytes in the blood), in contrast with the Rh-associated form, is often observed in the first incompatible child, and increasing severity of disease is not regularly seen in subsequent incompatible infants.

For a long time, the relative infrequency of hemolytic disease of the newborn from pregnancies involving Rh-negative mothers and Rh-positive fathers was unexplained. When such pregnant women, especially those who have already had an Rh-positive child, are tested for Rh antibodies in their serum, fewer than half show evidence of sensitization. It turns out that ABO incompatibility of mother and child is strongly protective against Rh immunization. An O, Rh-negative mother is rarely sensitized by an A or B, Rh-positive fetus. Immunization to the relatively weak Rh antigen depends on substantial exposure to fetal red cells by "leakage" at the time of parturition; A, B, or AB cells entering an O mother are so quickly destroyed by the naturally occurring antibodies that the associated Rh antigens fail to reach the lymphoid centers in sufficient quantity. This same protective mechanism prevents sensitization to the Kell antigen—another potential source of hemolytic disease. However, if Rh

antibodies have once been induced by pregnancy or blood transfusion, ABO incompatibility is no longer protective. Thus, an *OO,* Rh-negative woman with a *BO,* Rh-positive husband might be immunized against Rh by her first child if he or she happened to be *OO.* A second child, even if it were *BO,* would not be protected because even slight secondary Rh immunization leads to substantial, renewed antibody production. One may note that *AB,* Rh-negative women and *O,* Rh-positive men are most likely to have erythroblastotic children. The probable consequences of Rh maternal–fetal incompatibility in relation to ABO-compatible and ABO-incompatible marriages are illustrated in Figure 4–3.

A notable circumvention of Rh hemolytic disease has now been achieved by passive administration of Rh antibodies (anti-Rh 1 or anti-D), which apparently blocks active immunization against this antigen. Thus, none of several hundred nonimmunized Rh-negative women given Rh antiserum at the time of delivery subsequently showed active immunization against Rh antigens. This

FIGURE 4–3. Second or later Rh-positive children of Rh-negative mothers may be severely anemic or die due to Rh antibodies induced by passage of fetal blood cells into maternal circulation in the absence of ABO incompatibility. Usually milder ABO erythroblastosis may occur in first or later pregnancies mainly in O type mothers with A- or B-positive fetuses, irrespective of presence or absence of Rh incompatibility.



fully effective protection, compared to about 80 percent protection by natural ABO incompatibility, may depend upon sequestration of fetal Rh antigen or some form of feedback inhibition of active antibody production. Evidently the risk of primary Rh immunization prior to delivery is quite low or even nonexistent. The potential danger of hemolytic disease is, of course, much greater if the husband is homozygous, especially for Rh 1 or D; if he is heterozygous, only half the children will be positive. The actual incidence of hemolytic disease referable to particular systems is a function of gene frequencies, a point that may be illustrated with respect to the gene frequencies of *Rh* genes ($R(D) = 0.6$, $r(d) = 0.4$) and *Kell* genes ($K = 0.05$, $k = 0.95$) in Caucasian populations. The frequencies of the genotypes are:

Rhesus	Kell
RR 0.36	*KK* 0.002
Rr 0.48	*Kk* 0.095
rr 0.16	*kk* 0.903

As calculated from the genotype frequencies, the proportions of marriages in which hemolytic disease may occur are:

♂ ♀		♂ ♀	
$RR \times rr$	$0.0576 \times 4 = 0.230$	$KK \times kk$	$0.0018 \times 4 = 0.007$
$Rr \times rr$	0.077	$Kk \times kk$	0.086
	0.307		0.093

The homozygous mating frequencies above are multiplied by four since homozygous fathers are four times more likely than heterozygous fathers to have two Rh+ or K+ children in sequence. In other words, the chance of a heterozygous father having two successive Rh+ or K+ children is only $1/2 \times 1/2 = 1/4$ that of a homozygous father who must pass on the trait to all his children. Apart from consideration of the poorer antigenic efficacy of K, the summations of incompatible marriage frequencies indicate that about three times more Rh-related than Kell-related disease may be expected.

Some family studies indicate that ABO maternal–fetal incompatibility reduces fertility by about 6 percent and causes loss of about 9 percent of incompatible zygotes. ABO incompatibility tends to manifest its deleterious effects mainly in early pregnancy, often before pregnancy is recognized. However, the data on this point are not conclusive. Although the existence of maternal–fetal incompatibility may lead to selection against heterozygotes in mothers incompatible for the *ABO* locus, heterozygotes appear to have an advantage in compatible mothers. It may be noted that human leukocyte antigens are also a source of maternal–fetal incompatibility. Because of preoccupation with well-known blood groups, the potential significance of leukocyte alloantigens in this connection is yet to be fully explored.

The ultimate goal of measuring the relative fitness of each genotype in each

blood group system is complicated not only by poorly understood interactive effects of an immunological nature but also by differential effects on reproductive performance at various stages of the life cycle. Reproductive performance in relation to blood groups has been measured as a function of number of pregnancies, probability of sterility, proportion of pregnancies terminating in spontaneous abortions, stillbirths or fetal deaths, and proportion of liveborn children dying nonaccidentally under five years of age. Blood group phenotypes in six systems (*ABO, Rh, MN, Kell, Duffy, P*) and ABH secretor status were checked in one extensive study by a University of Michigan team for possible effects of husband's and wife's blood groups and types of matings. The highly significant correlations discerned are not amenable to simple explanation: Group O fathers and P_2 group mothers are associated independently with more deaths in liveborn children; Kell-negative women, independent of husband's group, have more pregnancies while more group-N women are sterile. ABO- and Rh-mating types also affect the numbers of pregnancies and spontaneous abortions, but the nature of the associations is still not clear because of conflicting evidence from different studies. Perhaps 20 percent of ABO-incompatible zygotes are eliminated early in pregnancy, but superimposed on this effect is a probable 20-percent differential in Rh selection pressure favoring ABO-incompatible zygotes. Rh-negative daughters of Rh-positive women may possibly acquire some tolerance to the Rh antigen in utero, compared with Rh-negative daughters of Rh-negative women. The impact of ABO-Rh interactions on allele frequencies in future generations is not yet predictable. Infertility also appears to be related in part to occurrence of antibodies of the *ABO* system in uterine secretions and to the presence of sperm agglutinins in the serum of certain married women.

Among patients with duodenal ulcer there is a 17 percent greater frequency of group O than in normal controls, whereas among gastric carcinoma or pernicious anemia patients, group A is 10 to 13 percent greater. However, these disease associations may have little selective value because of the relatively advanced age of the affected persons. On the other hand, the absence or extremely low frequency of Rh negatives in many regions where malaria is, or was, endemic strongly suggests effective natural selection. Natural selection should not be confused with genetic drift. The unusual blood group frequencies found in isolated Eskimo and aboriginal tribes are largely attributable to genetic drift—the random departure of gene frequencies from the norm caused by interbreeding in very small populations.

BLOOD GROUP POLYMORPHISMS IN ANIMALS

Genetic Systems in Cattle

The blood group systems of cattle have been more extensively characterized than those of any other species. Longstanding interest in bovine blood groups stems from their relevance to the breeding and maintenance of desirable beef

and dairy cattle. Moreover, important immunogenetic insights have emerged from analysis of bovine systems. Alloimmune hemolysis in the presence of rabbit complement has proved to be the most sensitive and reliable technique for cattle blood typing. In contrast with most other species, cattle erythrocytes are not prone to agglutinate even when coated with antibodies in excess. Among the eleven systems or genetic loci known (Table 4–7), all degrees of immunogenetic complexity have been revealed, ranging from simple two-allele systems such as *L* and *T'* to the amazingly polymorphic *B* system with more than 300 alleles and/or haplotypes determining different combinations of multifarious antigenic products or specificities. The number of possible diploid combinations or phenotypes of the *B* system is at least 60,000 according to Stormont. The number of distinguishable blood types in cattle systems is of course limited by the quality and quantity of monospecific antiserums available. The lack of correspondence between the numbers of alleles and alloantigens in nearly all genetic systems reflects the fact that different alleles may determine the production of none, one, or several antigens. The ten A-system alleles, for example, are designated a^{A_1}, a^{D_1}, a^{H_1}, $a^{A_1D_1}$, $a^{A_2D_1}$, a^{A_1H}, a^{D_1H}, $a^{A_1D_1H}$, $a^{A_1D_2Z'}$ and $a^{A_2D_1H}$ with the superscripts indicating six distinguishable antigenic components in various combinations. Note that the specificities D_2 and Z' occur only together and in conjunction with A_1 in the allele $a^{A_1D_2Z'}$, suggesting D_2 and Z' are interaction products. The distinction between D_1 and D_2 (or A_1 and A_2) is based on slight differences in reactivity. Absorption of

TABLE 4–7. Eleven Genetic Systems of Blood Groups in Cattle[a]

Genetic system	Number of alleles or haplotypes (minimum)	Minimum number of antigenic markers[b]	Number of distinguishable phenotypes or blood types[c]
A	10	6	20
B	300+	60	60,000+
C	40+	10	800+
F–V	4	5	8
J	9(?)	many intergrades	2
L	2	1	2
M	3	2	4
S	5+	7	64
Z	3	2	5
R'–S'	2	2	3
T'	2	1	2

[a]Clyde Stormont, personal communication. The total phenotypic variation that can be realized by tests for these markers is in the neighborhood of 236 trillion.

[b]Many of these markers occur in two or more serological subtypes which are taken into consideration when estimating the number of possible blood types.

[c]Dosage reagents are available for typing certain markers such as Z. Thus, anti-Z_1Z_1 permits the distinction between genotypes Z_1Z_1 and Z_1z.

a rabbit anti-D_1 serum with D_2 cells removes most of the D antibodies, but leaves unabsorbed antibodies specific for D_1. An exactly analogous situation obtains with the several A subtypes in man.

In the *B* system of cattle, factor K is another example of an interaction antigen since it appears only in combination with both B and G. The five known haplotypes (phenogroups) in this subsystem are BGK, BG, B, G, and "—"(or no-BGK). The number of haplotypes and alloantigens in a genetic system is usually less than the number of distinguishable phenotypes for the simple reason that the phenotypes reflect diploid combinations of codominant alleles. If the possible phenotypes shown for each of the eleven systems in Table 4–7 are combined, about 236 trillion different blood types could occur, which is probably more than all the cattle that have ever lived!

The *J* blood group system of cattle is especially interesting because the J substance naturally occurs in solution in body fluids and is secondarily absorbed onto erythrocytes. An animal with J on its cells and no J in its serum has never been found. The J character is inherited as a dominant trait and was detected until recently only with naturally occurring antibodies found in serums of cattle lacking J. Curiously, J-negative (j^aj^a) cattle may or may not possess naturally occurring antibodies, apparently depending upon the season. Clyde Stormont and associates at the University of California, Davis, and W. H. Stone and coworkers at the University of Wisconsin have extensively investigated this system from both immunogenetic and immunochemical standpoints. Wide variations in amounts of cell-bound J in different animals are apparent from the disparate reactivities of cells with given anti-J serums. Two J-positive classes (J^{CS} and J^S) were distinguished, each with four probable alleles determining different concentrations of cellular and serum J. Serums from J^{CS} cattle contain much higher concentrations of J than those of J^S cattle. Moreover, red cells from J^S cattle possess low levels of J detectable only by their ability to absorb anti-J. About twenty times as many J^S cells as J^{CS} cells are required to absorb antibodies from an anti-J serum. Progeny tests involving J^{CS} and J^S classes revealed significant, genetically determined differences in concentrations of J attributable to multiple alleles. The j^a allele, yielding no J substance, does not contribute in heterozygous individuals to the variation found among progenies in the expression of the J^{CS} and J^S phenotypes. In these studies, the amounts of cellular J were determined by titration of cells with a standard anti-J serum while levels of serum J were determined by inhibition tests. Although the presence and concentration of cellular J is largely governed by the level of J substance in the serum, additional unidentified genes may also be involved.

Coating experiments in vitro indicate that the concentration of serum J rather than the nature of the cells is decisive in determining whether erythrocytes become J-positive. However, this simple relationship is partially negated by exceptional cattle that have high levels of serum J with little cellular J evident. The J activity in purified preparations obtained from serum is associated with carbohydrate and protein, whereas that derived from gastric mucosa appears

to be carbohydrate alone. The determinant group of J specificity could well be an oligosaccharide bound to different macromolecules in different tissues since the best studied human blood groups suggest such molecular relationships.

Although erythrocyte J and S system antigens are also detectable on leukocytes, lymphocyte-defined antigens have only recently been studied in cattle. By use of alloimmune lymphocytotoxic serums from animals injected with whole blood or isolated leukocytes, at least two highly polymorphic loci governing lymphocyte-associated antigens have been identified. Some of the corresponding antibodies occur in parous cows as a result of fetal immunization, but this apparently does not lead to maternal–embryo incompatibility reactions.

Overtones of the classic multiple alleles versus closely linked genes controversy surrounding the human *Rh* system also persist in relation to the similarly complex *B* system in cattle. A provisional ordering of fifteen to twenty-eight genetic determinants of the *B* system as a linear array of subloci or gene clusters, based on putative recombinants, has been put forward by Bouw and coworkers in the Netherlands. Analogies with the more intensively analyzed *H-2* complex in mice and *HLA* in man are attractive. Indeed, the *B* system now appears to be part of the major immunogene complex in cattle. However, Stormont and coworkers in California have recently found that multiple antigenic specificities of a given *B*-system haplotype occur on a single molecule as determined by antibody-blocking tests. This result is consistent with the assumption of multiple alleles at a single locus, but of course does not preclude involvement of other loci, closely linked or not. Both sides of this "debate" may ultimately emerge as correct in the sense that extensive allelism is demonstrable at individual loci within a coordinated cluster or complex of separate immunogenes.

Blood Groups in Diverse Domestic Animals

Every vertebrate species that has been extensively studied, ranging from teleost fishes to primates, has shown multiple alloantigen systems with allelic diversity. Syrian hamsters were formerly thought to be nonconforming, but recent studies of wild-caught hamsters and their progenies now reveal substantial polymorphism of leukocyte and histocompatibility antigens. However, restricted blood group diversity and corresponding alloantibody responsiveness may represent unusual features of hamsters. Because of their economic importance, cattle, horses, pigs, sheep, and chickens have received much attention. Eight independent autosomal loci, each with two to ten alleles, are implicated in the control of at least twenty red cell antigens in horses. Among some thirteen blood group loci and four serum group loci identified in pigs, three autosomal linkages have been detected. Indeed, the loci for the C and J blood groups are closely linked. Moreover, the main histocompatibility complex in

pigs, designated *SLA* and governing both transplantation-defined and serologically-defined antigens, is closely linked to the *C* and *J* loci. Numerous instances of genetic linkage among blood group loci are now known in species of birds and mammals, but the functional significance, if any, is unknown. Cellular alloantigen systems of domestic or laboratory animals, approximating the totals demonstrable as erythrocyte, leukocyte, histocompatibility, and/or differentiation antigens are enumerated for comparison in Table 4–8. Insofar as detection of new alleles and antigens is concerned, the total number discernible in a species appears limited only by the resourcefulness and persistence of interested investigators. This is why the laboratory mouse now takes first prize in apparent immunogenetic complexity. Instructive characteristics of bird, rabbit, and sheep erythrocyte alloantigens will be considered further in the context of gene interactions.

Hemolytic disease of the newborn occurs naturally or may be produced experimentally in diverse species of domestic animals (Table 4–8). Intrauterine selection caused by maternal–fetal incompatibility of blood types has also been found in whales. In horses, pigs, and dogs, the antibodies reach the newborn through the mother's milk rather than the placenta. In the newly hatched chick, hemolytic disease may result from the passage of antibodies into the egg yolk before the shell is formed. The absence of hemolytic disease in cattle or sheep is probably attributable to digestion of relevant antibodies during their passage through the gut after ingestion of the mother's milk. Why the antibody-containing colostrum ingested immediately after birth leads to hemolytic disease in some species and not in others is puzzling. Antibodies protective

TABLE 4–8. Cellular Alloantigen Systems Known in Various Domesticated Animals

Species	No. of separate systems (loci) described[a]	Minimum number of cellular alloantigens detectable	Total number of linkage groups (chromosome pairs) in species	Maternal–embryo incompatibility reactions reported
Mouse	~55	120	20	yes
Pig	14	27	20	yes
Cattle	11	98	30	no
Chicken	10	23	39	yes
Horse	8	20	33	yes
Sheep	7	34	27	no
Dog	6	8	39	yes
Rabbit	5	13	22	yes
Rat	4	12	21	yes
Turkey	3	17	39	—

[a]All of these loci are not on separate chromosomes; some are closely linked. The number and complexity of the systems described reflect in general the extent of investigative effort; additional systems probably remain to be discovered in most of these species.

against infectious agents passively derived in this manner are of obvious importance. Possibly only certain molecular species of antibodies are passed to the young during a short period after birth via the digestive tract, and selective mechanisms may be involved.

Gene Interaction in Antigen Specification

We have already considered examples of blood group antigens in cattle such as K and Z' that appear to be interaction products dependent upon the presence of certain other alloantigens specified by the same allele. Thus, K antigen of the *B* system occurs only in association with B and G as products of the allele b^{BGK}; Z' in like manner has been found only in conjunction with the allele $a^{A1D2Z'}$. The molecular basis of the interaction between the independent *H* and *Le* genes yielding the Le^b antigen was the addition of a single fucose (H unit) to the Le^a trisaccharide. This is the prime instance known at present in which the chemical nature of the interaction product is defined. Further consideration of examples of hybrid antigens, suppression of antigens, and epistatic action of genes is essential for a full grasp of known blood group systems.

An instance of apparent gene interaction resulting in a "hybrid" antigen was noted by Irwin in the hybrid progeny of two species of doves of the genus *Streptopelia.* Rabbit antiserum against hybrid cells after exhaustive absorption with cells of both parents or parental species was still strongly reactive with hybrid cells. This finding revealed a new antigen characteristic of the hybrids that was absent from the cells of either parent. This result may be symbolically diagramed as follows:

$$\begin{array}{lll} P_1 & = & AA \times BB \\ & & \downarrow \\ F_1 & = & AB(C) \\ \text{hybrid} & & \end{array}$$

The new antigen (C) is dependent upon the concurrence of two or more genes (antigens) derived from the parents. As expected, hybrids between *Streptopelia chinensis* and *Streptopelia risoria* show both antigens that are common and those that are specific to the parental species. Backcross tests for allelism reveal that independent loci control nine different antigens specific to *S. chinensis. Inter se* matings of backcross birds demonstrate that progeny homozygous for *ch-8* of *S. chinensis* lack the hybrid antigen whereas heterozygotes with the contrasting *ri-8* of *S. risoria* possess it. The hybrid antigen then may be attributed to the interaction of the *ch-8* and *ri-8* alleles or their products. Actually, the backcross birds with ch-8/ri-8 antigens possess a minimum of five antigenic specificities of a hybrid substance not present in either parental species. Parallel instances of hybrid antigens have been identified in other interspecific hybrids among species of doves and ducks. However, hybrid antigens are not universal among species hybrids. Antibodies to hybrid antigens have been elicited by immunizing the actual parents with the hybrid cells, and mixtures of cells from

the parental species fail to induce antibodies against the hybrid antigens in rabbits. Moreover, treatment of parental cells with trypsin or papain does not expose determinants reactive with hybrid antiserum. Thus, no hybrid antigens are discernible in the parental species.

Comparable deviations from the one-gene–one-antigen relationship have been identified within species as well as in hybrids between species. In sheep, the appearance of the alloantigen R on red cells and in secretions requires the presence of two dominant genes, *R* and *I*, associated with different loci. Similarly in pigs, the A, O, and "–" (negative) phenotypes of the *A-O* blood group system are controlled by genes at two loci. Here the independent genes designated *S* and A^A are necessary for the production of soluble A substance and its acquisition by red cells. Certain hybrid antigens in rabbits depend upon particular allelic combinations in heterozygotes rather than separate loci. The hybrid specificity I has been found only on the cells of rabbits heterozygous for the Hg^A and Hg^{DJK} alleles of the *Hg* blood group system. The Hg^A and Hg^{FK} alleles of the same system likewise yield an antigen J in heterozygotes. The *Hg* system and other blood group systems in rabbits are summarized in Table 4–9. The agglutinability of erythrocytes of different phenotypes, when compared by quantitative hemagglutination tests, suggests that interaction antigen I appears at the expense of A and D. No such competition or interference, however, is apparent with J.

Several examples of interaction or epistatic action of genes in the suppression of antigenic products have been discovered. The term *epistasis* (epistatic)

TABLE 4–9. Blood Group Systems and Antigens in Rabbits

System	Genotype	Blood type
Hg	Hg^A/Hg^A	A
	Hg^{DJK}/Hg^{DJK}	DJK
	Hg^{FK}/Hg^{FK}	FK
	Hg^A/Hg^{DJK}	ADI[a]JK
	Hg^A/Hg^{FK}	AFJ[a]K
	Hg^{DJK}/Hg^{FK}	DFJK
Hb	Hb^B/Hb^B	B
	Hb^M/Hb^M	M
	Hb^B/Hb^M	BM
Hc	Hc^C/Hc^C	C
	Hc^L/Hc^L	L
	Hc^C/Hc^L	CL
He	*He/He* or *He/he*	E
	he/he	Lack of E
Hb	*Hb/Hb* or *Hb/hb*	H
	hb/hb	Lack of H

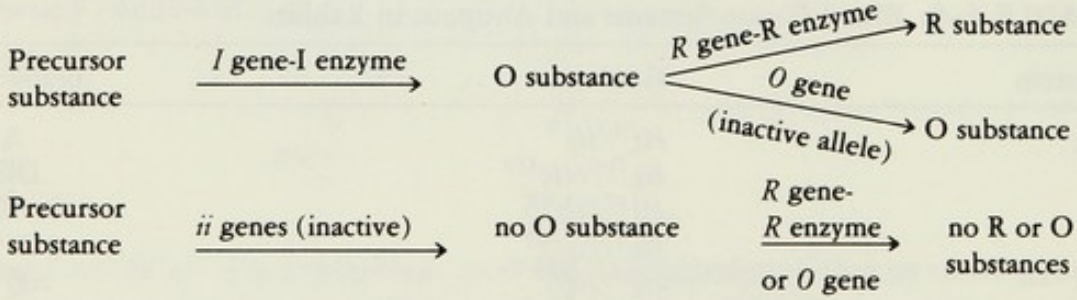
Modified from Cohen and Tissot, *J. Immunol.* 95:152, 1965. © 1965 The Williams and Wilkins Co., Baltimore, MD.

[a]I and J occur as interaction antigens in these heterozygous genotypes.

refers to nonreciprocal interaction of nonallelic genes. Studies by Rendel in Sweden among others indicate that the R and O (or r) alloantigens controlled by alleles in sheep depend upon an independent dominant gene *I* for their expression. In the recessive (*ii*) genotype, both antigens are suppressed. Erythrocytes from sheep of the *ii* group do not react with either anti-R or anti-O serums produced in cattle. The situation may be summarized as follows:

Group	Genotype	R antigen on erythrocytes and in serum	O substance on erythrocytes and in serum	Naturally occurring anti-R in serum
R	*I*–, *R*–	+	–	–
O	*I*–, *rr*	–	+	+ or –
i	*ii*, *R*– or *rr*	–	–	+ or –

Although *R* is dominant to *O*(*r*), recessive *OO* sheep nevertheless produce a distinctive antigen—another exception to the rule that only dominant genes yield antigens. The O substance is completely absent from heterozygous *R*/*O* individuals. However, O-cells incubated in serum from R individuals become positive for both R and O, and erythrocytes from group i may be similarly converted into R- or O- positive cells. Since R is present only in the serum at birth and later appears on the cells, it appears that R and O are acquired by the cells from the plasma, like the J antigen in cattle. A possible basis of the gene-product interactions in this sheep system is as follows:



If this sequential relationship, so analogous to the ABO and H dependence in man, proves correct, the O antigen would have to be regarded as a product of the dominant *I* gene rather than the recessive *O* gene.

A parallel example of gene interaction in man resulting in suppression of blood group B is known. A woman who transmitted the *B* and *Se* genes to her offspring nevertheless possessed the cellular phenotype of a group O nonsecretor. A recessive "suppressor" gene, like *i* in sheep, provides the simplest explanation for the lack of B in this mother. This supposition is in accord with the relevant phenotypes and genotypes for three generations of this family as determined by the late Philip Levine. Another very unusual family with eight members of three generations showed the *Rh* allele $R^{2.5}$ (r^{1} or *Cde*) suppressing the expression of Rh 1 (D) on its partner chromosome, resulting in a variant Rh 1 designated D^{u}. This effect was demonstrated in two individuals of ge-

notype $R^{1,2,5}/R^{2,5}$ (*CDe/Cde*) and a third of the uncommon genotype $R^{1,4,5}/R^{2,5}$ (*cDe/Cde*). Additional studies support this finding: whenever $R^{2,5}$ (*Cde*) is paired with the allele $R^{1,2,5}$ (*CDe*) or other alleles mediating Rh 1 (D), a weakly reactive Rh 1 or D^u specificity is produced. Strong reactions with anti-Rh 1 saline agglutinins can be demonstrated with Rh 1 positive cells, provided that Rh 2 (C) is not available to modify or suppress the reactivity of Rh 1. These and similar effects involving other *Rh* alleles have been attributed to cis-trans position effects (i.e., change in gene expression depending on chromosomal position of nearby genes) on the assumption of closely linked genes.

Known hybrid blood group antigens do not usually result from interaction of genes at the nucleic acid level, but from the specific enzymic attachment of saccharide units to macromolecules in linkages that lead to new stereochemical configurations. To the extent that this conception is generally valid, unusual antigens or masking of antigens primarily reflect complex properties of the final macromolecules including proteins. The concept of gene interaction is best understood in terms of sequential pathways, such that one gene and its functional enzyme require a precursor product of another gene(s). There is ample evidence from the *ABO, Lewis,* and *P* systems in man that final synthesis of an antigenic determinant occurs in a stepwise manner and can be governed by genes at several independent loci.

Serological Typing Patterns

In animals which cannot be readily bred and tested in captivity, such as large ocean fish or mammals, the genetic basis of antigens distinguishing individuals in wild populations can sometimes be deduced from serological typing patterns alone. Clyde Stormont has called attention to this promising strategy for blood-typing. This approach may be illustrated with five serological patterns detected with two antiserums (anti-X and anti-Y). It is assumed that each antiserum distinguishes individual differences and is reasonably monospecific as ascertained by absorptions.

Pattern *a*

Antigenic types	Anti-	
	X	Y
1	+	+
2	+	0
3	0	+
4	0	0

This is the classic four-phenotype pattern exhibited by human red cells in tests with anti-A and anti-B. Because this pattern has a maximum expectation for randomly selected pairs of antiserum reagents and because it is typified by the well-known *ABO* system of blood groups, many students are hardly aware

that there could be other patterns. Either the independent pairs of genes *X,x* and *Y,y* or the alleles X^x, X^y, and X^0, can explain Pattern *a*. Independent gene pairs and multiple alleles are usually distinguishable by gene frequency analyses.

Pattern *b*

Antigenic types	Anti-	
	X	Y
1	+	0
2	0	+

This two-phenotype pattern is rare. About the only good example is in typing certain populations of sheep with anti-O and anti-R reagents. One of the two types is inherited as a recessive. Although this pattern can best be explained by a single pair of alleles with one being dominant, other explanations are not excluded.

Pattern *c*

Antigenic types	Anti-	
	X	Y
1	+	0
2	0	+
3	0	0

This three-phenotype pattern, which is merely an extension of Pattern *b* to include a "negative" phenotype, is also rarely encountered. It is best exemplified by the reactions of human red cells with anti-Le^a and anti-Le^b, and the reactions of sheep red cells in certain populations with anti-O and anti-R. The patterns of *b* and *c* have been invariably associated with serologically reactive, soluble substances elaborated in the tissues and acquired in the plasma by the red cells. Although a single pair of alleles with one being dominant accounts for types 1 and 2, a second locus with epistatic effects is invoked to explain type 3.

Pattern *d*

Antigenic types	Anti-	
	X	Y
1	+	0
2	0	+
3	+	+

There are many examples of this three-phenotype pattern, the classic one being the reactions of human red cells with anti-M and anti-N. This pattern is

readily explained by a pair of codominant alleles, but such systems must often be expanded by invoking more alleles when additional antibodies are considered.

Pattern *e*

Antigenic types	Anti-	
	X	Y
1	+	+
2	+	0
3	0	0

There are also many examples of this three-phenotype pattern. The classic one is the reaction of human red cells with anti-A and anti-A_1 serums. Thus, individuals of subgroups A_1, A_2, and neither may be distinguished because only unabsorbed anti-A serum reacts with cells having either A_1 or A_2. This pattern is the simplest of all linear subtyping patterns. Like all linear and nonlinear subtyping relationships, this pattern connotes multiple alleles. Three alleles (X^{xy}, X^{x}, and X^{0}) can explain this example.

Even if the genetic basis of a typing pattern is not deducible with certainty, the relative frequencies of antigenic types in separate breeding populations may differ sufficiently to allow the investigator to identify the source of a population sample. For example, differentiating between American and Asian species of salmon caught in the Pacific Ocean has been done in this manner. Among cetaceans, blood groups in sperm whales and in the Atlantic bottlenosed porpoise have been assigned by cross-matching using naturally occurring alloagglutinins.

Gene–Antigen Relationships Deduced from Phenotype Frequencies

How much genetic information can one derive from phenotype frequencies alone? Two-by-two tables as defined below can be useful in distinguishing between allelism and nonallelism based on phenotypes found in random populations of a species. The question of allelism arose regularly in early studies of both cellular and soluble antigens. Although the following problem-solving techniques have been applied mainly to blood group studies, they may be used to advantage in many other systems involving antigenic differences within a species. Here again alleles are considered to be alternate units of a chromosomal locus in contrast to genes at noncontiguous loci. In the usual situation, two antigens, X and Y, are detectable by anti-X and anti-Y serums in four phenotypic classes: X+Y+, X+Y−, X−Y+, and X−Y−, where the + and − signs indicate presence or absence, respectively, of the antigen. These two antigens are assumed to be derived from either dominant or codominant genes. Also, a total of N random individuals from a population in Hardy-

Weinberg equilibrium must have been typed with anti-X and anti-Y. Such data may then be considered in terms of the following hypotheses:

Hypothesis	Number of loci	Allelic sets of genes
A. Nonallelism	2	*X*, *O* and *Y*, *O'*
B. Three alleles	1	*X*, *Y*, *O*
C. Four alleles	1	*X*, *Y*, *XY*, *O*

A distinction among these genetic hypotheses was attempted especially by Andresen and Baker at Iowa State University using actual data.

Initially a two-by-two table may be constructed as follows, where a, b, c, and d represent the observed number of individuals in each of the four phenotypic classes.

Phenotype	Y+	Y−	Totals
X+	a	b	a + b
X−	c	d	c + d
Totals	a + c	b + d	*N*

Assuming allelism versus nonallelism where p, q, and $1-(p+q)$ are the frequencies for three genes *X*, *Y*, and *O*, respectively, the expected numbers of phenotypes in these four classes are determined as follows: $1-(p+q)$ represents the null *O* gene(s) that fails to yield a detectable antigenic product.

	Numbers expected	
Numbers observed	Allelism (3 alleles)	Nonallelism
a (X+Y+)	$N \cdot 2pq$	$(a+b)(a+c)/N$
b (X+Y−)	$N \cdot [p^2 + 2p(1-p-q)]$	$(a+b)(b+d)/N$
c (X−Y+)	$N \cdot [q^2 + 2q(1-p-q)]$	$(a+c)(c+d)/N$
d (X−Y−)	$N \cdot (1-p-q)^2$	$(b+d)(c+d)/N$

Testing of a four-allele hypothesis is more complicated since there are ten genotypes corresponding to the four phenotypes. For purpose of chi-square analysis of the most probable genetic relationship, the two antigens X and Y must be independent, or referable to alleles. No other possibilities are inherent in the mathematical treatment of two-by-two tables. Given a total of four phenotypes, p equals $1-\sqrt{(X-Y+)+(X-Y-)/N}$ and q equals $1-\sqrt{(X+Y-)+(X-Y-)/N}$, for purposes of estimating allele frequencies.

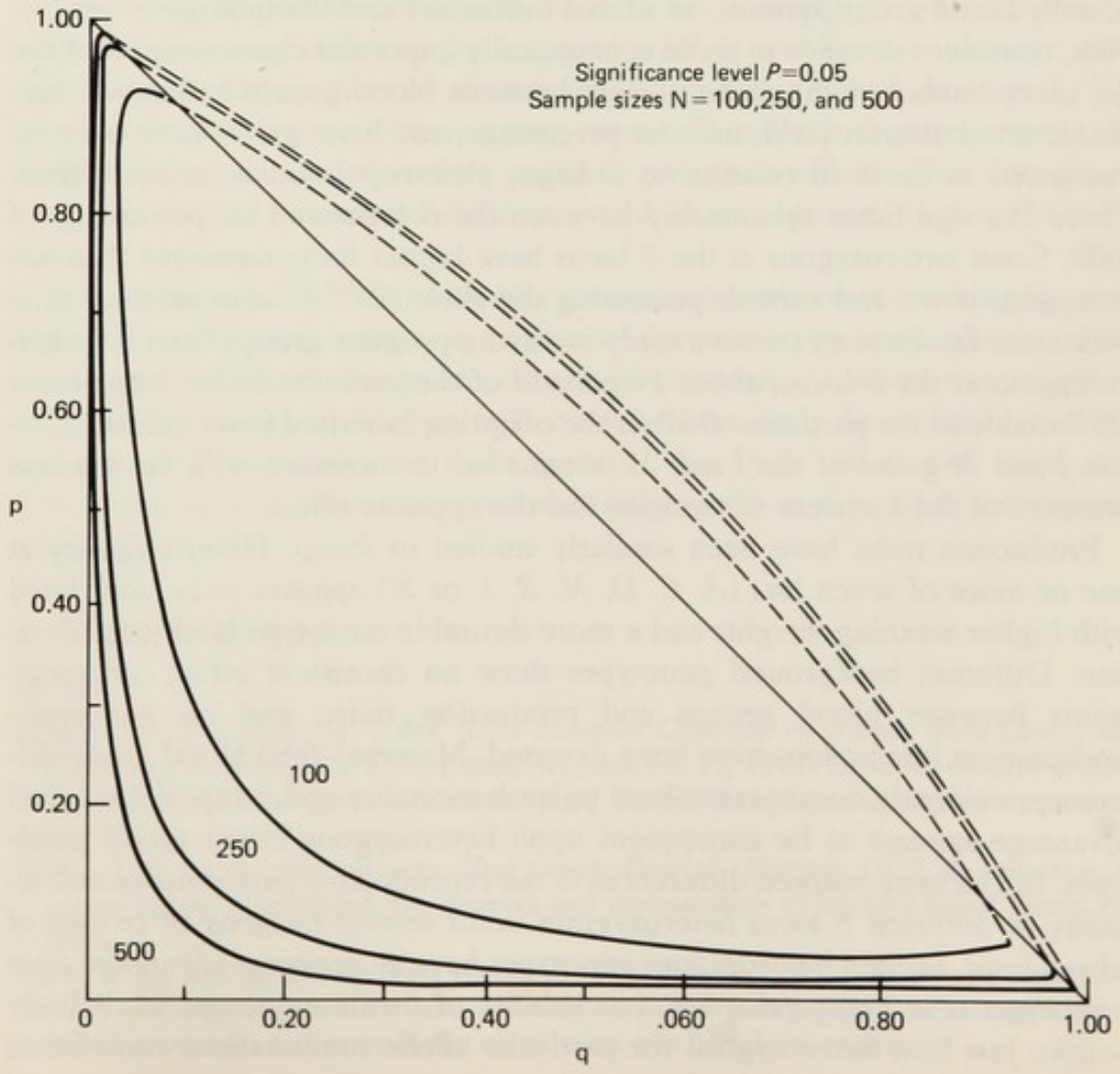
Considering a distribution of one hundred phenotypes as 4 X+Y+, 11 X+Y−, 38 X−Y+, and 47 X−Y−, p is calculated to be 0.08 and $q = 0.24$ using the above formulas. The student may wish to confirm his under-

standing of the above by calculating these values. The conventional chi-square formula for testing independence in this situation is $X^2 = (ad - bc)^2N/(a + b)(c + d)(a + c)(b + d)$. The calculated chi-square value of 1.70 for nonallelism corresponds to $0.10 < P < 0.20$. Expected numbers of phenotypes do not differ significantly from observed numbers, but a hypothesis of allelism fits the observed distribution much better than one of independence at the 5 percent significance level, as shown in Figure 4–4. Thus, if a straight edge is laid across the p scale at 0.08 parallel to the q scale, the $N = 100$ curve is intersected at q values of 0.39 and 0.92.

Note then, that the hypothesis of three alleles for $p = 0.08$ cannot be rejected for $N = 100$ unless $0.39 < q < 0.92$. However, about 250 random individuals would be needed in this instance to disprove independent segregation. Thus, a rather large sample size is required to disprove independence

FIGURE 4–4. Sample sizes required to disprove independent segregation for genes of frequencies p and q by means of the chi-square test and assuming a three-allelic relationship yielding two identifiable antigens with one null allele.

Source: Adapted from Andresen et al., *Immunogenetics Letter*, 3:17, 1963.



with respect to low gene frequencies. If the chi-square value is not elevated, independence cannot be claimed, because failure to disprove a hypothesis does not constitute proof of the same hypothesis.

Because long series of multiple alleles are commonly responsible for specification of antigens, two-by-two tables often tend to oversimplify reality! To avoid erroneous conclusions, family studies should be undertaken whenever possible. When a codominant locus has more than three alleles in substantial frequencies, family data may involve many possible types of matings. The maximum number of genotypes which can result from any mating is four, and this would involve unlike heterozygotes. This restriction is being used to advantage in family pedigrees for histocompatibility typing in man (Chapters 5 and 6).

USEFULNESS OF ALLOANTIGEN MARKERS

The reader now appreciates that most red cell alloantigens occur in glycoproteins or glycolipids intrinsic to the cell membrane. These molecules obviously have functional as well as structural roles. We conclude this chapter with a brief commentary on the multiple applications of cellular alloantigens, and especially blood group systems, to animal husbandry and the biology of people. First, consider relevance to some economically important characteristics of cattle, sheep, and chickens. Relationships between blood groups and production characters (butterfat yield, milk fat percentage, and heart girth) have been investigated in cattle in relation to linkage, pleiotropy, and heterotic effects. There is a significant relationship between the *B* locus and fat percentage of milk. Cows heterozygous at the *B* locus have higher fat percentages than homozygous cows, and animals possessing the allele $B^{BO_1V_2D'}$ also produce milk with more fat. In one extensive study involving progeny groups from sires heterozygous at the *B* locus, about 14 percent of the variation in fat content was attributable to the particular *B* allele the offspring inherited from the sire. Certain *J* and *M* genes of the *J* and *M* systems led to increased milk fat whereas presence of the *L* system alloantigen had the opposite effect.

Production traits have been similarly studied in sheep. Heterozygosity at one or more of seven loci (*A, C, D, M, R, I,* or *X*) appears to be correlated with higher weaning weights and a more desirable meat-type lamb conformation. Different background genotypes show no consistent effect on associations between blood groups and production traits, and no genotype–environment interactions have been detected. Maternal–fetal blood group differences evidently do not contribute to lamb mortality and no special survival advantage appears to be consequent upon heterozygous blood group genotypes. In chickens, marked differences in the reproductive performance and viability of different *B* locus heterozygotes occur among progeny of crosses of inbred lines. Several heterozygous genotypes have an apparent advantage over homozygotes in egg production, hatchability of fertile eggs, and adult body weight. Just how heterozygosis for particular allelic combinations contributes

to "hybrid vigor" is unknown. It is clear, however, that some blood group loci, such as the *B* loci in cattle and chickens, may influence morphologic or physiologic traits.

Anthropologists and ecologists have increasingly taken advantage of blood group and other biochemical polymorphisms in population studies. We have already cited numerous examples of unusual blood group frequencies in certain human populations attributable to genetic drift or to natural selection. Strongly suggestive correlations with fertility, maternal–fetal incompatibility, autoimmune disease, certain types of cancer and resistance to particular infectious agents have been identified in relation to various blood group specificities. In the infectious disease category, correlation of the Duffy blood group system to malarial resistance is convincingly instructive. Red cells lacking Fy^a(Fy 1) or Fy^b(Fy 2) determinants are resistant to infection in vitro by *Plasmodium knowlesi* merozoites. Moreover, African blacks of Fy (a−b−) phenotype, who constitute the vast majority of the population, fail to develop malaria when bitten by *P. vivax*-infected mosquitoes. Thus, these common Duffy antigens probably constitute a receptor required for attachment of subsequent penetration of malarial parasites. On the other hand, Rh negativity is somehow correlated with heightened susceptibility to malaria.

The scope of possible causes and effects has recently grown substantially with the discovery of leukocyte (especially *HLA*) and immunoglobulin allotype systems. Although a few disease associations with blood group and *HLA* specificities are now quite convincing, most such individual genes are not decisively influential and therefore point to polygenic effects. This now much emphasized field of immunodeficiency and immunogenetic diseases will be pursued in Chapter 8.

From a therapeutic standpoint, blood transfusion reactions resulting from allogeneic erythrocyte, leukocyte, or platelet destruction by immunologic mechanisms continue to challenge blood bank laboratories. At worst, transfusion of mismatched blood can lead to fatal immune hemolysis. In addition to ABO and Rh, induced antibodies to antigens of the *Duffy, HLA, Kell, Kidd,* and *MNSs* systems have been associated with either early or delayed transfusion reactions.

KEY REFERENCES

Books

Kovacs, G., and Papp, M. (eds). *XIIth European Conference on Animal Blood Groups and Biochemical Polymorphism* The Hague: W. Junk N. V., 1972. Includes diverse articles dealing with blood groups in cattle, pigs, horses, sheep, goats, rats, mice, poultry, and fishes.

Mohn, J. F., et al. (eds). *Human Blood Groups* Basel: S. Karger, 1977. A collection of recent papers concerning the genetics and immunology of various blood group systems.

Race, R. R., and Sanger R. *Blood Groups in Man.* 6th ed. Philadelphia: J. B. Lippincott, 1975. A long-time favorite text focusing on blood group inheritance, tests used in blood grouping, and steps in the elucidation of a blood group system.

Zmijewski, C. M. *Immunohematology.* 3d ed. New York: Appleton-Century-Crofts, 1978. A quite useful, reasonably up-to-date coverage of human blood group systems.

Articles

Human Blood Groups

Abraham, C. V., and Bakerman, S. Isolation and purification of Rh receptor component from human erythrocyte membrane by isoelectric focusing. *Science Tools* 24:22–24, 1977. Specific Rh antigens have been isolated and all have been associated with similar proteins rather than oligosaccharides.

Booth, P. B. Some serologic observations on I subdivisions. In *Human Blood Groups* (book ref. above), pp. 287–295. An update on the *Ii* blood group system.

Furthmayer, H. Structural comparison of glycophorins and immunochemical analysis of genetic variants. *Nature* 271:519–524, 1978. The *MNSs* system determines two structurally similar proteins. This study proves that *M* and *N* alleles specify a difference in amino acids in two positions only of glycophorin A, whereas the allelic *S* and *s* antigenic determinants are carried on glycophorin B.

Giblett, E. R. Genetic polymorphisms in human blood. *Annu. Rev. Genet.* 11:13–28, 1977. A brief overview of newer discoveries.

Koscielak, J. Chemistry and biosynthesis of erythrocytic membrane glycolipids with A, B, H, and I blood group activities. In *Human Blood Groups* (book ref. above), pp. 143–149. Provides new insights concerning sequential biosynthesis and gene actions.

Miller, L. H., Mason, S. J., Clyde, D. F., McGinniss, M. H. The resistance factor to *Plasmodium vivax* in blacks. New Engl. J. Med. 295:302–304, 1976. The convincing relationship between the *Duffy* blood group system and malarial resistance in blacks is summarized in this report.

Tippett, P. Some recent developments in the Kell and Lutheran systems. In *Human Blood Groups* (book ref. above), pp. 401–409. With multiple alleles, null phenotypes, and unlinked suppressors, both *Kell* and *Lutheran* must now be regarded as complex loci similar to *Rh* and *MNSs.*

Animal Blood Groups

Auditore, K. J., and Stormont, C. The cell-surface distribution of alloantigens on the bovine erythrocyte. *Immunogenetics* 6:547–552, 1978. Find that multiple antigenic determinants of given *B*-system haplotypes or phenogroups are carried on a single molecule as if specified by a single multiallelic locus.

Caldwell, J., Bryan, C. F., Cumberland, P. A., and Weseli, D. F. Serologically detected lymphocyte antigens in Holstein cattle. *Animal Blood Grps. Biochem. Genet.* 8:197–207, 1977. Relates newer findings of polymorphism of cattle lymphocyte antigens to systems of erythrocyte antigens.

Cohen, C., and Tissot, R. G. Blood groups in the rabbit. *J. Immunol.* 95:148–155, 1965. The five known rabbit blood group loci and the antigens they control are summarized. The I and J antigens of the *Hg* system occur as interaction products in certain heterozygous genotypes.

David, C. S., Stimpfling, J. H., and Shreffler, D. C. Identification of specificity H-2.7 as an erythrocyte antigen: control by an independent locus, *H-2G,* between the S and D regions. *Immunogenetics* 2:131–139, 1975. An instructive contribution to the continuing study of *H-2*-associated alloantigens in genetically defined mice. See also *Immunogenetics* 6:489–512, 1978, for an up-to-date review of this fast developing subject.

Hala, K., Vilhelmová, M., and Hartmanova, J. Probable crossingover in the *B* blood group system of chickens. *Immunogenetics* 3:97–103, 1976. A good entrance to the substantial recent literature on chicken blood groups.

Hildemann, W. H. The Syrian hamster in immunologic perspective. *Federation Proceedings* 37:2102–2105, 1978. A recent overview of immunogenetic studies of this unusual mammal.

Levine, P. The influence of the *ABO* system on Rh hemolytic disease. *Human Biology* 30:14–28, 1958. A classic study revealing the protective role of ABO incompatibility in reducing the incidence of Rh disease of newborns.

Myhre, B. A., Simpson, J. G., and Ridgway, S. H. Blood groups in the Atlantic bottlenosed purpoise, *Tursiops truncatus. Proc. Soc. Exp. Biol. Med.* 137:404–407, 1971. An instructive introduction to the problems of blood grouping in marine mammals.

Rasmussen, B. A. Gene interaction and the *A-O* blood group system in pigs. *Genetics* 50:191–198, 1964. The phenotypes in this system in pigs like the *R-O* system in sheep are found to be controlled by genes at two loci.

Ruiterkamp, W. A., Spek, C. M., and Bouw, J. Gene clusters in the blood group system B of cattle. *Animal Blood Grps. Biochem. Genet.* 8:231–240, 1977. A provisional genetic map of the *B* system is presented as a linear array of subloci or gene clusters on the basis of putative recombinants.

Stormont, C. Blood typing cattle and horses. In *Beef Cattle Science Handbook,* 14. Clovis, Calif.; Agriservices Foundation, 1977. Pages 572–591. See *Immunogenetics* 6:1–15, 1978, for historical perspective on immunogenetics of cattle blood groups.

Sullivan, P. T., Blystad, C., and Stone, W. H. Immunogenetic studies of rhesus monkeys. X. The O,P,Q,R and S blood group systems. *Immunogenetics* 7:125–130, 1978. A useful entrance to the developing literature on primate blood groups.

5

IMMUNOGENETICS OF TISSUE TRANSPLANTATION

RULES OR LAWS OF TRANSPLANTATION

Uniqueness of the Individual

Successful transplantation of allogeneic tissues, especially of intact organs in attempted replacement of diseased or atrophic organs, has long been a major life-saving goal. Unfortunately, from an immediate medical standpoint, rejection of grafts between unrelated individuals has been the rule. Such rejection, as we shall see in this chapter, is a prime manifestation of the "uniqueness of the individual"—a fundamental biological attribute. This uniqueness manifested by tissue or cellular incompatibility has the immediate significance of enabling the individual to cope with a multitude of pathogens, potentially malignant cells, and foreign substances. Indeed, the very integrity of the whole animal must depend on recognition of foreignness and reaction to it.

The terminology to indicate various donor-recipient relationships in tissue transplantation was given in the introductory chapter (Table 1–5). Transplants of skin and organs can be made successfully from one part of the body to another (autografts) and from an identical twin or equivalent highly inbred animal to another (isografts or syngeneic grafts), but are *not* normally successful between different individuals (allografts) or between different species (xenografts). Highly discriminating recognition of nonself applies to multicellular animals in general, both invertebrates and vertebrates. The extensive polymorphism of histocompatibility molecules in species ranging from sponges and corals to fishes and mammals is analyzed in Chapter 9. First, we need to understand the fundamentals established for genetically defined, inbred strains of mammals.

The now generally accepted genetic basis of transplantation incompatibility was first demonstrated about 1916 by C. C. Little, a geneticist, and E. E. Tyzzer, a pathologist, as a result of tumor transplantation experiments with closely inbred lines of mice. In succeeding years, Little and co-workers showed

that the same principles applied to transplants of normal tissue in mice. Thus, parents (closely inbred waltzers and albinos) uniformly failed to support implants of the splenic tissue of their hybrid progeny, whereas F_1 progeny (waltzer X albino) regularly grew the splenic tissue of either parent strain. Many subsequent studies have confirmed this unidirectional compatibility between the F_1 and inbred parent strains of mammals. The full interpretation of these results, however, requires additional test graftings and correlations between the genetic constitutions, sexes, and ages of donors and recipients. Numerous studies of skin, kidney, heart, and tumor transplants among F_1 and F_2 hybrid progenies as well as backcross progenies of various inbred strains have now been published. As a result, certain immunogenetic rules of transplantation established with diverse species of experimental animals have emerged (Figure 5–1).

These rules have been confirmed not only in mammals such as mice, rats, hamsters, rabbits, and guinea pigs, but in certain birds, amphibians, and fishes. These classic rules apply to donors and recipients of the same sex; a few other qualifications and exceptions will be considered later. Note that F_1 hybrids should behave as "universal recipients," accepting grafts from either parents or subsequent progenies. In other words, *H* genes are codominantly inherited and members of the F_1 generation appear to express all the antigens of the parent lines and no more. The relationship "one dominant allele → one antigen specificity" is consistent with the evidence. Existence of a large number of dominant histocompatibility genes is indicated by high frequencies of rejection of parent or F_1 hybrid grafts by F_2 progeny. A recipient will accept a graft only if the donor tissue has no genes for transplantation alloantigens not also possessed by the recipient. This finding brings us back to the first principle that a normal recipient will react only against antigens not present in his own cells.

Although graft rejection may be highly variable in its time course and severity, an immunological rather than a mere biochemical incompatibility is evident. The primary immunological causation of allograft rejection is revealed by test grafting hosts previously exposed to donor cells or tissues. Second-set or repeat test grafts made between an incompatible donor and recipient regularly show accelerated rejection in comparison with the first-set or initial graft. Thus, anamnesis or immunological memory is a characteristic feature of allograft reactions. Lymphoid cells transferred from graft rejectors to normal adults of the same inbred strain enable these animals to reject skin grafts from the original donor strain in an accelerated or immune fashion. The *adoptive immunity* is specific and referable to living, immunocompetent cells. Although specific serum antibodies are also regularly induced in conjunction with allograft and xenograft (interspecies) destruction as a further manifestation of immunity, the functional role(s) of such antibodies remain controversial. For present purposes, it will suffice to realize that the immunological events surrounding allograft rejection remain to be fully elucidated. Apart from special cases involving nonviable hard tissue such as bone or such immunologically privileged sites as the brain or anterior eye chamber, allograft rejection is uni-

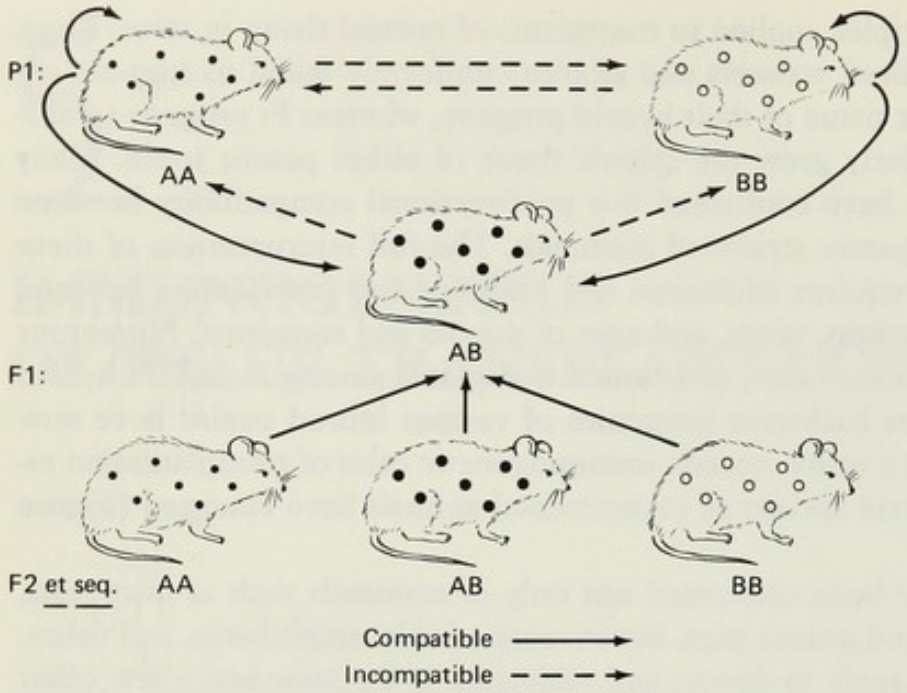


FIGURE 5–1. Diagram of genetic rules of transplantation on assumption that each histocompatibility antigen is determined by a single, autosomal, dominant gene (allele). Given separate inbred strains in the parental generation, A and B represent different alleles at each *H* locus in diploid adults. Note that F_1 hybrids should accept grafts from each other, from either parent strain, and from progeny of subsequent generations.

Source: Modified from Hildemann, in *Advances in Transplantation*, Copenhagen: Munksgaard, 1968, p. 19. © Munksgaard International Publishers Ltd., Copenhagen, Denmark.

versal among random-breeding populations of adult vertebrates, including man. Transplantation reactions have been extensively studied in diverse species of fishes, amphibians, reptiles, birds, and mammals (Chapter 9). Strong rather than weak incompatibility appears to be more common. However, chronic rejection of skin allografts reflecting "weak" histoincompatibility appears to be characteristic of caecilian and urodele amphibians, turtles, and certain mammals such as pigs and Syrian hamsters. The "uniqueness of the individual" and the consequent "uniqueness of separate breeding populations" in terms of alloantigenic constitution have emerged as fundamental immunogenetic concepts.

In *Xenopus* toads derived by nuclear transplantation, reciprocal skin grafts between members of the same clone survive like autografts, regardless of whether the recipient egg cytoplasm comes from the same or from different (outbred) females. Since reciprocal grafts between toads from separate clones are rejected, compatibility between toads derived by nuclear transplantation must depend on their genetic identity at the nuclear level. Preexisting cytoplasmic or episomal differences in the egg or zygote evidently do not affect later allograft compatibility in immunologically mature recipients. Incidentally, clonal histocompatibility is naturally occurring in certain species of Poeciliid fishes and confirms their gynogenetic origins. In these all-female species, inheritance is entirely matroclinous. Sperm from males of closely related, sym-

patric species merely serve to stimulate embryogenesis. With this background, let us now take a closer look at the genetic aspects of tissue transplantation.

Histocompatibility Genes and Corresponding Antigens

The indefinite acceptance of grafts from either inbred parent strain by F1 hybrids tends to rule out the possibility of alloantigens determined by recessive genes. If any antigen "a" present in one parent strain were determined by recessively functional alleles *aa,* this antigen would not be expressed in *Aa* F1 hybrids where the other parent strain contributed a dominant *A* allele. This situation should, of course, lead to rejection of grafts from one of the two parent strains by the F1; this result has not been observed when sex-associated incompatibilities have been avoided. That alloantigens must be foreign to the recipient to evoke an immune response accounts for the *acceptance* of grafts which carry no histocompatibility alleles alien to the host and the *rejection* of grafts which do carry alien alleles. How many such genes must then be invoked to account for the low frequency of successful allografts from parent strain to F2 or backcross progeny? The theoretical expectations are set out below with the simplifying assumption that the *H* genes are segregating independently from a Mendelian standpoint.

Expectation of Successful Allografts on F2 Progeny from Either Parental Strain:

$$\begin{aligned} F_1&: \quad AB \times AB \\ &\qquad\downarrow \\ F_2&: \quad \tfrac{1}{4}\,AA : \tfrac{1}{2}\,AB : \tfrac{1}{4}\,BB \end{aligned}$$

3/4 of progeny accept grafts from either AA or BB donors if only *two* alleles or one locus are involved; the proportion of successful grafts (S) = $(3/4)^n$, where n is the number of independent *H* loci involved.

Expectation of Successful Allografts on Backcross (BC) Progeny from Opposite Parental or F1 Donors:

Parental or F1 Donors:

$$\begin{aligned} BC:&\quad AB \times AA \\ &\qquad\downarrow \\ &\tfrac{1}{2}\,AB : \tfrac{1}{2}\,AA \end{aligned}$$

AB half of progeny accept grafts from either BB or AB donors if only two allelic genes are involved; the proportion of successful grafts (S) = $(1/2)^n$, where n is the number of independent *H* loci involved.

In one early series of mouse skin grafting experiments done at the University of Birmingham in England, Barnes and Krohn found that two of 120 A-strain and one of 154 CBA-strain grafts survived transplantation to F_2 generation mice for at least 100 days. On the now well-supported assumption that each separate antigen is capable of causing graft breakdown, at least fifteen independent loci must be invoked to account for the results. Since late rejections after 100 days often occur in response to weak antigens, even the minimum number of loci estimated is probably too low. Similar experiments by others involving many hundreds of F_2 backcross recipients derived from other strains of mice and rats yield estimates ranging from nine to twenty genetic loci influencing histoincompatibility. Many of these experiments involved 200-day observation periods. More recent studies, done by Donald Bailey of the Jackson Laboratory in Maine, led to a higher estimate of a minimum of twenty-nine to thirty-three *H*-locus differences in the BALB/c and C57BL/6 strains of mice. In one ingenious experiment, more than forty lines were derived by backcrossing (BALB/c ♀ × C57BL/6♂) F_1 females and their derivatives to C57BL/6 males through five successive generations. Tail-skin grafts from twenty sibs of each line in the fifth backcross generation were placed on C57BL/6 hosts of the same sex. The number (n) of histocompatibility loci still showing segregation of BALB/c alleles was estimated by the proportion (S) of grafts surviving through eleven weeks such that $S = (1/2)^n$ or $n = \log S/\log 0.5$. With a calculated mean of 1.8 loci per line, the original two strains should have differed by sixteen times this value or twenty-nine loci. This procedure is diagramed in Figure 1–4 of the first chapter. Even the latter estimate must be considered too low as a potential total for the following reasons: (a) numerous *H* loci, at least in mice, are linked and therefore do not segregate independently as assumed in the calculations; (b) many alleles will be held in common by any two strains under test and the loci involved will remain undetected; (c) *H* loci with weak antigenic products may remain undetected unless the graft observation period is more than 200 days.

Analogous studies involving tumor transplantation have used host death as the criterion of graft compatibility. A transplantable mammary tumor indigenous to strain A grew in all animals of this strain but failed to grow progressively in strain DBA. Tests of hybrid progenies with this strain A tumor yielded results as follows:

Strain A	All susceptible → death
Strain DBA	All resistant → mice survived
F_1 hybrid	All susceptible
F_2 hybrid	60 of 219 ($\sim 1/4$) susceptible
$F_1 \times$ A	All susceptible
$F_1 \times$ DBA	10 of 116 ($\sim 1/10$) susceptible

If one equates the proportion of successful grafts (i.e., susceptible mice) as $(3/4)^n$ for F_2 and $(1/2)^n$ for backcross recipients, where n is the number of loci, n must equal at least 4 to account for the above results. Higher and more

realistic estimates of *H* loci involved may be obtained by appropriate preimmunization. When mice derived from crosses of BALB/c and C57BL were tested with a C57BL transplantable leukemia, most of the recipients succumbed. When, however, F_2 and backcross mice were preimmunized with 0.1 ml of C57BL blood six to ten days prior to implantation of a C57BL leukemia, nearly all became resistant. In this situation, at least nine *H* loci distinguishing the two strains needed to be assumed to account for the low frequency of hybrid susceptibility. Other data reveal that malignant tumors often override weak transplantation barriers, even following preimmunization. Conversely, sensitive skin grafts are much more vulnerable to immune rejection and therefore more likely to yield meaningfully higher estimates of *H* gene differences.

In a larger sense, use of inbred lines oversimplifies the genetic basis of allograft incompatibility with respect to outbred populations such as human beings because widespread multiple allelism is not taken into account. Indeed, the allelic diversity found at loci such as *Rh* and *HLA* in man, the *B* and *C* systems of bovine blood groups, and the *H-2* system in mice points up the inevitable involvement of numerous alleles in the genetic control of normal tissue compatibility. Whereas the maximal number of alleles for each locus with any given parents is four, in view of the lack of restriction on the number of alleles that may be present in a random sample of a population, relatives and especially siblings should give better estimates of the nature of genetic diversity than unrelated individuals. Even goldfish *(Carassius)* exhibit complex transplantation genetics, such that numerous independent *H* loci must be invoked to account for the high frequency of incompatibility observed among siblings. As might be expected, successful grafts in people are generally more probable between relatives than between random pairs. The probability of histocompatibility is of course different for various genetic relationships between donor and recipient. In general, this probability is highest among siblings, intermediate between parents and children, and lowest between unrelated individuals. For each locus and any number of alleles, the lowest probability of compatibility is obtained when the alleles are equally frequent. Also, the chance of compatibility will decrease as the number of effective *H* loci increases. With unrelated combinations of donors and recipients, in particular, the chance of compatibility tends rapidly toward zero as the number of alleles per locus becomes larger. Now the prospects of prolonged or indefinite allograft survival in human patients are actually better than most quantitative genetic considerations imply, but only through recourse to histocompatibility matching and immunosuppression, as we shall see.

PROPERTIES OF TRANSPLANTATION REACTIONS

Strong Incompatibilities and the Major Histocompatibility Complex

Allografts exchanged between animals from random-breeding or feral populations typically provoke acute inflammatory reactions leading to early rejec-

tion. Such prompt tissue destruction is mediated primarily by T-lymphocytes and granulocytes, usually within two weeks after grafting. Similar cytotoxic sequelae occur in diverse target issues, including skin, heart, kidney, liver, pancreas, and solid tumors. The same considerations apply to most vertebrate classes, even to bony fishes and frogs maintained at elevated temperatures (25° C). Allografts between separately derived inbred strains of laboratory mammals regularly succumbed to acute rejection in early studies. Moreover, the very low success rates of parental grafts on F_2 hybrid or backcross progenies revealed complex genetic control by numerous *H* loci. With the selective development of new strains of congenic mice (see Chapter 1) by George Snell in the 1950s, it became possible to study the effects of individual *H* loci. This soon revealed the existence of few strong but many weak *H* loci governing bioincompatibility. Indeed, the *H-2* system, originally discovered by Peter Gorer on the basis of tumor transplantation and red cell typing, became recognized as the primary source of strong reactivities and was therefore called the major histocompatibility complex. H-2 disparities were readily detected by both histogenetic and serological methods, since the same immunogens that caused rapid allograft rejection also evoked serum alloantibodies in high concentration. This experimentally favorable situation gradually revealed a large array of *H-2* haplotypes (see Chapter 6).

Individual antigens specified by *H-2* loci are not all equally potent in transplantation incompatibilities, as we shall see. First, we need to describe briefly the constituent loci comprising the *H-2* complex; a genetic map of the *H-2* complex, a cluster of loci positioned near the middle of chromosome 17, is depicted in Figure 5–2. The borders of *H-2* in a liberal sense may be demarcated by *H-2K* (centromeric) and Tla (telomeric), two loci nearly 2.0 centimorgans (cM) apart. The intervening loci have been mapped by typing *H-2* recombinants derived fom congenic heterozygous parents. This is illustrated in Table 5–1 for nine strains, all of C57BL/10(B10) background except for the genes between *H-2K* and *H-2D*. The alleles of constituent *H-2* loci of one chromosome ($H\text{-}2^b$ haplotype) of C57BL/10 parental mice are designated *b b b b b b b b b*. Congenic strains carrying other *H-2* haplotypes such as *k* and *d* have been serologically typed and found to possess different antigens corresponding to distinctive alleles. Crosses have then yielded recombinant progeny showing crossing over at different positions within the *H-2* cluster. Thus, the recombinant strain B10.A (4R) derived from an $H\text{-}2^a \times H\text{-}2^b$ cross has *k* alleles from $H\text{-}2^a$ in the K and IA regions, but otherwise possesses *b* alleles extending from *IB* to *H-2D*. The $H\text{-}2^d$ haplotype characteristic of A strain mice is a combination of *k* and *d* alleles originating from a crossover between the E and C regions. Hence, $H\text{-}2^a$ as found in B10.A congenic mice is equivalent to $H\text{-}2^{kd}$. These recombinants have facilitated mapping additional genetic traits such as *Ir* loci regulating antibody responsiveness to allogeneic and xenogeneic determinants (Chapter 7).

Four distinctive classes of genes are identified with the *H-2* complex: (a) *H* genes (*H-2K* and *H-2D*) coding for membrane-bound glycoproteins involved

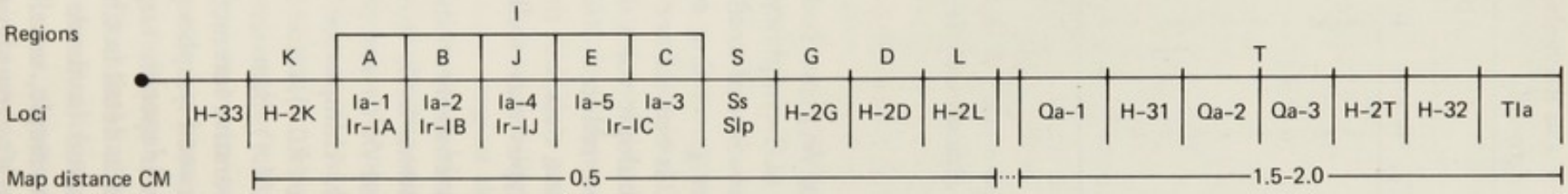


FIGURE 5–2. Genetic map of portion of mouse chromosome 17 emphasizing the *H–2* complex and its vicinity. The order of loci under brackets is uncertain. The narrow borders of *H–2* may be demarcated by *H–2K* (centromeric) and *H–2D,L*, a map distance of 0.5 CM. The gene order of *H–2D* and *H–2L*, and of the genes of the *H–2T* system are uncertain. If other closely linked immunogenes are included to *Tla* or *H–32* (telomeric), the *H–2* region occupies about 2CM (see also Figure 1–6.

TABLE 5–1. *H-2* Haplotypes of Congenic and Recombinant Strains, All of C57BL/10 (abbr. B10) Strain Background

H-2 haplotype	Strain designation[b]	Parental haplotypes	Alleles at H-2 regions[a]								
			K	A	B	J	E	C	S	G	D
b	B10	*b/b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
k	B10.BR	*k/k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*
d	B10.D2	*d/d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
a	B10.A	*k/d*	*k*	*k*	*k*	*k*	*k* |	*d*	*d*	*d*	*d*
bl	B10.A (1R)	*a/b*	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d* |	*b*
i3	B10.A (3R)	*b/a*	*b*	*b*	*b*	*b* |	*k*	*d*	*d*	*d*	*d*
b4	B10.A (4R)	*a/b*	*k*	*k* |	*b*	*b*	*b*	*b*	*b*	*b*	*b*
i5	B10.A (5R)	*b/a*	*b*	*b*	*b* |	*k*	*k*	*d*	*d*	*d*	*d*
il8	B10.A (18R)	*b/a*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b* |	*d*

Modified from Urba and Hildemann, *Immunogenetics* 6:437, 1978. © 1979 Springer Verlag, Inc., New York, NY.

[a]Vertical bars indicate postulated crossover positions.

[b]Strains designated with R numbers in parentheses identify different recombinants on the same B10.A ($H\text{-}2^a = H\text{-}2^{ka}$) background.

in allogeneic immunorecognition, (b) *Ia* genes coding for membrane-bound glycoproteins involved in activation of T-lymphocytes, (c) *Ir* genes involved in macrophage or helper T-cell regulation of antibody responses to diverse antigens, and (d) complement genes governing synthesis of certain serum complement components. The MHC is now better designated as the *MIC* or *major immunogene complex,* given its multiple roles in immunorecognition and immunoregulation. Some authors have reduced *MIC* genes to three classes by lumping *Ia*-like loci and *Ir* loci into one class, but this does not yet appear justified because the resulting phenotypes may well be coded by distinctive genes.

Although mice have been most extensively studied, every other species of eutherian mammal that has been systematically investigated possesses a multigene complex similar to *H-2.* These *MIC*s according to species have been designated as follows: man (*HLA*), chimpanzee (*ChLA*), rhesus monkey (*RhLA*), dog (*DLA*), pig (*SLA*), horse (*ELA*), cattle (*BLA*), sheep (*ShLA*), goat (*GLA*), rabbit (*RLA*), guinea pig (*GPLA*), hamster (*Hm-1*), and rat (*Rt-1*). In essence, MIC products promote cooperative interactions between subsets of T-cells, between T-cells and B-cells, between lymphocytes and macrophages, and between cytotoxic killer cells and their specific targets. The integrated function of these genes will be considered in detail in Chapter 6. We presently emphasize *H* genes and antigens involved in tissue transplantation reactions. Various H-2K region antigens, for example, usually provoke more rapid allograft rejection and more severe graft-versus-host reactions than do H-2D disparities. Before we proceed further with distinctions among *H* genes and their functions, the principle methods of detecting relevant reactions must be outlined.

Methods of Detecting Bioincompatibility Reactions

The methods of gene manipulation and tissue transplantation that permit identification of individual *H* loci were outlined in the first chapter and further elaborated at the beginning of this chapter. These so-called histogenetic methods hinge mostly on analysis of congenic resistant strains and recombinant inbred strains. Basic experimental approaches in vivo involve tissue grafting, parabiosis (i.e., natural union of two individuals), and graft-versus-host reactions mediated by immunocyte inoculation. Malignant tumor transplants were much used in early work to detect and select for alloantigenic differences. Tumors can be expected to grow progressively in the strain of origin, but not in strains lacking one or more potent antigens characteristic of the tumor. This expectation has often been subverted in two ways: (a) tumors may override moderate H barriers and continue to grow in spite of host immunity; (b) tumor-associated antigens distinct from normal tissue specificities may contribute to erratic results depending on cell dosage, route of inoculation, and other variables. Test grafts of normal tissues such as skin, kidney, heart, ovary, and pancreas avoid the complications of malignancy, although tissue-specific differentials can decisively influence the outcome. Given the same or similar H barriers, skin and pancreas are more vulnerable to early rejection than are heart or kidney allografts. Skin grafting has been most widely used in immunogenetic studies. In small mammals, a body skin graft, usually 0.5 to 1.0 cm in diameter with hair direction reversed, is orthotopically (i.e., naturally placed or joined) secured over the thorax with a plaster bandage until healing occurs. H-2 disparate skin allografts in mice are usually rejected in eight to twelve days as indicated by necrosis and scab formation. Moderate to weak H barriers typically yield slower or chronic rejection indicated by gradual graft hair loss and cicatrization. Tail-skin grafting is preferred by many, but more subtle criteria in scoring graft viability must be applied. Orthotopic kidney grafts demand microvascular surgical skills; kidney function and viability can be nicely monitored by measuring blood urea nitrogen or creatinine levels. However, the uremia which accompanies incipient rejection is nonspecifically immunosuppressive. It therefore contributes to prolonged survival unless a very strong H disparity is present, such as a homozygous *Rt-1* difference in rats. Whole neonatal heart grafts transplanted beneath the ear skin of adults can be unequivocally monitored by electrocardiographic recording. Cessation of electrical activity is used as a survival end point. Survival of ovarian allografts in ovariectomized mice has been indirectly monitored by taking vaginal smears—a measure of sex hormonal function. Similarly, survival of pancreas allografts (or islets of Langerhans) can be quantitated in terms of blood or urinary glucose levels, thereby measuring persistence of insulin production. All of these approaches, of course, assume that control autografts or isografts survive and function indefinitely under the same test conditions.

Parabiosis of mammals, literally joining the blood-vascular systems of two animals (Figure 5–3), allows the investigator to test for concurrent incompatibilities at multiple tissue levels. This can facilitate detection of moderate to

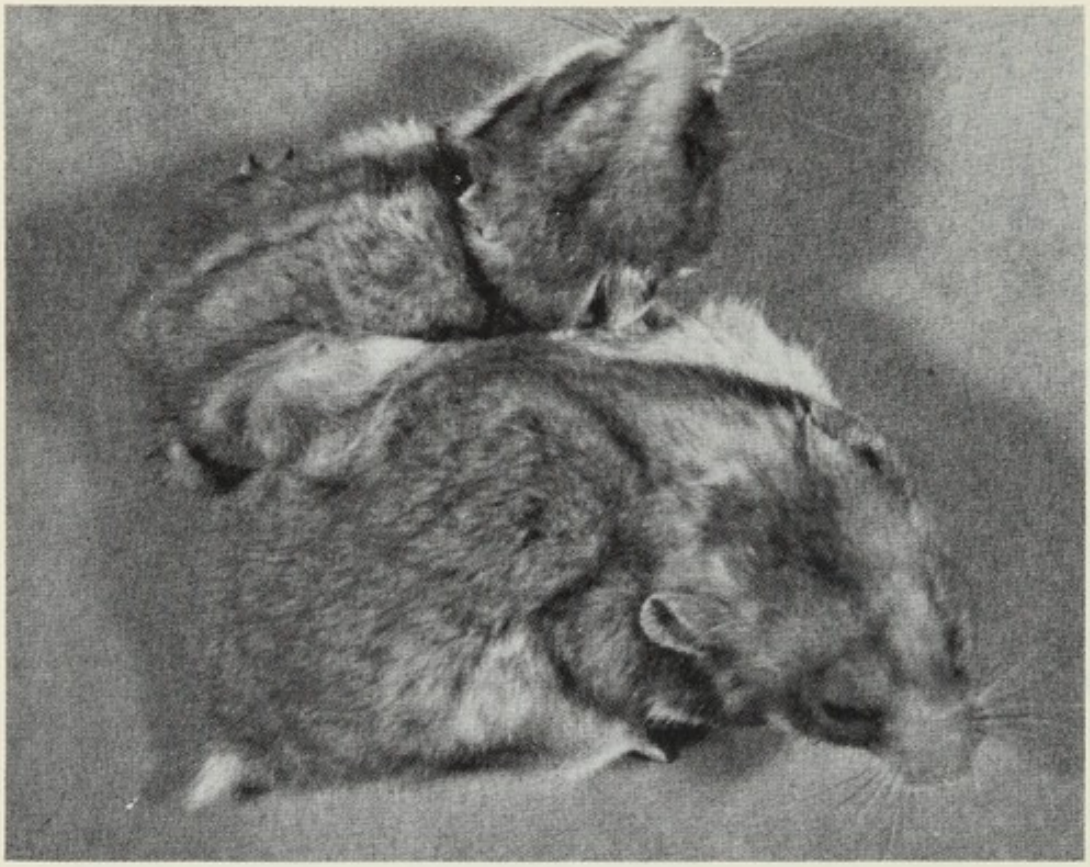


FIGURE 5–3. Allogeneic parabiosis in Syrian hamsters revealing unilateral rejection well underway after eight weeks. Animals initially were of same size and weight. There is marked atrophy of one partner.

Source: After Walford and Hildemann, *Transplantation* 2:101, 1964. © 1964 The Williams and Wilkins Co., Baltimore, MD.

weak H disparities that may selectively affect different organ tissue systems. This model is obviously complicated by an ongoing "immunological battle" between two intact animals, unless of course a unidirectional incompatibility is assured by parental strain ↔ F_1 hybrid parabiosis, for example.

Unidirectionality of alloincompatibility in vivo can be more neatly assessed in terms of both systemic and localized graft-versus-host (GVH) reactions. When immunocompetent cells in substantial numbers are inoculated into infant, juvenile, or even adult allogeneic animals incapable of destroying these cells, a syndrome called GVH disease, runt disease, or allogeneic disease usually ensues. More severe or accelerated disease is regularly observed if lymphoid cells are obtained from specifically preimmunized donors rather than normal donors. The conditions required for acute GVH reactions can be put in rather precise immunogenetic terms: when two individuals or inbred strains differ with respect to one or more strong *H* genes, transfer of mature lymphoid cells from one strain to perinatal recipients of another strain (or F_1 hybrids of the two strains) commonly leads to systemic, often fatal disease. There are several qualifications to this generalization that warrant further scrutiny. Among other experimental variables, the course and severity of allogeneic dis-

ease are substantially affected by the species-strain combination tested, by the age of the hosts, and by the type and number of donor cells. The acute forms of GVH disease in various mammalian and avian species are associated with complex pathologic features, including inhibition of growth, emaciation, diarrhea, hepatomegaly, splenomegaly, and terminal atrophy of lymphoid tissue. Three requirements for induction of allogeneic disease may be cited: (a) the host cells must possess one or more H antigens absent from the donor cells; (b) the donor cells must include immunocompetent T-lymphocytes; (c) the host must be incapable of rapid rejection of the donor cells. Although F_1 hybrids, regardless of their age, should be genetically incapable of reacting against inbred parent strain cells, such hybrids become increasingly resistant to GVH disease induction beyond the first few days after birth. Hybrid resistance can have a genetic or physiological basis, but may be overcome by use of irradiated adult F_1 recipients.

Moderate H differences associated with slow allograft rejection usually evoke weak GVH reactions which fail to produce overt cytotoxic manifestations of allogeneic disease. However, the whole range of strong to weak GVH reactions can be quantified in terms of host spleen or lymph node weight changes. Such localized GVH reactions in either newborn or adult hosts lead to early spleen or node enlargement reflecting a proliferative stimulus that may or may not lead to cytotoxic sequelae. Indexes or quotients representing experimental spleen or node weights over the mean of the respective weights of littermate controls are determined. Thus, indexes greater than one indicate organ enlargement whereas indexes less than one indicate an organ weight less than that of the controls. Whole body weight gains can be similarly compared between experimental and control newborn littermates, since acute allogeneic disease is reflected in overall curtailment of weight increase. This approach does not require killing animals to sample nodes or spleen, but the method is less sensitive in detection of weak reactions. The popliteal lymph node weight assay has gained favor because of its sensitivity and applicability to normal adult mammalian recipients. A test suspension of donor strain immunocytes is inoculated intradermally into a hind foot pad, while a control suspension of isogeneic cells or medium is inoculated into the other hind foot. One week later the draining popliteal lymph nodes are removed and weighed with the expectation that differential enlargement of the experimental node will measure local GVH reactivity. An even more sensitive assay is available in chickens and ducks. Immunocytes are simply inoculated over the chorioallantoic membrane of embryonated eggs. Each reactive donor cell then produces a proliferative lesion, thereby allowing easy enumeration. Note that the early proliferative component of GVH reactivity may or may not lead to cytotoxic sequelae characteristic of stronger incompatibilities.

We now turn briefly to major in vitro methods of detecting bioincompatibility. These include tests of mixed lymphocyte culture (MLC) reactivity and cell-mediated cytotoxicity or immunity (CMI) both depending primarily on specific T-cell activation. Other tests that depend on serum antibodies include

agglutination, complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). Students should bear in mind that in vitro methods artificially designed to detect H differences may not be assumed to yield the same end results in vivo. This applies especially to tests requiring xenogeneic sources of complement to produce target cell killing.

In direct CMI tests, immunocytes, often in the form of purified T-lymphocytes, are added to monolayers of adherent target cells in microtest tissue culture plates. Doubling dilutions to achieve effector to target cell ratios from 100:1 to about 5:1 are usually tested to measure degrees of CMI activity in the absence of serum complement. In addition to immune cell-target cell mixtures, each microtest plate should include control wells with normal immunocyte-target cell mixtures and wells with target cells alone. Only living cells remain attached to the floor of the wells after washing with culture medium and these can then be counted. Such tests in genetically-defined mammals usually correlate well with reactions of transplantation immunity obtained in vivo. Both depend mainly on specifically sensitized, killer T-cells. MLC testing also hinges on the specific stimulation of T-cells, but mitogenic stimulation from alloantigens on foreign lymphocytes is measured by new DNA synthesis (Figure 5–4). Functionally, MLC reactivity is similar to the proliferative phase of GVH reactivity demonstrable in vivo. The MLC test does appear to have predictive value in assessing the duration of both skin and kidney allograft survival. If potential donor lymphocytes are treated with mitomycin C before culture, they retain the capacity to stimulate recipient lymphocytes, even though their own ability to transform and divide is effectively inhibited. In effect, treated cells cannot respond by incorporating ^{3}H-thymidine (i.e., DNA synthesis), but they can still stimulate untreated allogeneic cells to do so. Low stimulation or absence of stimulation is equated with MLC compatibility in this "one-way" test. In one study, MLC incompatible human skin allografts between siblings matched by one-way stimulation survived only eleven to thirteen days, whereas MLC compatible grafts persisted for fifteen to thirty-six days. High MLC reactivity in man has been correlated mainly with differences at the *HLA-D* locus of the *MIC.* Both high MLC reactivity and acute allograft rejection in rats have regularly been correlated with differences at the major *Rt-1* histocompatibility complex. However, certain strong non-Rt-1 disparities, perhaps attributable to cumulative effects of otherwise weak antigens, are detectable by MLC testing. Multiple non-H-2 differences in mice can produce MLC proliferation comparable to that observed when H-2 differences are involved. Disparities at one locus, *Mls* on chromosome 1, in particular evoke strong MLC reactions.

An MLC assay can easily be converted into a cell-mediated lympholysis (CML) assay (Figure 5–4). Instead of assaying ^{3}H-thymidine incorporation after several days of MLC incubation, one asks in CML tests whether some of the responder lymphocytes acquire the ability to kill or lyse target cells that carry the antigens of the stimulator. One tests for killer activity by measuring release of radioactive sodium chromate (^{51}Cr) from labeled target cells; target

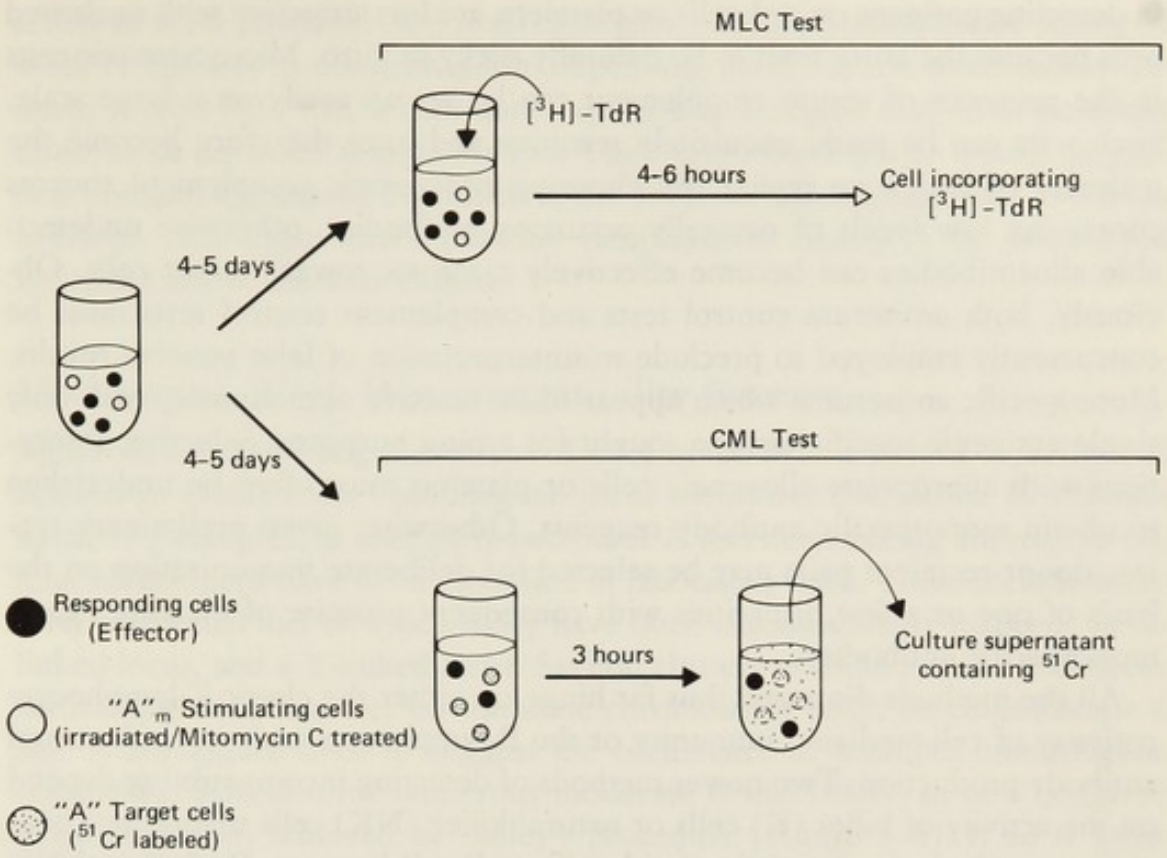


FIGURE 5–4. Comparative representation of the MLC and CML tests for alloantigenic disparities. Proliferative activity as measured by radioactive thymidine incorporation during new DNA synthesis is assayed after 4 to 5 days in the MLC test. Cytotoxic activity of the initial responding lymphocytes against ^{51}Cr labeled target cells is measured in the CML assay.

Source: Based on Bach, *Annu. Rev. Genet.* 10:321, 1976. Reproduced, with permission, by *Annual Review of Genetics*, © Annual Reviews, Inc.

cells are killed if they share certain antigens with the original stimulating or sensitizing cells. A recent variation of the MLC/CML assays is called the primed lymphocyte typing or PLT test. If lymphocytes stimulated in an MLC are left for ten days or well beyond their peak proliferative activity, they will exhibit a rapid and stronger (secondary-type) proliferative response when restimulated with cells from the original donor source. This secondary response can be assayed with radioactive thymidine or uridine very early—within twenty-four hours. Findings in man indicate that HLA-D antigens are mostly responsible for effective restimulation and the responding cells are T-lymphocytes. PLT responder cells from different sources show promise in typing the lymphocytes of people for HLA-D. Curiously, serologically-defined alloantigens such as those determined by the *HLA-A, HLA-B,* and *HLA-C* loci appear to be neither essential for, nor capable of, causing a secondary-type response in the PLT assay.

Serological typing methods have already been considered in connection with blood group alloantigen systems in Chapter 4. Agglutination tests, useful

in detecting antigens on red cells or platelets, are less attractive with nucleated cells because the latter tend to be naturally sticky in vitro. Microcytotoxic tests in the presence of serum complement can be set up easily on a large scale. Such tests can be made exquisitely sensitive and have therefore become the mainstay of leukocyte typing. By choosing xenogeneic complement sources containing low levels of naturally occurring antibodies, otherwise undetectable alloantibodies can become effectively cytotoxic toward target cells. Obviously, both antiserum control tests and complement control tests must be concurrently employed to preclude misinterpretation of false positive results. Monospecific antiserums which appear to be reactive operationally with only single antigenic specificities are sought for typing purposes. Selective absorptions with appropriate allogeneic cells or platelets must often be undertaken to obtain monospecific antibody reagents. Otherwise, given preliminary typing, donor-recipient pairs may be selected for deliberate immunization on the basis of one or a few disparities with consequent promise of evoking nearly monospecific antibodies.

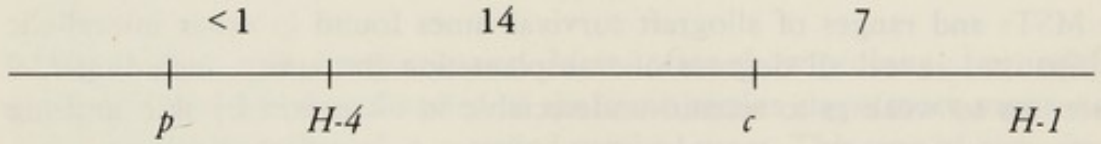
All the methods discussed thus far hinge on either the classic T-lymphocyte pathway of cell-mediated immunity or the B-lymphocyte pathway of humoral antibody production. Two newer methods of detecting incompatibility depend on the activity of killer (K) cells or natural killer (NK) cells that do not bear surface markers characteristic of either T- or B-cell lineages. Both K and NK cells possess Fc receptors for antibodies and may thus become armed or triggered to react with target cells carrying the corresponding antigens. The K cells are nonphagocytic and capable of interacting with Ig-coated foreign cells to cause contact lysis. This antibody-dependent cell-mediated cytotoxicity (ADCC) is independent of the complement system. NK cells may pick up free antibodies of diverse specificities and thereby become activated or armed to kill target cells such as virus-transformed cells displaying the appropriate cell-surface antigens. The specificity and mode of NK-cell action remain unknown. This type of reaction is also complement independent. Presumably the specific antigen–antibody reaction at the target cell surface merely serves to trigger localized release of nonspecific, nonantibody effector molecules from the K or NK cells. K and NK cells have similar properties, are under common genetic control, and may belong to the same cell lineage. At this stage, these methods are more useful in delineating mechanisms of allogeneic or tumor immunity than in tissue typing.

We now return our attention to detection of gene products of constituent loci in the mouse *MIC* (see Table 6–1). Whereas only about 7 *H-* and *I-* region loci are detectable by tissue transplantation immunity, a total of some 13 *H-*, *I-*, and *T*-region loci yield products that are serologically detectable. This latter group can be further subdivided according to the occurrence and intensity of proliferative stimulations (MLC or GVHD) in congenic test combinations. Still other distinctions are emerging in the occurrence of *MIC* gene products on subsets of T- and B-lymphocytes. The uniqueness of the *MIC* evidently resides in the clustering of particular genes and the coordinated inter-

action of their products. The individual genes can determine either strong or weak H barriers to transplantation, depending on the allelic combination. Indeed, several *MIC* loci within the *I* region and *T* region may have no direct effect at all on tissue transplantation. Their importance partly resides in general immunoregulation, especially at the levels of macrophage and T-cell activation. This topic has numerous ramifications that will be considered systematically in the next chapter.

Moderate to Weak Histocompatibility Barriers

Again we focus on congenic strains of mice as the best source of experimental animals to evaluate the immunogenetics of tissue transplantation. In addition to the *H-2* complex, at least forty-two other *H* loci determining alternative cellular alloantigens have been identified in laboratory mice. These include some forty autosomal loci of which thirty have been chromosomally mapped, an X-linked locus, and a Y-linked locus. Several clusters of these *non-H-2* loci occur sufficiently close together on the same chromosome (e.g., on chromosome 4 and 7, see Figure 1–6) to suggest the occurrence of multiple immunogene complexes. Selection for numerous moderate *H* loci (*MoH*) in new congenic strains has been achieved by Bailey's procedure (Figure 1–4). If an *H* locus can be shown to be chromosomally linked to other genetic markers, this can serve to identify the locus in a unique and useful way. Thus, the *H-1* and *H-4* loci of mice occur on chromosome 7 in proximity to the genes for albinism (*c*) and pinkeye (*p*).

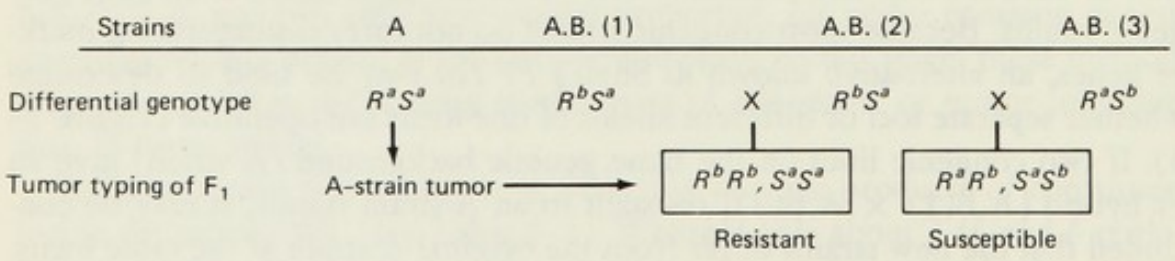


The chromosome map distances in centimorgans or observed crossover frequencies are shown above the line. The close linkage between *H-1* and *c* as well as between *H-4* and *p* became evident in the process of developing congenic strains. Because most congenic strains do not carry distinguishing marker genes, an alternative known as Snell's *F1 Test* may be used to determine whether separate loci or different alleles of one locus are operative (Figure 5–5). If two congenic lines on the same genetic background (A strain) give an F1 hybrid [A.B(1) × A.B(2)] resistant to an A-strain tumor, it may be concluded that the new strains differ from the original A strain at the same locus. In other words, these strains carry *H* alleles of non-A-strain origin that distinguish them from the A strain. If, however, the F1 hybrid [A.B(2) × A.B(3)] is susceptible to the test-transplant, a two-locus difference from the common partner is indicated. If three lines of common background give susceptible hybrids in all three possible F1s, then three *H* loci are involved. The F1 test may also be applied to the identification of alleles. If A.B(2) and A.B(3) in Figure 5–5 were inbred strains unrelated to the other congenic strain pair and even

to each other, their alleles could be determined at any locus for which A and A.B(1) are known to differ (e.g., R^a and R^b). Thus, if the F1 hybrid between A.B(1) and A.B(2) is resistant, the unknown allele in A.B(2) differs from that in the A strain. F1 susceptibility, however, as in A.B(1) × A.B(3) progeny, would indicate the same or similar alleles in A.B(3) as in the A strain. In studies of allelic diversity, the main value of the F1 test is in typing various strains for already identified alleles. One should note that skin rather than tumor transplants might be as well or better employed in the F1 test. The frequent occurrence of tumor-associated antigens and of substantial variations in tumor virulence are at least theoretically objectionable in relation to "weak" transplantation barriers.

Histoincompatibilities as a function of different *H*-locus disparities are found in all degrees of strength as determined by allograft survival times under defined experimental conditions. Median survival times of reciprocal body skin allografts between congenic strain pairs of mice are set out in Table 5–2 for ten autosomal *non-H-2* loci in relation to recipient sex. The strengths of the barriers are strikingly variable, with MSTs for the various loci ranging from fifteen to more than 300 days. The H-1 difference indicated gave an MST of fifteen days with narrow fourteen to sixteen day 95 percent confidence limits in either sex—a survival time characteristic of the twelve to sixteen days found for H-2K or H-2D disparities on the same C57BL/10 strain genetic background. However, the reciprocal H-1 combination yielded very prolonged graft survival in excess of 250 days, a markedly weak incompatibility. Note in this connection that the congenic strains listed were originally selected on the basis of resistance to a B10 tumor, not the other way around. The MSTs and ranges of allograft survival times found in other interallelic combinations reveal all degrees of transplantation immunity, including *H-9* differences so weak as to remain undetectable in either sex by skin grafting

FIGURE 5–5. F1 test for distinguishing different *H* alleles from different *H* loci in congenic strains having the same background genes.



	Strain A (tumor donor)	F_1 Hybrid	F_1 Response to transplant
One locus difference A.B. (1) × A.B. (2)	$H\text{-}R^a/H\text{-}R^a$	$H\text{-}R^b/H\text{-}R^b$	Resistant
Two locus difference A.B. (2) × A.B. (3)	$H\text{-}R^aH\text{-}S^a/H\text{-}R^aH\text{-}S^a$	$H\text{-}R^aH\text{-}S^a/H\text{-}R^bH\text{-}S^b$	Susceptible

TABLE 5–2. Median Survival Times in Days of Skin Allografts Between Congenic Strain Pairs, All of the Same C57BL/10 Background, but Carrying Different Alleles at Various Non-H-2 Loci

	Unimmunized recipients				Preimmunized recipients[c]	
	C57BL/10 recipients		C57BL/10 donors		C57BL/10 donors	
Locus[a]	Female	Male[b]	Female	Male[b]	Female	Male
H-1	>250	>250	15	15	7	7
H-3	52	46	21	30	10	11
H-4	25	24	120	119	22	>250
H-7	33	47	23	25	10	11
H-8	37	>200	32	47	22	19
H-9	>300	>300	>400	>300	23	>250
H-10	71	>300	91	>250	45	>250
H-11	164	>300	78	105	26	101
H-12	>300	>300	259	>300	15	>250
H-13	73	116	38	67	16	17

Data from Graff, Hildemann, and Snell, *Transplantation* 4:429, 1966. © The Williams and Wilkins Co., Baltimore, MD.

[a]Note that H barriers (MSTs) range from strong (15d) to very weak (>400d) and depend on interallelic combination rather than *H* locus as such. Body skin allografts of precisely 1 cm diameter were used.

[b]Note that male alloimmune reactions are usually less vigorous than those of otherwise identical adult females.

[c]Mice preimmunized by three weekly injections of 4×10^6 donor thymocytes ip; body skin grafts of a standard 1 cm in diameter applied seven days after last immunization. Test grafts invariably showed accelerated rejection in females, but prolonged survival suggestive of specific immunosuppression occurred in males under conditions of very weak histoincompatibility.

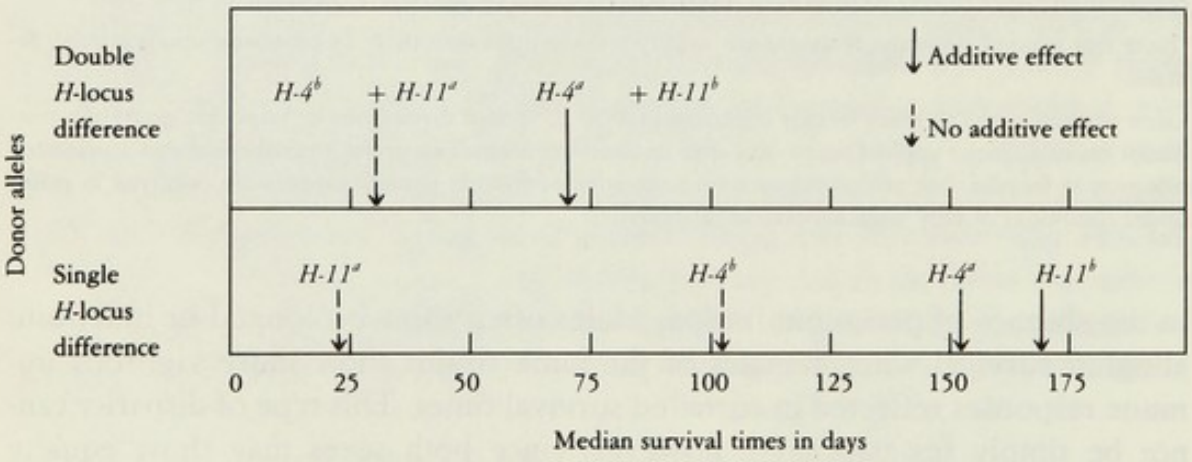
in the absence of preimmunization. Males often show prolonged or indefinite allograft survival when females of the same strains show more vigorous immune responses reflected in curtailed survival times. This type of disparity cannot be simply sex-associated, however, since both sexes may show equally weak reactions toward certain antigens, as illustrated by the reciprocal *H-4* results in Table 5–2. Note that the "strength" of histoincompatibility between congenic lines of mice is a function of the interallelic combination rather than the genetic locus involved. This may be clearly seen at four separate loci as diagramed below:

	Interallelic histoincompatibility					
	Stronger				Much weaker	
$H\text{-}1^c$	⟶ $H\text{-}1^b$	(15)	>> $H\text{-}1^b$	⟶	$H\text{-}1^c$	(>250)
$H\text{-}3^a$	⟶ $H\text{-}3^b$	(21)	>> $H\text{-}3^b$	⟶	$H\text{-}3^a$	(52)
$H\text{-}4^b$	⟶ $H\text{-}4^a$	(25)	>> $H\text{-}4^a$	⟶	$H\text{-}4^b$	(120)
$H\text{-}11^a$	⟶ $H\text{-}11^b$	(78)	>> $H\text{-}11^b$	⟶	$H\text{-}11^a$	(164)

The numbers in parentheses are the MSTs in days of female to female allografts. The same relationships were found for males in these combinations.

In certain weak congenic incompatibilities at least, heterozygous grafts survive longer than homozygous grafts, indicating that allelic dosage may also be a significant factor. Clearly, the quantity and distribution of cellular antigen as well as its quality may be important. The longer the median survival time, the greater was the range in survival times of individual grafts. The wide variation in survival times within supposedly homogeneous populations of grafts is only partly explicable in relation to slight differences in graft dosage. Variation in the time required for adequate host sensitization and a delicate balance between graft cell destruction and replacement may be involved. The latter assumption is supported by the repeated finding that second-set skin grafts may be destroyed during the vulnerable healing-in phase even though nearly fully viable first-set grafts continue to survive on the same hosts.

Multiple weak histocompatibility antigens may also have additive or augmentative effects leading to curtailed allograft survival. Double (and multiple) H differences between strain pairs show a cumulative effect if the discrepancies between the MSTs of the constituent pairs are not large. This point may be epitomized with respect to two reciprocal allelic combinations in congenic lines of mice as follows:



In this and other strain combinations not shown, an additive effect occurred only with similarly weak *H*-locus differences. In essence, otherwise weak immunogens may become substantially stronger through their cumulative effect. However, once a recipient exhibits its maximum response to an allograft, the addition of alloantigens may not further accelerate graft rejection. The nature of the cumulative effect is unknown. Absence of synergism could be attributed to interference or preemption by a strong antigen of an immune response otherwise available for a similar but weaker immunogen. The distinction between "strong" and "weak" immunogens at the molecular level is also unknown. Although the data cited refer only to skin allografts, the strength and manifestations of rejection of skin and tumors across non-H-2 histocompatibility barriers parallel one another. Weak histoincompatibility barriers have also been investigated, though less extensively than in mice, in many other species including Syrian hamsters, rats, urodele amphibians, and even invertebrate

species. Certain rules and generalizations may now be stated in relation to weak histoincompatibility and associated chronic rejection, especially of skin transplants.

1. Interallelic Combination Rule

The "strength" of histoincompatibility is a function of the interallelic donor-recipient combination rather than the particular *H* locus involved. There has long been a tendency to refer to strong or weak *H* loci as if all alleles at a given locus determined products having similar immunogenic potencies. This notion arose from the finding that most *H-2* alleles, or, more often, clusters of alleles within the *H-2* complex, yielded stronger incompatibilities than other *H* loci in mice. We are now confronted with extensive evidence that alleles rather than loci determine the quality and impact of immunogenetic disparities. Data were just cited to illustrate this point for *H-1, H-3, H-4,* and *H-11* congenic combinations with respect to skin allografts. The same allelic effect is evident in GVH reactivities and even for individual loci within the *H-2* complex. For example, a decisive effect of the interallelic combination on *H-2IC* subregion-induced GVH reactions was found with *H-2* congenic and recombinant C57BL/10 mouse strains as summarized in Table 5–3. Note that IC^d recipients showed GVH responses running the gamut from none to weak to moderate to relatively strong, depending on the donor *IC* allele of the inoculated (i.e., responding) lymph node cells. The strength or severity of the allogeneic reactions induced also varied as a function of the *IC* alleles in other strain combinations and was particularly influenced by properties of the recipient IC determinants.

Similar differences in antigen strength or immune responsiveness have been repeatedly found for other individual regions within the *H-2* complex. *H-2K* region and *H-2IA* region donor-recipient differences, either individually or in

TABLE 5–3. Effect of Interallelic Combination on H-2IC-Induced GVH Reactions[a]

Donor→recipient combination	*IC* alleles	GVH response
B10.HTT → B10.S(9R)	$IC^k \rightarrow IC^d$	None
(7R × HTT)F1 → B10.S(9R)	$IC^{s/k} \rightarrow IC^d$	Weak
B10.S(7R) → B10.S(9R)	$IC^s \rightarrow IC^d$	Moderate
B10.A(4R) → B10.A(2R)	$IC^b \rightarrow IC^d$	Strong
B10.S(9R) → B10.HTT	$IC^d \rightarrow IC^k$	None
(7R × 9R) → B10.HTT	$IC^{s/d} \rightarrow IC^k$	Weak
B10.S(7R) → B10.HTT	$IC^s \rightarrow IC^k$	Moderate
B10.S(9R) → B10.S(7R)	$IC^d \rightarrow IC^s$	Strong
B10.HTT → B10.S(7R)	$IC^k \rightarrow IC^s$	Strong

Modified from Clark and Hildemann, *Immunogenetics* 5:309–324, 1977. © Springer-Verlag, Inc., New York, NY.

[a]Newborn recipients injected with 20×10^6 normal donor lymph node cells by intracardiac route and recipient GVH splenomegaly assayed at day ten.

concert, usually cause acute allograft reactions. However, other *H-2* region (*H-2D, IB, IC, S,* and *G*) differences may yield a broad range from weak to moderately strong alloincompatibilities depending on the allelic combination. Obviously, the uniqueness of the *MIC* resides in its multigenic totality, rather than in properties peculiar to individual loci. Studies with congenic strains of rats point to the same conclusion. Although kidney allografts usually suffer acute rejection at seven to eleven days across the major Rt-1 barrier in rats, grafts in the $Rt\text{-}1^d \rightarrow Rt\text{-}1^l$ direction consistently survive longer than those made in the reciprocal interallelic combination. Heterozygosity for Rt-1 specificities as well as for Rt-1 plus non-Rt-1 specificities (semiallogeneic kidneys) favors long-term renal allograft survival. Across strictly non-Rt-1 barriers, striking differences in survival times of both skin and kidney allografts have been found in reciprocal strain combinations. Among four congenic sublines of chickens differing at the *B* locus, the *MIC* of this species, skin allograft survival times were clearly influenced by the genotype combination. A similar situation obtains for both *MIC* and non-*MIC* differences in species of amphibians. Allelic dosage effects have even been found with fin and heart allografts exchanged between inbred lines of platyfish and their hybrid progenies. Notwithstanding multiple sources of genetic diversity inherent in human beings, skin allografts from Al donors to O recipients exhibit substantially curtailed survival compared to other combinations of *ABO* alleles.

2. Additive Effect Rule

Multiple histocompatibility differences will exhibit additive or cumulative effects leading to curtailed allograft survival whenever the constituent differences are of similar potency. The validity of this assertion initially depended upon finding median graft survival times significantly shorter with respect to two H differences that would obtain if only one or the other difference alone were operative. In the early 1960s, McKhann found that two combinations of H-1 and H-3 antigens led to shorter skin allograft survival times than the single gene differences alone on mice of otherwise similar genetic backgrounds. Existence of cumulative effects of mouse H antigens has now been studied in diverse donor-recipient combinations involving 2, 3, 4, or more *H* loci. Multiple H differences regularly showed a cumulative effect whenever the component disparities were of similar strength. Perhaps the most critical finding is that otherwise weak immunogens can become much stronger through their synergistic interaction. Viewed the other way around, recipient *Ir* genes controlling macrophage and T-cell functions may require multiple antigenic determinants for activation. This type of interpretation has gained favor for two reasons: (a) molecular distinctions between "strong" and "weak" antigens as such have not been discerned; (b) *Ir* genes, especially within the *MIC,* are closely linked to *H* genes, and some *H* genes may actually function as *Ir* genes. Known *Ir* gene effects (Chapter 7) undoubtedly contribute to apparent differ-

ences in antigenic strength. Since diverse H antigens are probably components of the same cell-surface continuum, their concurrent processing by host immunocytes could well affect the response to multiple determinants. In human beings, the life span of skin and kidney allografts is often a function of the number of interacting incompatibilities. This number tends to be smaller in siblings which typically show prolonged allograft survival compared to unrelated donors and recipients. If individual transplantation antigens in humans are intermediate or weak in strength, as HLA typing evidence suggests, additive effects of multiple weak H antigens could prove decisive in allogeneic transplantation.

3. Sex-Rule

Allograft survival times on females are usually shorter than on males of the same strain or genotype. Indeed, females generally give more vigorous immune responses than males to a wide array of antigens. Of the twenty-four congenic mouse strain combinations tested by Graff, Hildemann, and Snell, five showed indeterminate MSTs (>250 days) in both sexes. Even in these groups there were many more rejections recorded for female than for male recipients. Among the remaining nineteen combinations, fourteen clearly showed curtailed skin allograft times on females compared to males of the same strain. Here the sex differences ranged from just a few days for $H\text{-}7^a \rightarrow H\text{-}7^b$ to >229 days for $H\text{-}10^b \rightarrow H\text{-}10^a$. In four additional strain combinations there was no significant difference in allograft survival times. Preimmunization leading to heightened alloimmunity either accentuated the more vigorous responsiveness of females or caused both sexes to react with similar promptness (Table 5–2). In congenic mice of quite different genetic background with weak H-1 disparities, male to male skin allografts survived considerably longer than comparable female to female grafts. Substantial sex differences in alloimmune responsiveness are less frequently evident with stronger incompatibilities reflected in MSTs under thirty days. Even given a strong immunogenetic disparity in mice, Brent and Medawar found significantly longer skin allograft survival on males than on females following whole-body irradiation at all dose levels. Turning from T-cell to B-cell effects, Batchelor and Chapman found consistently higher primary and secondary hemagglutinin responses against allogeneic sarcoma cells in female mice than in males of the same strain. Moreover, antibody production was sustained at a higher rate in females than in males over a long period of time.

In sum, the alloimmune response of male recipients is almost never better than that of females of the same strain. Sex-influenced responsiveness evidently hinges on corticosteroid levels, since adrenalectomy-gonadectomy increases allograft rejection rates in both sexes, but more so in male mice. Many investigators have noted that specific immunoblocking by antibody, as opposed to immunity, is more readily produced in males. Female mice and rats also dis-

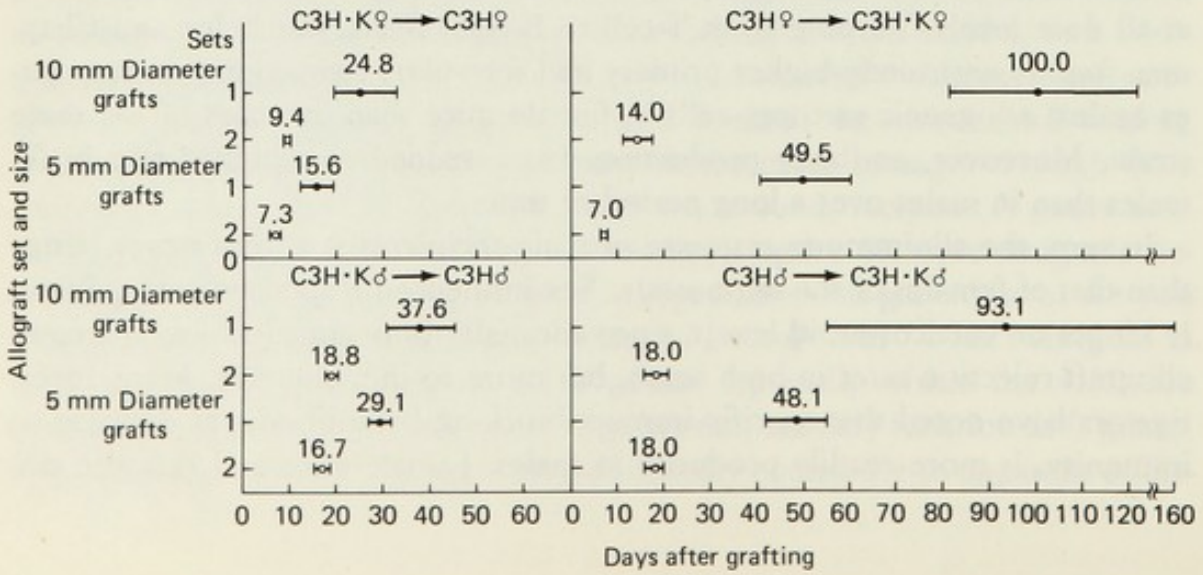
play a tendency to give higher antibody responses to defined haptens and xenogeneic antigens. Women apparently pay a price for their immunologic superiority as young adults; this is reflected in an increased probability of autoimmune disease after the age of menopause.

4. Preimmunization Rule

The weaker the H barrier, the greater is the potential efficacy of preimmunization in curtailing subsequent allograft survival times. In other words, many just perceptible incompatibilities may be made very much stronger by suitable preimmunization. The hooker in this important assertion surrounds the nature and timing of the exposure to specific antigens. The immune status of the recipient clearly depends on the source and tactics of antigen stimulation. The asymmetric H-1 barrier distinguishing the congenic C3H and C3H.K strains of mice was studied as a function of preimmunization, interallelic combination, graft dosage, and recipient sex (Figure 5–6). In the stronger C3H.K ($H\text{-}1^b$) → C3H ($H\text{-}1^a$) disparity, second-set rejections were accelerated by a factor of 2 to 2.5 in both sexes, but this factor rose to three- to sevenfold in the opposite combination. Thus, preimmunization was proportionately more effective in curtailing subsequent allograft survival with the weaker incompatibility. Preimmunization can easily convert a weak incompatibility into a strong one, especially in female recipients. This is illustrated for various *H* loci in congenic mice in Table 5–2. For moderately strong H-1, H-3, H-7, and H-8 barriers,

FIGURE 5–6. Comparative survival times of first- and second-set skin allografts in C3H ⟷ C3H.K mice as a function of preimmunization, interallelic combination, sex, and graft dosage. MSTs are indicated by numbers over solid (first-set) and open (second-set) circles with 95 percent confidence limits shown by horizontal lines. Each entry is based on single grafts placed on ten to eighteen mice.

Source: From Hildemann, *Transplantation Rev.* 3:6, 1970, © Munksgaard, International Publishers Ltd., Copenhagen, Denmark.



skin allograft survival times were curtailed by a factor of about 2 in female recipients of C57BL/10 donors. For much weaker H-4, H-9, and H-12 disparities, however, similar preimmunization evoked a striking six- to twenty-fold acceleration of allograft rejection. With very weak incompatibilities there may be few or no rejections in the absence of prior immunization. In terms of potential quantitative impact, preimmunization has more influence on allograft survival than do interallelic combination, recipient sex, or graft dosage.

5. *Dosage-Tolerance Rule*

The weaker the H barrier, the greater is the tendency for larger allografts to show prolonged survival. In other words, allograft survival times across weak barriers tend to increase with increasing graft dosage. Given a very weak initial histoincompatibility, induction of immunity by preexposure of recipients to donor cells becomes difficult, whereas the converse probability of inducing indefinite survival (i.e., tolerance or negative memory) is high. In essence, the chance that tolerance will be acquired increases with both the weakness of the histoincompatibility and increasing dosage of donor cells. At first sight, this rule appears to be at odds with the preimmunization rule which states that preimmunization becomes more effective the weaker the H barrier. The present dosage-tolerance rule usually applies only to *very weak* histoincompatibilities. In genetically defined strains of mice, this rule has been most convincingly demonstrated with respect to skin allografts in male recipients as illustrated by the data in Table 5–2 and Figure 5–6. Thus, preimmunization by injection of allogeneic thymocytes across very weak H-4, H-9, H-10, or H-12 disparities led to permanent survival of subsequent body skin allografts of 10 mm diameter in male recipients, but caused accelerated rejection in corresponding female recipients. Since the donor antigens and their dosage were the same, the marked difference in induction of "negative" memory (males) versus "positive" memory (females) must be attributed to differences in recipient *Ir* gene functions. The issue of graft dosage per se was tested in congenic adult recipients differing at the *H-1* locus. In all four strain-sex combinations (Figure 5–6), the first-set MSTs of single 5-mm grafts were shorter than those of the fourfold larger 10-mm grafts. This effect was most impressive in the weaker C3H → C3H.K disparity where large grafts survived twice as long (100 days) as small grafts (50 days). The influence of graft dosage was substantially greater with first-set than with second-set allografts. Only in the C3H ♀→ C3H.K♀ combination did curtailed second-set survival occur as a consequence of lower graft dosage (7 vs. 14 days). Among preimmunized males, repeat grafts evoked similar responses regardless of graft dosage. Note that across these C3H ⇌ C3H.K barriers of intermediate or moderate strength, effective preimmunization rather than tolerance induction consistently occurred.

Essentially the same outcome obtained across weaker H-X and H-Y barriers as a function of sex-dependent antigens and graft dosage. However, when preimmunization was attempted by various schedules of injection of donor

lymphoid cells, rather than first-set skin grafting, complete tolerance of test skin allografts resulted. Thus, the manner or route of exposure to specific antigens can be decisive, even across the weakest known H barriers. In rats, for example, orthotopic kidney allografts across H-X or H-Y barriers remain fully viable indefinitely, whereas skin allografts tend to be rejected. Although we have emphasized congenic strains of mice because of their sharp genetic definition, the same rules concerning preimmunization and dosage-tolerance effects apply to other vertebrate species ranging from primitive hagfish to urodele amphibians and mammals such as rats and Syrian hamsters. The success of so-called antigen loading in the promotion of prolonged kidney allograft survival may actually hinge on graft dosage in relation to degree of foreignness. Unfortunately, nothing is known about the genetic control of immunologic memory. In essence, weak histocompatibility interactions may yield a delicate balance between positive and negative components of immune memory. The preimmunization rule (i.e., positive memory) holds under most circumstances, whereas the dosage-tolerance rule (i.e., negative memory) depends far more on developmental and homeostatic variables. The presence or absence of particular alleles at *Ir* loci, or recipient *H* loci functioning as *Ir* loci, appear more important than donor *H* gene products as such.

6. Late Onset/Late Rejection Rule

The later the time of onset of foreign graft rejection, the greater is the interval between onset and complete rejection. The longer the median or average survival time, the greater is the range or spread in survival times of individual grafts. In other words, the range in survival times of individual grafts increases as the immunogenetic disparity decreases. These quantitative statements have proved valid in all species in which allogeneic incompatibilities have been evaluated. The dual relationship between late onset and late rejection was first discerned in Syrian hamsters by Hildemann and Walford about 1960 and was later found to apply to diverse moderate H differences in congenic strains of mice and rats. This rule has important predictive value: the longer an allograft remains fully viable, the higher is the probability that it will show prolonged survival. The interval between onset of rejection and the survival end point provides a measure of the rate of the rejection process. Although survival end points of prolonged chronic rejections are difficult to determine precisely, complete baldness of mammalian skin grafts associated with formation of a dermal collagen pad may be unequivocally scored. In hamsters, there was an approximately fourfold increase in the interval between onset and complete rejection of skin allografts in the transition from short to very prolonged survival times. Closely similar results have been obtained in mice, rats, and several species of urodele amphibians in which slow reactions regularly occur.

Slow mobilization and expression of host immunity toward moderate to weak immunogens has another important consequence. As the immunogenetic disparity between grafts and hosts decreases, the spread of allograft survival times around the median increases, especially in the direction of longer sur-

vival times. For example, at three disparate levels of congenic incompatibility involving C57BL/10 female donors (Table 5–2), the MSTs with their 95 percent confidence limits reflecting increasingly wider ranges of individual survival times were: *H-8* = 32(26–39) days; *H-10* = 91(67–122) days; *H-12* = 259(132–510) days. The actual ranges of the individual graft survival times were, of course, proportionately greater than the statistical confidence limits indicated. Studies of hamsters and rats as well as amphibians and fishes are in accord with the mouse data given. Although this rule surely has important predictive value, it may be regarded as merely descriptive of observed time-mortality distributions. Effects rather than causes of immunogenetic differences are evident.

Exceptions to the "One Autosomal, Dominant Gene → One Transplantation Antigen" Theory

Sex-Associated Specificities

The most straightforward exceptions are transplantation antigens associated with both X- and Y-linked genes in mice. Rejection of male skin grafts by adult female recipients is commonly found within many inbred strains, although comparable female to female, male to male, and female to male grafts are accepted. It is remarkable that this finding was not made until 1955 by the pathologist E. J. Eichwald, then working in Great Falls, Montana. Many earlier workers in their zeal to push on to new frontiers had simply not bothered to do these elementary experiments. The male-specific antigen(s) is moderately weak with MSTs of about twenty-five days (range ≅ 14–100 days) in mice of C57BL/6 background and considerably longer within many other inbred strains. This antigen appears to be present in all males and absent in all females although females of certain strains (e.g., A/J and BALB/c) have rejected only a fraction of skin grafts from males of their own strain. As noted earlier, minor differences may decisively modify responses to weak alloantigens. The male-specific antigen has been found in all tissues except testis and some tumors. The assumption that this *H* locus is nonautosomal and located on the unpaired segment of the Y chromosome is supported by consistent failure to detect the antigen in females, even under conditions of prolonged treatment with androgens. Indeed, testosterone treatment or castration of female hosts in one series of experiments failed to alter their capacity to reject male grafts. Nevertheless, the suspicion that autosomally determined but sex-limited antigens might be involved has persisted.

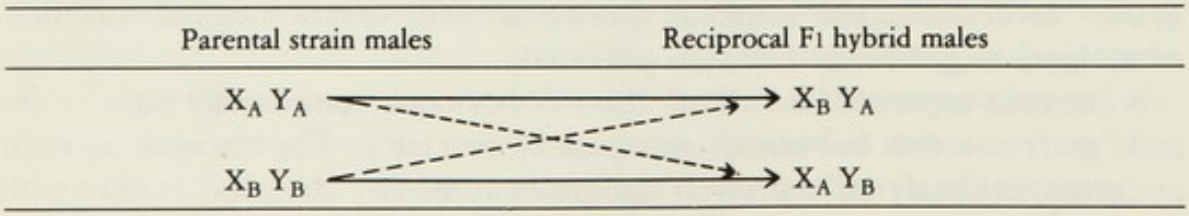
Numerous experiments indicate that sex hormones can modify male → female graft rejection but cannot eliminate or reverse it. The response to male antigen(s) is clearly influenced by the genes in the *H-2* complex. Studies with intra-*H-2* recombinants have mapped the regulation of male skin graft rejection to the *I-A* subregion. Among *H-2* haplotypes, *b* and *s* are strong responders, whereas *a* and *f* are weak or nonresponders in the usual congenic male → female experimental model. The H-2 molecules with which H-Y antigens

are associated may also influence the occurrence or rate of rejection. For example, H-Y on *H-2*^{k} grafts (B10.BR) evokes a greater allogeneic reaction than H-Y on *H-2*^{b} grafts (B10) in (B10 × B10.BR) F1 female hosts.

Quite possibly, male specific antigens of both autosomal and H-Y origins may exist, a supposition supported by evidence suggesting mouse sperm-specific antigens are influenced by the *T/t* complex on chromosome 17; moreover, the *Slp* locus in the *H-2* complex governs a serum antigen restricted to male mice. Existence of a structural *H-Y* gene yielding an antigenic product is indirectly supported by studies of exceptional XO female and XXY male mice. Whereas XXY tissue can immunize congenic females against male-specific antigens, XO tissue apparently cannot. Also, loss of the Y chromosome has been correlated with absence of H-Y antigen in testicular teratomas. In these situations, male-specific antigen is neither a result of the single-X condition normal to males, nor is it suppressed by the double-X condition normal to females. Still, the *H-Y* gene could be autosomal, with expression of H-Y (i.e., male) antigens being controlled by Y-linked regulatory genes or dependent on the male hormonal environment.

Serum antibodies induced in C57BL (*H-2*^{b}) female mice against *H-2*^{b} male cells react with sperm or male leukocytes from other strains of mice, rats, and even humans. Moreover, male cells from some but not all strains of rats were effective in sensitizing female mice against male mouse skin grafts. These results suggest molecular similarity or cross-reactivity between male antigens of diverse mammals. The question therefore arises whether *H-Y* is a highly conservative or monomorphic locus yielding the same product in diverse species. Alternatively, *H-Y* could be polymorphic with numerous alleles, but yielding a macromolecule with both public (i.e., heterogenetic) and private specificities. H-Y polymorphism could be detectable as a function of either serologic or transplantation reactivities. The assays commonly in use have not given sufficiently consistent results for this purpose. However, a new *Staphylococcus aureus* binding technique demonstrating H-Y ('male') antigen on male leukocytes reveals cross-reacting but not identical H-Y antigens among humans and mice of different strains.

Use of the parental strain male → reciprocal F1 hybrid male design not only avoids objections to the male → female design, but provides a test for allelism and nonidentity of products of both *H-X* and *H-Y* loci. The essential design may be diagramed as follows:



The different strain sources of X and Y chromosomes are designated by the letters A and B. Solid arrows indicate potential H-X differences and dashed arrows indicate potential H-Y differences, respectively. Skin grafts in all four

directions have been tested in several strain combinations of mice and rats. *H-X* polymorphism, but not *H-Y*, has been consistently demonstrated by graft rejection. In one series of experiments, both A/J (*H-2*^{a}) and BL/6 (*H-2*^{b}) male skin grafts → (A/J ♀ × BL/6 ♂) F1 male or reciprocal F1 male hybrids yielded chronic rejection. Note that control parental female to reciprocal F1 hybrid female grafts must survive permanently for this experiment to be valid. X-linked incompatibilities were found to be stronger than the Y-linked ones in relation to otherwise identical male recipients. H-Y barriers in these and other male to male combinations of mice and rats appeared so weak that many large (8 to 12 mm diameter) grafts remained viable for nearly the entire life span of the recipients. In accordance with the dosage-tolerance rule, earlier rejections were usually found with smaller first-set grafts across either H-X or H-Y barriers.

Although accelerated second-set rejection has been observed across both H-X and H-Y disparities, other forms of attempted preimmunization have tended to produce tolerance. Moreover, acceptance of CBA and C57BL male skin by their F1 hybrid males and successful interstrain male → female tolerance induction experiments suggest that male mice of many strains have the same H-Y antigen. The latter experiments involved injection of juvenile females of one strain with male cells of other strains (e.g., B ♂ → A ♀); when these females became adults, they were found to accept grafts from males of their own strain (A ♂ → A ♀) which would otherwise have been rejected. Since supposed *H-Y* allelism evident in parental male → F1 hybrid male graft rejections has involved *H-2* heterozygotes, these rejections might be attributable to *H-2* controlled hybrid resistance instead of H-Y incompatibility. However, standing against this possibility was the compatibility of parental female → F1 hybrid female control grafts in the studies cited. Nevertheless, the issue of the existence and extent of *H-Y* allelism remains unsettled. If the *H-Y* locus were immutable or monomorphic, it would be the only known *H* locus having this property.

H-X polymorphism has been repeatedly and independently demonstrated even among H-2 identical mice. X-linked *H* genes were first discerned about 1963 by the geneticist Don Bailey using an ingenious experimental approach. Complete rejection of grafts of orthotopic tail skin of (BL/6 ♀ × BALB/c ♂) F1 hybrid males on reciprocal type F1 hybrid males was observed, but only partial rejection of reciprocal F1 hybrid female skin on male hosts as shown here:

$X_A Y♂$	$X_B Y♂$	Complete rejection
$X_A X_B ♀$ →	$X_A Y♂$	Partial rejection
$X_A X_B ♀$ →	$X_B Y♂$	Partial rejection

Again, the two parental sources of X chromosomes are distinguished as X_A and X_B. Slow rejection was the rule, quite predictable for weak immunogens in male recipients. Partial rejection was scored in terms of mosaic survival pattern of hair crops on female grafts, as opposed to obliteration of all hair in

complete rejection. Although the partial rejections are consistent with the Lyon hypothesis of random X-chromosome inactivation, similar rejection patterns are also seen on grafts in relation to prolonged weak reactions associated with autosomal *H* genes. Three alleles of the *H-X* locus in mice have been identified so far. Curiously, H-X and H-Y specificities present on the same skin cells promoted anergy or immunoblocking leading to prolonged or indefinite survival of small skin allografts exchanged between reciprocal (A/J × BL/6) F1 hybrid male mice.

Male-specific antigens similar to H-Y of the mouse have been identified in numerous mammalian species: cattle, guinea pig, human, rabbit, rat, and wood lemming, but not Syrian hamsters. Since female-to-male skin grafts within highly inbred lines of chickens show slow rejection, one must invoke an H antigen characteristic of the heterogametic female which is analogous to the H-Y antigen of mammals. Similarly, the heterogametic sex in frogs and teleost platyfishes expresses H-Y type antigens.

Hybrid Antigens and Hybrid Resistance

According to the "one dominant gene → one antigen" theory, grafts from backcross, F2, or F3 progeny of inbred parental lines made to F1 individuals should succeed. In other words, all transplantation antigens possible in a pedigree should be expressed in the F1 generation. Progeny in subsequent generations should then acquire new antigens only by gene mutation or by exceptional *H* gene-product interactions. This assumes, of course, that sex-associated incompatibilities are avoided by sticking to a female-to-female design of test grafting. Partial dominance or recessive inheritance of specific immune responsiveness in F1 hybrids is now known for certain *H-2* associated *Ir* genes (Chapter 7). Possibly some of the same genes also function as *H* genes. In any event, rejection of skin grafts from F2 and F3 hybrid donors by F1 hybrid mice has been observed in females of a few strain combinations. In one study, about 3.5 percent first-set grafts from F2 → F1 hybrid females derived from A/J × BL/6 matings were rejected within six months, while nearly one-third of F3 → F1 grafts exhibited variable, chronic rejection, also reflecting weak histoincompatibility. However, lower frequencies of rejection have been obtained in different laboratories in this and other strain combinations. Frequent "cosmetic crises" observed in female parental skin grafts placed on female F1 hybrids in some strain combinations may represent a hybrid effect, possibly reflecting one or more weak antigens not fully expressed in F1 hybrids.

Only a very low incidence of F1 rejectors of F2 or F3 grafts has been found in most mouse strain combinations. The exceptions could be attributed to atypical gene-product interactions, cumulative mutations among numerous "weak" *H* or *Ir* loci, and chronic virus infection. A substantial mutation rate of 1.35 percent per zygote was found for skin isograft rejections (9/667) among female (BALB/c × BL/6) F1 mice. The prevalence of apparent mu-

tations leading to antigen gains in contrast to antigen losses in additional studies has suggested the possibility that these histocompatibility changes resulted from incorporation of viral genomes into parental germinal cells, analogous to lysogenic conversion in bacteria. Alternatively, the rarity of detectable mutations restricted to H antigen losses may usually be a result of lethality early in development because these molecules may be essential components of structural proteins of cells.

Parental tumors of diverse types are commonly transplantable with a higher frequency of success and a shorter latent period in the strain of origin than in their F1 hybrids. Parental bone marrow, spleen, and fetal liver cells also exhibit deficient growth in irradiated F1 recipients compared to irradiated syngeneic recipients. Natural cell-mediated cytotoxicity and F1 antiparent cell-mediated lympholysis are thought to be in vitro correlates of these forms of "hybrid resistance." NK-cells, the T-cells mediating antiparent cytotoxicity, and hybrid resistance display functional similarities including common genetic control by both *H-2*- and non-*H-2*-linked genes. In some parent-to-F1 combinations, resistance of F1 hybrids depends on heterozygosity at a locus close to or within the *H-2D* region designated *Hb-1* for hybrid histocompatibility (Table 6–1). Heterozygosity at the *Hb-1–H-2D* region accounts for hybrid resistance to $H\text{-}2^b$ lymphomas, fibrosarcomas, and bone marrow. Mice heterozygous elsewhere in the *H-2* complex, but homozygous for *Hb-1–H-2D,* do not exhibit hybrid resistance to $H\text{-}2^b$-bearing cells. *H-2K*-associated genes, however, may control F1 antiparent T-cell-mediated cytotoxicity to certain tumor cells.

The effect of heterozygosity at defined portions of the *H-2* complex has been examined by mating C57BL/10($H\text{-}2^b$), congenic B10.A($H\text{-}2^a$), and selected $H\text{-}2^a/H\text{-}2^b$ recombinant mice. Hybrid resistance was then assessed by comparing mean survival times and tumor incidence in *H-2* heterozygotes versus homozygous mice. Hybrid resistance to both fibrosarcomas as well as lymphomas was found to depend on heterozygosity at *Hb-1–H-2D* in the twenty recombinants tested. *Hb-1*-controlled hybrid resistance is usually reduced by preimmunization of recipients with parental donor lymphoid cells. This effect is specific, suggesting that *Hb-1* codes for or somehow regulates the expression of cell-surface antigens. Both *Hb-1*-controlled resistance and its in vitro correlate, NK-cell reactivity, appear to be most strongly expressed in the abdomen, that is, in spleen and mesenteric lymph node cells. With some tumors, effective resistance is more readily detected when tumor cells are injected by the intraperitoneal route rather than subcutaneously. That *Hb-1* is not the whole story is evident from the finding that *H-2I* region genes also influence fibrosarcoma growth in F1 hybrid hosts. Several loci within the *H-2* complex thus influence the polygenic control of the immune response to fibrosarcomas.

The mechanisms responsible for hybrid resistance are not known. Since both normal and tumor cells of several types are affected by *Hb-1, Hb-1* probably does not code for an *Ir* gene conferring resistance to a tumor antigen. *Hb-1* could be a structural gene coding for a cell-surface antigen expressed in *H-*

2^b parental mice but not in F1 offspring. This theory would account for the rejection of *Hb-1*^{b}-bearing tissues by F1 mice, but requires postulation of a recessive histocompatibility gene. *Hb-1* could be a structural gene for a surface molecule which upon association with certain membrane determinants (e.g., endogenous viral antigens) makes them more antigenic. In the heterozygous state, these determinants may preferentially associate with the *Hb-1*^{d} molecules and not *Hb-1*^{b} structures. F1 recipients, therefore, would be tolerant only of those determinants associating with *Hb-1*^{d}. Alternatively, *Hb-1* may be a regulatory gene which controls expression of certain antigenic structures. Genes regulating viral gene expression in cells, such as the *Fv-1* locus on chromosome 4, like *Hb-1* show recessive inheritance of the state permitting antigen expression. Other recent evidence of hybrid histocompatibility molecules in the F1 not present in either parent has been reported. In conclusion, three kinds of exceptions to the classic genetic rules of transplantation have been identified: (1) sex-associated specificities governed by *H-X* or *H-Y* genes, (2) autosomal, donor-associated changes in H antigens or appearance of hybrid antigens, and (3) autosomal, recipient-associated changes in immune responsiveness or appearance of hybrid resistance.

GENE REGULATION OF TRANSPLANTATION IMMUNITIES

Acquired Tolerance

If an individual is exposed to an allograft or other kinds of antigens in sufficient quantity during embryonic or early postnatal life, the capacity to give an immune response to these antigens (e.g., to reject an allograft from the same donor source) is usually lacking when the individual becomes an adult. Many readers will already be aware that specific tolerance and acquired immunity represent opposite poles of immunologic responsiveness. The capacity of immunologically competent cells to distinguish "self" from "not-self" in a functional way is often fully acquired or matures near the time of birth. Thus, if an animal is exposed to an antigen before it has developed the capacity to react against it, the development of this capacity is delayed and, with continued presence of antigen, can be indefinitely postponed. In this sense, tolerance may be defined as a state of essential nonreactivity of the individual at the biosynthetic level of lymphoid cells.

The roots of present understanding of immunological tolerance go back to Ray Owen's shrewd perception of persistent erythrocyte mosaicism in dizygotic twin cattle. It had been noted by the immunogenetics group at the University of Wisconsin in the early 1940s that twin cattle showed identical blood types much more often than expected on the basis of apparent identical twinning. By differential immune hemolysis tests, Owen showed that most twin cattle are not only born with, but generally retain a stable mixture of, each other's erythrocytes. Since the mixed populations of red cells were found to retain their separate antigenic identities well into adult life, it followed that

red cell precursors exchanged via placental anastomoses persisted as new self-constituents in reciprocally tolerant hosts. R. E. Billingham and P. B. Medawar not only extended Owen's results by successful skin grafting between cattle twins but were able to obtain allogeneic tolerance in perinatal mice by direct injection of cells from a foreign strain. During this period in the early 1950s, M. Hasek produced specific tolerance in chickens by simply joining chick embryos across the shell in synchorial parabiosis. At hatching, such birds were not only chimeras or erythrocyte mosaics, but they also permanently accepted skin grafts from each other. Fraternal twin chick embryos regularly become reciprocal chimeras. Naturally occurring tolerance has also been repeatedly found in twin sheep and more rarely in dizygotic human twins. The first human example of allogeneic tolerance was rather dramatic: a woman known as Mrs. McK was shown to have in her circulation red cells descended from her twin brother who had died as an infant more than two decades earlier.

Immunological tolerance has been produced experimentally in diverse vertebrates ranging from teleost fishes through mammals. In general, tolerance is much more easily procured early in development than in mature individuals. The simplistic concept of acquired tolerance as an absence of specific immune responsiveness in adults who had previous embryonal or perinatal contact with an antigen has required revision. Indeed, production of blocking antibodies and suppressor cells accounts for many forms of specific immunosuppression in adults. This dynamic immunoregulation as a positive response more often accounts for unresponsiveness in adults than does acquired tolerance defined as an absence of any detectable response to an antigenic stimulus. Transplantation tolerance is distinctive in that chimerism attributable to a persisting population of intact donor cells appears to be required. The strength or immunogenic potency of H antigens as well as their dosage are critical in determining whether tolerant states may be obtained in juveniles or adults. The capacity to reject allotransplants, reflecting in part developmental activation of *Ir* genes, matures at or near the time of birth or hatching in many vertebrates. However, species and even strain differences in this respect are considerable. Cattle, sheep, and human beings are already capable of vigorous immune responses long before birth. Complete transition from a tolerance-type to an immune-type response against strong alloantigens is achieved in most strains of mice, rats, rabbits, and man at or within a few days after birth.

Either tolerance or immunity, or both concomitantly, may be induced in perinatal or adult life depending on immunogenetic disparity and antigen-dosage relationships. Newborn mice may be immunized with allogeneic cells which in higher dosage will induce tolerance. Neonatal A/J *(H-2^a)* and BL/6 *(H-2^b)* mice which received allografts of neonatal skin reciprocally as early as the day of birth rejected such grafts vigorously with median survival times of ten to eleven days—only slightly longer than those determined for adults. Preimmunization with 50 to 100 $\times$ 10^3 allogeneic thymocytes at birth led to accelerated rejection of test skin allografts placed four days later. Similar preimmunization effectively protected BL/6 neonates against an otherwise fa-

tal inoculum of A/J tumor cells (Sarcoma I) injected four days later. The capacity to respond to alloantigens by humoral antibody production matures more slowly than the capacity for graft rejection.

Failure to obtain allogeneic tolerance in adult animals is attributable mostly to strong histocompatibility barriers such as *H-2* in mice, *Rt-1* in rats, and *B* in chickens. Under these circumstances, even a profound assault on an adult animal's immune response capacity achieved by means such as persistent antigen overloading, parabiosis, and whole-body radiation has led to only partial tolerance. Tolerance toward weaker H antigens not only can be more readily achieved in adults, but tolerance responsiveness is directly proportional to effective immunogenic weakness. Female mice of the C57BL strain may easily be made tolerant of a moderately weak H-Y antigen as late as seventeen days postpartum; very high doses of male cells will produce tolerance in adult females. Parabiotic union between adult males and females may also induce a high degree of tolerance. Weakly disparate H-1 or H-3 congenic strains of mice may also become reciprocally tolerant as a consequence of adult parabiosis. With quite weak H-9 or H-12 differences in congenic mice, large skin allografts alone appear to induce prolonged tolerance in adult recipients. Since tolerated graft cells retain their antigenic identity, the tolerant state clearly reflects a systemic alteration of the host. Mice derived from pairs of conjoined, cleavage-stage embryos of different histocompatibility genotypes can remain chimeras retaining their characteristic antigenic products. These "allophenic" or tetraparental animals are fully and reciprocally tolerant. Notwithstanding H-2 disparities, they apparently remain free of allogeneic disease but otherwise show normal immune responses. Allogeneic tolerance is usually plural in the sense that a donor normally contains multiple antigens that are lacking in the recipient.

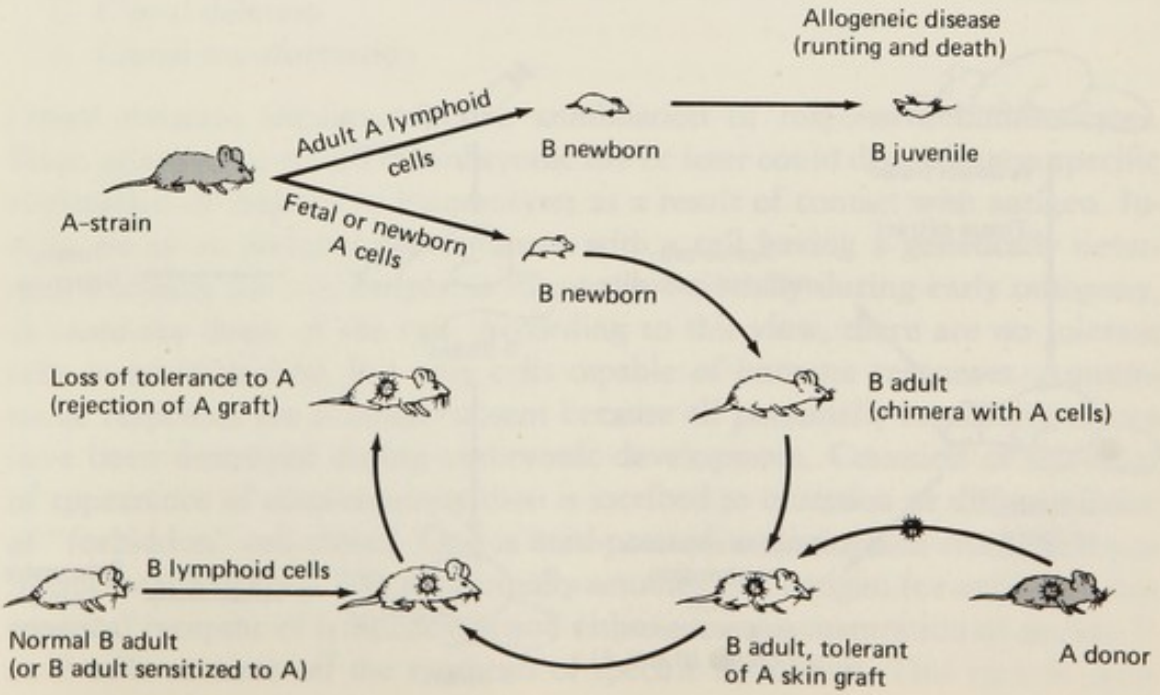
Tolerant states often appear to be partial rather than complete. For example, slow rejection of skin allografts between dizygotic cattle co-twins often occurs in spite of persistent red cell chimerism established in utero. This suggests existence of tissue-specific H antigens. E. L. Triplett showed that frog embryos deprived of their pituitary gland are later capable of rejecting their own hypophysis. In these experiments the organism and gland were grown apart from one another until the tadpoles were immunologically mature and the glands were differentiated. We now know that this type of result in mice is attributable to an *Sk* locus determining an alloantigen limited to skin and brain. Although neonatal inoculation of marrow cells from one strain to another can produce permanent chimerism for lymphocytes, subsequent adult tolerance of donor skin grafts may or may not occur.

When adult BL/6 hosts are heavily irradiated and reconstituted with *H-2*-disparate (BL/6 × A/J)F1 bone marrow cells, they recover and are blood-cell tolerant displaying (BL/6 × A/J)F1 lymphoid cells, yet they reject strain A/J skin grafts and produce antibodies reactive with donor epithelial cells. Evidently, skin has alloantigens not present on lymphoid tissues; two allelic specificities SK-1 (A/J) and SK-2 (BL/6) have been identified. The (BL/6 × A)F1

bone marrow cells maturing in BL/6 hosts never "learn" to be tolerant of SK-1 antigens since they have matured in an environment free of SK-1. However, if A/J skin grafts are placed on hosts early while chimerism is being established, the hosts become tolerant to such grafts. Thus, tolerance for the SK molecules appears to be acquired during immunocyte maturation. Tolerance toward the liver-specific F antigen also appears to be acquired.

Finally, let us consider abrogation of tolerance by an experimental device known as adoptive immunity. The required design is diagramed in Figure 5–7. Newborns of one inbred strain (B) inoculated with several million nucleated cells of another strain (A), under conditions ensuring reciprocal graft-host tolerance, will later accept skin grafts from the donor (A) strain without deleterious consequences. The tolerated (A) graft can be destroyed within a week by an injection of (B) lymphoid cells from an adult preimmunized with (A) cells. Inoculaton of the tolerant (B) animal with *normal* rather than immune B cells will also terminate the tolerant state, but more slowly. The adoptive immunity achieved by initially nonimmune cells raises the question whether the tolerant state reflects a central failure of cell-mediated immunity or some form of specific interference with its inception or execution. Induction of al-

FIGURE 5–7. Diagram relating transplantation tolerance to allogeneic disease and abrogation of tolerance by adoptive immunity. Two antigenically disparate inbred strains are here designated as A and B. Newborn B injected with immature A cells leads to reciprocal graft-host tolerance and persistent chimerism in B adult. Such B adults will be tolerant of A skin grafts, but tolerance may be terminated adoptively by inoculation of normal or immune adult B lymphoid cells. Note requirement for cells isogenic with host to accomplish adoptive immunity. Newborn B injected with adult A lymphocytes across strong H barrier allows graft-versus-host reactions which usually lead to fatal allogeneic disease.

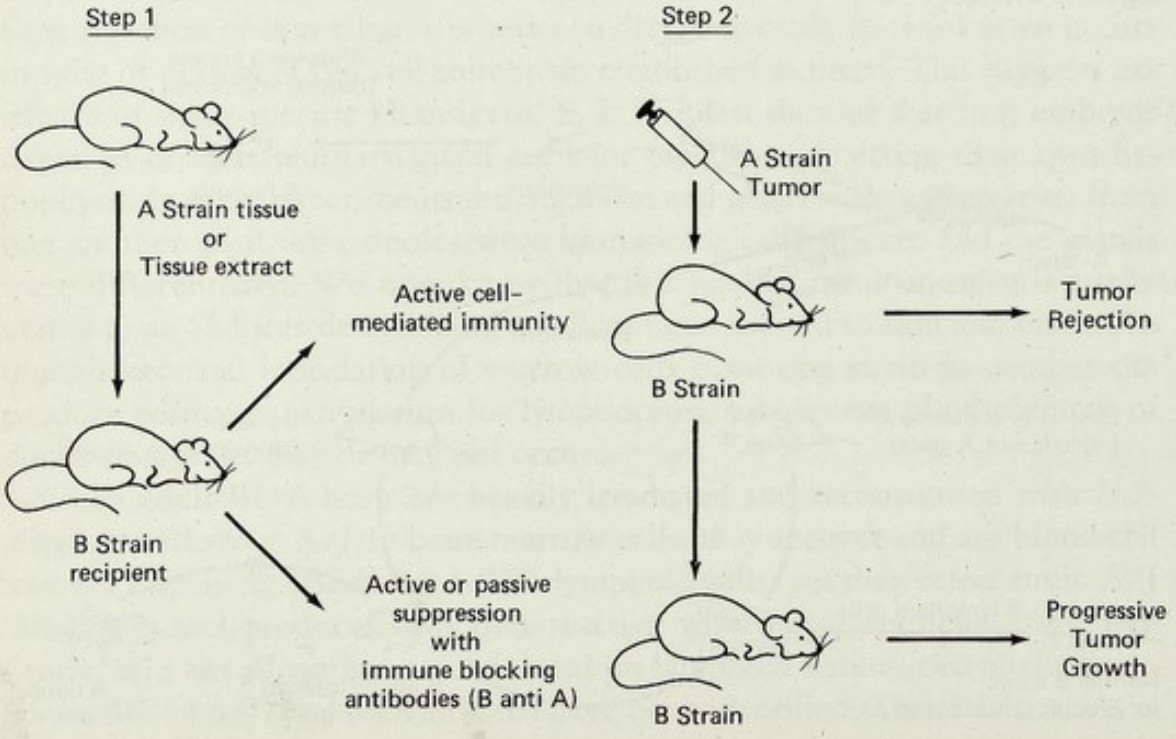


logeneic tolerance clearly depends on three major factors: (a) the degree of immunogenetic disparity between donor and recipient, (b) the recipients' stage of immunomaturation or competence, and (c) the antigen dosage.

Specific Immunoblocking and Suppression

Several forms of specific immunosuppression formerly attributed to acquired tolerance now appear to be mediated by blocking antibodies or suppressor cells instead. Even allophenic mice made chimeric at the eight-cell stage by joining two embryos from histoincompatible parental strains have yielded adults with lymph node cells capable of destroying parental fibroblasts and with serum capable of blocking this cellular immunity. It had long been known that tumor allografts could become successfully established and grow progressively to the death of the host as a consequence of the tumor's contact with specific antiserum in the host. This promotion of growth or failure to reject a virulent tumor as a function of (or in spite of) the host's specific immune response became known as immunological enhancement or specific immunoblocking (Figure 5–8). Since active, cell-mediated immunity or effective rejection of foreign cells is suppressed rather than enhanced, the term "enhancement" is undesirably confusing in this connection. *Specific immunoblocking,* a preferable designation, hinges on the presence of specific antibodies

FIGURE 5–8. Immunologic enhancement of tumor growth or specific immunoblocking hinges on the presence of specific antibodies or antigen–antibody complexes in a recipient. Normal tissues as well as tumors, including syngeneic neoplasms carrying tumor-associated transplantation antigens, may avoid rejection as a consequence of this form of immunoregulatory suppression of cell-mediated immunity.



or antigen–antibody complexes in a recipient. A prime example of this phenomenon is the highly effective suppression of Rh immunization by injection of anti-Rh IgG (but *not* IgM) into Rh-negative women soon after the birth of Rh-positive children (Chapter 4). Whether such an antibody works by feedback suppression of maternal lymphocytes, peripheral "masking" (sequestration) of Rh+ target cells, or some other mechanism is debatable.

The common denominator in specific immunoblocking is active suppression of cell-mediated immunity (i.e., T-lymphocyte/macrophage killing). By contrast, acquired tolerance usually implies a total absence of specific immunity rather than a redirection or selective inhibition of a particular pathway. Rejection of normal tissue allografts is not easily suppressed in adults across strong histocompatibility barriers. Administration of blocking antiserum or putative suppressor immunocytes tends to be far more effective in prolonging survival of kidney, ovarian, and heart allografts than skin or pancreas allografts. Apart from such tissue differentials, ease of specific immunoblocking, like ease of tolerance induction, increases the weaker the H barrier is. The weaker the immunogenetic barrier, the greater is the effectiveness of specific immunoblocking antibodies in facilitating prolonged allograft survival. In rats, for example, passive IgG immunoblocking antibodies extended renal allograft survival from twofold in the Buffalo → Lewis strain combination to twenty-fold [(Lewis × Buffalo) F_1 → Lewis] to permanent acceptance (Fischer → Lewis). This reflects a transition from a strong homozygous *Rt-1* to a heterozygous *Rt-1* to a weak *non-Rt-1* disparity, respectively. Although kidney failure eventually occurred across stronger Rt-1 incompatibilities despite impairment of transplantation immunity, non-Rt-1, H-X, and H-Y differences permitted persistent functioning of enhanced renal grafts.

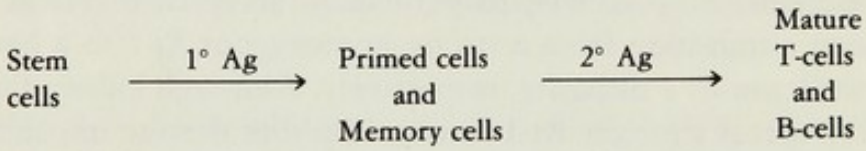
Two theories of specific immunosuppression may be contrasted from a genetic standpoint:

1. Clonal deletion
2. Clonal transformation

Clonal deletion implies selective annihilation of responsive immunocytes. Thus, tolerance acquired in embryonic life or later could depend upon specific elimination of responsive lymphocytes as a result of contact with antigen. Interaction of an antigenic determinant with a cell having a genetically determined affinity for this antigen is supposed, especially during early ontogeny, to cause the death of the cell. According to this view, there are no tolerant cells in an individual, but only cells capable of immune responses. Autoimmune responses are normally absent because all potentially responsive clones have been destroyed during embryonic development. Cessation of tolerance of appearance of autoimmunity then is ascribed to mutation or differentiation of "forbidden" cell clones. One is hard-pressed to distinguish clonal deletion from clonal blocking. Blocking usually assumes that antigen (or antibody) hits a critical receptor of lymphocytes and either prevents maturation of certain T- or B-cells or turns off the synthesis of specific antibodies. This view is more

in accord with the finding of various degrees of incomplete tolerance induced even in previously sensitized mature animals. Exposure of embryos to potential antigens is supposed to establish complete tolerance provided sufficient antigen reaches all reactive stem cells, thereby eliminating them or preventing their further differentiation. Tolerance or immunosuppression in adults would represent the replacement of immunocompetent mature cells by a specifically nonreactive population. Possibly, given cells become either tolerant or immunoreactive, depending on the nature of their encounter with antigen, antibody, or specific blocking factors. Permanent allogeneic tolerance at the level of the whole animal or person is the only form of specific immunosuppression explicable entirely by clonal deletion or equivalent inactivation. States of partial or transient tolerance when some manifestation of specific immune reactivity persists are obviously inconsistent with the clonal deletion theory.

Clonal transformation theories accept the premise of divergent responsiveness to antigen contact: some cells become tolerant (or are eliminated) and other cells become immune (or transform into reactive T- and B-cells). In other words, antigen can either paralyze ("tolerize") or prime, depending on the status and genotype of the cells encountered. At least three levels or successive stages of cell maturation may be assumed, with numerous possible variations, as follows:



Here, 1° Ag and 2° Ag symbolize initial exposure and renewed contact with an antigen, respectively. Transformation of stem cells into primed or positively activated cells may be either blocked or promoted, in embryos or adults, depending on both the *Ir* genotype of the recipient and the antigen encountered. Even if primed or memory cells are produced in moderate numbers, further contact with sufficient antigen may elicit terminal differentiation of mature T- and B-cells. Such immune exhaustion or unresponsiveness following an earlier period of significant antibody production may result from depletion of a population of primed or memory cells. Proponents of clonal transformation theory tend to view specific immunosuppression as a favorable balance between relatively few "immune" cells and many "unresponsive" cells. This approach has the potential virtue of coping with selective blocking of separate T- or B-cell pathways. Each of the steps in these interconnecting pathways is now known to be controlled by *Ir* genes with numerous allelic alternatives governing high-versus-low and all-or-none responsiveness. The clonal transformation approach also provides for cognizance of memory and major differences in primary versus secondary responsiveness. The whole concept of dynamic immunoregulation—blocking-versus-cytotoxic antibodies and suppressor-versus-helper T-cells—is more in accord with clonal transformation than clonal deletion. It is becoming increasingly evident that suppression versus immunity

hinges more on the *Ir* gene library of the recipient than on the molecular properties of antigenic determinants. Tolerance appears more often referable to subpopulations of lymphoid cells than to a condition of the entire organism.

CLINICAL TRANSPLANTATION

ABO and HLA Typing

Blood grouping by standard serological techniques allows identification of characterization of individual people to a remarkable extent (Chapter 4). The need for accurate tissue typing in whole blood transfusions has long been recognized. Other human applications range from establishing correct parent–offspring relationships and recognition of monozygotic versus fraternal twins to identification of blood stains in criminal cases. In addition to red cell typing, identification of alloantigens restricted to nucleated cells or their products has proved highly desirable for matching potential donors and recipients of allotransplants. The clinical goal of tissue typing, however accomplished, is to minimize histoincompatibilities. Early studies demonstrated that A and B erythrocyte antigens can also act as strong transplantation antigens. Volunteers sensitized by repeated injections of ABO-incompatible erythrocytes regularly showed accelerated rejection of skin grafts from other AB-incompatible donors compared with grafts from compatible (O) donors. Additional group (O) recipients pretreated with purified A glycoprotein subsequently showed very rapid rejection of grafts from A donors while concurrent grafts from (O) donors showed slow first-set type reactions. The interallelic combination was also decisive in the sense that $A_1 \rightarrow O$ incompatibility was much stronger than $A_2 \rightarrow O$. Only ABO-compatible and preferably ABO-identical donors and recipients are now used in clinical transplantation. Among other blood group systems, only P antigenic disparities appear to influence skin allograft survivals, and then only slightly.

Human leukocyte typing was first achieved by lymphocyte agglutination, but now more sensitive microcytotoxicity tests are preferred. In the usual cytotoxic test, rabbit complement (preabsorbed or preselected for low naturally occurring cytotoxicity) is added to a blood lymphocyte-alloantiserum mixture and cell killing is scored on the basis of trypan blue or eosin dye uptake. A total of some sixty antigenic specificities are now serologically detectable as products of one major *H* complex designated *HLA* (HL = Human Leukocyte; A = the first such system). The serologically defined antigens, determined by multiple alleles at each of three closely linked loci, *HLA-A, HLA-B,* and *HLA-C* (Figure 5–9), act individually as weak transplantation antigens. A fourth locus designated *HLA-D* has greater influence on alloimmune responsiveness toward tissue grafts. Its allelic variants are identified in terms of MLC reactivity of T-lymphocytes (Figure 5–4). Another locus very closely linked or identical to *HLA-D,* designated *HLA-DR,* yields antigens expressed on bone marrow-derived lymphocytes (B-cells) and monocytes that can be detected se-

rologically by complement-mediated lymphocytotoxicity using sets of nearly monospecific antiserums. The DR antigens appear to be the same as most of those identified with the *HLA-D* locus defined by the cumbersome MLC assay. However, the finding of positive MLC reactivity between HLA-DR identical sibs suggests that -DR and -D products may be separate entities, perhaps attributable to closely linked loci. From the clinical standpoint, donor-recipient compatibility for HLA-D or -DR specificities now appears more predictive of prolonged allograft survival than HLA-A, -B, or -C matches. The term "matches" implies identity of donor and recipient for the given antigens. HLA-DR typing may become especially useful in bone marrow transplantation for repair of immunodeficiency diseases. Additional loci within the *HLA* complex on the sixth chromosome code for erythrocyte antigens (Chido and Rodgers blood groups) which are also polymorphic variations of certain complement components (Bf, C2, C4). This last group of genes does not appear to influence the outcome of clinical transplantation. The three or four classes of genes identified with *HLA* appear remarkably similar to those associated with the *H-2* complex of mice.

Sibling Versus Cadaver Donors

HLA-identical siblings have consistently proved to be more compatible donors of kidney and other allografts than HLA-disparate siblings, parental, or cadaver donors (Figure 5–10). Although HLA-identical sibs have a three-year survival rate of about 80 percent, immunosuppressive therapy must be maintained. Transplants between sibs with no *HLA* haplotypes in common yield survival rates little better than those of unrelated cadaver transplants. Sharing of one *HLA* haplotype between sibs or in parent-to-child transplants has given intermediate three-year survival rates of about 65 percent. Failure has almost invariably followed bone marrow transplantation (where graft-versus-host disease is more probable than graft rejection), except where transplantation is performed between HLA-identical sibs. Even with complete HLA compatibility, success is not assured.

The extensive renal allograft data are revealing on two counts. First, the potential importance of HLA gene products as transplantation antigens or as determinants of alloimmune responsiveness is demonstrated. Second, the importance of other *H* genes or *Ir* genes independent of *HLA* is also evident. Other than for siblings, HLA identity alone will not assure extended allograft survival, even with continued administration of immunosuppressive drugs such as azathioprine and prednisone. Obviously, non-*HLA* genes likely to be shared by siblings can become decisive when mismatched, as in the case of unrelated cadaver donors. The importance of non-HLA determinants is emphasized in Figure 5–10 by the finding that fewer than half of cadaver donor kidneys survived for two years in a large series despite all efforts to minimize HLA mismatches.

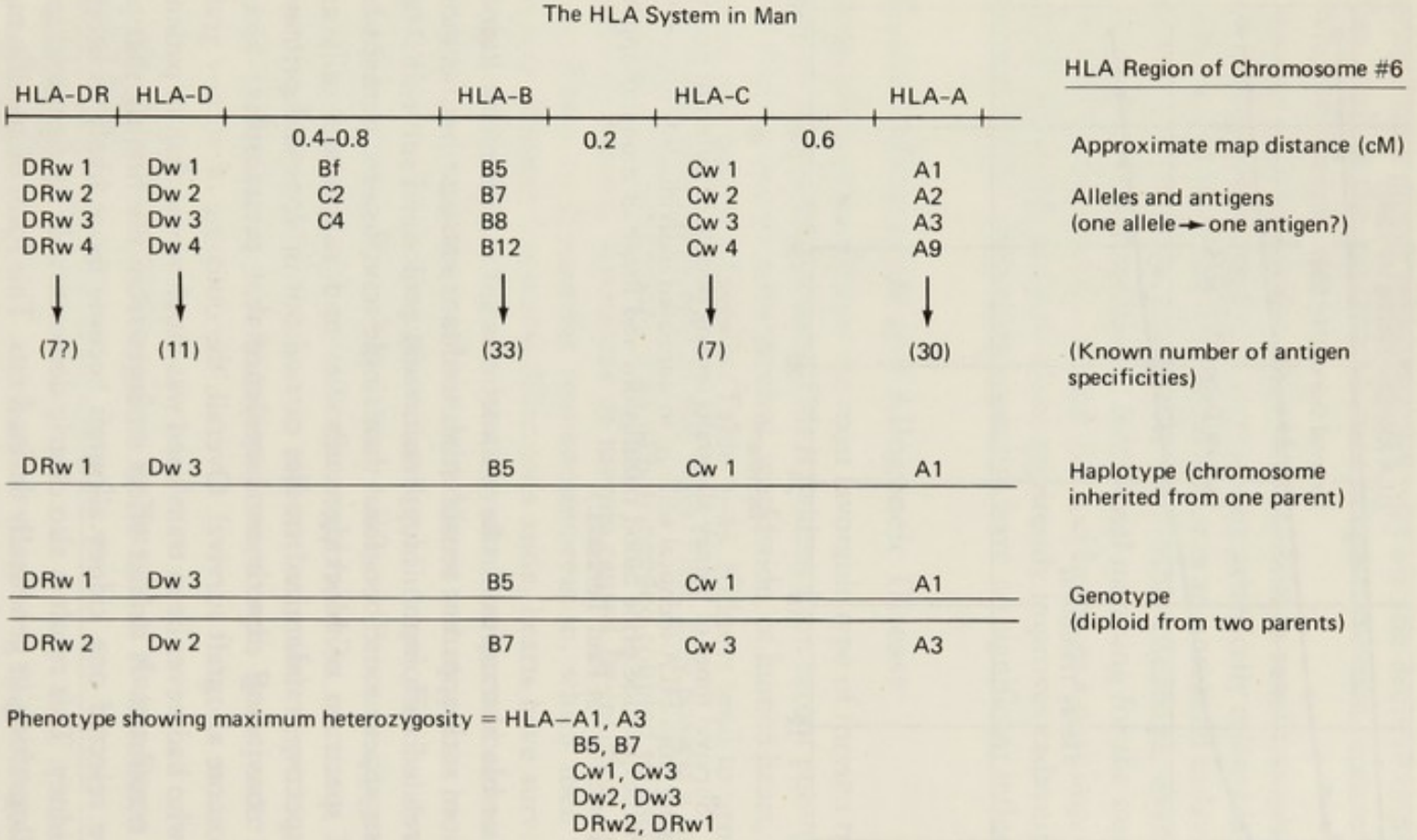


FIGURE 5–9. Schematic diagram of *HLA* system showing sequential correspondence between chromosomal genes, alternative alleles at each locus, and expression of antigenic products. Some specificities assigned separately to *D* and *DR* may be coded by allelic genes at the same locus. The prefix DRw indicates identification at a D-related workshop. The *w* for workshop designates less defined specificities and is included in HLA nomenclature as the first step in official recognition by the World Health Organization.

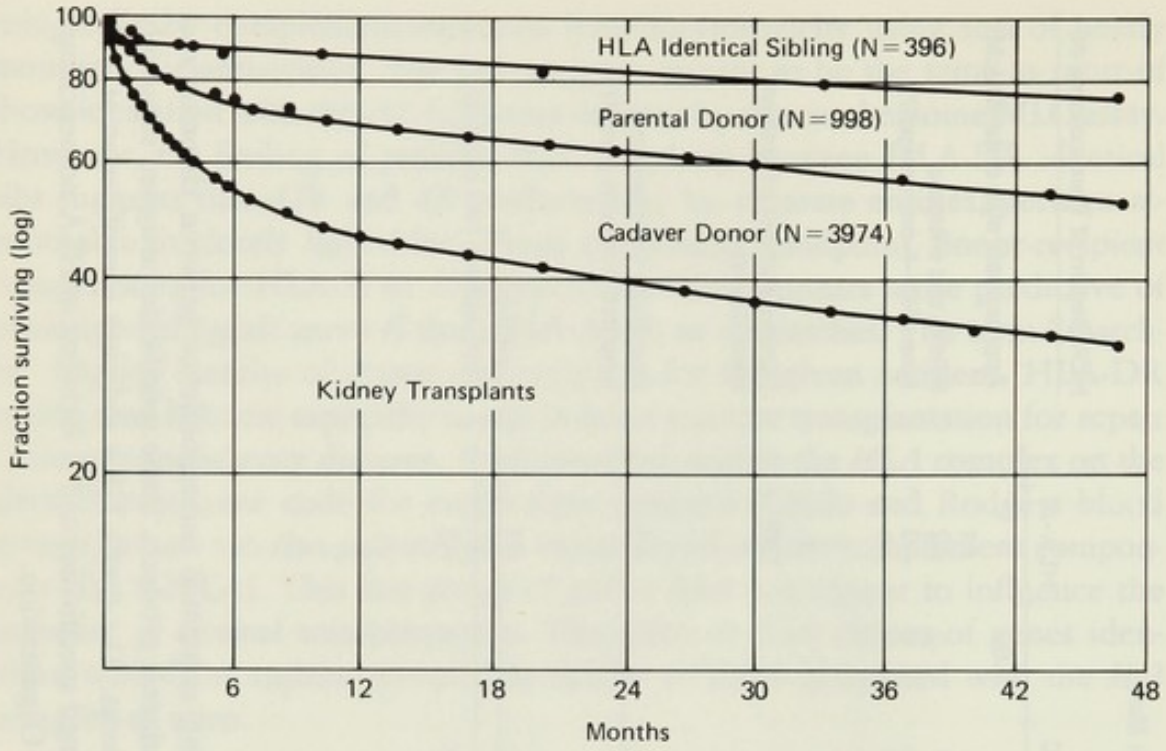


FIGURE 5–10. Survival times of kidney allografts among people of different genetic relationships ranging from HLA-identical siblings to unrelated individuals (cadaver donors). Other variables include prior blood transfusions and usage of immunosuppressive drugs. (Data courtesy of Paul Terasaki.)

Although prior blood transfusions have sometimes led to hyperacute kidney allograft rejection, such apparent sensitization to donor antigens has far more often been correlated with immunosuppression and prolonged survival. Multiple transfusions appear more beneficial than single transfusions, perhaps because a wider spectrum of blocking antibodies and suppressor cells are produced. Prospective, randomized studies carried out in dogs and primates have provided convincing experimental evidence that pretransplant blood transfusions promote allograft survival. Overall, the outcome of kidney grafting in patients who had never been transfused was much worse than in patients who had been transfused. A similar effect emerges from the finding that patients who have rejected one kidney allograft become more likely to accept a subsequent kidney. This result is also clearly demonstrable by first- and second-set renal allografting in genetically defined rats. This form of specific suppression becomes more effective the weaker the genetic H barrier. It is noteworthy that HLA-DR matching appears to overcome the prospect of poorer graft survival in certain nontransfused patients. Even in previously transplanted or transfused patients, at least partial HLA matching appears necessary to assure prolonged renal allograft survival. If the transfusion or pregrafting effect is indeed attributable to specific immunoblocking, it becomes important to determine which *H* genes or *Ir* genes are involved. Otherwise, the risk of inducing active immunity remains unpredictable. It is already known, for example, that blood group O recipients have a better chance of prolonged graft

survival than non-O recipients. Sex may also be decisive under certain conditions, such as the improved kidney allograft survival reported in multiparous female recipients.

Exact matching for the whole *HLA* system in renal transplantation can substantially improve results, at least among genetically related individuals. In the usual clinical situation, where the donor is an unrelated cadaver, matching for HLA-A and -B alone becomes a formidable challenge, even with extensive lists of prospective recipients. Additional matching for the numerous antigens specified by the *HLA-C, -D,* and *-DR* loci becomes a near impossibility. Matching for HLA-DR antigens alone apparently improves cadaveric graft survival, whereas HLA-C disparities appear to have no significant influence.

Tissue Differentials and Allogeneic Disease

Renal allografts constitute the most favorable type of foreign transplant among various vascularized tissues. This is evident from recent published statistics indicating the comparative survival, worldwide, of human heart, liver, lung, pancreas, and kidney allografts (Table 5–4). Kidneys tend to promote their own continued survival in at least two ways. First, they possess a much higher glomerular filtration capacity than usually needed coupled to excellent reparative capacity. Second, appearance of uremia caused by incipient rejection quickly contributes to nonspecific immunosuppression, which then inhibits further kidney damage. Technical difficulties aside, hearts have survived better than livers, while lung and pancreas allografts have proved almost entirely unsuccessful. Although few well-matched donors and recipients have been reported in cardiac and liver transplantation for HLA specificities, in neither instance has any influence of such matching emerged. Pancreas allografts have usually provoked particularly vigorous and early rejection reactions. This same result has been repeatedly obtained in rats not only with respect to the major *Rt-1* complex but across non-Rt-1 disparities as well. Perhaps the pancreas, more

TABLE 5–4. Tissue Differentials in the Survival of Human Organ Allografts (Circa 1979)

Organ transplant registry data	Heart	Liver	Lung	Pancreas	Kidney
Transplant teams involved	64	41	22	15	301
Number of allograft recipients	301	255	37	47	22,261
Long-term survivors with functioning grafts	59	33	0	1	10,300
Longest survival with functioning graft	8 yr	7 yr	10 mo	4 yr	21 yr[a]

[a]Identical twin

than other tissues, expresses potent tissue-specific transplantation antigens. In any event, pancreas transplantation will not soon solve the enormous problem of human diabetes.

Use of skin allografts in badly burned people, where insufficient undamaged autogenous skin is available for grafting, has long been practiced in burn centers. While many variables affect graft survival in these patients, prolonged allograft acceptance has been positively correlated with more extensive *HLA* matching. However, the relative importance of different HLA specificities is unclear in this connection. In genetically defined animals, skin allografts are more vulnerable to early rejection than kidney or heart grafts in the same donor-recipient combinations.

Corneal transplants under ideal conditions do not evoke alloimmune reactions in the absence of vascularization. Their immunological isolation means that recognizable rejection episodes occur infrequently. Among some 300 corneal allografts recently performed in England, less than half of those that became vascularized survived beyond eighteen months, whereas 80 percent of the grafts with no vascularization survived this long. Because nonvascularized corneas exhibit a low failure rate, the degree of *HLA* matching reveals no influence on the outcome. However, matching for HLA-A and -B antigens has been connected with better survival of vascularized allogeneic corneas. Most HLA disparate corneal grafts with marked vascularization survive less than one year.

Allogeneic bone marrow transplantation is being tried extensively to repair serious diseases ranging from aplastic anemias to congenital immunodeficiencies. Since the donor bone marrow contains an abundance of immunocytes and recipients are usually lacking in immunocompetence, the stage is unfortunately set for severe graft-versus-host reactions, which often lead to fatal allogeneic disease. Because GVH disease inevitably occurs in conjunction with HLA incompatibility, bone marrow transplantation is usually restricted to HLA identical sibs. MLC matching for HLA-D and related specificities is claimed to improve prospects in marrow cell grafting, but complete *HLA* matching is not an absolute prerequisite for successful marrow transplantation. Even with *HLA* matching, fatal allogeneic disease frequently ensues, presumably because of other *H*-gene and *Ir*-gene differences between graft and host. Indeed, the high incidence of GVHD in HLA identical sibs surely points to involvement of *non-HLA* loci.

Although definitive diagnosis of GVHD in man can be uncertain, the incidence is at least 60 percent in both aplastic anemia and acute leukemia following allografting. When allogeneic marrow grafting has been promptly undertaken in the therapy of severe aplastic anemia, some 40 percent of recipients showed long-term disease-free survival. Most aplastic anemia is caused by drugs and X-irradiation that kill marrow cells in a dose-related manner, so the recipient's own immunocytes may gradually restore competence with or without tolerance of allogeneic donor cells. Use of allogeneic marrow grafting in acute leukemia or congenital immunodeficiency is more controversial be-

cause of generally unfavorable results. Improved tissue typing to provide sharper identification of strong incompatibilities of both *HLA* and *non-HLA* origins appears particularly desirable. Less promising is selective removal of potential killer T-lymphocytes from bone marrow cell suspensions prior to inoculation. Hematopoietic reconstitution following transplantation of cryopreserved autogenous bone marrow holds promise for patients with leukemia or lymphomas subjected to chemoradiotherapy, but of course this approach would be of no avail for repair of congenital deficiencies.

KEY REFERENCES

Books

Gotze, D. (ed.). *The Major Histocompatibility System in Man and Animals.* Berlin: Springer-Verlag, 1977. This book reviews MHCs of various species in terms of gene action, regulation, evolution, and possible role in the pathogenesis of disease. Individual articles reflect the biases of diverse authors.

Snell, G. G., Dausset, J., and Nathenson, S. *Histocompatibility.* New York: Academic Press, 1976. Deals admirably with the concepts and methodologies of transplantation immunogenetics, especially in experimental animals.

Articles

Animal Emphasis

Brent, L., et al. Transplantation tolerance. *Br. Med. Bull.* 32:101–106, 1976. A succinct review contrasting classical transplantation tolerance leading to chimerism with states of partial tolerance or selective immunoblocking.

Clark, E. A., and Harmon, R. C. Genetic control of natural cell-mediated cytotoxicity and hybrid resistance. *Adv. Cancer Res.* 31:227–285, 1980. Hybrid resistance and natural cytotoxicity are regulated by both *H-2*- and non-*H-2*-linked genes. Models accounting for the mechanism of hybrid resistance are described.

Clark, E. A., and Hildemann, W. H. Genetics of graft-versus-host reactions. II. Interallelic effects and regulation of GVHR by antirecipient alloantibodies. *Immunogenetics* 5:309–324, 1977. Studies reveal that individual *MIC* loci can have the impact of moderate (*MoH*) loci. Moreover, the intensity of allogeneic reactions varies as a function of the interallelic combination and is responsive to specific immunoregulation.

Gasser, D. L. Genetic studies on the *H-3* region of mice and implications for polymorphism of histocompatibility loci. *Immunogenetics* 3:271–276, 1976. Demonstrates close linkage of *H-3, Ir-2,* and *H-13* on chromosome 2. Also reveals that extensive allelism at *H-1, H-3, H-4,* and *H-8* loci is similar to that found at *H-2D* and *H-2K.*

Gill, T. J. Report of the first international workship on alloantigenic systems in the rat. *Transplant. Proc.* 10:271–285, 1978. Ten haplotypes of the rat *MIC,* now named *Rt-1*, have been identified. Three additional systems of non-MIC antigens have also been revealed by either histoincompatibility reactions or alloantibody reactions.

Hildemann, W. H. The Syrian hamster in immunologic perspective. *Fed. Proc.* 37:2102–2105, 1978. The *MIC* equivalent *Hm-1* in this species governs alloantigens or *Ir* genes restricted to T-cell functions, i.e., acute allograft rejection, GVH reactivity, MLC reactions, and cell-mediated cytotoxicity in vitro. No alloantibody response to Hm-1 determinants is detectable.

Klein, J. The major histocompatibility complex of the mouse. *Science* 203:516–521, 1979. An update on the *MHC* or *MIC* of the most-studied laboratory mouse, with emphasis on polymorphism and separate gene functions.

Klein, J., Flaherty, L., VandeBerg, J. L., and Shreffler, D. C. Review: *H-2* haplotypes, genes, regions, and antigens. *Immunogenetics* 6:489–512, 1978. Some 110 identified *H-2* haplotypes comprise various combinations of about sixty different antigenic determinants, and this is by no means a final tally.

Mullen, Y., Raison, R., and Hildemann, W. Cytotoxic versus immunoblocking effects of specific alloantibodies. Effects of IgM, IgG and IgG2 on rat kidney allograft survival. *Transplantation* 24:99–105, 1977. The strikingly opposite effects of specific cytotoxicity (IgM) versus immunoblocking (IgG) as a function of antibody isotype occur across both strong Rt-1 and moderate non-Rt-1 barriers. Underlying mechanisms remain obscure.

Nash, J. R., Peters, M., and Bell, P. R. F. Comparative survival of pancreatic islets, heart, kidney and skin allografts in rats, with and without enhancement. *Transplantation* 24:70–73, 1977. Although islet allografts are more susceptible to rejection than other tissues, their survival can be prolonged by specific immunosuppression.

Schultz, J. S., Beals, T. F., and De Mott-Friberg, R. Tissue graft rejection in mice. V. Survival, rejection, or proliferation across minor barriers. *Immunogenetics* 6:585–595, 1978. Multiple non-H-2 differences can cause rejection at least as rapidly as H-2 differences. H-2K differences tend to be more potent in causing tissue rejection than H-2D differences. Degrees of incompatibility vary with different types of donor tissue.

Snell, G. D. Recent advances in histocompatibility immunogenetics. *Adv. Genet.* 20:291–355, 1979. Recent review of *H* loci and other alloantigen loci in mice.

Snell, G. D. T cells, T cells recognition structures, and the major histocompatibility complex. *Immunol. Rev.* 38:3–69, 1978. Evidence bearing on the nature of T-cell recognition structures is related to the functions and evolution of the *MIC*. See also Cunningham, B. A., *Sci. Am.* 237:96–107, 1977, for other interpretations of the receptor/recognition functions of *MIC* cell-surface antigens.

Wachtel, S. S. H-Y antigen: genetics and serology. *Immunol. Rev.* 33:33–58, 1977. H-Y antigen expression in mammals is governed by a Y chromosomal gene and occurs in the heterogametic sex of all vertebrates so far tested. *MIC* genes in mice influence both expression of H-Y and responsiveness to H-Y. Controversy persists concerning the extent of H-Y polymorphism and involvement of autosomal gene products sex-limited to males.

Human Emphasis

Bach, F. H. Genetics of transplantation: the major histocompatibility complex. *Annu. Rev. Genet.* 10:319–339, 1976. Focuses lucidly on newer methods of human tissue typing with emphasis an *HLA* genes. Also considers questions of genotypic identity, selection, and linkage disequilibrium.

Bodmer, W. F. (ed.). The HLA system. *Br. Med. Bull.* 34:213–320, 1978. Covers the whole gamut of *HLA* genetics, cellular typing, clinical transplantation, and disease associations. Includes new work on *HLA-DR* genes and antigens.

Carpenter, C. B. (ed.). Clinical histocompatibility testing. *Transplant. Proc.* 10:687–999, 1978. An extensive series of articles dealing with recent advances.

Gale, R. P., and Opelz, G. (eds.). Immunobiology of bone marrow transplantation. *Transplant. Proc.* 10:1–268, 1978. The perils, pitfalls, and promise of allogeneic bone marrow transplantation are considered from different perspectives.

Van Rood, J. J., Balner, H., and Morris, P. J. Blood transfusion and transplantation. *Transplantation* 26:275–277, 1978. The benefit versus hazard of pretransplant exposure to H antigens is considered from immunogenetic and clinical standpoints.

6

MAJOR IMMUNOGENE COMPLEXES AND LYMPHOCYTE DIFFERENTIATION GENES

IMMUNOGENE FAMILIES

Specialized Molecules at Cell Surfaces

The gene products expressed on the external surface of all cells, including lymphocytes, can be divided into several broad classes. First, there are molecules that are the membrane scaffolding and the enzymes responsible for membrane construction and maintenance. Second, there are surface molecules involved in transport of ions and nutrients and other systems necessary for general cell growth and maintenance. Finally, there are surface gene products specific for a given cell type, which play a role in the specific physiologic development and function of the cell. In this chapter we will deal especially with the group of these molecules found on lymphocytes.

During the formation and development of blood cells—a process called hematopoiesis—a cell lineage progressively becomes committed to differentiate into cell types with characteristic phenotypes. Although much of this process is most certainly preprogrammed, external factors may also play a role: cells are influenced in their microenvironment by hormonal signals or cell-cell surface interactions. Some cell-surface molecules may participate in specific recognition events which mediate developmental changes. For example, they may serve as receptors for diffusible differentiation signals or mediate specific cell-cell interactions. These molecules are gene products of cell-surface differentiation; the genes that code for them at a particular stage of development are called *differentiation genes.*

Lymphoid cells express a number of other surface structures, including several kinds of recognition molecules. As we have seen in Chapter 2, B-lymphocytes bear surface immunoglobulin for specific recognition of foreign antigens. T-lymphocytes also have receptors for "nonself" but the nature of these receptors remains elusive. Lymphocytes are group-oriented cells—they do not usually act alone; the immune response to a foreign antigen is a group

effort requiring cooperation and interactions between different lymphoid cell types. Thus, lymphocytes must also have surface molecules to facilitate cell-cell interactions and regulatory signals among cell subsets during the generation of the immune response. Within this group of molecules are structures that either promote or restrict the function of differentiated lymphocytes. Finally, the immune system is unique in having to discriminate between "self" and "nonself." Certain molecules on the cell surface control self-recognition in order to regulate responses to foreign antigens.

Some of these cell-surface molecules can be distinguished by serologic means. When cells of one strain of animals are injected into another strain, antibodies could theoretically be made against all distinctive membrane components because respective donor and recipient immunogenes yield polymorphic determinants. However, much of the membrane scaffolding is not alloimmunogenic; genes coding for membrane proteins vital for general cell growth and survival tend to be highly conserved and thus display little polymorphism. Accordingly, many of the antibodies to lymphoid cell-surface components are directed against lymphocyte-associated antigens that show intraspecific variation. Vital lymphocyte-specific molecules that are not polymorphic will probably not be distinguished by intraspecies or even by interspecies immunizations.

Frequently, when a lymphocyte surface gene product is defined, its underlying function is not apparent; therefore, cell-surface gene products on lymphocytes are often grouped according to characteristic phenotypes. The type of immune response generated by surface molecules in alloincompatible animals has been a commonly used classification scheme. Thus, those genes responsible for the rejection of tissue grafts are termed *histocompatibility* (*H*) genes, as we saw in the last chapter, and are further subdivided into "major" *H* genes or "moderate" *H* (*MoH*) genes according to the strength of the reaction they evoke. Some alloantigens such as Thy-1 only induce an alloantibody response; in contrast, *H-15* disparities in the mouse are detectable only by skin graft rejection. Another approach is to divide surface molecules according to their tissue and cell distribution or their expression during differentiation. In Chapter 4 we discussed a group of surface molecules, blood group antigens, which are grouped together because they all have the common properties of being antigenic and expressed on erythrocytes. Similarly, the Lyt surface antigens are those found exclusively on T-lymphocytes. As with most classification schemes, there are overlapping groups of immunogenes with respect to phenotypic manifestations.

The Major Immunogene Complexes

A cluster of genes found in all higher vertebrates, often called the major histocompatibility complex (*MHC*), codes for cell-surface glycoproteins that cause acute tissue allograft rejection and thus is the principal transplant barrier of the species. This gene complex is now better designated the major immun-

ogene complex or *MIC,* because the constituent genes have important roles in immunorecognition and immunoregulation. The *MIC* has special characteristics and probably unique properties: it is extremely complex genetically, typically containing at least two *H* loci separated by 0.5 to 1 cM. Perhaps hundreds of other loci exist within this chromosomal segment. Many of the constituent loci have numerous alleles, therefore the *MIC* is highly polymorphic and can exist in innumerable gene combinations. The gene products of the *MIC* have been detected by various methods including serology, allograft rejection, and assays measuring cell proliferation or allocytotoxicity. Genes controlling the immune response to a variety of antigens, resistance to disease, and serum complement components are also contained within the *MIC.* The *major immunogene complex,* therefore, consists of a group of immunogenes controlling diverse phenotypic traits associated with immunoresponsiveness.

The major immunogene complexes of the mouse (*H-2*) and of humans (*HLA*) have been examined most thoroughly and will be discussed in detail here. Knowledge of such systems in other species has also been valuable and has contributed in particular to our understanding of comparative and evolutionary aspects of the *MIC* (see Chapter 9). Studies in mouse and man have taken quite different approaches: whereas murine research has utilized particular inbred strains, congenic lines, and intra-H-2 recombinants, the outbred human population has required a combination of family studies and statistical correlations.

THE H-2 COMPLEX IN MICE

The *H-2* complex was first defined as an erythrocyte antigen and a strong transplantation barrier by Gorer in 1936, who postulated that a single locus controlled both phenomena. Later serologic and genetic studies revealed that *H-2* is not a single locus since it is divisible by crossing-over. Today our knowledge of the complexity of *H-2* has expanded rapidly with an ever-increasing number of genetic regions being distinguished (see Figure 5–2). The products of *H-2* appear to be associated with membrane alloantigens, regulation of immune responsiveness, and complement components. Table 6–1 lists some of the genes and traits known to be controlled by the different *H-2* regions.

Nomenclature

A frequent barrier to understanding *H-2* has been mastery of the nomenclature, with all its superscripts and subregions. The polymorphism of *MIC*s by necessity makes their nomenclature difficult; however, the standardized nomenclature of the mouse based on classic *Drosophila* usage is now generally employed and provides a model for application to other species. The chromosome region embracing the *MIC* on chromosome 17 of the mouse is termed the *H-2 complex.* The gene regions which are defined by recombination are

TABLE 6–1. Loci and Phenotypes Associated with Distinct Regions of the *H-2* Complex[a]

	H-2 regions									*T* regions			
Locus or phenotype	K	IA	IB	IJ	IE	IC	S	G	D	Qa-1	Qa-2	Qa-3	Tla
Histocompatibility (H)	*H-2K*	*H-2IA*	–	–	–	*H-2IC*	–	*H-2W?*	*H-2D* *H-2L*	–	|←*H-31, H-2T, H-32*→|		
Immune response (*Ir*)		*Ir-IA*	*Ir-IB*			*Ir-IC*	–		*Ir-TlA*				*Ir-ferritin*
Serologically detectable alloantigen loci	*H-2K*	*Ia-1*	*Ia-2*	*Ia-4*	*Ia-5*	*Ia-3*	*Slp*	*H-2G*	*H-2D* *H-2L*	*Qa-1*	*Qa-2*	*Qa-3*	*Tla*
MLC-GVH proliferation[b]	+	++	–	±	?	+	–	±	+	u	u	u	–
CML-GVH disease[b]	++	+	–	–	?	±	–	–	++	+?	+	+	–
Lymphoid cell distribution	all	B,T,MØ	?	T_S some T_H	|←B,T,MØ→|		–	RBC	all	Thymocytes + T-cells	T+?	T	Thymocytes
Serum substances		T_H factor macrophage factors		T_S factor			Ss (C4)						
Restricted T-cell types	T_K	T_H T_{DTH} MØ		T_S		MLC suppression		*H-2R*	T_K				
Other associated loci		*Rgv-1*							*Rfv-1* *Hb-1*				

[a]Symbols are defined as follows: *H* = histocompatibility; *Ia* = I region-associated antigen; *Ir* = immunoregulatory or immune response gene; *Ss* = serum serological; *Slp* = sex-limited protein; *Qa* = Q-region antigen; *Tla* = thymus-leukemia antigen; T_K, T_H, and T_S = thymus-derived killer, helper, and suppressor lymphocytes; MØ = macrophage.

[b]Observed reactivity very strong (++), strong (+), weak (±), absent (–), unknown or unclear (u).

designated by capital letters, *K, I, S, G,* and *D,* as are *subregions,* distinct segments of a region defined by recombination, e.g., *I-A, I-J.* Individual histocompatibility *loci* are distinguished by appending the corresponding capital letter, e.g., *H-2K, H-2IC.* Different allelic genes at defined loci are noted by superscript small letters, e.g., *I-C*^{d}, *H-2K*^{s}. The *H-2* complex occupies a chromosome segment of approximately 0.5 cM, and since *H-2* loci are therefore closely linked, a given sequence of alleles tend to be inherited as a unit; this set of *H-2* genes on a single chromosome is called an *H-2 haplotype* and is also designated by small superscript letters, e.g., *H-2*^{f}, *H-2*^{m}. Inbred strains of mice have a characteristic *H-2* type or haplotype, e.g., C57BL/6 mice are *H-2*^{b}, B10.A(4R) mice are *H-2*^{b4}. Each haplotype has a characteristic *genotype,* that is, a sequence of alleles, at known *H-2* loci. The genotype of the *H-2*^{b} haplotype, for example, can be expressed *K*^{b}, *I*^{b}, *S*^{b}, *D*^{b}, *T*^{b}. With recombinant *H-2* haplotypes, the I region must be further subdivided according to the distinctive alleles at the separate *A, B, J, E,* and *C* subregions as shown in Tables 5–1 and 6–1.

Numbers are used to distinguish the *H-2*-coded serologic specificities. These broad groups of specificities have been designated as H-2, Ia (for *I* region-associated), and Tla (for Thymus, leukemia antigen). The classic "H-2" specificities detectable by hemagglutination are expressed H-2.1, H-2.2, and so on; Ia antigens are designated Ia.3, Ia.4, and so on. Note that the italics are used for *H-2* genes but are not used for the actual H-2 antigens or gene products.

Before continuing, the reader should verify his understanding of the above section. Some care is required when using this nomenclature, particularly when it is spoken. For example, *H-2D* refers to a histocompatibility locus in the *D* region; *H-2*^{d} refers to a particular *H-2* haplotype characteristic of certain inbred strains. The term *I-A* designates a subregion within the *I* region; "Ia," however, is used to describe antigens coded for by various *I* region genes.

The K and D Regions

Every region in the *H-2* immunogene complex codes for serologically detectable products. Two loci, *H-2K* and *H-2D,* were the first to be defined by serologic and histogenetic methods; therefore, the antigens coded for by these loci are sometimes called "classic H-2" or simply H-2 antigens. These H-2 antigens are transmembrane glycoproteins with a molecular weight of approximately 45,000 daltons (Figure 6–1). They associate noncovalently on the cell surface with a 12,000 molecular weight protein, β_2 microglobulin, which is coded for by an unlinked gene. *H-2K* and *H-2D* loci are remarkable because they are expressed in all nucleated somatic cells, and they are the most polymorphic loci known. In late 1978 there were already thirty-seven defined alleles at *H-2K* and thirty-two alleles at *H-2D* Any theory purporting to explain the biologic role of H-2 antigens must account for these distinctive features.

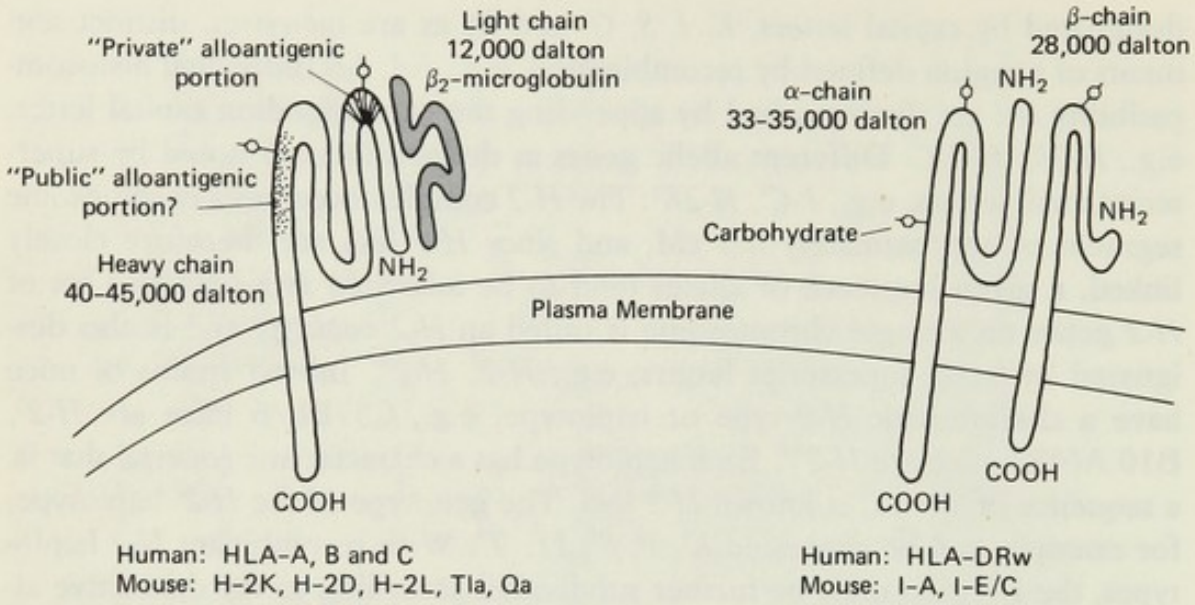


FIGURE 6–1. Hypothetical model of *MIC*-coded cell-surface antigens. Major H- and Ia-like molecules have an external globular region and are transmembrane proteins.

Serology

From the earliest serologic studies it was clear that the *H-2* genes coded for complex determinants. Initial studies characterizing H-2 antigens used alloantiserums raised simply by immunizing inbred mice with cells from other inbred strains. An important observation was that two kinds of specificities could be distinguished by using absorption analysis (see Chapter 4). Some H-2 specificities were identified on cells from a number of inbred strains (Table 6–2). For example, antiserum X reacts with the immunizing strain C, as expected, and several other strains, indicating these strains share with strain C a common determinant not expressed in Strain A. Such a determinant, expressed in more than one parental haplotype, is called a *public specificity.* These public specificities were found to vary considerably in their haplotype distribution. For example, antigen 1 is present in all strains except strain A; antigen 3, however, is found only in haplotypes of strains A, C, and D. Another group of specificities could only be detected in a characteristic strain; for instance, absorption of antiserum X with strain B cells leaves an antiserum that reacts exclusively with strain C. This antiserum is detecting a *private specificity,* which is expressed in a single *H-2* haplotype. Because private specificities are unique for a particular haplotype, they serve to define that haplotype. A continuing effort to define the H-2 specificities present in diverse inbred strains has led to an imposing array of private and public antigens as summarized in abbreviated form in Table 6–3. The two-locus model of Shreffler and Klein divides antigenic H-2 determinants into three general classes: the K- and D-specific private specificities; public specificities found only on *K* or only on *D* region products; and public antigens shared by the *K* and *D* regions. Some antigens (not shown) have not yet been assigned to either region. Each *H-2* allele or haplotype has a characteristic group of specificities, or *serotype.* For example, $H\text{-}2^k$ strains express the following H-2 antigens: 1, 3, 5, 8, 11, 23, 25, 32, 45, 47, 49?, 52,

and 60; mice with the *H-2*^{b} haplotype have H-2 antigens 2, 6, 27, 28, 29, 33, 35, 36, 39, 46, 53, 54, 56, and 62.

Separation of K- and D-private specificities was made possible once intra-*H-2* recombinants were characterized (Table 6–3). This was greatly facilitated by the discovery of the C4 complement locus *Ss* between *K* and *D* (see below). For example, erythrocytes from *H-2*^{a} strains (an *H-2*^{k}/*H-2*^{d} recombinant) are hemagglutinated by both anti-H-2^{k} and anti-H-2^{d} serums. Absorption of an H-2^{k} antiserum with H-2^{a} recombinant cells removes some H-2 antibodies (anti-H-2.23) and allows the remaining anti-H-2D^{k}-private specificity H-2.32 to be distinguished. In parental haplotypes where recombinants have not been isolated, such as *H-2*^{r}, the private specificities cannot yet be assigned to either the *K* or *D* region. Over sixty distinct H-2 specificities have been distinguished in inbred strains of mice and many more are being identified, especially in mice derived from feral populations.

The complexity of specificities coded by one region raises the question of whether similar determinants are coded by different loci or different alleles of the same locus. Many public specificities appear to be present on the same membrane glycoprotein as the private specificities. This has been shown by "cocapping" experiments. Antibodies to cell-surface antigens can cause the antigens to move and cluster or "cap" together on the membrane. Molecules that move or cocap as a unit are assumed to be part of the same macromolecule. If molecules bearing public specificities are removed from the cell surface by cocapping with appropriate antiserum, the treated cells are no longer susceptible to lysis with antiserums directed against the private specificities; immunochemical studies using sequential precipitation technology also indicate that the same 45,000 M.W. glycoprotein usually carries both public and private determinants (Figure 6–1). One public *D* end specificity, H-2.28, however, has been shown sometimes to be expressed on a structure distinct from the H-2D^{d} private alloantigen. The gene coding for this antigen has been designated *H-2L* and maps within the *H-2D* region. No homologue for *H-2L* has been detected in the *K* region. This could reflect some specialization of *K* and *D* region functions.

TABLE 6–2. Serologic Analysis of Alloantiserums Produced in *H-2* Incompatible Strains of Mice

		Tested against						Antigen identified	
Antiserum	Absorbed by	A	B	C	D	E	F	No.	Type
X (A anti-C)	—	0	+	+	+	+	+	1	public
	B	0	0	+	0	0	0	2	private
Y (B anti-A)	—	+	0	+	+	0	0	3	public
	C	+	0	0	0	0	0	4	private
Z (C anti-B)	—	+	+	0	+	+	+	5	public
	A	0	+	0	+	0	0	6	public?

Strains A, B, C, D, E, and F are six hypothetical inbred strains with different *H-2* haplotypes.

+ = positive; 0 = negative as detected by hemagglutination.

TABLE 6–3. An Abbreviated *H-2* Chart of Inbred Strains

Inbred strains	*H-2* Haplotypes	H-2K																		
		Private	Public																	
			K Region-associated											Shared with *D*						
			8	11	25	34	39	45	46	47	52	53	54	1 28	3	5	35	36	42	49
A.BY, C57BL, B10, 129	*b*	33,62					39		46			53	54	28		5	35	36		
DBA/2, BALB/c, B10.D2	*d*	31	8			34			46	47				28	3		35	36	42	
A.CA, B10.M	*f*	26	8				39		46			53		28?						
AKR, C3H, CBA, B10.BR, CE, C58 RF/J	*k*	23,60	8	11	25			45		47	52			1	3	5				49?
P/J, B10.P	*p*	16	8			34			46	u				1		5	35			49?
DBA/1, B10.G, C3H.Q	*q*	17		11		34		45	u		52		54	1?	3	5				49?
RIII, B10.RIII	*r*	K18	8	11	25			45	u	47	52		54	1	3	5				49?
A.SW, B10.S, SJL	*s*	19				u		45?	u					1		5		36?	42?	u
SM/J	*v*	K21				u		u	u	u				1		5				
RECOMBINANTS A,B10.A (*k/d*)	*a*	23	8	11	25					47				1	3	5				
C3H.OL (*d/k*)	*ol*	31	8			34			46	47				28	3		35	36	42	

Adapted from Snell et al., *Histocompatability*, pp. 108–110, Academic Press, New York, 1976 and Klein et al., *Immunogenetics* 6:496–497, 1978.

u = unknown; ? = uncertain. Recombinants in two parental haplotypes have not been reported (*H-2^r*, *H-2^v*); therefore, the K and D end private specificities have not been clearly distinguished and are designated K18, D18, and K21, D21. The specificities H-2.1 and H-2.28 are placed together because they may be different alleles at the same locus.

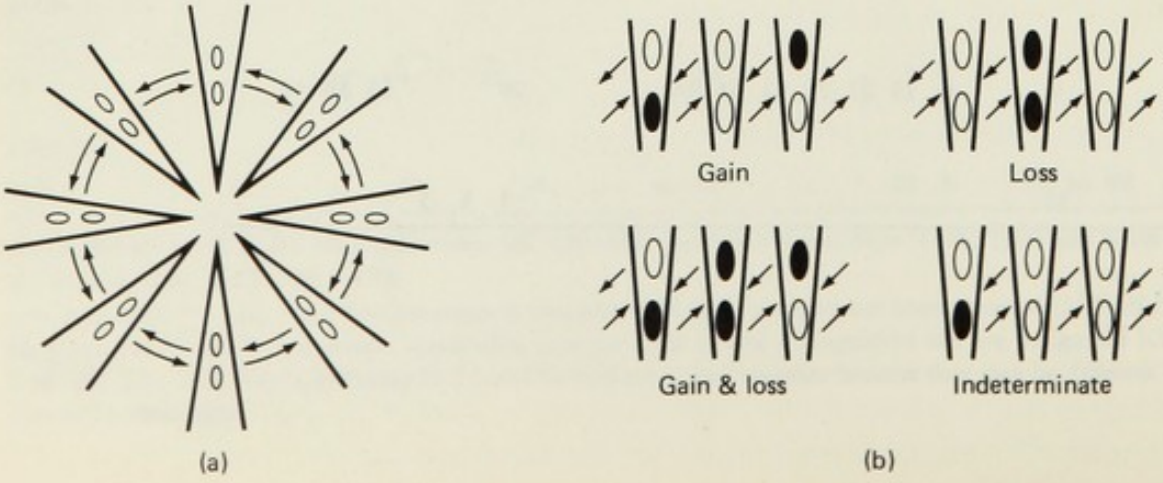
H-2D																	
Private	Public																
	D Region-associated									Shared with *K*							
	6	13	27	29	41	43	44	55	56	1 28	3	5	35	36	42	49	
2	6		27	29					56	28			35	36			
4	6	13	27	29	41	43	44		56	28			35	36	42	49	
9	6		27							28							
32										1	3	5				u	
22	6				41					1	3	5?	35	36		49?	
30	6	13	27	29				55	56	28	3			36		49?	
D18	6									28						49?	
12	6									28	3			36	42	49?	
D21	u									28	3			36		u	
4	6	13	27	29	41	43	44			28			35	36	42		
32										1	3	5					

Mutations

The major immunogene complex is remarkably pleiotropic; a recent compendium listed sixty traits associated with the *H-2* complex. Identification and application of mutations at *H-2* loci offers a powerful means for examining this pleiotropism and determining if particular *H-2* loci control individual traits or groups of traits. Some twenty-eight *H-2* mutations have been identified: sixteen occur in the *K* region, three in the *D* region (*H-2D* and *H-2L*), and nine have not been precisely mapped. These mutations were detected by tissue allografting such as the reciprocal circle system of Bailey and Kohn (Figure 6–2). Tail-skin grafts are exchanged among groups of ten to twenty mice so each mouse receives and donates two grafts. Three types of mutations in antigenic determinants, *gain, loss,* and *gain-loss* mutants, can be distinguished by the pattern of graft rejection observed. F_1 hybrid mice are generally used to facilitate detection of loss-type mutations; because *H* genes are codominantly expressed, loss-type mutants can be detected at loci only in the heterozygous state. Once a putative mutant is identified, the genetic nature is established by a progeny test. If the *H* gene mutation is found by histogenic tests to be transmitted to progeny, mice carrying the mutation are mated *inter se,* and a new line homozygous for the mutation is established from their offspring. Because mutation at any of numerous *H* loci may lead to skin graft rejection, a linkage test must be done to determine if the mutation occurred in the *H-2* complex. Alternatively, a complementation test can be performed. In this case, one produces F_1 hybrids between the mutant and various intra-*H-2* recombinant strains. These mice are tested for their ability to reject skin from the parent strain of the mutant. If *H-2* genes complement the mutation, the grafts are not rejected.

FIGURE 6–2. The reciprocal circle system for detecting mutations at *H* loci. (a) Tail-skin grafts are exchanged among small groups of mice so that each mouse donates and receives two grafts. (b) Three types of mutations *gain* (addition of a new antigen), *loss* (loss of a determinant), or *gain-loss* (addition of a determinant and loss of another) mutants can be distinguished by the pattern of graft rejection. ○ graft accepted. ● graft rejected.

Source: Reproduced from Bailey and Kohn, *Genet. Res.* 6:330–340, 1965, with permission of Cambridge University Press.



By selecting for complementation analysis those recombinant strains that share distinct parts of the *H-2* complex with the parental strain of the mutant, the precise location of the mutation can be mapped.

Most of the *H* gene mutants detected have been of the gain or gain-loss type. Preliminary biochemical data of three *H-2* mutants suggests that these mutants may be the result of a single point mutation. One mutation $H\text{-}2^{dml}$ was induced by an alkylating agent, diethyl sulfate; $H\text{-}2^{dml}$ as shown by peptide maps comparing mutant and normal molecules is clearly of a complex nature and must have at least six amino acid changes. It is possible that diethyl sulfate induced a transversion leading to a frame-shift mutation. Another possibility is that $H\text{-}2^{dml}$ gene is a fusion of portions of two closely linked genes (i.e., *H-2D* and *H-2L*) caused by unequal crossing over.

Current estimates of mutation rates at *H-2* loci are based on limited sampling because of the difficulty of detection methods. Yet the mutation rate of *H-2* genes appears remarkably high compared to rates observed for most visible recessive or dominant mutations in mice. Spontaneous *H* gene mutations at the $H\text{-}2K^b$ allele occur at a rate of about 2×10^{-4} per locus per gamete compared to only $0.4\text{–}4.0 \times 10^{-6}$/locus/gamete for most mouse mutations. Other *H-2* alleles have more modest mutation rates ($2 \text{ to } 5 \times 10^{-5}$/locus/gamete). The relatively high mutation rates for *H-2* loci may have been detected because the techniques used picked up more mutations than those employed to identify mutants at other mammalian loci. Or a high mutation rate may be a special feature of *H-2* genes related to their considerable polymorphism, which could be favored by natural selection. There may be special mechanisms that allow *H-2* genes to be hypermutable or to be influenced by mutator genes which thereby generate and maintain *H-2* polymorphism.

Functional studies with *H-2* mutants have revealed that single, constituent loci control numerous immunologic traits. For example, alloincompatibility caused by mutation at *H-2K* can evoke allograft rejection, MLR and GVH proliferative responses, CML and GVH disease reactions, and alloantibody production (Table 6–1). Serological analysis of *H-2* mutants has also confirmed that both private and public specificities are present on the same molecule. As the amino acid sequences of *H-2* mutant gene products become known, a number of intriguing questions about H-2 antigenicity, the functional regions of H-2 molecules, and the mechanisms leading to high mutation rates may be answered.

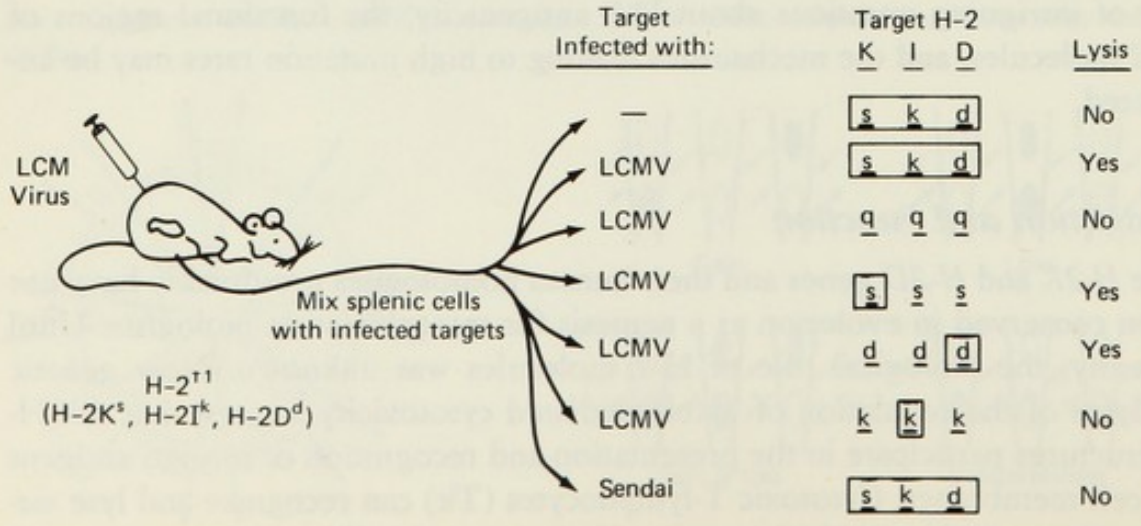
Restriction and Function

The *H-2K* and *H-2D* genes and their human homologues presumably have not been conserved in evolution as a nemesis for transplantation biologists. Until recently, the biological role of H-2 molecules was unknown. Now genetic analyses of the regulation of T-cell-mediated cytotoxicity suggest that self-H-2 structures participate in the presentation and recognition of foreign antigens on cell membranes. Cytotoxic T-lymphocytes (Tk) can recognize and lyse target cells expressing viral antigens, moderate non-*H-2* alloantigens, or coupled

haptens, but for a high level of killing to occur, the target cells must express both the immunizing antigen and the same H-2K or H-2D antigens of the lymphocyte donor strain. An example of such "restriction" of T_K-cell activity is illustrated in Figure 6–3. Immune T-cells from mice infected with lymphochoriomeningitis virus (LCMV) or other viruses kill infected target cells in vitro having the same *H-2* haplotype but not infected cells from H-2 disparate strains. Identity at *H-2K* or *H-2D* but not *H-2I* is sufficient for cytolysis to occur. The killing appears specific for self-H-2K or H-2D plus virus since uninfected cells in culture or targets infected with unrelated viruses are not killed. *H-2* heterozygous immunized mice will kill infected targets from either parent strain. Further studies with $(H\text{-}2^k \times H\text{-}2^b)F_1$ mice indicate that *H-2* heterozygotes have at least four populations of T_K specific for $H\text{-}2K^k$ virus, $H\text{-}2D^k$ virus, $H\text{-}2K^b$ virus, and $H\text{-}2D^b$ virus, respectively. Experiments with *H-2* mutants have shown that the same genes coding for H-2K or H-2D transplantation antigens code for the H-2 component involved in the T-cell-virus-infected target interaction. Note that the recognition inherent in this restricted cytotoxicity applies to primed or secondary responses, but not to initial or primary responsiveness. The source of antigen and the interval between priming and challenge often determine whether a specific immune response is *MIC* restricted or unrestricted.

Two models with several variations have been proposed to account for the requirement for H-2 compatibility: "single recognition" and "dual recognition" (Figure 6–4). The single recognition model assumes that cytotoxic T-cells express one receptor site which either recognizes a complex between self-H-2 (S) and the foreign antigenic determinant (X) or recognizes self-H-2 only

FIGURE 6–3. *H–2* restricted T-cell-mediated cytotoxicity toward lymphochoriomeningitis virus (LCMV) of infected target cells. $H\text{–}2^{tl}$ recombinant mice ($H\text{–}2K^s$, $H\text{–}2I^k$, $H\text{–}2D^d$) inoculated with LCM virus develop T-cells which lyse LCMV-infected syngeneic target cells. Compatibility at *H–2K* or *H–2D* alone is sufficient for cytolysis to occur; *H–2* incompatible or *I* region compatible cells are not killed. The killing in this instance is specific for virus plus self-H–2K or H–2D. Uninfected targets or syngeneic cells infected with other viruses are not killed.



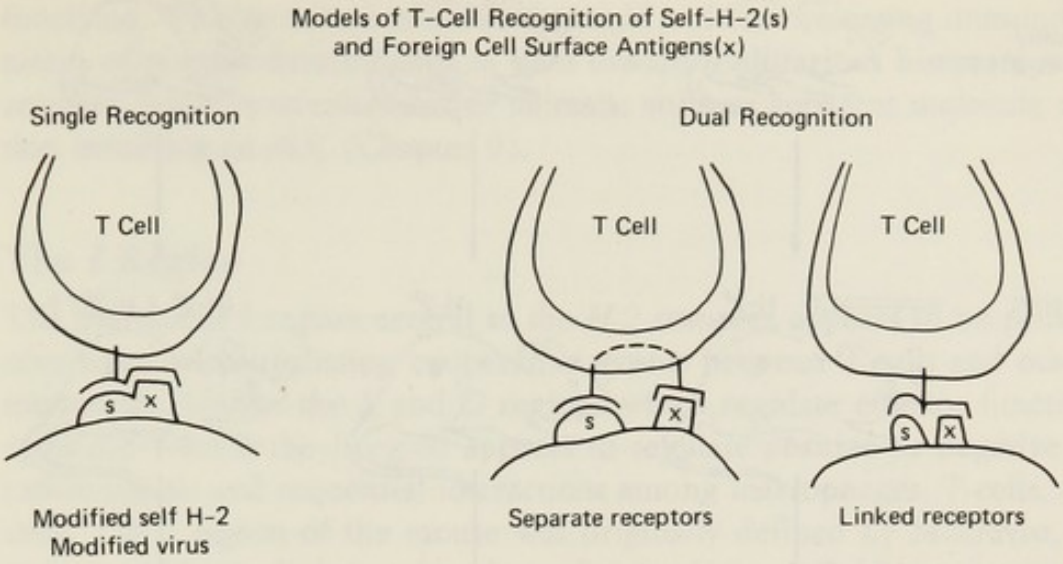


FIGURE 6–4. Models of T-cell recognition of self-H–2 (S) and foreign cell-surface antigens (X).

after it has been altered somehow by viral infection. Single T-cells would not interact with either S or X alone but only with new antigenic determinants formed upon interaction of X with S. According to the alternative model, T-cells have two receptors, one for self-H-2 and one for X. The receptors may be on one or two molecules but are coded for by distinct genes. Either model is conjectural because the array of receptors on cytotoxic T-cells is unknown. T-cells do express idiotypes that cross-react with B-cell idiotypes, therefore at least a portion of T-cell receptors probably contain variable regions similar to those of immunoglobulin.

The dual recognition concept predicts that the specificity of T-cells for self is selected independently of antigen. The single recognition model supposes that the development of restriction should be antigen-dependent because the complex of S and X is recognized together. To examine whether T-cells are restricted in their specificity prior to contact with antigen or at the level of antigen presentation, chimeric animals with bone marrow, thymus, peripheral lymphocytes, or macrophages of different *H-2* origin have been constructed. After inoculation with virus, they are then tested for their ability to lyse infected targets of different H-2 types. When irradiated A or B parental mice are reconstituted with immature (A × B)F1 bone marrow cells *(F1→P chimeras),* the T_K that develop can only kill infected targets of host parental cell phenotype (i.e., A or B, not both). This restriction is maintained even when mature T-cells from this chimera are transferred into secondary (A × B)F1 hosts. Normal F1 bone marrow cells maturing in F1 animals in contrast can lyse both infected A and B targets. The host environment in which T-cells mature, therefore, dictates the restricted specificity. This does not appear to reflect active suppression but rather results from a process termed "adaptive differentiation" which is known to be thymus dependent.

The effect of thymus *H-2* genotype on the restriction of cytotoxic T-cell

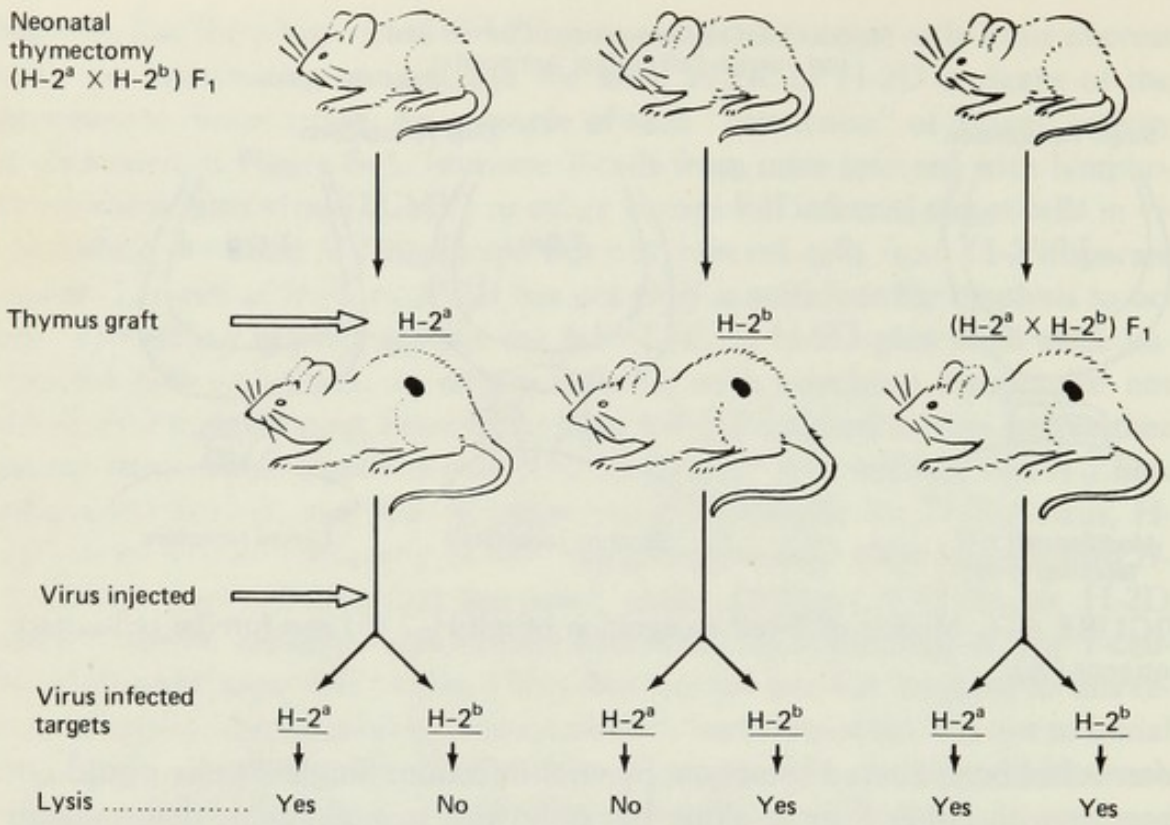


FIGURE 6–5. The *H–2* genotype of the thymus dictates the restriction specificity of cytotoxic T (T_k) cells. ($H\text{–}2^a \times H\text{–}2^b$) F1 mice were thymectomized when neonates and were reconstituted with thymus transplants from either $H\text{–}2^a$, $H\text{–}2^b$, or $H\text{–}2^{a/b}$ donors. These mice were then inoculated with virus and their T_k-cells were subsequently tested for their ability to kill virus-infected $H\text{–}2^a$ or $H\text{–}2^b$ target cells. The specificity of T_k-cell killing corresponded to the H–2 type of the original thymocyte donor.

specificity is outlined in Figure 6–5. If newborn ($H\text{-}2^a \times H\text{-}2^b$)F1 mice are thymectomized and given a compatible neonatal $H\text{-}2^a$, $H\text{-}2^b$, or F1 hybrid thymus, the cytotoxic T-cells which develop acquire the corresponding H-2 specificity of the thymus donor. Thus, the restriction specificity or cytotoxic T-cells is dictated by somatic events occurring during maturation in the thymus. How the thymus affects anti-self (S) specificity is not known. Appropriate clones could be selected for, or "forbidden" clones deleted, or the recognition of self could be learned during differentiation in the thymus. Jerne has proposed that the generation of diversity and selection of the anti-X specificity toward foreign determinants also occurs in the thymus. Whether or not concurrent somatic mutation and/or selection of germ-line genes in the thymus functions to generate anti-X diversity is as yet an unanswered question.

Any model of the generation of diversity of T-cell receptors must account for the very high levels of T-cells (at least 5 percent) which can react with foreign H-2 alloantigens. Current models do not account for T-cell-mediated cytotoxicity that is not H-2 restricted, for example, against *I*-region or *Qa*-region associated antigens. Nor do they explain why athymic nude mice, after reconstitution with allogeneic thymus grafts, display a number of T-cell-dependent

functions. We also call attention to the rapid and discriminating immunorecognition of nonself determinants in vivo toward multifarious histocompatibility antigens, even in so-called lower animals, without apparent molecule restriction involving an *MIC* (Chapter 9).

The *I* Region

The multilocus *I* region central to the *H-2* complex appears to be principally concerned with mediating cooperative events between T-cells and other immunocytes. Unlike the *K* and *D* regions which regulate effector functions of cytotoxic T-cells, the *I* region appears to regulate positive or negative maturation signals and sequential interactions among macrophages, T-cells, and B-cells. The *I* region of the mouse was originally defined by McDevitt, Snell, and co-workers. Using a number of intra-*H-2* recombinant strains, they mapped the *Ir-1* locus, which controls immune responsiveness to several synthetic polypeptides (Chapter 7), to a region between the *H-2K* and *H-2S* regions (Table 6–4). The region was designated *I* for immune response with *Ir-1* as its marker locus. Soon it became apparent that more than one locus in the *I* region controlled levels of antibody responses to various antigens. Based on differential responsiveness of intra-*H-2* recombinants to IgA and IgG myeloma proteins, a new locus distinct from *Ir-1* was soon distinguished and the *I* region was subdivided into two *subregions, I-A* and *I-B.* Subsequently, three additional subregions *I-J, I-E,* and *I-C,* were defined using serologic and biochemical methods. Approximately twenty traits have already been reported to be controlled by the *I* region, some of which are listed in Table 6–1. We will not describe all of these phenotypes, but rather will select several key phenotypes under known gene control which provide some clues to I region functions.

TABLE 6–4. Mapping the *H-2I* Region with Intra-*H-2* Recombinants[a]

	H-2 region				
	K	*I*	*S*	*D*	Response to
H-2 haplotype		*Ir-1*			(H,G)-A—L
a	*a*	*a*	*a*	*a*	high
b	*b*	*b*	*b*	*b*	low
b4 (a/b)	*a*	*a*	*b*	*b*	high
a	a	a	a	a	high
q	q	q	q	q	low
Yl (q/a)	q	a	a	a	high

Based on McDivitt et al., *J. Exper. Med.* 135: 1269, 1972. © The Rockefeller University Press.

[a]Intra-*H-2* recombinant strains were typed as either high or low responders to synthetic polypeptide chains such as (H-G)-A—L and assigned alleles for *Ir-1* based on responses in parental haplotypes. The *b4* recombinant defined one boundary of the *I* region indicating *Ir-1* was centromeric to the *S* region. Two recombinants including *yl* separated *Ir-1* from *H-2K* and defined the other boundary.

Alloreactivity

Allogeneic disparities across the *I* region evoke strong macrophage-dependent proliferation by T-cells demonstrable by MLC reactivity (see Figure 5–4). Because this reaction is so pronounced, it was initially thought that *I* region gene products were solely responsible for producing MLC and in vivo graft-versus-host responses. However, it is now clear primarily from studies using *H-2K* and *H-2D* mutants that *K* and *D* region products can also evoke T-cell proliferation. The cells responding to *I* region products are principally Lytl+ cells (see below), which suggests they may be related or identical to helper T-cells.

I region incompatibilities can promote both antibody and cytotoxic T-cell responses. Although strong T-cell proliferative responses are evoked by *I* region disparities, very weak cytotoxic responses are usually generated. *D* region incompatibility provokes some cytotoxicity. T-cells responding concurrently to *I* and *D* region products present on different cells develop pronounced CML reactions. In essence, the *I-A* region codes for alloantigens or "allogeneic effect" factors which can augment cytotoxic T-cell responses to H-2K and H-2D antigens or T-cell-dependent antibody responses without benefit of linked or dual recognition. In contrast, the *I-C* subregion regulates factors that often suppress alloreactivity. Thus, different *I* regions gene products can activate T-cells and factors which can either augment or suppress immune responses.

Ia Antigens

Identification of *Ir* genes in the *I* region motivated efforts to define *I* region products serologically. Several laboratories simultaneously noted that reciprocal antiserums raised in strains which differed in the *I* region contained antibodies capable of killing subpopulations of splenic lymphoid cells. The percentage of cells killed was often as low as 15 percent above control levels and, therefore, sometimes not readily noticeable. Because the alloantigens were detected by antiserum against *I* region incompatibilities, they were called Ia (*I*-region-*a*ssociated) antigens. Amino acid sequence or peptide differences associated with a particular haplotype have been observed in Ia polypeptides from various *H-2* congenic strains. This implies that the structural genes that control synthesis of the Ia antigens actually map within the *I* region.

Over thirty Ia specificities had already been identified within five years after their discovery, and there is every indication that some *H-2I* loci will turn out to be as complex and polymorphic as the *H-2K* and *H-2D* loci (Table 6–5). This polymorphism is particularly evident for *I-A* subregion genes. As with H-2K and H-2D antigens, both public and private specificities can be distinguished: Ia.2 is found only in $I\text{-}A^k$ strains, whereas the public antigen Ia.3 is expressed on gene products of a number of *I-A* alleles. Most private and public specificities have been mapped to a specific subregion, but as with K and D determinants, certain Ia specificities coded by different subregions have not yet been detected serologically. For example, Ia.7, associated with the *E/C* region, has not been serologically defined in $H\text{-}2^b$, $H\text{-}2^q$, and $H\text{-}2^s$ mice.

TABLE 6–5. An Abbreviated Ia Chart

H-2 Haplotype	Strain	Region	Private specificities								Public specificities																		
			2	4	11	14	20	21	22	23	1	3	5	6	7	8	9	10	12	13	15	16	17	18	19	24	W25	W29	W30
			IA	*IA*	*IA*	?	*IA*	*IE*	*IE*	*IE*	*IA*	*IA*	*IA?*	*IC*	*IE/C*	*IA*	*IA*	?	?	?	*IA*	?	*IA*	*IA*	*IA*	?	*IE?*	*IA*	*IA*
b	C57BL/10		—	—	—	—	20[a]	—	—	—	—	3	—	—	—	8	9	—	—	—	15	—	—	—	—	—	—	W29	W30
d	B10.D2		—	—	11	—	—	—	—	23[a]	—	—	—	6	7	8	—	—	—	—	15	16	—	—	—	—	—		W30
f	B10.M		—	—	—	14[a]	—	—	—	—	1	—	5	—	—	—	—	—	—	—	—	—	17	18	—	—	W25	W29	W30
k	B10.BR		2	—	—	—	—	—	22[a]	—	1	3	—	—	7	—	—	—	—	—	15	—	17	18	19	—	W25	—	—
p	B10.P		—	—	—	—	—	21[a]	—	—	—	—	5	6	7	—	—	—	—	13	—	—	—	—	—	—	?	?	?
q	B10.Q		—	—	—	—	—	—	—	—	—	3	5	—	—	—	9	10	—	13	—	16	—	—	—	—	—	—	W30
r	B10.RIII		—	—	—	—	—	—	—	—	1	3	5	—	7	—	—	—	12	—	—	—	17	—	19	24	W25	W29	—
s	B10.S		—	4	—	—	—	—	—	—	—	—	5	—	—	—	9	—	12	—	—	—	17	18	—	24	—	W29	W30
RECOMBINANTS																													
a	B10.A *(k/d)*		2	—	—	—	—	—	22	—	1	3	—	6	7	—	—	—	—	—	15	—	17	18	19	—	W25	—	—
q2	D2.GD *(d/b)*		—	—	11	—	—	—	—	23	—	—	—	—	—	8	—	—	—	—	15	16	—	—	—	—	?	?	?
b2	B10.A(4R) *(a/b)*		2	—	—	—	—	—	—	—	1	3	—	—	—	—	—	—	—	—	15	—	17	18	19	—	—	—	—
i5	B10.A(5R) *(b/a)*		—	—	—	—	20	—	22	—	—	3	—	6	7	8	9	—	—	—	15	—	—	—	—	—	W25	W29	W30
i4	B10.S(9R) *(s/a)*		—	4	—	—	—	—	22	—	—	—	5	6	7	—	9	—	12	—	—	—	17	18	—	?	?	?	?
y2	B10.T(6R) *(q/k)*		—	—	—	—	—	—	—	—	—	3	5	—	—	—	9	10	—	13	—	16	—	—	—	?	?	?	?

[a]Not tested on some parental haplotypes.

The loci within the *I* subregions coding for Ia antigens are tentatively called *Ia-1, 2, 4, 5,* and *3* corresponding in sequence to the A, B, J, E, and C subregions (Table 6–1). Most of the Ia determinants characterized thus far have mapped to the *I-A* subregion (Table 6–5). No Ia antigens have been definitively assigned to the *I-B* subregion, which is defined by *Ir-IB* immune response genes. The *Ia-3* and *Ir-IC* loci serve to define the *I-C* subregion. The precise relationship between *I-C* and *I-E* subregion specificities awaits detailed characterization of new recombinants and further biochemical studies. The *I-J* subregion now associated with the *Ia-4* locus was mapped by testing the ability of cells from selected intra-*H-2* recombinants to absorb out antibodies specific for suppressor T-cells. Two recombinant strains B10.A(3R) and B10.A(5R), previously indistinguishable in their *H-2* genotypes, showed differential activity. Other congenic and recombinant strains have revealed allelism or distinctive loci at each of the five *I* subregions as determined by *Ir* gene effects (see Table 5–1).

Unlike H-2K and H-2D molecules which appear to be present on all somatic cells, Ia antigens have an intriguing restricted tissue distribution. They are present on about 90 percent of mature B-cells and 10 to 15 percent of peritoneal macrophages. They are also found on minor populations of T-lymphocytes, notably T-cells activated by the mitogen concanavalin A. Ia antigens are also expressed on fetal liver cells, thymic epithelium, sperm, and epidermal cells. However, Ia determinants are not readily detectable on erythrocytes, platelets, fibroblasts, or brain, kidney, lung, or muscle tissue. The evidence revealing Ia molecules on nonlymphoid cells requires qualification of theories suggesting an exclusive role of Ia molecules in regulating immune responses. Preliminary evidence suggests each subregion may code for Ia antigens expressed on distinct subpopulations of lymphoid cells. For example, some *I-A* genes may code for Ia molecules expressed on B-cells, T-cells, and macrophages; certain *I-J* genes code for Ia molecules found on suppressor T-cells; *I-E/C* gene products appear to be expressed on both T- and B-cells. The lack of Ia antigens on erythrocytes and platelets has made them useful for absorbing out unwanted H-2 antibodies while preparing anti-Ia serums.

Ia molecules are integral, external cell-surface glycoproteins (Figure 6–1). They have two subunits of approximately 35,000 daltons (the α subunit) and 28,000 daltons (the β subunit). The subunits are noncovalently associated in the mouse, but in some other species they may be covalently bound. Ia molecules move about the membrane independently from H-2K and H-2D molecules and do not associate with β_2-microglobulin. It is not clear if Ia antibodies are directed against determinants on the α or β subunits or against configurations resulting from interactions of the two chains. Removal of carbohydrate from Ia molecules does not affect the binding of most Ia specificities. Only limited amino acid sequence data of Ia antigens are currently available, but preliminary evidence indicates that Ia molecules show no homology with H-2K or H-2D antigens. The α subunits of various alleles in general are more similar than β subunit alleles. However, the α and β subunits coded

by distinct subregions are quite different; for example, the β subunits coded by $I\text{-}A^k$ and $I\text{-}A^r$ have very few peptide differences, whereas the β chains coded by *I-A* or *I-C* subregions are structurally diverse. The N terminal amino acid sequence of the α chain of the *I-E* subregion shows striking homology with its putative human counterpart encoded by *HLA-D* region genes, and $I\text{-}E_\beta$ polypeptides also show some homology to human and guinea pig β subunits. This suggests that these genes have retained similar functions in mammalian evolution.

The E/C α and β subunits are evidently coded by two different *I* subregion loci, one in *I-E/C* and the other in *I-A*, according to recent studies. Immunoprecipitates with antiserums specific for E/C were examined using two-dimensional gel analysis, and two distinct polypeptides, α and β, were revealed. Further gel and peptide analyses of E/C antigens from intra-*H-2* recombinants has demonstrated that the β subunit is controlled by a locus outside *I-E/C* probably in *I-A*.

Functions of Ia Molecules

The first indications that I region molecules are instrumental in regulating cell interactions between lymphoid subsets came from studies examining the ability of histoincompatible T-cells, B-cells, and macrophages to cooperate in T-cell or antibody responses. In general, T-cells were shown not to be able to cooperate with *H-2* incompatible B-cells and macrophagelike cells to yield antibody responses. The site of this restriction has been mapped to the *I-A* subregion. Paul, Rosenthal, Schwartz, and Shevach at the U.S. National Institutes of Health have reported evidence suggesting that Ia molecules may be *Ir* gene products which affect presentation of antigens by macrophages to T-cells and perhaps to B-cells. The proliferative response of T-lymphocytes from peritoneal exudates of immunized mice to antigens engulfed, processed, and "presented" by macrophagelike cells was measured. The interaction between antigen-presenting cells and primed T-lymphocytes leading to a proliferative response required identity of the two cells at the *I-A* subregion.

In addition, the donor of the antigen-presenting macrophages must have a responder allele for the *Ir* gene controlling response levels to the antigen. The need for genetic identity at *I-A* indicates that T-cells recognize both the antigen and *I* region gene product on the antigen-presenting cell. Whether this is done by complex multiple receptors or by some form of dual recognition is unknown (see Figure 6–4). It is quite possible that the I region products involved are Ia molecules, because Ia antibodies specifically block the T-cell proliferation. The Ia molecules may play an active role in orienting and presenting antigens, and this property may be related to *Ir* gene function (Chapter 7). Ia antigens may even be *Ir* gene products that act as receptors to bind and orient antigen fragments. Another possibility is that *I* region products, perhaps Ia antigens, code for a portion of the T-cell receptor(s) that recognizes antigen and/or self-molecules. This view is supported by studies showing that antigen-

specific and nonspecific T-cell factors bear both Ia antigens and V_H-gene-associated idiotypes. Alternatively, Ia molecules could be part of the T-cell signal that triggers lymphocyte subsets rather than the region involved in antigen presentation and recognition.

Virus-specific cytotoxic T-cells appear to be specifically activated by *H-2K*- or *H-2D*-coded self-markers; in contrast, T-cells that contribute to delayed type hypersensitivity responses or protect against intracellular bacteria such as *Listeria* are predominantly activated or turned on by *H-2I* molecules. Zinkernagel has suggested that this dichotomy may have been selected for in evolution to deter infections by intracellular parasites. According to this theory, K and D antigens are expressed on all cells as receptors for cytolytic signals to destroy ubiquitous pathogens such as viruses. Indeed, the K and D molecules themselves could be the weak point in cells where lysis may be mediated, thereby curtailing further viral replication. The more selectively expressed I-coded molecules could be receptors for cell-specific differentiation signals which promote T-cell recruitment and macrophage-mediated destructive activities. Thus, Ia antigens appear to play a key role in immunocyte signalling and in interactions between lymphocyte subsets. Each step in the generation of the immune response requiring cell-cell interactions may be under *I* region control. Regulatory *Ir* genes are likely to be acting at several levels including presentation of antigen by macrophages, response of T-cells to antigen, and signalling of B-cells and regulatory T-cell subsets.

The *S* Region

The *S* region is defined by two markers, *Ss* and *Slp,* which have not been separated by recombination and may in fact be one complex locus. The *Ss* (*s*erum *s*erological substance) locus determines the amount in serum of a beta-globulin, now shown to be the C4 component of the murine complement system. Levels of serum Ss are detected by rabbit antiserum. Inbred strains have either high (Ss-H) or low (Ss-L) levels of this serum protein. The *Slp*-alloantigen marker (*s*ex-*l*imited *p*rotein) defines an allotypic variant on a subclass of Ss protein. The Ss and Slp antigens though cross-reactive are not identical, as shown by absorption analysis. However, not all Ss molecules in strains expressing Slp bear the Slp allotype. Biochemical analyses of radiolabeled Ss proteins synthesized by peritoneal macrophages have shown that Ss produced by Slp-negative strains has a molecular weight of about 180,000 to 200,000 daltons with three constituent polypeptides α, β, and γ of 93,000, 75,000, and 33,500. Slp-positive molecules also consist of three chains, but with molecular weights of 105,500, 75,000, and 32,000. It is not clear if these differences in polypeptide sizes, particularly in the Ss and Slp α subunits, are attributable to structural gene differences or to variation in the post-translational processing of the subunits. Strains vary considerably in quantitative expression of Slp allotype, and these differences along with Ss protein levels are used to define *Ss* haplotypes (Table 6–6). There are at least seven known *Ss* alleles; thus, the *S* region, like other *H-2* regions is remarkably polymorphic.

Ss was found to be linked to *H-2* by segregation analysis; all recombinants between *H-2* and *Ss* were found to be intra-*H-2* crossovers. Ss typing of intra-*H-2* recombinants has shown *Ss* to be near the middle of the *H-2* complex (Figure 5–2 and Table 6–1). This has made *Ss* a crucial marker for precisely locating *H-2* genes and for establishing that two separate loci, *H-2K* and *H-2D,* were responsible for the complex of specificities of "classic" H-2 antigens. No *H* genes or *Ir* genes have yet been found in the *S* region (Table 6–1). However, the actual response to Slp antigen is controlled by *H-2*-linked *Ir* genes. The expression of *Slp* and to a lesser degree the amount of serum Ss are under hormonal control. Levels of Ss are greater in males than in females, but females treated with testosterone develop the same levels of Ss as normal males. The sex-limited expression of *Slp* is unusual in that only males express this antigen and then only after sexual maturity. Females express Slp only if they are injected with testosterone. The hormone appears to be required for synthesis and maintenance of Slp. Further evidence for hormonal control comes from studies using the X-linked *tfm* (testicular feminization) mutation. The *tfm* mutant blocks expression of androgen-regulated traits and renders mice unresponsive to testosterone. Slp expression is also affected by *tfm:* Ss^d normal males usually express Slp. However, $Ss^{d/d}$-*tfm*/Y males do not. Therefore, the presence of *tfm* prevents Slp allotype production. The expression of Slp is not sex-limited in all *Ss* alleles. Both male and female B10.WR7 mice with Ss^w (an allele first isolated in a female wild mouse) express Slp. In this case the Slp produced has an α subunit of 98,000 daltons and is not under hormonal control; it is present in immature mice not secreting androgens, and is not affected by the *tfm* mutation.

Hansen and Shreffler have suggested complex regulation of Slp controlled by ongoing operator-regulator interactions (Figure 6–6). According to this hypothesis, *Ss* codes for an operator (O), which activates the structural gene coding for the Slp-bearing protein. A repressor (R), coded for by the wild-type of the *tfm* locus, can bind O and thereby block Slp synthesis. However, in males, an inducer (I) testosterone is made which blocks the repressor and allows Slp to be made (Figure 6–6a). Because females lack testosterone, O is bound by the repressor and Slp is not produced (Figure 6–6b). The *tfm* mu-

TABLE 6–6. Properties of Seven Ss Alleles

Ss allele	Type strain (Males)	Relative Ss level	Relative Slp level
Ss^w	B10.WR7	1.12	1.51
Ss^d	B10.A	0.90	0.68
Ss^s	B10.S	0.84	0.18
Ss^p	B10.P	0.73	0.03
Ss^{bf}	B10.M	0.91	0
Ss^b	B10	0.65	0
Ss^k	B10.K	0.06	0

From Hansen and Shreffler, *J. Immunol.* 117:1511, 1976. © 1976 Williams and Wilkins Co., Baltimore, MD.

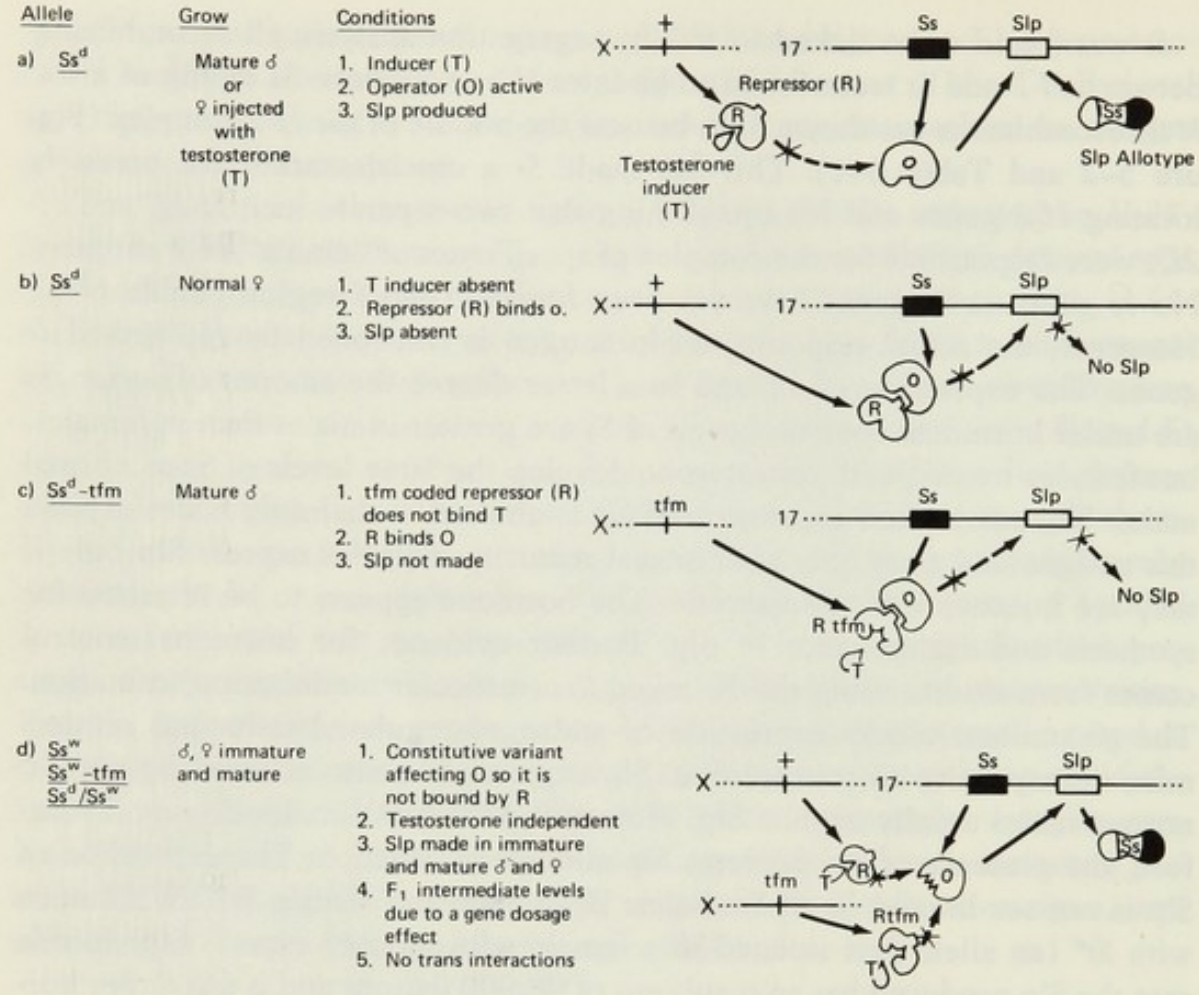


FIGURE 6–6. An operon model for control of expression of the Slp allotype.

Source: Based on Hansen and Shreffler, *J. Immunol.* 117:1507–1513, 1976. © The Williams and Wilkins Co., Baltimore, MD.

tation alters the repressor so it is not bound properly by testosterone; this in turn leads to inactivation of the operator by R. The Ss^w allele is most likely not sex-limited because it is a *constitutive* variant that precludes inactivation of O. This could explain why Slp production in Ss^w mice is hormone-independent and occurs regardless of whether the normal or mutant *tfm* repressor is made. Quantitative differences in Slp levels could reflect *Ss* allelic differences in operator-repressor or operator-structural gene interactions. This model is attractive because it accounts for the observed phenomena and is one of the first definitive models for an operon in eukaryotes. Other explanations for Slp regulation are possible, of course; for example, the actual function of the *tfm* gene product is unknown and thus speculative. Why a serum component of complement and an allotypic variant are under hormonal control remains a mystery.

Other *H-2* Regions and Phenotypes

The chromosomal segment between *Ss* and *H-2D* designated the *G* region comprises about half of the *H-2* complex. It is relatively unexplored territory,

fertile ground for pioneers. Though several loci have been linked to this end of the *H-2* complex, only one marker locus, *H-2G,* appears unique among *H-2* genes in that its H-2.7 antigenic product is more abundant on erythrocytes than on lymphocytes. The precise location of many *S–D* region recombinants remains unclear for want of a more polymorphic marker. Loci determining weak MLC responses have been detected near the *G* region (Table 6–1). Recently, a locus designated *H-2R* (for rosetting) has been mapped between the *G* and *D* regions; it controls the ability of splenic lymphocytes to form rosettes with syngeneic erythrocytes. B- and T-cells bind only erythrocytes that are compatible at this genetic region.

H-2-linked loci have been implicated in the control of a diverse array of phenotypes. *H-2*-linked genes control natural cytotoxicity and hybrid tumor resistance (Chapter 5), the expression of complement receptors, and even levels of testosterone. Components of aging in mice are affected by *H-2* haplotype; *H-2* congenic resistant strains differ significantly in their life spans (see Table 8–7). One of the most curious observations of *H-2* control concerns the regulation of what Lewis Thomas and his colleagues have termed "mating preference." If a male mouse of one *H-2* haplotype is caged with a syngeneic female and an *H-2* congenic female, both in estrus, the male will consistently prefer one or the other *H-2* haplotype. If a male selects an *H-2* incompatible female for mating, in subsequent matings he consistently displays a preference for *H-2* disparate females. The choice is made by the male and not the female. Such mating preferences could increase the incidence of particular *H-2* haplotypes or serve to maintain *H-2* heterozygosity in natural populations. Thus, the array of distinctive traits controlled by the *MIC* is almost as extensive as the degree of *MIC* polymorphism.

THE HLA COMPLEX IN MAN

HLA Genetics

The *HLA* major immunogene complex on chromosome 6 in humans shares a number of features with the mouse *H-2* complex. But because humans are an outbred population, different approaches have been necessary to characterize *HLA* gene products. Serologic analyses in particular have been made possible by numerous international collaborations and workshops, application of computer technology, and standardization of serotyping assays and reagents. Antiserums for HLA typing are usually obtained from multiparous women who have developed antibodies to HLA antigens on fetal cells or from persons who have received blood transfusions. By testing blood lymphocytes from numerous sources with many antiserums, and by absorption analyses, nearly monospecific antiserums have been selected for routine typing.

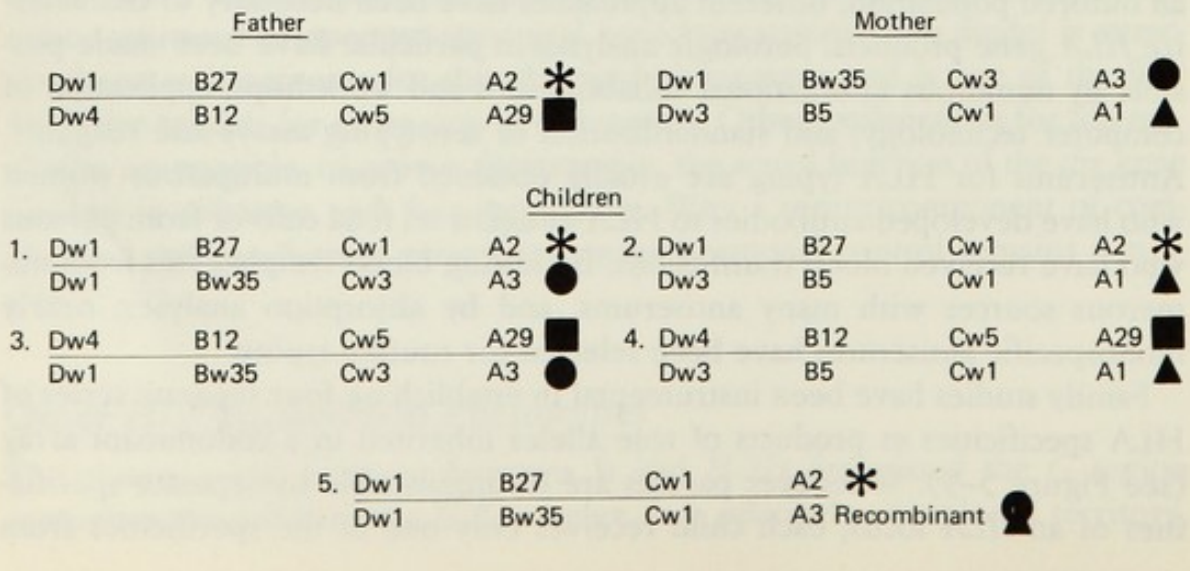
Family studies have been instrumental in establishing four separate series of HLA specificities as products of true alleles inherited in a codominant array (see Figure 5–9). Whenever parents are distinguishable by separate specificities of an *HLA* locus, each child receives only one of the specificities from

each parent. With rare exceptions, any given family has no more than four different types of children (Figure 6–7). This fact is important for clinical transplantation because it means the chance of finding an identical HLA match among siblings is only 25 percent. Rare recombinants in families helped to establish that *HLA-A,B,C, and D* loci are distinct. As yet only one putative recombinant between *DR* and *D* has been detected.

The cell membrane molecules determined by *HLA* fall into two general classes. The HLA-A, B, and C molecules, like the H-2K and H-2D molecules, are transmembrane structures consisting of one variable glycosylated polypeptide chain of about 43,000 daltons noncovalently linked to a constant β_2-microglobulin chain of about 12,000 daltons (Figure 6–1). The gene coding for β_2-microglobulin is located on chromosome 15, far removed from the *MIC.* Thus, the subunits of HLA-A, B, and C antigens, like other proteins composed of different subunits (e.g., hemoglobin, immunoglobulin), are coded by unlinked genes. These antigens are expressed on the surface of almost all cells, though weakly on erythrocytes and spermatozoa. Each molecule carries both public and private specificities. The HLA-DRw(D) molecules are analogous to the mouse Ia antigens. They have a similarly restricted tissue distribution and biochemical structure. It is not yet clear if the DRw and D specificities are coded for by identical or tightly linked genes.

Serologic definition of HLA specificities is complicated by frequent cross-reactions between a number of allelic products of each of the two loci *HLA-A* and *HLA-B.* The cross-reactive specificities at first glance suggest H-2 type "public" specificities. However, few determinants appear to be shared between HLA-A and HLA-B molecules. HLA intralocus "cross-reactions" frequently display asymmetry; that is, they are completely or predominantly unilateral. For example, anti-A11 activity is completely absorbed by A3-bearing cells, but A3 antibodies are only partially removed by A11 cells.

FIGURE 6–7. Inheritance of *HLA* haplotypes. Only four *HLA* genotypes are observed in offspring unless a recombination or mutation occurs (Child 5). Symbols *, ■, ●, ▲ serve to mark different haplotypes.



Cell-line variants lacking certain HLA antigens have been isolated by Pious and co-workers using a method termed *immunoselection.* Cells are treated with a specific corresponding antiserum, such as anti-HLA-A2, or anti-*DRwl,* plus complement. Nearly all the cells are killed by this treatment, but occasionally spontaneously arising variants, which do not express the HLA antigen, survive and can be isolated. Based on several criteria, many such variants appear to be due to loss mutations rather than epigenetic changes or mitotic crossing over. The frequency of the spontaneous loss mutation rate of one HLA antigen, B27, has been estimated to be 8×10^{-7}/cell/generation. HLA-A2 loss variants concomitantly lose the cross-reactive HLA-A28 specificity, which suggests that the A2 and A28 cross-reactive determinants are on the same molecule.

The distribution of HLA antigens has been measured in large populations of unrelated individuals in order to establish phenotype and gene frequencies. If one assumes the test population is in Hardy-Weinberg equilibrium, the gene frequency can be estimated by the formula $g = 1 - \sqrt{1-f}$, where g is the gene frequency and f is the phenotype frequency as a fraction of one. The gene frequencies of *HLA-A, B, C,* and *DRw* loci vary considerably among different ethnic groups. Some alleles such as *A2* are present at relatively high frequencies in all populations. Other alleles are present in most populations but clearly absent in one or more groups; for example, *A1* and *A3* and *DRw3* are virtually absent from Japanese. Finally, there are certain alleles found exclusively in one race or population, such as *Bw42* in blacks. Because of their extraordinary polymorphism and geographic variations, HLA antigens are particularly useful for anthropological studies. Migration patterns and studies of random genetic drift have been facilitated by HLA typing. For example, a clear *founder effect* has been noted in Guatemalan American Indians among whom 91 percent of the population has the *Bw35* allele. When a newly isolated population is established, its gene pool may soon diverge from that of the parent population because of sampling differences in choice of mates. This founder effect may become further exaggerated because different evolutionary pressures will operate in different environments. The Japanese population, for example, has a low degree of HLA polymorphism compared to African blacks or European Caucasoids.

The HLA antigens belonging to different loci are inherited as a haplotype in coupling. Since the haplotype is thus the unit of inheritance, the distribution of *HLA* genes in a population is often expressed as a group of haplotype frequencies. This, of course, is only possible when the genotypes of individuals can be ascertained by family studies. Analysis of *HLA* haplotype frequencies has revealed that some haplotypes are much more frequent than would be expected on the basis of the gene frequencies of the alleles involved. The tendency for alleles at different linked loci to occur together more often on the same chromosome or haplotype than expected by change is called *linkage disequilibrium.* For example, in one European population, the gene frequency of *A1* is 0.17 and that of *B8* is 0.11; if there were free association between the alleles, the expected frequency of *HLA-A1, B8* haplotypes would be 0.17 $\times$

0.11 = 0.019. In reality, however, the *A1, B8* haplotype frequency is 0.088. The difference (Δ) between the observed and expected frequencies (0.088 − 0.019 = 0.069) is a measure of the extent of linkage disequilibrium. Strong linkage disequilibrium has been observed in haplotype pairs for *HLA-A* and *B, HLA-B,* and *D (DR)* loci and between the closely linked *HLA-B* and *C* loci.

What then might account for this phenomenon? Linkage disequilibrium can exist for *very* closely linked loci in a random mating population even in the absence of natural selection, simply because the low frequency of recombination impedes the establishment of equilibrium. This could be the case for the *HLA-B* and *C* loci. Alternatively, certain alleles may have arisen too recently for equilibrium to have been achieved. However, Bodmer has suggested that the *A* and *B* or *B* and *D* loci are sufficiently distant so some form of natural selection must be operating to maintain linkage disequilibrium. It is tempting to speculate that the overall coordinated functions of the *MIC* are more efficient in certain allelic combinations. Alternatively, some haplotypes may be selected against because they promote increased susceptibility to autoimmune or immunologic diseases. The relation of HLA to certain diseases will be considered in Chapter 8.

MIC Loci in Other Species

A major immunogene complex has been identified in chickens and in every mammalian species examined in detail. Anuran amphibians and teleost fish may also have *MIC* equivalents, but relatively little is known about these systems in lower vertebrates and invertebrates (see Chapter 9). Here we briefly compare MIC-associated traits in several well-studied species (Table 6–7). One is immediately struck by the numerous similarities among the various mammalian *MIC*s and the chicken *MIC.* Extensive polymorphism at *MIC* loci appears to be a general rule. Both major *H* loci and *Ia*-like loci have been found in all species studied in detail. Furthermore, preliminary comparisons of the biochemical properties of these gene products suggest strong homology. The major *H* gene products of man, rhesus monkey, guinea pig, rat, mouse, and chicken all are glycoproteins with two polypeptide moieties of 37,000 to 45,000 and 10,000 to 12,000 daltons. The Ia molecules of man, guinea pig, and mouse are also very similar. *MIC* loci regulating certain complement components (especially C2 and C4) have also been detected in several species of mammals.

Apparent distinctions among the *MIC*s of various species may simply reflect limited investigative effort. However, several striking incongruities should be noted. First, the actual number of constituent *H* loci is uncertain for most species. The prototype mouse *MIC* has two or three such *H* loci, *H-2K, H-2D,* and *H-2L;* the dog and man supposedly have three major *H* loci. The less well-defined macaque and rat *MIC* probably have at least two counterpart loci. While the *B* complex in chickens is clearly polygenic, only one major *H* locus has been identified. Second, species differences in the proximity and arrange-

TABLE 6–7. Phenotype Traits of Loci of the Major Immunogene Complex in Various Species

Phenotype or locus[a]	Man *(HLA)*	Rhesus monkey *(RhLA)*	Dog *(DLA)*	Pig *(SLA)*	Guinea pig *(GPLA)*	Hamster *(Hm-1)*	Rat *(Rt-1)*	Mouse *(H-2)*	Chicken *(B)*
Major *H* loci	+	+	+	+	+	+	+	+	+
Serologically detectable	+	+	+	+	+	−	+	+	+
Number	3	2	3	2	U	U	2	3	U
CML or GVH disease	+	+	+	U	+	+	+	+	+
Ia-like loci	+	+	+	+	+	+	+	+	+
Serologically detectable	+	+	+	+	+	−	+	+	U
MLC or GVH proliferation	+	+	+	+	+	+	+	+	+
Ir genes	+	+	+	U	+	−	+	+	+
Serum complement components	+	U	−?	+	+	−?	U	+	U

[a]Phenotype or locus present (+), absent (−), or unknown or unclear (U).

ment of strong *H* and *Ia*-like loci are evident. In the mouse, the *Ia* genes map between the two major *H* loci. In all other species studied thus far (dog, rhesus, macaque, man, and perhaps the rat), the *Ia* loci are outside but linked to the major *H* loci. Third, some MIC alloantigens are expressed on chicken, rat, and mouse erythrocytes, but they are not readily detected on human, macaque, or dog RBC. Finally, the *MIC* of the Syrian hamster *Hm-1* has several unique features: acute allograft rejection and MLC, CML, and GVH reactions are evoked by Hm-1 disparities, but alloantibodies have not been detected. Although this may seem remarkable to mammalian immunogeneticists, it would hardly surprise invertebrate immunophylogenists, who have detected allograft rejection without concurrent alloantibodies in numerous species. Recent results suggest *Hm-1* alloantibodies may be raised in wild hamsters; hence, apparent peculiarities of *Hm-1* may in part reflect the restricted origin of inbred hamster strains. Whereas the *Ir* gene controlling antibody responses to bovine serum albumin is not linked to the *MIC* in the hamster, it is *MIC*-linked in the guinea pig and under polygenic control in the mouse.

Perhaps the *MIC* genetic organization differs even among closely related orders or taxonomic families. Further comparative studies with chemically defined antigens are needed to clarify this point. Apparently, rearrangements within the *MIC* are possible without seriously affecting *MIC* functions. The *MIC* may reflect specific functional adaptations during evolution. Frequent mutations, or gene rearrangements by duplications and deletions, could have repeatedly altered the mono- and bi- or multipartite organization of the *MIC.*

MIC Polymorphism

Certain constituent loci of the major immunogene complexes are remarkably polymorphic, more so than almost all other genes. For example, about 60 percent of the loci controlling allozymes are monomorphic; that is, only one active allele can be found in a population. The remaining allozyme loci that are polymorphic have two or rarely three identifiable alleles. Wild mouse populations in contrast have at least twenty alleles each at *H-2K* and *H-2D* with individual gene frequencies ranging from 12 percent to less than 2 percent. Some 110 identified *H-2* haplotypes comprise diverse combinations of about sixty antigens, and this is by no means a final tally. The extent of *MIC* polymorphism can also be ascertained by comparing the overall frequency of heterozygotes in a population of various systems. The overall average frequency of heterozygotes per locus is about 15 percent in man. For the *ABO* system in man, the frequency of heterozygotes is approximately 50 percent, whereas more than 90 percent of Caucasians are heterozygous at *HLA-B.* Limited amino acid sequence analyses of various gene products indicate that constituent *H* and *Ia* loci of the *MIC* are structural genes, whereas associated *Ir* loci provide regulatory genes. However, certain *Ir* genes may be the structural genes for Ia antigens expressed on antigen-presenting macrophages and helper T-cells.

What then might account for the remarkable polymorphism of *MIC* loci? One possibility is that the various alleles are neither selectively advantageous or disadvantageous, and as a result multiple alleles are well tolerated by the species. This neutralist view of *MIC* polymorphism is probably incorrect because *MIC* genes are unique in the extent of polymorphism exhibited. Perhaps some *MIC*-linked loci such as *H-2S* are essentially neutral genes but are held polymorphic in a population by the effects of selection on closely linked extensively polymorphic *H-2D* or *H-2K* loci. A high level of *MIC* polymorphism may exist because new variants or heterozygosity itself may be selectively advantageous. The *MIC* appears to play a major role in resistance to infectious diseases. It is well known that hosts and parasites have a marked reciprocal effect on each other's evolution. Thus, adaptive changes by a pathogen may need to be countered by changes in immune response genes or antigen recognition and processing by the host. Several mechanisms can be envisioned whereby *MIC* polymorphism may operate in resistance to pathogens. The *molecular mimicry* theory first proposed by Snell assumes that viruses or bacteria often mimic *MIC* structures and thereby avoid being recognized as foreign. Heterogeneity in the host population would ensure that some individuals would not have the corresponding microbial antigens and would accordingly resist infection. However, *MIC* heterozygosity might also provide pathogens a larger choice of molecules to mimic. The *virus receptor* theory assumes as a first step in infection that viruses attach to specific receptors on the host cell membrane. Some of the receptors may be associated with or be *MIC* molecules. In both of the above theories, *MIC* heterozygotes providing more receptors or structures to mimic would be at a disadvantage, whereas new variants would be at a selective advantage over their preexisting counterparts. This mechanism for producing polymorphism is termed frequency-dependent selection.

To the extent that killer T-cells recognize antigens in association with self-*MIC* molecules, another explanation for extensive *MIC* polymorphism is suggested. *MIC* polymorphism could accordingly provide for *preferential associative recognition.* The fate of a particular virus may be determined by its immunogenicity, which in turn may depend on its preferential association with products of certain *MIC* alleles. Hosts with given *Ir* alleles will respond vigorously to contain certain viruses, but other hosts without the necessary *MIC* type will be more susceptible. From the virus' point of view, in order to be an effective but not uniformly lethal parasite, both types of alleles are necessary for its survival as a species. Thus, selective pressures from pathogens as well as hosts could favor some sort of "balanced polymorphism." This type of polymorphism would be selected for by heterozygote advantage rather than frequency-dependent selection. The *MIC* might also act in this way as an immune surveillance mechanism. Structures on newly arising tumors may be more immunogenic in association with certain *MIC* molecules and thus evoke a better tumor protective response.

Why has a requirement for associative recognition of antigens evolved at

all? Direct recognition independent of other molecules would seem preferable and may indeed be the rule in initiation of primary responses. As noted earlier, *MIC*-associated restriction of T-cell cytotoxic activity may simply facilitate immunosurveillance to rid the body of undesirably altered, but otherwise syngeneic cells. Linked recognition could be an adaptive specialization of higher vertebrates to ensure that processing of antigens with subsequent activation of lymphocyte subsets occurs in a coordinated fashion. Perhaps, as numerous investigators have suggested, Ia molecules involved in antigen presentation may also be sources of regulator signals, whereas H-2K and H-2D may provide lytic signals. A requirement for concurrent antigen recognition with the signaling could assure that the signals are specific.

LYMPHOCYTE DIFFERENTIATION GENES

Different lymphocyte populations exist in subsets of functionally specialized cells. Not only do these cells look alike, but they also recirculate together in the blood and lymph. Thus, special approaches are necessary to discriminate one cell type from another. The characteristic array of cell-surface structures and phenotypes of a particular lymphocyte subpopulation can be used for identification. For example, B-lymphocytes have surface immunoglobulin receptors for complement, and are nonadherent and nonphagocytic. Responses to mitogens (e.g., Concanavalin A [Con A] or lipopolysacchaside [LPS]), binding to erythrocytes, and specific functional activities are also used to characterize lymphocyte subsets. A particularly powerful approach has been to serologically define structures that are characteristic of certain lymphocytes. As distinct subpopulations have been defined by their cell-surface phenotype, it is becoming possible to construct maturation pathways and to investigate interactions among subsets.

As lymphocyte subpopulations differentiate from common stem cell precursors, discrete sets of genes are expressed. One set of genes—those controling the expression of lymphocyte surface molecules—has been studied extensively as a model of cell differentiation. Studies that have monitored changes in cell-surface phenotype by serologic methods have shown that the array of surface gene products changes in a regular way through development. The role of these cell-surface differentiation gene products in ontogeny is just beginning to be unraveled. Cell surface molecules according to one view are involved in specific recognition during growth and development; they provide key signals for cell-cell interactions or serve as receptors for diffusible differentiation signals. Moreover, unique structures on lymphocyte subsets may participate in mediating their specific function. Lymphocytes are particularly suitable models for studying cell differentiation. They can be specifically triggered to differentiate; changes in their morphology, surface phenotype, or function can then be readily measured. Little is known about the lymphocyte membrane phenotype—the surface has literally just been scratched. Some genes coding for lymphocyte-associated membrane molecules display limited polymorphism and certain allotypic differences have consequently been detected by serologic

techniques. This set of molecules is designated "lymphocyte alloantigens." The murine differentiation alloantigens associated with T- or B-cells are summarized in Tables 6–8 and 6–9.

T-Lymphocyte-Associated Systems

T-cell precursors originate in the bone marrow and migrate to the thymus where they mature under some crucial influence in the thymic microenvironment. Subsequently, they circulate in the blood and lymph and into peripheral lymph nodes and spleen. As we have seen, T-cells can be divided roughly into several functional subpopulations including mature killer T-cells (T_k), helper T-cells (T_H), suppressor T-cells (T_s), and their precursors. The maturation of these subsets and identification of further subdivisions has been made possible by characterization of T-cell surface phenotypes.

Thy-1

The *Thy-1* locus (chromosome 9 in the mouse) codes for a glycoprotein of approximately 28,000 daltons which is expressed on T-lymphocytes but not B-lymphocytes and on fibroblasts, neurons, and mammary and skin epithelial cells. Two alleles, $Thy\text{-}1^a$ and $Thy\text{-}1^b$, have been identified which code for the Thy-1.1 and Thy-1.2 determinants, respectively. Congenic strains of mice differing only at *Thy-1* have been developed. The Thy-1.1 specificity is also detected on rat thymocytes and brain. Thy-1 was one of the first lymphocyte-associated differentiation antigens to be defined and has served as the principal marker to define mouse T-cells. It may also be present on a subpopulation of natural killer cells. Thy-1 in the rat is not found on mature T-cells, but is expressed on T- and B-cell precursors in the bone marrow. Thy-1 equivalents have also been found in dogs and man, which suggests that lymphocyte differentiation genes are conserved in mammals; however, unlike rodent Thy-1 canine and human Thy-1 may be found in kidney cells. Although Thy-1 is a prime marker for mouse T-cells, it should be used in conjunction with more restricted T-lymphocyte markers. *Thy-1* functions as a differentiation gene by several criterions. The expression of Thy-1 in the brain increases during early development. Thy-1 is induced on T-cells once pre-T-cells enter the thymus, but upon maturation much less is expressed. Further, antibodies to Thy-1 can act to redirect the in vitro differentiation of mammary myoepithelial cells. Thy-1 incompatibility in congenic strain combinations leads to a weak T-cell independent antibody response; however, in the presence of additional *MoH* locus disparities, a strong anti-Thy-1 response is evoked. This suggests that Thy-1 alone on the membrane may be "helpless" and requires other structures to make it immunogenic. Thy-1 differences also elicit weak T-cell proliferative responses but no cytotoxic T-cells.

A number of genes necessary for synthesis and expression of Thy-1 on the cell membrane have been identified by the techniques of somatic cell genetics. $Thy\text{-}1^-$ mutant cell lines derived by immunoselection, have been grouped

TABLE 6–8. T-Lymphocyte-Associated Alloantigens of Mice

Locus (chromosome)	Allele	Specificity	Distribution in common strains	Cell distribution
Lyt-1 (19)	*Lyt-1*^{a}	Lyt-1.1	CBA, C3H, DBA/2	Thymocytes, T-cells in general, some B-cells (?)
	Lyt-1^b	Lyt-1.2	A, AKR, BALB/c, C57BL/6, CE, NZB, RF, RIII, SJL, SWR	
Lyt-2 (6)	*Lyt-2*^{a}	Lyt-2.1	AKR, C58, CBA, C3H, CE, DBA/2, RF	Thymocytes, T_k-cells and T_s-cells for antibody production
	Lyt-2^b	Lyt-2.2	A, BALB/c, C57BL/6, NZB, SJL, SWR	
Lyt-3 (6)	*Lyt-3*^{a}	Lyt-3.1	AKR, C58, RF	Thymocytes, T_k-cells and T_s-cells for antibody production
	Lyt-3^b	Lyt-3.2	A, BALB/c, CBA, C3H, CE, C57BL/6, DBA/2, NZB, SJL, SWR	
Ly-5 (Lyt-4) (1)	*Ly-5*^{a}	Ly-5.1	A, AKR, BALB/c, CBA, C3H, CE, C57BL/6, DBA/2, RF, SWR	Thymocytes, peripheral T-cells, NK-cells
	Ly-5^b	Ly-5.2	RIII, SJL	
Ly-6 (Lyt-5)	*Ly-6*^{a}	Ly-6.1	A, BALB/c, CBA, CE, C3H	Some T_k precursors, mature T-cells, activated T-cells, and B-cells
	Ly-6^b	Ly-6.2	AKR, C57BL/6, C58, DBA/2, RF, SJL, SWR	
Thy-1 (9)	*Thy-1*^{a}	Thy-1.1	AKR, RF, RIII	All immature and mature T-cells, neurons, fibroblasts, epithelial cells
	Thy-1^b	Thy-1.2	Most other strains	

Tla (17)	*Tla*^{a}	Tla 1,2,3,5	A, C58, NZB, SJL, SWR (Leukemias Tla 1,2,3,–,5)	Thymocytes and T-cell leukemias
	Tla^b	Tla 0	AKR, CBA, C3H, CE, C57BL/6, DBA/1, RF (Leukemias Tla 1,2,–,4,–)	
	Tla^c	Tla 2	A.TL, BALB/c, DBA/2 (Leukemias Tla 1,2,—Tla 1,2,–,4,5)	
	Tla^d	Tla 1,2,3	A.CA, B10.M	
Qa-1 (17)		Qa-1+	A, A.TH, C58, SJL	Thymocytes, some T_H- and T_S-cells
		Qa-1–	AKR, A.TL, BALB/c, C57BL/6, C3H, DBA/2, RF	
Qa-2 (17)		Qa-2+	A, A.TH, A.TL, BALB/c, B10.D2, C57BL/6, DBA/1, DBA/2	Some thymocytes Mainly mature T-cells; some non-T-cells
		Qa-2–	AKR, C58, C3H, CBA, RF	
Qa-3 (17)		Qa-3+	A, BALB/c, C57BL/6, DBA/2	Mature T-cells
		Qa-3–	AKR, C58, C3H, DBA/1, RF, SWR	
Gix		Gix +	A, AKR, CE, CeH, C58, DBA/2, SJL	Thymocytes, other tissues depending on age and strain
		Gix –	BALB/c, C57BL/6, CBA, DBA/1, RF	
Ia-4 (17)	*Ia-4*^{k}		B10.A(5R), B10.A(9R), etc.	T_S-cells
	Ia-4^b		B10.A(3R), etc.	

into five complementation classes, all of which are recessive in crosses to the wild type. At least one and perhaps several of the mutants directly affects the glycosylation of the Thy-1 polypeptide. Hybrids of a Thy-1.1^+ lymphoma and a Thy 1.2^- myeloma suggest that expression of Thy-1 may also be under negative control. Although Thy-1^- clones which express both Thy-1.1 and Thy-1.2 antigens. Myeloma cells evidently express a gene that operates in hybrids to extinguish the expression of Thy-1 on the cell surface. In the "revertant" subclones, the gene may be lost or mutated.

Lyt Loci

The *Lyt-1* locus on chromosome 19 has two alleles, *Lyt-1*^{a} and *Lyt-1*^{b}, which specify Lyt-1.1 and Lyt-1.2 antigens, respectively. The precise structures that constitute Lyt-1 determinants have not been determined. Lyt-1.1 has been found on two glycoproteins of approximately 67,000 and 87,000 daltons. Lyt-1 determinants are expressed only on T-lymphocytes. Initial studies suggested immature T-cells (Lyt-$1^+2^+3^+$) could be distinguished from T_k- and T_s- cells (Lyt-$1^-{}_2{}^+{}_3{}^+$) and T_H-cells (Lyt-$1^+2^-3^-$) and that Lyt-1 might be a specific marker for amplifying helper T-cells. This now appears to be an oversimplification because some T_k-cells may be Lyt-1^+ and some T_H-cells may be Lyt-2^+. Differences in Lyt phenotypes in T-cell subsets may be more quantitative than qualitative or depend on the antigen used and how it is recognized. The *Lyt-2* and *Lyt-3* loci are closely linked to each other on chromosome 6. No genetic recombinations between them have been observed among 380 progeny tested. However, sequential immunoprecipitation studies suggest that Lyt-2 and Lyt-3 are distinct glycoproteins. Both are about 35,000 daltons in size and can be found either alone or in association on the membrane. *Lyt-2* and *Lyt-3* are also closely linked to a locus (or loci) governing the expression of V_k-light chain variants and to one and perhaps several *MoH* loci. This raises the intriguing possibility that a second immunogene complex distinct from *H-2* may be found on chromosome 6.

The Lyt-2 and Lyt-3 determinants are found only on T-lymphocytes, principally on immature T-cells and mature T_s- and T_k-cells. Certain T_H-cells, which augment allogeneic cytotoxic responses, are also Lyt-2^+. Expression of these genes is apparently restricted to these functional subsets; this suggests that the Lyt 2, 3 antigens participate in the functions of the cells which bear them. Killer T-cell activity is blocked by antibodies specific for Lyt-2 or Lyt-3 on the T_k-cell surface but not by anti-Lyt-1 serum, even when Lyt-$1^+2^+3^+$ effector cells are used. The Lyt-2 and Lyt-3 determinants may therefore be adjacent to or part of the T-cell receptor or be structures that facilitate the lytic activity of T_k-cells.

The Qa System

Three serologically defined loci, *Qa-1, Qa-2,* and *Qa-3,* which map telomeric of the H-2D region have been identified. Recently, two additional specific-

ities, Qa-4 and Qa-5, have been identified with monoclonal antibodies and several more are likely to be characterized. The products of these loci are lymphocyte differentiation antigens which, like the *Lyt* system, are restricted in their expression to certain lymphocyte subpopulations. But unlike Lyt antigens, Qa antigens are not expressed on all thymocytes and in general appear at a later stage of T-cell differentiation. *Qa-1* and *Qa-3* code for molecules of approximately 45,000 daltons which associate with a β_2-microglobulin subcomponent. The striking similarity of these membrane components to those specified by *H-2K, H-2D, H-2L,* and *Tla* genes suggests they may all be part of the major immunogene complex. The *Qa* and *Tla* regions in particular may be important in the regulation of T-cell differentiation. Serologically distinctive *Qa* alleles have not yet been identified.

Qa-1 is expressed on thymocytes and about two-thirds of peripheral T-cells. A subpopulation of LPS activated cells are also Qa-1$^+$. Cytotoxic T-cells and many T-cells that proliferate in response to alloantigens do not express Qa-1. However, Qa-1 is present on a subpopulation of Lyt-1$^+$ cells which induce Ts activity. Qa-2 is found predominantly on peripheral T-cells but is also expressed on a subpopulation of thymocytes and some B-cells and bone marrow cells. In contrast, Qa-3 is absent from thymus and bone marrow, but found on a fraction of peripheral T-lymphocytes. Mitogen-responsive T-cells express Qa-1, Qa-2, and Qa-3. The Qa-2 locus, and perhaps Qa-1 as well, may code for antigens that can serve as targets for cytotoxic cells. In the same region, several *H* loci such as H-31 and H-32 have been identified. The relationship of these loci to the *Qa* genes is unknown; Qa determinants have not been detected on skin epithelium. Homologues of *Qa* have been identified in the guinea pig.

The Tla Region

The *Tla* region is 1-2 cM telomeric of *H-2D* on chromosome 17 (Figure 5–2). *Tla* codes for a glycoprotein of about 50,000 daltons, which like H-2K and H-2D is associated with β-2 microglobulin on the membrane. Four *Tla* alleles have been identified with alloantigenic products (Table 6–8). Tla is expressed on thymocytes but is lost after T-cells migrate from the thymus. An intriguing property of Tla antigens is that many T-cell leukemias derived from strains with Tla negative thymocytes surprisingly do express Tla determinants. No Tla specificities are found on C57BL/6 thymocytes; yet Tla.1, 2, and 4 are expressed on many C57BL/6 leukemias. Indeed, Tla.4 is restricted to T-leukemic cells. Thus, Tla-coding structural genes are present in all strains and their expression is regulated by a *Tla* regulatory gene. Tla.3 and Tla.4 may be allelic products of *Tla* structural genes, although Tla.4 has been found only on leukemic cells. Since Tla.1 and Tla.2 specificities occur on normal cells or leukemias of all strains thus far examined, the corresponding structural locus or loci have not been mapped.

Tla-associated gene products have several unusual membrane-associated properties. First, the amount of Tla antigen expressed on F_1 heterozygous

thymocytes varies according to the allelic combination. *(Tla^b × Tla^a)*F1 or *(Tla^b × Tla^c)*F1 heterozygotes express only half the amount of the Tla specificities detected on *Tla^a* homozygous thymocytes, as expected for a conventional gene dose effect. However, *(Tla^a × Tla^c)*F1 thymocytes have twice as much Tla.1 and half the amount of Tla.2 expected. *Tla* may also influence the amount of H-2D antigens on thymocytes. Tla− thymocytes have more detectable H-2D molecules than Tla+ thymocytes but have the same amounts of H-2K and Thy-1 molecules. Tla antigens are also distinctive in that anti-Tla can induce the phenotypic loss of Tla from the cell surface—a process called *antigenic modulation* (see Chapter 3). Antigenic modulation represents a phenotypic suppression of Tla antigens triggered by specific antibodies. New Tla does not reappear on the membrane for at least one to two cell divisions. In contrast to modulation of protozoan ciliary antigens, removal of Tla antigens is not accompanied by the appearance of alternative antigens. Tla antigenic modulation does not result from capping. H-2D associated with Tla is not modulated from the surface by Tla antibodies; nor does anti-H-2D serum remove Tla determinants. The complex gene-product interactions inherent in the *Tla* region remain to be sorted out.

Other T-cell-Associated Determinants

The Ly-5 alloantigen is coded by the *Ly-5* gene on chromosome 1, which has two known alleles. Ly-5 is expressed on several lymphocyte subsets including thymocytes, most mature T-cells, and NK-cells, but not on mature B-lymphocytes or macrophages. The Ly-6 antigen is present predominantly on peripheral T-cells and, in contrast to other Ly specificities, is sparsely represented on thymocytes. Ly-6 may be a T-cell differentiation antigen characteristic of mature T_k effector cells. *Ly-6, Ala-1,* a marker of mitogen-stimulated lymphocytes, and another lymphocyte alloantigen locus, *Ly-8,* do not segregate in backcross progeny and have virtually identical strain distribution patterns, indicating these loci are closely linked or perhaps identical.

Certain Ia antigens are characteristic of T-cells; for example, the *Ia-4* code for determinants found on T_s-cells. The *I-E/C* subregions of *H-2* may also code for T-cell specific markers. The products of the *Tla, Qa,* and *Lyt* loci have served as identification markers of T-cells at discrete stages of differentiation and of functionally separable subpopulations. About 90 percent of thymocytes are Tla$^+$, Lyt-1$^+$2$^+$3$^+$. Upon migration to the periphery, these T-cells cease to express Tla and mature into at least three subpopulations: Lyt-1$^+$2$^+$3$^+$ cells, Lyt-1$^+$2$^-$3$^-$ cells, and Lyt-1$^-$2$^+$3$^+$ cells. Some peripheral Lyt-1$^+$2$^+$3$^+$ cells may mature into Lyt-1$^+$ and Lyt-2$^+$3$^+$ cells. These functional subsets probably interact in a "regulatory network" involving various feedback signals. The muddy water surrounding the cellular events occurring during an immune response should begin to clear as new T-cell markers and new immunologic mutants (Chapter 8) are better characterized.

B-Lymphocyte-Associated Systems

The presence of surface immunoglobulins (Ig) of various isotypes, or complement receptors have served to distinguish B-cell subsets. Polymorphic determinants present on murine B-cell subsets have been distinguished mainly with alloantiserums (Table 6–9). These alloantigens differ in both their strain and tissue distribution. The *Lyb-1* locus on chromosome 2 codes antigens found on B-cells, antibody-producing cells, and myelomas but not on B-cell precursors. *Lyb-2* on chromosome 4 by contrast dictates a marker found on pre-B-cells as well as mature B-cells and plaque-forming cells. The *Lyb-3*, *Lyb-5*, and *Lyb-7* loci segregate independently but are related; they all code markers distinguishing mature B-cells of various strains. Absence of these markers is connected with an X-linked B-cell defect leading to the absence or dysfunction of a mature B-cell subset in CBA/N mice. *Lyb-3* codes for a polypeptide with a molecular weight of about 68,000 daltons found on approximately 35 to 50 percent of B-cells.

B-cells can be divided into subsets by their differential capacity to respond to T-cell independent antigens (TI) such as TNP-LPS (TI-1) or TNP-Ficoll (TI-2). Lyb-5 and Lyb-7 are expressed on mature B-cells which respond to the TI-2 antigens. Antibodies to Lyb-7 structures block the ability of B-cells to respond to TI-2 antigens. Lyb-5 is found on about half of all cells. Anti-Lyb-5 serum in the presence of complement selectively kills B-cells that respond to TI-2 antigens. *Lyb-7* is distinct from but loosely linked to C_H immunoglobulin genes. The Ly-Ml antigen is expressed on B-lymphocytes but not T-lymphocytes and is coded for by a gene linked to the *Mls* locus on chromosome 1. However, Ly-Ml is not B-lymphocyte-specific because it occurs on non-T- and non-B-cells in the spleen. Lyb-4 positivity is characteristic of more mature B cells, but is demonstrable only in certain strains. Another marker designated Pca.1 has been detected on antibody-secreting plasma cells and myeloma cells. This antigen, however, also appears in non-lymphoid tissues such as kidney, liver, and brain.

Certain Ia antigens are B-lymphocyte differentiation markers. A small proportion of cells in the fetal liver express Ia by day 16 of gestation and the amount increases during ontogeny. Press and co-workers have suggested that B-cells which can give rise to IgG-antibody-producing cells express Ia molecules, whereas Ia-negative B-cells may develop into IgM-producing cells. Perhaps Ia molecules play a role in the IgM to IgG switchover during antibody production.

Other Nucleated Cell-Surface Specificities

Lymphocyte specific antigens have also been detected in several species by xenogeneic antiserum induced by immunizing rabbits or goats with lymphoid cells and exhaustively absorbing the serum with appropriate tissues to remove species-specific and other unwanted antibodies. Unfortunately, xenoantiser-

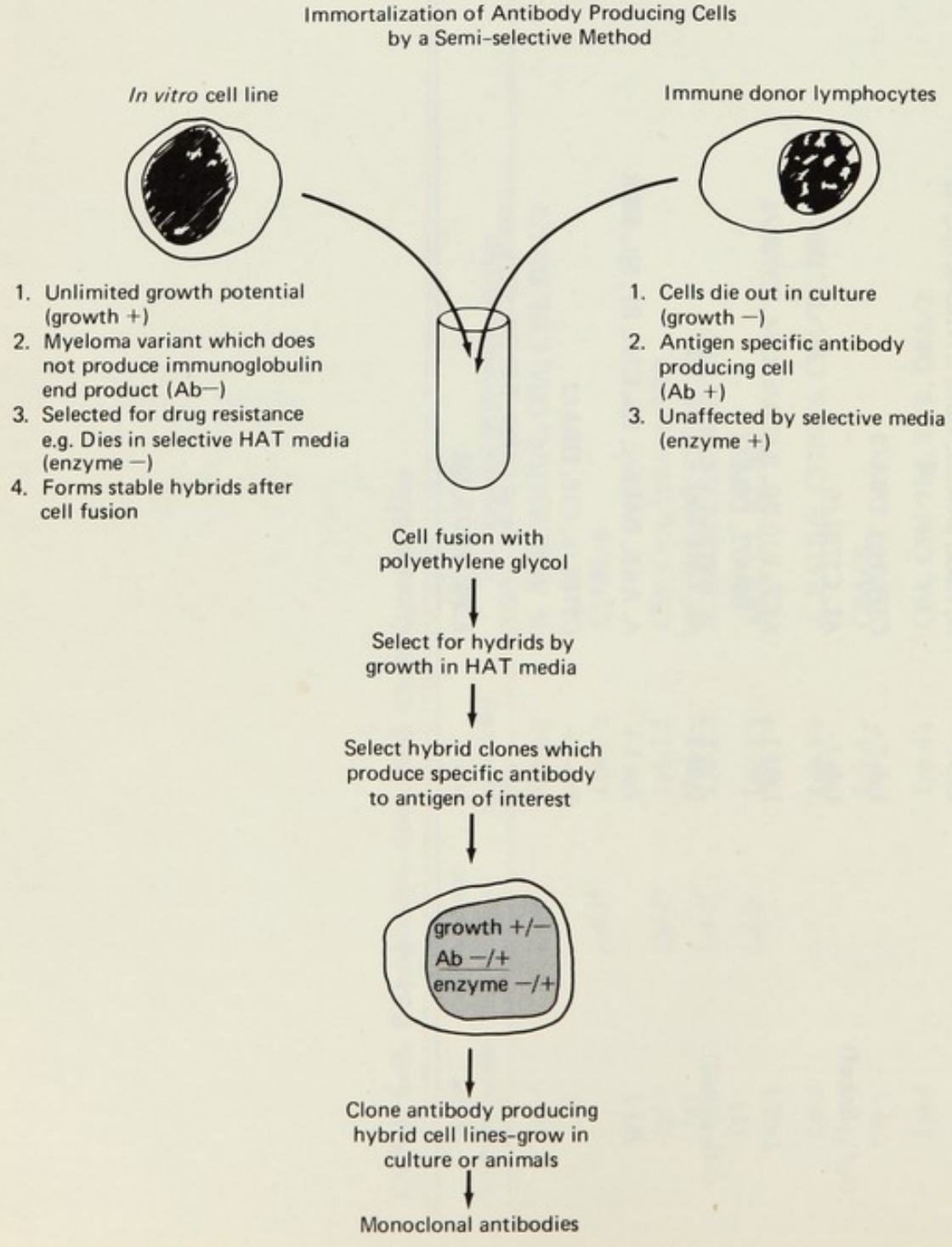
TABLE 6–9. B-Lymphocyte-Associated Alloantigens of Mice

Locus (chromosome)	Allele	Specificity	Distribution in common strains	Cell distribution
Lyb-1 (2)	*Lyb-1*^{a}	Lyb-1.1	A, AKR, BALB/C, CBA, C3H, DBA/2	B-cells, myeloma cells; antibody producing cells
	Lyb-1^b	Lyb-1.2	C57BL/6	
Lyb-2 (4)	*Lyb-2*^{a}	Lyb-2.1	C58, CBA, DBA/2, SWR	Immature and mature B-cells antibody producing cells
	Lyb-2^b	Lyb-2.2	A, BALB/c, C57BL/6, RIII	
	Lyb-2^c	Lyb-2.3	AKR, CE, RF, SJL	
Lyb-3		Lyb-3+	A, BALB/c, C57BL/6, CBA/T5, DBA/1	Mature B-cells MW 68,000
		Lyb-3–	CBA/N	
Lyb-4		Lyb-4+	CBA, C3H/HeJ, SWR, DBA/2	Mature B-cells
		Lyb-4–	A, AKR, BALB/c, B10.D2, C57BL/Ks, C57BL/6, SJL, RF, CE	

Lyb-5		Lyb-5+	AL, C3H/HeJ, CE, DBA/2	Mature B-cells T-independent antigen sensitive population
		Lyb-5−	A, AKR, B10.D2, CBA, C57BL/6, SJL, RF	
Lyb-7 (IgC_H-linked)		Lyb-7+	C3H/HeJ, DBA/2	Mature B-cells
		Lyb-7−	AL, C57BL/6	
LyM-1 (1) (Mls-linked)		LyM-1+	AKR, B10.BR, BALB/c, CBA, C57BL/6 DBA/1, DBA/2	B-cells, bone marrow cells antibody producing cells
		LyM-1−	AL, C3H/HeJ, CE, RF, SJL	
Pca-1		Pca-1+	A, AKR, BALB/c, CE, C3H, RF, SJL, SWR	B-cells plasma cells, myelomas
		Pca-1−	C57BL/6, C58, DBA/2	brain, liver, kidney
Ia-1 (7)	*Ia-1*^{k}	Ia.2	*H-2I-A*^{k} strains (AKR, B10.BR, CBA, etc.)	IgG-producing B cells
	Ia-1^s, etc.	Ia.4	A.SW, B10.S, SJL	

ums are usually directed against "backbone" or species-specific structures found in all individuals, therefore genetic analyses are impossible with these reagents. This and other constraints on procurement of discriminating antibodies can be overcome by utilizing somatic cell hyridization techniques to develop cell lines producing monoclonal antibodies. The principles behind this technique pioneered by Kohler and Milstein are shown in Figure 6–8. A nonsecreting myeloma cell line variant is selected for resistance to a drug such as

FIGURE 6–8. Immortalization of antibody-producing cells by a semiselective method.



8-azaguanine. Cells resistant to the drug lack an enzyme which is necessary for the nucleotide biosynthetic salvage pathway. After fusion with antibody-producing cells from a mouse, cell hybrids are selected by adding medium containing *h*ypoxanthine, *a*minopterin, and *t*hymidine, the so-called HAT medium. The aminopterin blocks the main biosynthetic pathways for purines and pyrimidines. Only cells that have the salvage pathway intact (i.e., enzyme-producing hybrids) will grow. The hybrid clones producing a specific antibody are selected, cloned, and the *monoclonal* antibodies retained. High-titered antiserum can be collected from animals in which the hybrid cell lines or "hybridomas" are growing. Already monoclonal antibodies to determinants such as H-2, HLA, Thy-1, Qa, and Lyt antigens, and new lymphocyte differentiation markers have been produced. This approach is revolutionizing serology and many new surface gene products should be discovered accordingly.

Natural killer (NK) cells have a cell-surface phenotype distinct from the usual B- and T-cells. The NK-1 antigen appears specific for NK-cells. This determinant was detected by contaminant antibodies in Lyt-1 and Ly-6 antiserums. A subpopulation of NK-cells bears the Qa-5 marker. Remarkably little is known about the surface antigens on NK-cells and macrophages. Ia^+ and Ia^- populations of macrophages have been characterized. The *Mph-1* locus on chromosome 7 in the mouse controls the expression of a determinant found on a subpopulation of peritoneal macrophages.

THE T/t COMPLEX

The *T/t* complex of the mouse is located on chromosome 17 close to the centromere approximately 12 cM from the *H-2* complex (see Figure 1–6). It is marked by two series of mutations that have a range of pleiotropic effects, semidominant *T* (Brachyury), and recessive *t*. The *T* mutations where heterozygous *(T/+)* may produce a short tail, but when homozygous *(T/T)* they are lethal. The *T* mutations can also interact with recessive *t* alleles within the complex to produce a tailless *(T/t)* phenotype:

Cross:	T/t^x	X	T/t^x
Progeny:	T/T	T/t^x	t^x/t^x
Phenotype:	lethal	tailless	viable, lethal, or semilethal

There are three categories of recessive *t* "alleles." The majority of alleles identified in the laboratory are *viable* as homozygotes. A few are *semilethal;* that is, they produce some homozygotes that die before birth and some that survive to become normal adults. The *lethal t* alleles in the homozygous state lead to embryonic death at particular stages of development.

The lethal *t* alleles have been most extensively studied. They have been subdivided into six complementation groups: t^9, t^9, $t+^{12}$, t^{w1}, t^{w5}, and t^{w73}. Tests for complementation among these groups have utilized crosses of balanced le-

thal tailless parents with different *t* alleles (e.g., t^x and t^y) and have the following expectations:

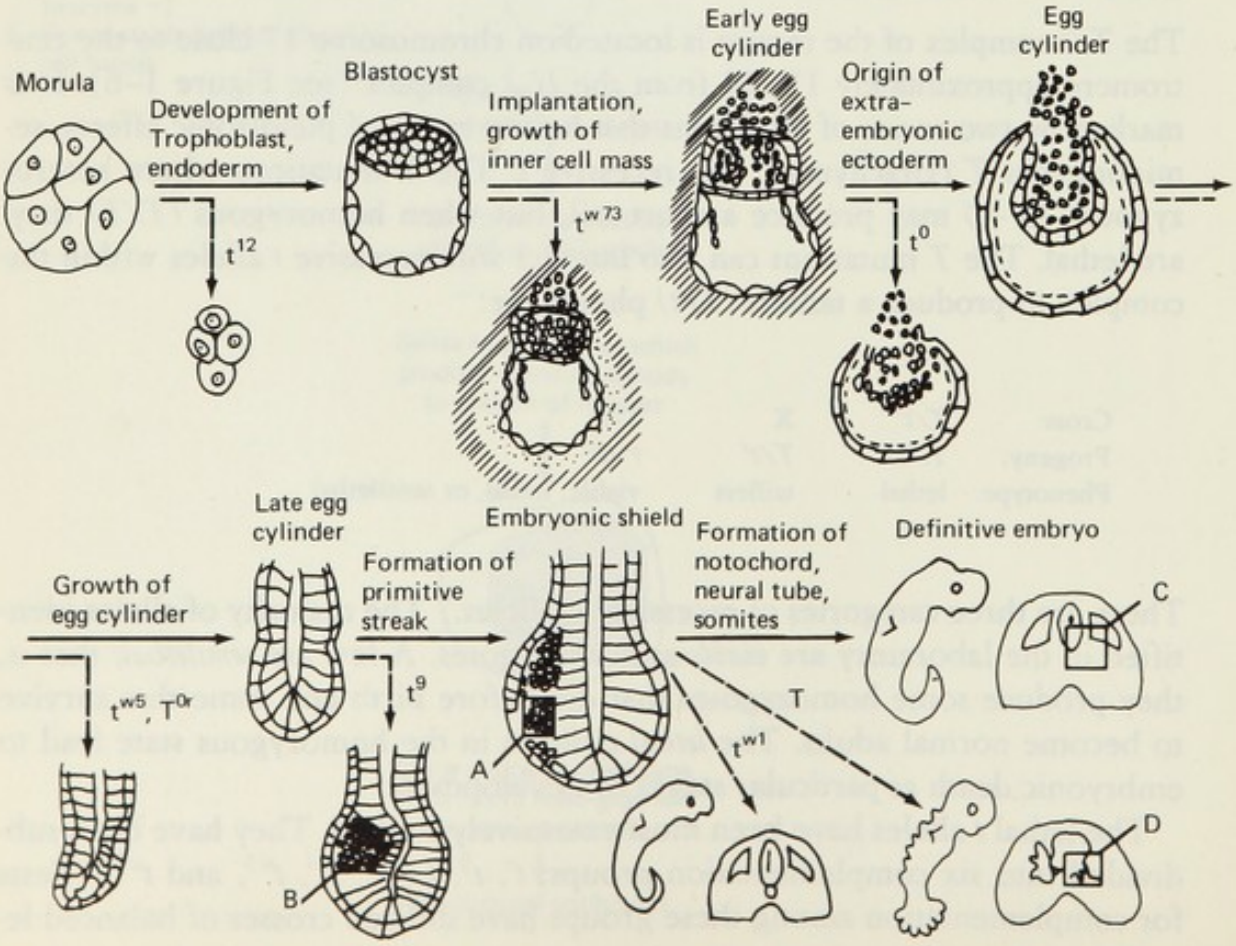
Cross:	T/t^x	X	T/t^y
Progeny:	T/T	T/t^x, T/t^y	t^x/t^y
Phenotype:	lethal	tailless	normal or lethal

If normal-tailed offspring are produced in such a cross, then the two alleles are placed in different complementation groups. If only tailless progeny survive, the alleles belong to the same group. Although we have been using the term "allele," *t* "haplotype" is perhaps a preferable term since closely linked loci or a segment of chromosome probably constitute an allele.

The *t* haplotypes of one complementation group have characteristic effects on embryonic development that are clearly distinct from the effects of other groups. Members of the same group produce grossly similar embryonic abnormalities (Figure 6–9). As a whole, the lethal *t* mutations appear to affect critical transitions during the progressive differentiation of ectodermal derivatives. For example, homozygotes at the t^{12} group reach the morula stage and then

FIGURE 6–9. A diagrammatic representation of the early developmental stages in the mouse. The defects seen in embryos homozygous for *T/t* complex mutations are indicated.

Source: From Bennett, *Cell* 6:441–454, 1975. © M.I.T. Press, Cambridge, MA.



subsequently die before blastocyst formation or implantation in the uterus. In contrast, t^0 homozygotes successfully implant in the uterus but then fail to organize the embryonic and extraembryonic ectoderm. The underlying nature of these *t* lethal mutants is controversial. According to the "organizational failure" hypothesis, *T/t* products are expressed on the cell surface and particular defects in mutant embryos are caused by malfunctions in cell-cell recognition and morphogenetic movements. Alternatively, some *t* mutations may affect all cells of the embryo, and generalized cell lethality then ensues. Some *t* mutants are probably general cell lethals. For example, no cell types from homozygous t^{w5} mutant embryos survive in culture under conditions allowing survival of normal embryo cells. Furthermore, even when t^{w5} and normal embryos are combined to form chimeras, the homozygous t^{w5} cells in the embryo mosaic die and are not rescued by contact with normal cells. Embryos of other haplotypes, however, such as the t^{w18} mutant of the later acting t^9 complementation group (Figure 6–9) do grow well in culture. Orthotopic t^{w18} embryo implants develop normal ectodermal derivatives but mesodermal elements fail to develop.

Support for the organizational hypothesis has come from serologic studies suggesting that some *t* haplotypes code for cell-surface gene products. Loci in the *T/t* complex affect principally embryonic cells and sperm. Thus, antibodies against putative "t antigens" have been evoked by immunizing mice with syngeneic embryonal carcinoma cells or with sperm. Antiserums to "*t* antigens" produced by immunizing wild-type mice with sperm from viable T/t^x mice and then absorbing the serum with *t/+* or *T/+* sperm or sperm from a different *t* haplotype (e.g., $t^9/+$) have detected several *t*-associated specificities. However, these assays with sperm have proved difficult to reproduce. In another approach, Bennett and co-workers developed antibodies to an embryonal carcinomal cell line F9. The "F9 antigen" detected is expressed in adults only on sperm and testicular cells. F9 is expressed in fertilized eggs and increases in concentration during cleavage up to the morula stage. It can be detected until the tenth day of embryonic development. The expression of F9 antigen is influenced by the *t* haplotype of embryos: t^{w32} and t^{w5} embryos are F9 negative but wild-type and t^{w18} embryos do express the F9 marker. It is not yet clear where the F9 structural genes are located; they could map within the *T/t* complex or on another chromosome. As yet there is no clear serologic evidence that t-coded "antigens" are expressed at a stage of development which the *t* haplotype affects.

The *t* series of haplotypes have unusual effects including segregation distortion or sterility in males and strong crossover suppression. The characteristic of segregation distortion is of interest because it runs counter to Mendel's law of segregation. *Segregation distortion* is a deviation from expected Mendelian ratios because a class of gametes is either lethal or unable to yield the usual meiotic segregation pattern. As a rule, lethal and semilethal *t* haplotypes are transmitted by *t/+* males to an excess of progeny (75 to 97 percent), whereas many but not all viable *t* haplotypes are transmitted at lower (0.1 to 0.4) than

normal (0.5) transmission ratios. This segregation distortion only affects males; the transmission ratio in heterozygous females is normal. Distortion is seen equally in *T/t* or *t/+* males. The distortion does not result from any obvious meiotic abnormalities since chromosome segregation occurs normally in the testes of *t/+* mice. Nor is it a result of degeneration of one kind of sperm or of events occurring after fertilization, because fetal death rates are similar in +/t♀×+/+♂ or +/♀×+/t♂ matings. Evidence has been obtained that certain *t* haplotypes enable sperm to maintain their fertilizing ability longer than wild-type sperm. Lethal *t* haplotypes also influence sperm differentiation. In general, the surviving t^x/t^y males of $T/t^x \times T/t^y$ crosses described above (in which t^x and t^y are from different complementation groups) are sterile. Males homozygous for any of the semilethal *t* haplotypes are always sterile and indeed aspermic. In this situation the early stages of spermatogenesis are normal, but the spermatids that are produced are abnormal and therefore are phagocytosed. Males with one lethal and one viable *t* haplotype display some impairment of fertility, that is, they are semisterile. In essence, the particular combination of *t* haplotypes determines whether the resulting phenotype is viable, semilethal, or lethal. Together, the segregation distorting *t* haplotypes in mice share a number of similarities with the segregation distorter locus in *Drosophila*.

Apart from *T* region-associated genes, other chromosome 17 loci contribute to *t* haplotype effects. A locus closely linked to *tf* affects male sterility and lethality. Another gene between *T* and *tf* influences transmission ratios. Thus, the phenotypes observed among various *t* haplotypes are under polygenic control. Most *t* haplotypes have the property of suppressing recombination over the segment of chromosome 17 from *T* up to the *H-2* complex. Could such a mechanism function to prevent segregation distorting genes from separating and spreading through the genome? Why does this effect extend up to but not beyond *H-2*? Is ther some special evolutionary or functional relationship between the *T/t* and *H-2* complexes? We do not yet have the answers. In the absence of *t*, the linkage map of chromosome 17 appears as shown in Figure 1-6. The presence of a *t* haplotype causes the linkage map to "shrink," that is, fewer chaismata are formed between nonsister homologous chromatids during the first meiotic division. The amount of crossover suppression varies slightly from one *t* haplotype to another. In balanced lethal crosses ($T/t^y \times T/t^x$), new *t* haplotypes often arise by recombination between *T* and *tf*. Most of these new *t* haplotypes are viable and usually permit some recombination between *T* and *tf*.

With few exceptions, *t* haplotypes of the same complementation group have identical *H-2* haplotypes. Even mice of the same *t* group from diverse geographic origins carry the same alleles at all *H-2* regions. This result is particularly surprising in light of the marked polymorphism of *H-2* in natural populations. How can this strong linkage disequilibrium be explained? A simple explanation is that certain *t/H-2* haplotype combinations are more advan-

tageous than others and thus are selected for in wild populations. Snell has suggested that the two complexes may comprise a "supergene" which functions to maintain *H-2* heterozygosity in feral populations. Several analogies between the *T/t* and *H-2* complexes support some such functional relationship. Both polygenic complexes are on chromosome 17 and are highly polymorphic. Serologic studies of t-associated antigens suggest they are also polymorphic like H-2 antigens. The F9 antigen which is influenced by *t* haplotype, also has a structure similar to H-2 antigens, but not associated with β2-microglobulin. There is some evidence to suggest reciprocal expression of *H-2* and *T/t* gene products; lethal haplotypes take effect during the first nine days of development, whereas *H-2* antigens are first detectable i seven-to-nine-day-old embryos. However, these connections or correlations could be misleading. Indeed, it is not certain if the serologically defined t-associated markers are in fact *T/t* complex gene products. Although *H-2* functions may be linked to regulation of development, all evidence to date indicates that *H-2* plays a principal role in immunoregulation. What role if any the *H-2* complex plays in cell recognition outside the immune sytem is unknown. Albeit the *H-2* and *T/t* complexes could both be regions governing cell recognition, perhaps even evolved from a common ancestral gene, this does not mean their functions are necessarily interdependent. The *t* haplotype and existing *H-2* complex could have remained together as a consequence of crossover suppression. Although lethal *t* alleles are polymorphic in natural populations, any selective advantage accruing from such heterogeneity has escaped notice. The intriguing relationship between *T/t* and *H-2* and the evolutionary role of crossover suppression both invite further exploration.

KEY REFERENCES

Books

Klein, J. *Biology of the Mouse Histocompatibility-2 Complex. Principles of Immunogenetics Applied to a Single System.* New York: Springer-Verlag, 1975. Excellent compilation of mouse cellular immunogenetics with emphasis on the *H-2* complex. Lymphocyte differentiation genes, the *T/t* complex, and basic immunogenetic concepts are also considered.

McDevitt, H. E. (ed.). *Ir Genes and la Antigens.* New York: Academic Press, 1978. Series of papers from the Third *Ir* Gene Workshop, focusing especially on the question whether la antigens are in fact *Ir* gene products.

Melchers, F., Potter, M., and Warner, N. L. (eds.) *Lymphocyte Hybridomas. Current Topics Microbiology and Immunology.* Vol. 81. New York: Springer-Verlag, 1978. Plasma cell-plasmacytoma hybrids secreting monoclonal antibodies specific for a variety of cell-surface antigens are finding wide application in immunology, biochemistry, and molecular and cell biology.

Moller, G. (ed.). *Immunology and Differentiation.* Vol. 33. Munksgaard, Transplantation Reviews, 1977. Contains cogent articles on embryonic antigens, *H-Y*, the *T/t* complex, and lymphocyte differentiation.

Articles

Bach, F. H., Bach, M. L., and Sondel, P. M. Differential function of major histocompatibility complex antigens in T lymphocyte activation. *Nature* 259:273–281, 1976. Distinct T-cell subsets react to H-2I or H-2K (D) antigens and interact during cytotoxic T-cell activation.

Bennett, D. The *T*-locus of the mouse. *Cell* 6:441–454, 1975. A good summary of the various pleiotropic effects of the *T/t* complex.

Bodmer, W. F., and Bodmer, J. G. Evolution and function of the *HLA* system. Br. Med. Bull. 34:309–316, 1978. A thoughtful discussion of HLA polymorphism, linkage disequilibrium, and associations of HLA alleles with various disease states.

Cantor, H., and Gershon, R. K. Immunological circuits: cellular composition. *Fed. Proc.* 38:2058–2067, 1979. Summarizes recent ideas concerning the regulatory network operating in humoral responses. Other articles in the same symposium address topics in immune regulation.

Crow, J. F. Genes that violate Mendel's rules. *Sci. Amer.* 240:134–146, February 1979. Description of genes that "cheat" such as segregation distorter (SD) in Drosophilas. Timely discussion pertinent to those interested in the *T/t* complex.

Flaherty, L. Genes of the *Tla* region: the new Qa system of antigens. In *Origins of Inbred Mice*. H.C. Morse, III (ed.). New York: Academic Press, 1978, pp. 409–422. Description of the lymphocyte differentiation genes in the region between *H-2D* and *Tla*.

Hansen, T. H., and Shreffler, D. C. Characterization of a constitutive variant of the murine serum protein allotype, Slp. *J. Immunol.* 117:1507–1513, 1976. Evidence and model for operon control of Slp expression.

Klein, J., Flaherty, L., Vandeberg, J. L., and Shreffler, D. C. *H-2* haplotypes, genes, regions, and antigens: First listing. *Immunogenetics* 6:489–512, 1978. Useful tables listing alleles and sepcificities of *H-2*-associated loci in various inbred strains of mice.

Kohn, H. I., Klein, J., Melvold, R. W., Nathenson, S. G., Pious, D., and Shreffler, D. C. The first *H-2* mutant workshop. *Immunogenetics* 7:279–294, 1978. A sumary of the genetics, serology, biochemistry nomenclature, and functional studies of *H-2* mutants.

Lyon, M. F., Evans, E. P., Jarvis, S. E., and Sayers, I. *t*-haplotypes of the mouse may involve a change in intercalary DNA. *Nature* 279:38–42, 1979. Speculations on the organization and basis of crossover suppression in the *T/t* complex.

McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpfling, J. H., and Snell, G. D. Genetic control of the immune response. Mapping of the *Ir-1* locus. *J. Exp. med.* 135:1259–1278, 1972. Definition of the H-2*I* region.

McKenzie, I. F. C., and Potter, T. Murine lymphocyte surface antigens. *Adv. Immunol.* 27:179–338, 1979. Current and very comprehensive review of lymphocyte differentiation markers.

Paul, W. E., and Benacerraf, B. Functional specificity of thymus-dependent lymphocytes. *Science* 195:1293–1300, 1977. The specificity of T-cells for self-H-21 or H-2K or D antigens is related to their function and mechanisms of immunoregulation.

Pious, D., and Soderland, C. HLA variants of cultured human lymphoid cells: evidence for mutational origin and estimation of mutation rate. *Science* 197:769–771, 1977. Evidence is presented that some HLA variants are a result of true mutations.

Press, J. L., Klinman, N. R., and McDevitt, H. O. Expression of Ia antigens on hapten-specific B cells. I. Delineation of B-cell subpopulations. *J. Exp. Med.* 144:414–427, 1976. Ia antigens are found principally on IgG producing B cells.

Rosenthal, A. S., Barcinski, M. A., and Blake, J. T. Determinant selection is a macrophage dependent immune response gene function. *Nature* 267:156–158, 1977. Macrophages may function to "orient and present" antigens to T-cells in association with self-Ia molecules. *Ir* gene control may act at the level of the macrophage.

Snell, G. D. The *H-2* locus of the mouse: observations and speculations concerning its comparative genetics and polymorphism. *Folia Biol. (Praha)* 14:335–358, 1968. Provocative discussion of relations between *H-2* and *T/t* complexes and *H-2* polymorphisms. Different *H-2* alleles on cells are likened to different locks on different doors, making it increasingly difficult for "burglar" viruses to gain entrance.

Uhr, J. W., Capra, J. D., Vitteta, E. S., and Cook, R. G. Organization of the immune response genes. *Science* 206:292–297, 1979. Biochemical analyses of Ia antigen subunits suggest they display allele-associated variations and that subunits are encoded by distinct loci.

Williams, A. F. Differentiation antigens of the lymphocyte cell surface. *Cont. Topics Molecular Immunol.* 6:83–116, 1977. Discussion of immunology and biochemistry of mouse and rat lymphocyte surface-differentiation markers.

Zinkernagel, R. M. Major transplantation antigens in host responses to infection. *Hosp. Practice*. 13:83–92, July 1978. Cogent description of *MIC* restriction, maturation of restriction specificity in the thymus, and the role of *MIC* genes in resistance to infectious disease.

7

GENETIC CONTROL OF IMMUNE RESPONSIVENESS

Genetic influence on the immune response occurs at two distinct levels. Structural gene complexes govern the nature, specificity, and heterogeneity of adaptive immune responses. This level of genetic control, especially of antibody molecules, was emphasized in Chapter 2. The second level of gene involvement is that of regulation or modulation of the immune response. The term "immune response gene" (*Ir* gene) is commonly used to describe this latter class of genes. The immune response or immunoregulatory genes control processes such as antigen recognition, cellular cooperation, and regulatory mechanisms that can be clearly distinguished from the genes encoding immunoglobulin structure. The regulatory processes of the immune response influence the ability to respond at a high level or a low level, or the inability to respond to a given antigen. The genetic control may be expressed in the inability of macrophages to interact with T-cells or of T-cells to help activate B-cells, or the capacity to switch from IgM to IgG production during the normal course of the immune response. To date, *Ir* gene control has been identified mostly in responses to T-dependent antigens, suggesting that T-cells, or their interactions with other immunocytes, are a major target for expression of *Ir* genes. This concept will be elaborated later in this chapter.

Early studies on human blood groups and diseases of an immunologic nature indicated a correlation between the operations of the immune response and the genetic profile of the individual. Since these early studies, refinements in techniques and the development of inbred laboratory animals, especially genetically defined strains of mice, have led to the recognition of many specific immune response genes located at numerous chromosomal loci. Furthermore, these *Ir* genes have often been found within the major histocompatibility or immunogene complex in mice, rats, guinea pigs, chickens, rhesus monkeys, and humans. Such genes are known as *MIC*-linked *Ir* genes.

LEVELS OF EXPERIMENTAL DEFINITION OF HERITABILITY OF IMMUNE RESPONSES

Genotypes of Experimental Animals

The heterogeneous genotypes of outbred laboratory animals complicated the unequivocal demonstration of *Ir* genes in early experiments. Thus, although studies as early as 1905 demonstrated heritable differences in the susceptibility of guinea pigs to diphtheria toxin, it was not until inbred animals were developed that the nature of the genetic control of the immune response became apparent. A random population in Hardy-Weinberg equilibrium is most heterogeneous, or least defined, and could be expected to show a broad range of immune responses to nearly all antigens. Selected coisogenic or mutant strains differing at only one known gene locus would be best defined because significant differences in any measured immune response could be associated with one gene locus unmodified by other genetic differences. Intermediate between these extremes would be animals selected for certain phenotypic traits or particular genotypes, but otherwise heterogeneous, or inbred strains with multiple interstrain genetic differences on homozygous backgrounds.

Large numbers of inbred strains have been produced in mice, making them the best immunogenetically characterized of the mammalian species. Extensive breeding programs have yielded congenic mouse strains that differ only at single loci or at small groups of linked genes. Such strains are obtained by the prolonged backcrossing of progeny to one parental strain and selecting for the desired genotype at each backcross. This ultimately results in progeny being genetically identical to the parent strain except at the selected locus. These breeding techniques have been dealt with in detail in Chapter 1. Inbred strains of guinea pigs have also figured prominently in the demonstration of the basic principle of genes controlling immune responsiveness.

Complex Versus Chemically Defined Antigens

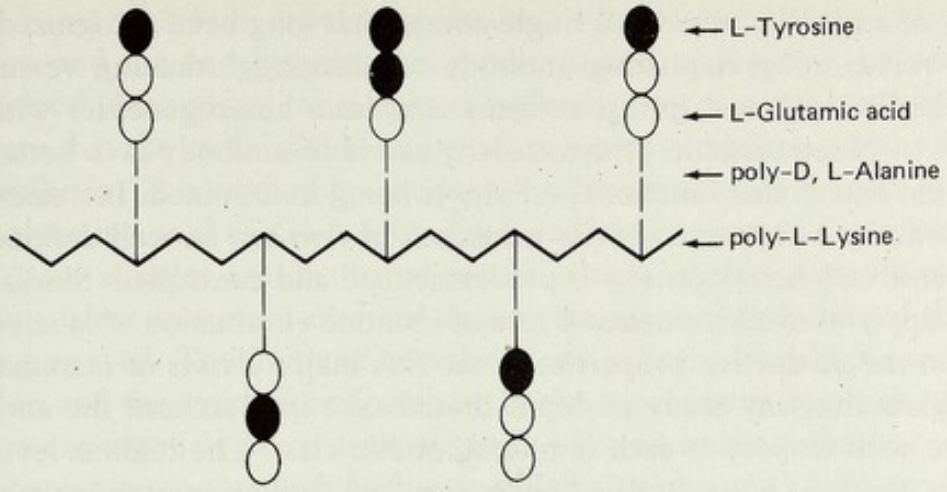
Although a species of *Salmonella* or blood cells from an individual are sometimes termed *the* antigen when used for immunization, a complex mixture of many antigens is really involved. Many distinct and overlapping immune or antibody responses are induced in the immunized animal under these conditions. When clones of a given microorganism or homogeneous subpopulations of cells are employed, one is still dealing with complex antigens with many determinant groups, but the source is better defined and the antigenic polymorphism is reduced considerably. Much better definition is achieved when highly purified or homogeneous preparations of proteins or polysaccharides are used for immunization, even though multiple antigenic determinants are still involved. One obtains greater sophistication when chemically defined haptens are conjugated to purified macromolecules. Theoretically, maximal definition is achieved when a hapten is conjugated to the responding animal's own

macromolecules. Single allelic or mutational variants of known macromolecules or alloantigens in inbred strains represent optimally defined sources.

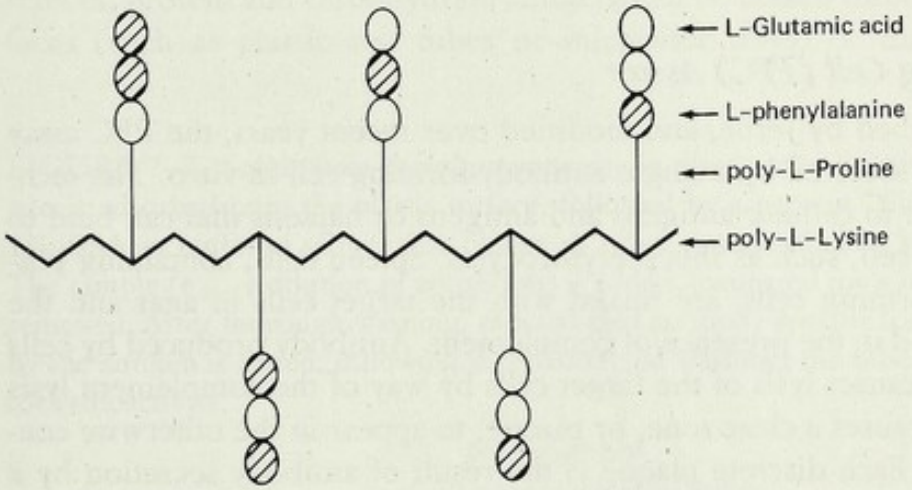
In order to undertake detailed genetic studies of immune responsiveness, one should obviously utilize antigens with a restricted number of determinants. This should, in turn, restrict the heterogeneity of the immune response elicited. This effect may be promoted in several ways:

1. Use of low antigen dosage leading to selective stimulation of clones of antibody-forming cells bearing high affinity receptors for the antigen.
2. Phylogenetic relatedness of antigen. An antigen more closely related to self such as an alloantigen will have fewer recognizable determinants.
3. The use of self-molecules as carriers of haptenic groups; for example, immunizing mice with TNP conjugated to autogenous serum albumin.
4. Chemically and structurally defined molecules such as insulins, lysozymes, or synthetic antigens.

Synthetic polypeptides have proved particularly popular and may be either random linear polymers of a small number of amino acids or branched copolymers consisting of side chains of one or two residues on a well-defined backbone (Figure 7–1). By selective substitution of residues in these synthetic antigens the specificity of *Ir* genes may be examined. Despite the partially defined chemical nature of these antigens they are clearly not homogenous and their conformational complexity leads to heterogeneous antibody responses. Complex immunogens such as xenogeneic erythrocytes, bacteria, and soluble protein antigens have repeatedly revealed polygenic control of corresponding immune responses. Selective breeding experiments in mice, by identifying high and low responders to a complex antigen and then breeding for the character of either high or low responsiveness, have clearly demonstrated the polygenic nature of antibody responsiveness to complex immunogens. For example, using adult outbred albino mice as a foundation population, Biozzi and co-workers selected animals with maximal or minimal antibody responses to various antigens in consecutive generations. Assortive mating of the highest responding mice produced high (H) lines and that of the lowest responding mice low (L) lines. Analysis of interline divergence and crosses between the H and L lines revealed that the optimal antibody response to sheep or pigeon erythrocytes is controlled by about ten independently segregating loci, including *H-2*- and *Igh-1*-linked genes. The number of loci involved in the regulation of antibody responses varied according to the immunization protocol and antigen used. Thus, the response to threshold doses of sheep erythrocytes is regulated by two loci, one of which is *H-2*-linked. Antibody responses to erythrocyte and bacterial antigens are influenced by as many as fifteen loci. As with any quantitative trait, some loci (usually relatively few) have a pronounced effect on the phenotype over a range of conditions. Only a few genes are usually found to regulate responsiveness to synthetic or haptenic determinants. Thus, the number of genes involved in the regulation of responsiveness to a given antigen appears to be a function of the complexity or number of antigenic determinants expressed on the molecule.



a) The synthetic copolymer:
(L-TYR, L-GLU)-poly-D, L-ALA-poly-L-LYS denoted (T, G)-A-L



b) The synthetic copolymer:
(L-PHE, L-GLU)-poly-L-PRO-poly-L-LYSINE denoted (PHE, G)-PRO-L

FIGURE 7–1. Diagrammatic representations of the structures of two branched chain copolymers of amino acids used extensively in studies of *Ir* gene control. (a) The synthetic copolymer (L-tyr, L-glu)-poly-D,L,ala-poly-L-lys; i.e., (T,G)-A-L. (b) The synthetic copolymer (L-phe, L-glu)-poly-L-pro-poly-L-lys; i.e., (Phe,G)-Pro-L. Both polymers are formed on a backbone of poly-L-lysine.

Assays for Specific Immunity or Antibody Responses

In conventional serology, the antibody response is commonly measured in whole serum titers assayed by agglutination, precipitation, or complement fixation. Although such tests are useful in the diagnosis of immune responsiveness to infectious agents, they are complex because heterogeneous populations of antibodies with divergent reactivities contribute to the overall reaction. The

heterogeneity of antibodies even to a single antigen has long been recognized. Precipitating versus nonprecipitating antibody or saline-agglutinating versus blocking antibodies to blood group antigens constitute heterogeneities with opposing effects. Neutralization or cytotoxicity assays of antibody give better definition to the extent that functional activity is being determined. Increased functional immunity or hypersensitivity measured in vivo can be well defined if cellular or antibody heterogeneity is predetermined and controlled. Similar qualifications apply to measurements of rate of immune elimination of labeled antigen. Given the distinctive properties of the five major classes of mammalian immunoglobulins, any study in depth should take into account the antibody response with respect to each immunoglobulin class. The highest level of definition is obtained when purified or fractionated antibodies of given molecular classes are measured by the most sensitive and reproducible techniques available. The recent elaboration of the genetic control of immune responsiveness has been, in part, a result of refinements in the methods used to assess specific immune responses. In particular, the increased sensitivities offered by radioimmunoassay systems have allowed accurate quantitation of the humoral response. Some of the most commonly used assay systems used to examine *Ir* gene control are listed below.

Plaque-Forming Cell (PFC) Assay

Originally described by Jerne, and modified over recent years, the PFC assay detects antibody secreted by a single antibody-forming cell in vitro. The technique is confined to cellular antigens and antigens or haptens that can bind to a suitable target cell, such as sheep erythrocytes. Spleen cells, containing possible antibody-forming cells, are mixed with the target cells in agar and the mixture incubated in the presence of complement. Antibody produced by cells from the spleen causes lysis of the target cells by way of the complement lysis pathway, which causes a clear zone, or plaque, to appear in the otherwise confluent cell lawn. Each discrete plaque is the result of antibody secretion by a single antibody-forming cell. The number of plaques obtained can be used as a quantitation of the humoral response. In this direct form of the plaque assay, only IgM-secreting cells are detected. If IgG antibody is to be detected, antiserum against IgG is added to the cell mixture prior to incubation with complement. These antibodies bind to the target cell/IgG complexes and enhance the activation of complement, thus promoting lysis of the target cell. This assay system has proved useful in quantitating humoral responses to haptens such as dinitrophenol that can be readily linked to erythrocytes and in the response to cellular antigens such as the murine alloantigen *Thy-1*.

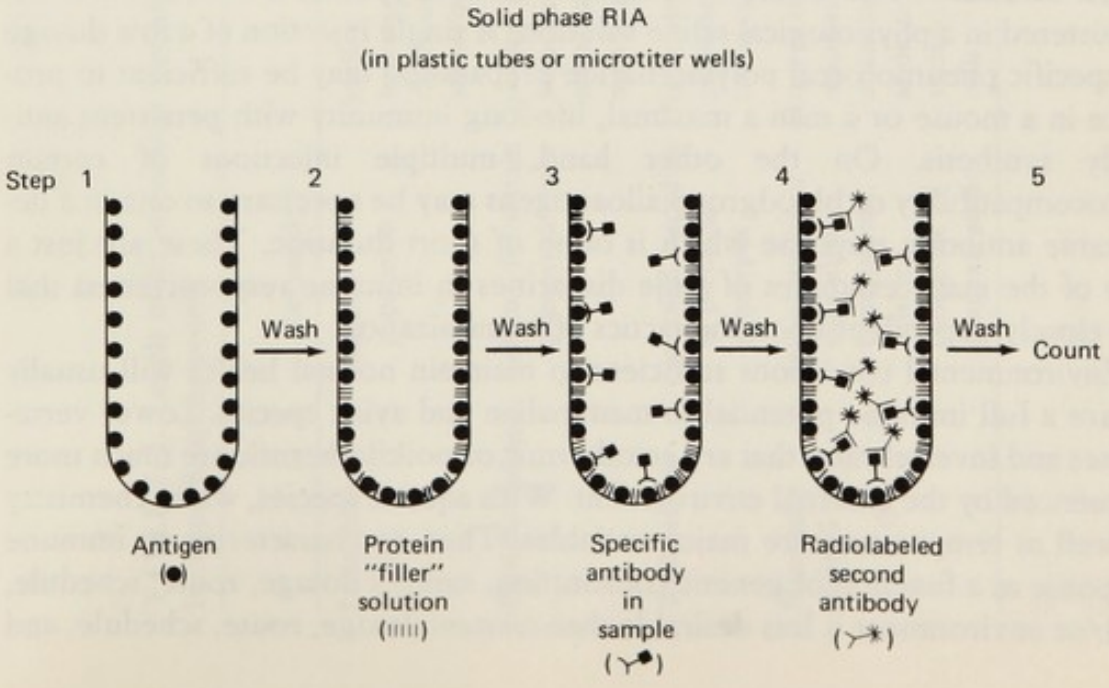
Antigen-Binding Assays

These methods offer the advantage of assaying antibody binding to antigen in the absence of secondary effector functions, such as the ability to fix complement.

Farr-type assays: Briefly, antibody is allowed to bind the radiolabeled antigen and the ensuing complexes precipitated with either ammonium sulfate, polyethylene glycol, or a second antibody system. Care must be taken to ensure that the radiolabeled antigen is not precipitated in the absence of bound antibody. This method had been applied to assaying antibodies to the synthetic polyamino acid (T,G)-A-L by direct radioiodination of the tyrosine residues. Serum samples are incubated with the radiolabeled ligand and then with a polyvalent antiserum directed against the immunoglobulins of the species being assayed. The immune complexes are pelleted by a high-speed centrifugation and the residual radioactivity in the supernatant determined. The results are expressed as percentage of antigen bound in the pelleted complexes. Antihapten responses such as that to the trinitrophenyl (TNP) group may be assayed by binding the hapten to a carrier protein (e.g., TNP-bovine serum albumin) and radioiodinating the conjugate. Immune complexes resulting from incubation of radiolabeled antigen and serum are precipitated with polyethylene glycol (10% w/v final concentration). Counting of supernatants again allows determination of the percent of antigen bound.

Solid phase radioimmunoassay: Direct binding of antibody to antigen can be readily assessed if the antigen can be bound in a solid phase. In many instances, protein and carbohydrate antigens can be bound directly to plastic surfaces (such as plastic test tubes or microtiter trays) or may be bound to

FIGURE 7–2. Solid phase radioimmunoassay in plastic tube or microtiter well. Antigen is adsorbed onto the plastic surface, followed by a protein "filler" solution (e.g., 1% BSA in buffered saline) that blocks all other protein-binding sites on the plastic. The sample (e.g., a dilution of antiserum) is added, incubated for a suitable period, and removed. After thorough washing, radiolabeled antibody specific for antibodies bound by the antigen is added; following incubation and washing, the tube or well is counted for radioactivity.



Sepharose beads or filter paper discs through cyanogen bromide activation of the supporting matrix. Serum samples are incubated with the solid phase-bound antigen, and the antibody bound is quantitated by a radiolabeled second antibody system (Figure 7–2). By using immunoglobulin class- or subclass-specific second antibodies, one can obtain both qualitative and quantitative assessments of the humoral response.

Hypersensitivity

The classic indicator of cell-mediated immune responsiveness is the delayed-type hypersensitivity (DTH) reaction. This assay has been well defined in guinea pigs and rats; however, it is only recently that DTH has been accurately quantitated in mice. Miller and his colleagues have established an ear assay that measures ^{125}I iododeoxyuridine uptake in the ears of mice. The antigen is administered topically to one ear; the other ear serves as a control. The DTH reactivity is assessed by the ratio of ^{125}I in the test ear compared to that in the control ear.

Tactics of Immunization and Testing

Every specific immune response is substantially influenced by the tactics and timing of immunization. The chief variables, in addition to the species selected for immunization, are dosage of antigen, route of inoculation, the schedule of immunization, and the external environment. The effective dosage of antigen depends on the inherent adjuvanticity or persistence of the preparation as well as the route of inoculation. For the induction of transplantation immunity, a skin graft is much more effective than an equivalent number of dissociated spleen or lymph node cells. Bovine serum albumin in a water-in-oil emulsion containing killed *Mycobacteria* (Freund's adjuvant) is a potent immunogen when inoculated into mice, but the same soluble protein is ineffective if administered in a physiological saline solution. A single injection of a low dosage of specific pneumococcal polysaccharide preparation may be sufficient to produce in a mouse or a man a maximal, life-long immunity with persistent antibody synthesis. On the other hand, multiple injections of certain histocompatibility or bloodgroup alloantigens may be necessary to obtain a detectable antibody response which is often of short duration. These are just a few of the many examples of wide disparities in immune responsiveness that are closely dependent upon the tactics of immunization.

Environmental conditions sufficient to maintain normal health will usually assure a full immune potential in mammalian and avian species. Lower vertebrates and invertebrates that are ectothermic or poikilothermic are much more influenced by the external environment. With aquatic species, water chemistry as well as temperature are major variables. Thus, to characterize an immune response as a function of genetic constitution, *variable* dosage, route, schedule, and/or environment is less desirable than *constant* dosage, route, schedule, and

TABLE 7–1. Levels of Experimental Definition of Major Variables Involved in Measurement of Inheritance of Specific Immune Responsiveness

	Responders	Antigens	Antibodies or immunity	Tactics of immunization and testing
Least defined	Random population	Complex: Species of microorganisms; heterogeneous mixtures of cell types; different macromolecules such as whole serum or cell extracts	Ab titer of whole serum measured by agglutination, precipitation, or complement fixation	Variable dosage, route, schedule, and/or environment
Partially defined	Selected for certain phenotypic traits	Complex: clones of microorganisms; homogeneous subpopulations of cells; macromolecules of one general type such as serum albumin fraction	Immune elimination of labeled antigen. Increased functional immunity or hypersensitivity	Constant dosage, route, schedule, and environment
Well-defined	Selection according to particular genotypes	Highly purified or homogeneous preparations of proteins or polysaccharides	Ab response measured in terms of particular immunoglobulin classes; neutralization or cytotoxity assays of Ab produced	Constant dosage, route, schedule, and environment
Maximally defined	Inbred strains or equivalent homozygotes. Selected coisogenic or congenic strains	Haptens conjugated to purified macromolecules or responders own macromolecules; synthetic polyamino acids; single allelic or mutational variants of defined macromolecules or cells	Purified or fractionated Ab of given molecular classes measured by most sensitive and reproducible techniques available. Monoclonal antibodies of selected hybridoma origin	Constant dosage, route, schedule, and environment, each predetermined to provide maximal sensitivity for assays employed

environment. The latter approach would yield the highest level of experimental definition if each factor is predetermined to provide maximal sensitivity for the assays employed. These relationships are given in Table 7–1.

The strategy for identifying genetic control of a given immune response will depend largely on the genetic profile of the population. In random or outbred populations, genetic control of an immune response must be sought by either association of responsiveness with some other known genetic trait or through inheritance patterns. In man, the association of susceptibility with certain diseases may correlate with distinct HL-A specificities and may be inherited accordingly in familial studies (see Chapter 8). This is usually indicative of *Ir* gene control of susceptibility to the disease. However, in outbred populations it is often difficult to assess the functional significance of such gene associations. Use of congenic or mutant inbred strains where large numbers of animals of identical genotype are available is usually necessary to identify particular *Ir* genes and to determine their functions.

In mice the identification and mapping of *Ir* genes linked to *H-2* is based upon the availability of numerous inbred, congenic resistant, and recombinant strains. A series of inbred strains covering a range of *H-2* haplotypes is tested for response to the desired antigen allowing the correlation of *H-2* genotype with responsiveness. To map the *Ir* gene within the *MIC,* congenic intra-*H-2* recombinant strains with defined crossover positions are used. A hypothetical example of mapping by this method is shown in Table 7–2. Since the *k* haplotype is a nonresponder, whereas *b* and *d* are responders, the nonresponsiveness of *a* (i.e., *k/d*) maps the responder gene to the left of *I-C.* This implies that *d* alleles to the right of *I-E* are not sufficient for responsiveness. The high responder haplotypes (*i3* and *i5*) map responsiveness to the left of *I-J* as *k* and *d* alleles to the right of *I-J* are associated with nonresponsiveness. Vertical lines mark crossover positions. The recombinant strain bearing the *b4* haplotype responds to the antigen: *k* alleles at *K* and *IA* must be identified with nonresponsiveness since *k* and *a* haplotypes are nonresponders. Therefore, h4 maps the *Ir* gene in question to the right of *I-A,* by exclusion to the *I-B* region of *H-*

TABLE 7–2. Use of Intra-*H-2* Recombinants for *Ir* Gene Mapping

	H-2 region									
		I								
H-2 haplotype	K	A	B	J	E	C	S	G	D	Immune response observed
k	k	k	k	k	k	k	k	k	k	–
b	b	b	b	b	b	b	b	b	b	+
d	d	d	d	d	d	d	d	d	d	+
a	k	k	k	k	k |	d	d	d	d	–
i3	b	b	b	b |	k	d	d	d	d	+
i5	b	b	b |	k	k	d	d	d	d	+
b4	k	k |	b	b	b	b	b	b	b	+

2. Such linkage should be confirmed with intra-*H-2* recombinants of different strain backgrounds. Conceivably, another locus mapping to the right of *H-2D* could influence responsiveness.

Many immune responses, particularly those directed against complex antigens, have been shown to be under *Ir* gene control only when the antigen is administered at limiting levels. This presumably allows presentation of only a limited number of determinants on the complex antigen that are present at a sufficient level to trigger high-affinity receptor-bearing cells. At higher doses, more determinants are capable of stimulating responses, thus increasing the heterogeneity of the response and probably masking genetic control of responses to single determinants on the antigen. Such an explanation is supported by the finding that different antigenic determinants on lysozyme are under separate *Ir* gene control.

SPECIFIC IMMUNE RESPONSE GENES IN ANIMALS AND MAN

Ir Genes in Guinea Pigs

One of the earliest established *Ir* gene controlled immune responses was that of guinea pigs to the hapten-carrier congugate 2,4-dinitrophenyl-poly-L-lysine (DNP-PLL). Initial investigations in outbred guinea pigs showed that the conjugate elicited DNP antibodies in only some of the animals immunized. Responder animals produced high levels of anti-DNP and showed strong, delayed-type hypersensitivity to the antigen. A second conjugate, DNP linked to a copolymer of glutamic acid and lysine (DNP-G,L) behaved similarly in experiments with outbred animals.

Later studies with inbred guinea pigs revealed strain 2 animals as responders to DNP-PLL and DNP-G,L and strain 13 animals as nonresponders. By testing the response of F_1 (2 $\times$ 13) and backcross (i.e., (F_1 $\times$ 2) and (F_1 $\times$ 13)) animals of both sexes, it was determined that the response to DNP-PLL was controlled by a single, autosomal, dominant gene. This was designated the *PLL* gene. Strain 2 guinea pigs were shown to respond to a number of DNP conjugates including DNP-poly-L-ornithine and DNP-poly-L-arginine. The genetic control of the carrier-specific response to these conjugates has also been localized to the *PLL* gene. It is unclear whether these responses are controlled by separate genes closely linked to *PLL* or whether different alleles of the *PLL* gene control individual carrier-specific responses. Carrier specificity of the PLL control is clear from the fact that DNP-PLL nonresponder animals are able to produce anti-DNP when immunized with DNP-PLL linked to an immunogenic carrier such as bovine serum albumin (BSA). Carrier specificity was also shown by putting different haptens on PLL or DNP on different carriers. As carrier recognition is an established T-cell function, these results suggested that the defect in nonresponder guinea pigs was at the T-cell level of carrier recognition. Further evidence of a T-cell defect in nonresponders was the demonstration that nonresponders could not mount a DTH reaction even

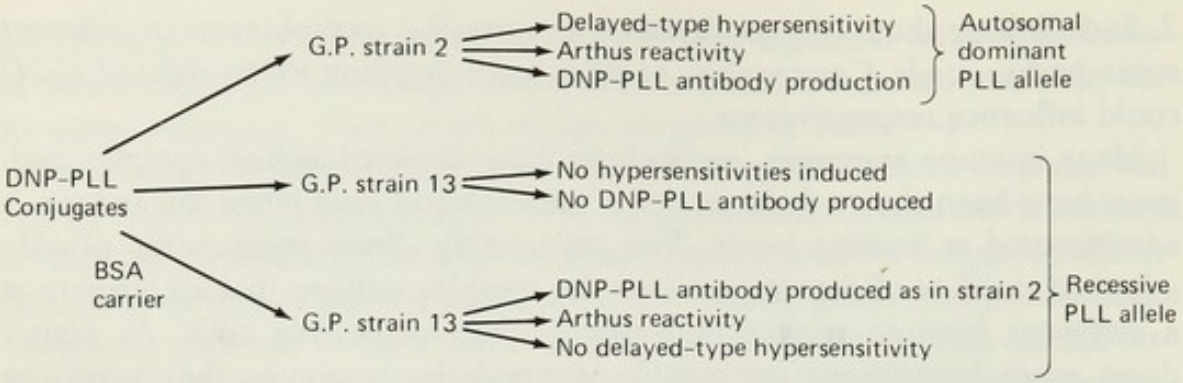
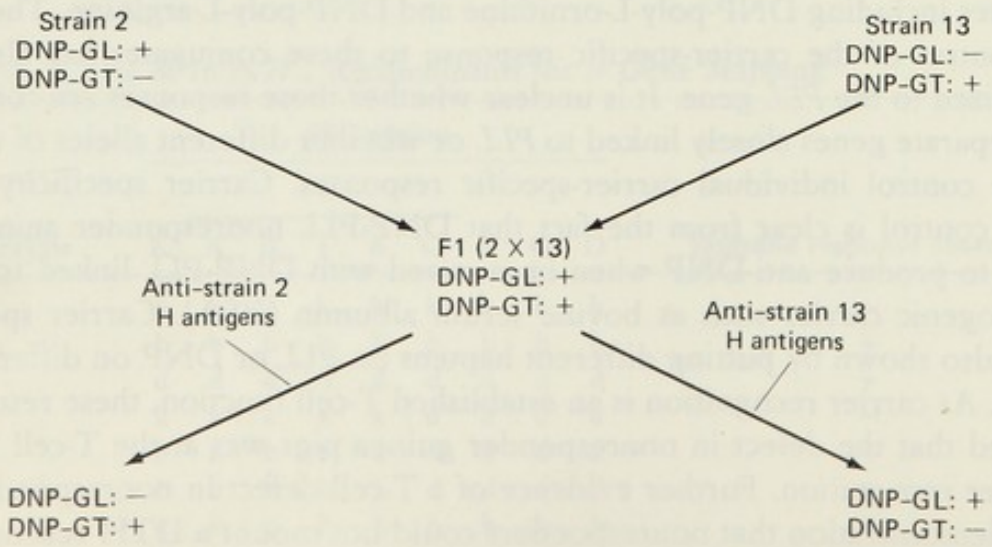


FIGURE 7–3. Responses of responder (strain 2) and nonresponder (strain 13) guinea pigs to DNP-PLL. Note that addition of DNP-PLL to immunogenic BSA carrier allows antibody responses but not development of delayed hypersensitivity in strain 13.

when DNP-PLL was administered linked to a BSA carrier or given with complete Freund's adjuvant. These results are summarized in Figure 7–3. The recessive *PLL* allele cannot activate the entire T-cell pathway even though helper T-cell cooperation is apparently instigated by the xenogeneic carrier. DTH reactions are T-cell-mediated and do not involve B-cells. Analysis of the heterogeneity of the anti-DNP response in responders and "turned on" nonresponders by isoelectric focusing (IEF) techniques indicated no significant differences in their IEF patterns. Moreover, several antibody clones, as identified by characteristic isoelectric points, were seen in common in antibodies produced by both responder and nonresponder. Such results suggest that the nonresponder defect does not lie at the mature antibody-forming B-cell level.

FIGURE 7–4. Demonstration in vivo of *MIC* linkage of *Ir-DNP-GL* and *Ir-DNP-GT* genes in inbred strain 2 and strain 13 guinea pigs. Treatment of (2 × 13)F1 hybrids with antiserum against either strain 2 histocompatibility (Ia) antigens or strain 13 histocompatibility (Ia) antigens inhibits functional expression of the *Ir* gene possessed by the parent strain carrying the corresponding histocompatibility molecules.



Finally, a correlation was established between the presence of the *PLL* gene and the presence of the major strain 2 histocompatibility (*H*) loci in an outbred population of guinea pigs. Similarly, the presence of strain 2 H antigens in responder ($F_1 \times 2$) backcross animals indicated linkage of the *PLL* gene to the guinea pig *MIC*.

Elegant experiments utilizing alloantiserums directed against guinea pig *MIC* loci have given some insight into the relationship between *Ir*-genes and the *MIC*. Strain 2 guinea pigs respond with antibody production to DNP-GL but not to DNP-GT. Conversely, strain 13 responds to DNP-GT but not to DNP-GL. F_1 progeny of the two strains (2×13) respond to both antigens. If F_1 hybrids are treated with antiserum specific for strain 2 H antigens, they are no longer able to respond to DNP-GL but maintain their responsiveness to DNP-GT. However, if hybrids are treated with antistrain 13, DNP-GT reactivity is lost while DNP-GL reactivity remains. The observed blocking apparently acted on macrophages rather than helper T-cells. These experiments, outlined in Figure 7–4, confirm the *MIC* linkage of the relevant *Ir* genes, but leave open the question whether the *Ir* gene products form part of the antigen receptors on macrophages or the T-cell surface.

Use of the synthetic polypeptides has promoted study of the fine specificity of *Ir* gene control. Responses to the copolymers of glutamic acid and alanine (GA) and glutamic acid and tyrosine (GT) are controlled by separate *Ir* genes in guinea pigs. However, the terpolymer of glutamic acid, alanine, and tyrosine (GAT), which contains both GA and GT determinants, is immunogenic in all guinea pig strains. If strains carrying the *Ir* gene for GA responsiveness are immunized with GAT, the resulting humoral and cellular responses exhib-

TABLE 7–3. Histocompatibility-Linked *Ir* Gene Controlled Responses in Guinea Pigs[a]

Antigen type	Antigen	Strain showing high responsiveness
Modified self	DNP-guinea pig albumin	13
	p-idiophenyl sulfonyl guinea pig albumin	13
	DNP-poly-L-lysine	2
	DNP-poly-L-ornithine	2
	DNP-poly-L-arginine	2
Synthetic	DNP-(glutamic acid, lysine)	2
	DNP-(glutamic acid, tyrosine)	13
	Copolymer of glutamic acid and alanine	2
	Copolymer of glutamic acid and tyrosine	13
Foreign proteins	Protamine	2
	Bovine serum albumin	2
	Human serum albumin	2

[a]See Green, I. *Immunogenetics* 1:4–21 (1974) for further details.

it cross-reactivity with GA but not with GT. Thus, a specific *Ir* gene controlling responsiveness to a defined antigenic determinant (in this case GA) can govern the specificity of the immune response to a more complex antigen (GAT) that carries the defined determinant.

A number of other antigen systems studied in guinea pigs have revealed *H*-linked *Ir* gene regulation (Table 7–3). In addition to the synthetic antigens already cited (e.g., DNP-PLL, DNP-G,L), several protein antigens have been examined. Strain 13 animals produce very low levels of specific antibody when immunized with 0.1 μg to 1.0 μg BSA, but strain 2 animals respond well at these dose levels. When larger doses (10 μg to 100 μg) of BSA are administered, the two strains respond similarly. Other protein antigen systems have confirmed *Ir* gene control when the antigen is administered at limiting doses.

MIC-Associated *Ir* Genes in Mice

Mice have notable advantages for genetic studies of immune responsiveness because of the large number of inbred and recombinant strains available and the short generation time that facilitates progeny analyses. One of the first *MIC*-linked *Ir* genes described in mice was designated *Ir-1;* McDevitt and coworkers found it to control the antibody response to the synthetic branched polypeptide (T,G)-A-L (see Figure 7–1). C57BL mice responded well to (T,G)-A-L whereas CBA mice were found to be low responders. F_1 hybrids between the two strains gave intermediate responses. In contrast, replacement of the tyrosine residue in this branched polymer with histidine or phenylalanine (giving (H,G)-A-L or (Phe,G)-A-L) resulted in high responsiveness in the CBA strain and low responsiveness in C57BL. Thus, *Ir-1* gene products can discriminate between tyrosine and histidine or phenylalanine in the antigenic determinant. This *Ir-1* gene regulation of high responsiveness is dominant, unigenic, and quantitative in that low responses rather than nonresponses were observed as the recessive trait. Cell transfer studies showed that responsiveness to (T,G)-A-L in F_1 mice could be transferred to lethally irradiated nonresponder parents, thereby indicating the lymphocyte source of the *Ir* gene product(s). Because these immunocyte reconstitution experiments were often complicated by graft-versus-host reactions due to *MIC* differences, experiments were undertaken with mice having similar H-2 types but differing in their responsiveness to (T,G)-A-L. Through these studies the surprising linkage of the *Ir-1* gene to the *H-2* region was confirmed. For example, C57BL/10 mice bearing the $H\text{-}2^b$ haplotype are high responders to (T,G)-A-L whereas B10.BR congenic mice of $H\text{-}2^k$ haplotype are low responders. Other congenic combinations confirmed the *H-2* linkage. Mapping of the *Ir-1* gene within the *I* region of the *H-2* complex has been determined by use of recombinant inbred mice (see Table 6–4). The gene appears to be located in the *I-A* region.

The *Ir-1* gene effect is exerted at the level of secondary IgG antibody production. $H\text{-}2^b$ and $H\text{-}2^k$ strain mice immunized initially with (T,G)-A-L respond slowly with high responders ($H\text{-}2^b$)producing only marginally higher titers

than low responders. After boosting, however, a rapid rise of IgG antibody is seen in high responder strains whereas low responders continue to produce low levels of IgM antibody and fail to switch to IgG production. Furthermore, neonatal thymectomy of high responder strain mice prevents the IgG response to (T,G)-A-L. Thus, the *Ir-1* gene action is T-cell dependent and presumably controls cell cooperation at the time of induction of IgG formation.

In contrast to the quantitative control exerted by the *Ir-1* gene in the response to branched multichain copolymers, linear polypeptide antigens usually evoke an all-or-none response. *Ir* gene control in this system was studied using a series of polypeptides based on a random linear polymer of glutamic acid (60 percent) and lysine (40 percent) (GL). GL was nonimmunogenic in all inbred strains tested. The addition of a third amino acid to GL yielded a number of terpolymers, each of which was potentially immunogenic in selected mouse strains. The terpolymers contained approximately 55 percent glutamic acid, 35 percent lysine, and 10 percent of either alanine (GLA^{10}), serine ($GLser^{7}$), proline ($GLpro^{10}$), leucine ($GLleu^{10}$), tyrosine (GLT^{5} and GLT^{15}), or phenylalanine ($GL\Phi^{9}$). The superscript indicates the percentage of the third amino acid in the polymer. The use of congenic and recombinant mice revealed these responses to be under *H-2*-linked *Ir* gene control. These experiments also yielded information that confirmed the independence of the *H-2*-linked *Ir* genes from the genes encoding immunoglobulin V regions. Responsiveness to the linear terpolymers was associated with distinct *H-2* haplotypes. Although the polymers varied in their ability to elicit antibody production in a given mouse strain, the antibodies thus formed were highly cross-reactive. In fact, antibody responses to all the polymers could be accurately determined by measuring antibody levels to GLT. Thus, antibody specificity in this system is independent of the ability to respond to the antigen.

The *Ir* genes controlling high responses to the synthetic antigens mentioned above plus those governing high responsiveness to numerous other antigens are expressed as dominant traits, i.e., the F_1 progeny of high responder $\times$ low responder strains behave as high responders. However, other antigen systems have revealed either recessive inheritance of high responsiveness or more complex partial dominance among multiple alleles. Responsiveness to both mouse liver F antigen and TNP conjugated autogenous serum albumin (TNP-MSA) fall into this category. Investigation of the latter system by Hildemann and his colleagues has led to identification of the locus *Ir-6*, mapping within the *I-A* region of the *H-2* complex and exhibiting either hierarchical dominance as described below or recessive inheritance of high responsiveness in different haplotype combinations. *H-2* linkage was demonstrated both by comparison of the responses of congenic strains differing only at *H-2* and by formal genetic linkage analysis in F_1 and F_2 generations. Production of TNP-MSA antibodies has been measured by antigen-binding (Farr) assays, solid phase radioimmunoassays, and enumeration of TNP-specific plaque-forming cells. *H-2*^{b} and *H-2*^{d} strains of mice are high responders, whereas *H-2*$^{a,k,or\ s2}$ strains are low responders. High responsiveness is inherited recessively in *H-2*^{b} haplotype

combinations but dominantly in $H\text{-}2^d$ combinations. Thus, low $(H\text{-}2^k) \times$ high $(H\text{-}2^b)$ F1 mice are low responders, and low $(H\text{-}2^k) \times$ high $(H\text{-}2^d)$ F1 mice are high responders. A hierarchy of haplotypes governing responsiveness to TNP-MSA ranging from high to low is evident as follows: $H\text{-}2^d > H\text{-}2^b > H\text{-}2^{k.a.\ or\ j2}$. The observed patterns of inheritance suggest graded regulation of responsiveness. Despite the unusual features of *Ir-6* gene action, this locus exhibits several important characteristics in common with other *H-2* linked *Ir* genes. Features shared by *Ir* genes of the *I* region include: (a) thymus-dependent regulation of production of different immunoglobulin classes, especially switchover from IgM to IgG production in high responders, (b) abolition of high responsiveness by adult thymectomy and treatment with anti-thymocyte serum, (c) induction of high responsiveness in mice of low responder genotype by complexing the antigen to a xenogeneic carrier, and (d) restricted heterogeneity of the antibodies produced as determined by isoelectric focusing (IEF). Similar restriction of IEF patterns has been observed in antibodies of high responder mice to the conjugate DNP-poly (glu, lys, phe). This reflects a restriction in the number of specific antibody clones expressed.

We have considered almost exclusively to this point the genetic control of humoral immune responses, but it should be borne in mind that cell-mediated immune reactions are also under genetic control. Resistance of mouse strains to virus-induced leukemogenesis and to group B togaviruses, for example, is determined by both *H-2* and non-*H-2* genes affecting T-cell and macrophage functions (Chapter 8). The cell-mediated immune response of F1 hybrid mice to an AKR tumor cell line (BW5147) is under *H-2*-linked control for both primary and secondary responses. Responsiveness is controlled by a gene mapping in the *I-J* region of *H-2* and resistance is associated with the $H\text{-}2^k$ genotype. High responsiveness appears to be recessive, as the hybrid genotypes $H\text{-}2^{q/k}$ and $H\text{-}2^{b/k}$ are low responders. Alternatively, suppression leading to unresponsiveness or susceptibility may be a dominant trait in this system.

Complementary Ir *Genes*

Although some of the immune responses described thus far appear to be regulated entirely by single *Ir* genes, many systems are influenced by two or more *MIC*-linked *Ir* genes. This occurs in the regulation of the response to the alloantigen H-2.2 and to the terpolymer GLΦ described above (Figure 7–5). Indirect indication of multigenic control occurred when F1 progeny of two low responder strains were shown to be capable of greater responses than either parent strain.

The GLΦ system has been elucidated by studying the responsiveness of recombinant mouse strains derived from crossovers between low responder haplotypes. Recombinant strains derived from low responder haplotypes $H\text{-}2^d$ and $H\text{-}2^b$ and from the haplotypes $H\text{-}2^s$ and $H\text{-}2^d$ respond well to GLΦ. Two gene loci, α and β with their respective alleles $\alpha(+)$, $\alpha(-)$ and $\beta(+)$, $\beta(-)$, were identified through the recombinant study. The α genes map in the *I-E/C* region and the β genes in the *I-A* region of the *H-2* complex. Low respon-

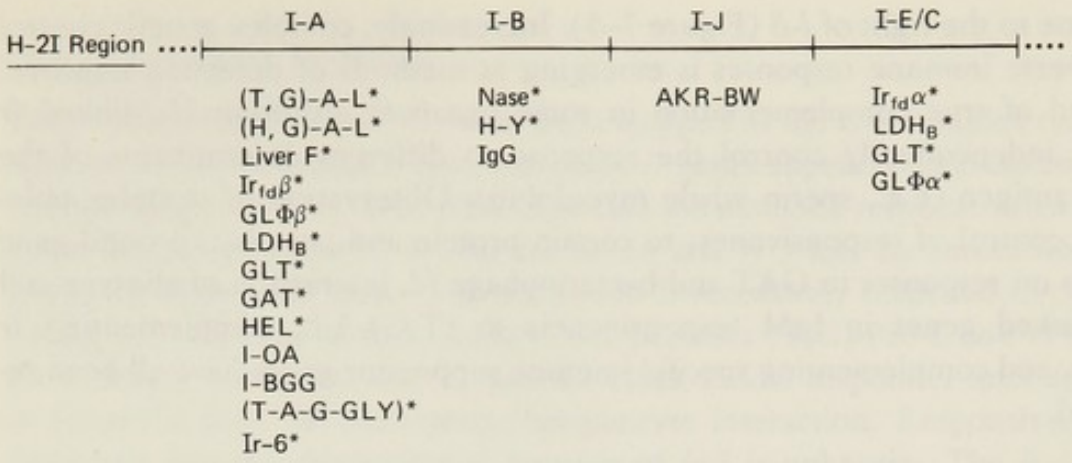


FIGURE 7–5. Mapping of *Ir* genes within the five *I* subregions of the *H–2* complex in mice. Responsiveness to many antigens marked by asterisks is known to be regulated by two or more gene loci either within the *MIC* as indicated, or by interaction with one or more non-*H–2* loci. Symbols for antigens are as follows: Branched copolymers of L-amino acids, (T,G)-A-L = (Tyr,Glu)-Ala-Lys; (H,G)-A-L = (His,Glu)-Ala-Lys. Random linear copolymers of L-amino acids, GlΦ = $(Glu^{53}Lys^{36}Phe^{11})^n$; GLT = $(Glu^{57}Lys^{38}Tyr^{5})^n$; GAT = $(Glu^{60}Ala^{30}Tyr^{10})^n$; (T-A-G-Gly) = $(Tyr\text{-}Ala\text{-}Glu\text{-}Gly)^n$. I-OA = low dose of chicken ovalbumin; I-BGG = low dose of bovine gammaglobulin; liver F = liver-specific alloantigen found in mice and other mammalian species; Ir_{fd} α *or* β = separate loci regulating responses to bacteriophage fd; LDH_B = porcine lactic dehydrogenase B; HEL = chicken (hen) egg lysozyme; IgG = alloantigen on BALB/c IgG myeloma; H-Y = murine male-specific alloantigen; *Ir–6* = locus controlling responsiveness to TNP conjugated to autogenous serum albumin; Nase = staphylococcal nuclease; AKR-BW = AKR tumor cell line BW 5147.

siveness may result when either one, or both, of the *Ir-GL*Φ (+) genes are absent. However, complementation leading to responsiveness may occur when the α and β genes are in either the *cis* (i.e., on the same chromosome) or *trans* (on separate chromosomes) position. This type of α-β complementation has been observed in the *Ir* gene control of three other polypeptide antigens: GLleu, GLT^5, and GLT^{15}. In the GLΦ system, a second form of gene interaction, that of β-β complementation, occurs in the absence of a functional α allele. It appears in this instance that the β genes must be derived from different haplotypes.

The immune response of mice to bacteriophage fd is under the control of two *H-2I* region loci plus non-*H-2* genes. C57BL/10 mice are high responders, however B10.BR and B10.D2 mice are low responders. Analysis of recombinant and F_1 responses indicates the presence of two complementing Ir_{fd} loci. $Ir_{fd}\alpha$ maps to the right of the crossover between the *IE* and *IC* regions, and $Ir_{fd}\beta$ maps to the left of *I-B*, presumably at *I-A*. Complementation occurs effectively with certain pairs of alleles only, a phenomenon termed α-β coupled complementation. Dual gene control has been demonstrated in several other antigen systems in the mouse. Antibody responses to the LDH_B and GLT antigens now appear to be regulated by two interacting genes, one in *IA*

and one to the right of *I-B* (Figure 7–5). Increasingly, complex genetic control of diverse immune responses is emerging as methods of detection improve. Instead of true complementation in some instances, different *H-2*-linked *Ir* genes independently control the response to different determinants of the same antigen (e.g., sperm whale myoglobin). Observation of complex polygenic control of responsiveness to certain protein antigens, background gene effects on responses to GAT and bacteriophage fd, interaction of allotype and *H-2*-linked genes in IgM responsiveness to (T,G)-A-L, complementing *Ir* genes, and complementing specific immune suppressor genes have all been reported.

MIC-*Associated Immune Suppressor Genes*

While *MIC*-associated *Ir* genes appear to specifically control the development of helper T-cell activity to T-dependent antigens, low or nonresponsiveness in some *Ir* gene controlled systems may be attributable to development of suppressor cells following antigen administration. The controlling genes are sometimes termed immune suppression or *Is* genes, though this could be misleading. The role of T suppressor cells in low or nonresponsiveness was first suggested by experiments with the terpolymer GAT. As in other antigen systems already described, low responders to GAT can be stimulated to produce GAT antibodies in high concentration when immunized with GAT coupled to a xenogeneic carrier such as methylated bovine serum albumin (GAT-MBSA). However, prior administration of GAT alone to nonresponder genotypes renders them incapable of producing antibodies to GAT-MBSA. Thus GAT can suppress GAT-MBSA responses in mice bearing GAT low responder haplotypes, and this suppression was found to be adoptively transferable by specific Lyt2,3-bearing T-cells. Similar findings were obtained in the GT system and extended to demonstrate that the *Is* genes responsible were: (1) inherited in a dominant manner, (2) *H-2*-linked and located in the *I* region, and (3) not present in all low or nonresponder mouse strains. Moreover, GT-specific immune suppression appears controlled by two complementing *Is* genes mapping in the *I* region.

In summarizing the properties of *MIC*-associated *Ir* genes in mice, the following points may be made:

1. They regulate immune responsiveness to T-dependent antigens; *H-2*-linked control of T-independent antigens has rarely been observed.
2. They appear to function independent of the immunoglobulin V region or idiotype genes.
3. They map in the *I* region of the *H-2* complex.
4. They usually regulate high-versus-low rather than all-or-none expression of specific immune responsiveness.
5. Although dominant inheritance of high responsiveness is common, other modes of inheritance include complex gene interaction, partial dominance, and recessive determination of high responsiveness.

Other *Ir* Genes in Mice

The *Ir* genes just discussed have all been mapped to the *H-2* complex on chromosome 17 of the mouse. However, many *Ir* genes appear to bear no essential relationship to the *MIC*. *Ir-2*, which controls the antibody response to an erythrocyte alloantigen (Ea-1), is near the *agouti* and *H-3* loci on chromosome 2. High responsiveness under *Ir-2* regulation is recessively inherited. *Ir-3*, governing the response to the synthetic polypeptides (T,G)-Pro-L and (Phe,G)-Pro-L also is not linked to *H-2*, and the block in low responder mice appears to be at the level of macrophage-lymphocyte interaction. Responsiveness is dominant, but the chromosomal location of *Ir-3* is unknown. The *Ir-4* locus was revealed by the antibody responses of different mouse strains to potent enterobacterial lipopolysaccharides. All strains tested were high responders except C3H/HeJ, and responsiveness was dominant. Nonresponsiveness of C3H/HeJ mice segregated in a backcross as a single gene trait independent of *H-2* and of Ig heavy chain allotype. *Ir-5* was located on chromosome 17 telomeric of the *Tla* locus (see Figure 1–6) as a consequence of complementation studies. Although the C57BL/6 and DBA/2 strains are both low responders to Thy-1.1 antigen, their F_1 hybrid is a high responder. Backcross analysis using the *T* locus as well as *H-2* as markers suggested complementary interaction of *Ir-1* and *Ir-5*, but these findings have recently been questioned. Apart from these autosomally scattered *Ir* loci, a sex-linked *Ir-X* locus is also evident. The IgM antibody response to type III pneumococcal polysaccharide, a T-independent immunogen, is determined in an almost all-or-none manner by an *X*-linked gene.

The genetic control of the immune response to cell-surface antigens has been studied using erythrocyte antigens, differentiation and *H* gene alloantigens, and tumor-associated antigens. Both *H-2* and non-*H-2* gene control have been implicated. Extensive studies by Lake, Mitchison, and Clark have been done with the Thy-1 alloantigen since the anti-Thy-1 response can be readily quantitated with a plaque-forming cell assay. Additional specific non-H-2 antigens augment the response, and foreign H-2 antigens diminish or block anti-Thy-1 responses. Self-H-2 molecules are also known to affect the immunogenicity of surface antigens. A major insight emerging from these studies is that other cell-surface gene products influence the response to Thy-1. Thus, the response to cell-surface molecules such as Thy-1 can be affected by genetic differences influencing donor cell immunogenicity and by recipient *Ir* genes which influence the host's ability to respond.

Both *MIC* and non-*MIC*-linked genes or their products have been frequently found to interact in controlling immune responses. Two separate genes control the immune response to the sequential polypeptide $(Tyr\text{-}Ala\text{-}Glu\text{-}Gly)_n$ in mice. *Ir-(T-A-G-Gly)-1* maps in the *IA* region of *H-2* and controls the ability to respond to this antigen. The magnitude of the antibody response is controlled by a second gene, *Ir-(T-A-G-Gly)-2*, that segregates independently of *H-2*. Genetic control of responsiveness to staphylococcal nuclease is mediated

by at least one *H-2*-linked and two non-*H-2*-linked genes. One of the non-*H-2* genes is allotype-linked (see below) and codes for idiotypic determinants on the nuclease antibodies. The other non-*H-2* gene appears to control the overall magnitude of the hyperimmune response. The interacting *H-2*-linked genes govern the early antibody response and the relative proportions of antibodies directed toward different antigenic determinants on the nuclease molecule.

Within the family of *Ir* genes not associated with the *MIC*, a group of genes exists in linkage to the genes controlling immunoglobulin allotypic determinants on the mouse heavy chain. These are known as allotype linked *Ir* genes. However, these genes control idiotypic determinants in the variable region of the heavy chain, the region of the antibody molecule that determines antigen-binding specificity. The allotype-linked *Ir* genes therefore appear to be immunoglobulin V region genes, a characteristic that distinguishes them sharply from the *H-2*-linked *Ir* genes. Such genetic control is usually expressed in responder strains as a particular idiotype that is absent from nonresponder strains. The heavy chain constant region allotype Ig-1e in strain A/J mice is linked to genes controlling the ARS idiotype in p-azophenylarsonate antibodies and the ASA idiotype in streptococcal antibodies. The T 15 idiotype in strain Balb/c antibodies to phosphorylcholine is similarly linked to allotypic markers. The IgM response to (T,G)-A-L is linked to the *Ig-1* allotype locus (see Chapter 2). In this system a second *Ir* gene linked to *H-2* regulates both IgG and IgM responses.

Ir Genes in Man and Other Species

Ir gene control in man is most obvious in certain hereditary immune deficiency syndromes. The most profound defects in the immune system preclude activation of either the T- or B-cell pathways. Hereditary thymic aplasia associated with atrophy of all lymphoid tissues and failure of immunoglobulin production is attributable to either X-linked or autosomal recessive genes. The defect lies in the failure of stem cells to differentiate into cells of the T- and B-lymphocyte lineage. On the other hand, sex-linked Bruton-type hypogammaglobulinemia is specifically a B-cell defect and normal T-cell functions such as DTH, allograft rejection, and resistance to viral infections are maintained. Only B-cell functions are impaired. Infants with the Di George syndrome (thymic hypoplasia) lack cellular immune competence, yet are capable of antibody production and usually possess normal serum immunoglobulin levels, the genetically controlled defect apparently being confined to T-cell lymphokine-mediated immune functions. Associations between disease susceptibility and HLA type in man represent examples of *Ir* gene control that are examined in more detail in Chapter 8 in conjunction with immunodeficiency and immunogenetic diseases.

The genetic control exerted in the case of congenital immunodeficiencies is obviously more broadly based than the antigen-specific *Ir* gene control evident in normal animals. The search for specific *Ir* genes in man has been hindered

by inability to challenge with synthetic immunogens possessing limited antigenic determinants. However, tentative identifications of antigen-specific *Ir* genes have been reported (Table 7–4). Immune reactions (either cellular or humoral) were assessed to the antigens following either natural stimulation (e.g., ragweed, gluten, avian proteins, Australia antigen, *M. leprae,* streptococci, and measles) or vaccination (rubella, influenza, and diphtheria toxoid). In most instances, apparent association of antibody or CMI levels could be made with particular *HLA* loci. Although the IgE response to ragweed antigens was reportedly linked to *HLA,* subsequent studies with this and other pollen antigens have failed to confirm *HLA*-linked *Ir* gene control. Perhaps the most concrete evidence for *HLA*-linked *Ir* gene control was obtained in a study of susceptibility to *Mycobacterium leprae* and the nature of the ensuing disease. Initial observations that the incidence of leprosy was associated with BW21 in Ethiopia, B14 in Spain, and A9 in India were substantiated in studies of families in which two or more siblings suffered from leprosy. It was found that siblings contracting the same form of leprosy (tuberculoid in this study) shared the same *HLA* haplotype at a frequency greater than that expected given non-HLA-association of the disease. It has been proposed that an *Ir* gene linked to *HLA* leads to an inappropriate immune response to *M. leprae* and thus precipitates the disease. Correlations between *HLA* and diverse diseases are considered in greater detail in Chapter 8.

Histocompatibility-linked *Ir* genes have been identified in rats and rhesus monkeys. As the rat *MIC* becomes better mapped, this species should serve to broaden and extend the studies carried out in mice and guinea pigs. Single *Ir* gene regulation linked to the *MIC* of rats has been found for lactic dehydrogenase and the synthetic polymer (T,G)-A-L. Genes controlling respon-

TABLE 7–4. Proposed *Ir* Genes in Man

Antigen	HLA association
Ragweed antigen E	?
Ragweed antigen Ra5	B7
Gluten	DW3
Avian proteins	DW6
Australia antigen	BW35
Mycobacterium leprae	+
Streptococci	B5
Rubella	BW17 + A28
Influenza virus	BW16[a]
Diphtheria toxoid	−
Measles virus	+

Adapted from von Rood et al., *Progress in Immunology III*, New York: Elsevier-North Holland, 1977, p. 341.

(?) linkage unconfirmed or questionable; (+) indicates association exists but not mapped to a given locus; (−) indicates no association with *HLA*.

[a]Associated with low responsiveness

siveness to the copolymers GT (*Ir-GT*) and GA (*Ir-GA*) have been shown to govern both humoral and cellular responses (DTH) to these antigens. Furthermore, the rat system has allowed studies at the T-cell level which show that only T-cells from high responder *Ir-GT* strains can undergo a proliferative response when stimulated with GT. Studies in rhesus monkeys are of special interest because of the obvious phylogenetic links with man. The capacity of rhesus monkeys to respond to either GA or DNP-GL has been shown in inheritance studies to be linked to *RhLA* haplotypes. *RhLA* is the monkey equivalent of the human *HLA* and murine *H-2* complexes (see Chapter 6).

Of the nonmammalian species, chickens have been shown to possess *MIC*-linked *Ir* genes. As in mice, high responses to GAT in chickens are controlled by a dominant autosomal gene linked to the *B* complex of loci representing the *MIC* of the chicken. However, unlike the GAT *Ir* gene system in mice, the defect in nonresponder chickens does not appear to lie at the T-cell level. Obviously, *Ir* gene regulation is not an exclusive property of mammals. *Ir* gene regulation almost certainly began in lower multicellular animals and may have evolved in complexity as the immune system evolved to the multicomponent integration of macrophage, T- and B-cell functions characteristic of mammals.

MECHANISMS OF *Ir* GENE ACTION

Cellular Expression of *Ir* Genes and Their Influence on Immunocyte Interactions

Macrophages have been shown to play an essential role in the initial phases of the immune response, particularly in antigen processing, and lymphocyte interactions leading to antibody production. *Ir* gene defects at the macrophage level leading to low or nonresponsiveness have been documented in guinea pigs and mice. Restriction of the interaction of macrophages and T-lymphocytes was shown in vitro to depend on the expression of appropriate *I* region gene products on the macrophage surface. Strain 2 guinea pigs possess the *Ir* gene for responsiveness to DNP-GL but not to DNP-GT. Strain 13 guinea pigs conversely respond to DNP-GT but not to DNP-GL. F_1 progeny (i.e., 2 $\times$ 13) respond to both DNP-GL and DNP-GT in vivo and in vitro when antigen is presented to sensitized peritoneal lymphocytes either alone or on previously stimulated F_1 macrophages. However, when induction of antibody production in F_1 cultures was attempted with antigen-stimulated macrophages from either parent strain, the ability to stimulate F_1 peritoneal lymphocytes hinged on the *Ir* gene(s) possessed by the strain from which the macrophages were derived. Thus, strain 2 macrophages pulsed with DNP-GL, or strain 13 macrophages pulsed with DNP-GT stimulated excellent F_1 responses to the corresponding antigens. Conversely, nonresponder strain 2 macrophages pulsed with DNP-GT or strain 13 pulsed with DNP-GL did not elicit an in vitro response in F_1 peritoneal lymphocytes. It was later found that alloanti-

serum directed against the Ia antigens of responder macrophages could block the in vitro F1 response. These studies demonstrated that *Ir* gene dysfunction may be expressed in macrophages and not exclusively in T-cells as earlier studies indirectly indicated. Furthermore, a strong correlation was established between *Ir* gene expression and function and the presence of certain Ia specificities.

That Ia molecules with *Ir* gene functions play a role in orienting and presenting antigens has been strongly suggested by Rosenthal and co-workers who examined the immune response of guinea pigs and mice to insulins from various species. The T-cells responding to these insulins are helper T-cells, since they can specifically augment antibody responses to haptens coupled to insulins. T-cells from both strains 2 and 13 guinea pigs and their F1 progeny can respond to whole porcine insulin. Strain 2 can only respond to the A chain, strain 13 responds preferentially to B chain, and (2 × 13) F1 animals can respond to both isolated A chain and B chain insulin determinants. The relevant *Ir* genes defining the response to the A and B chain determinants map to the *I* region homologue of the guinea pig. The ability of strain 2 T-cells to respond to A chain insulin is dependent on which amino acids are at positions 8, 9, and 10 in the A chain loop. Insulins from species with total identity in this area can induce strain 2 T-cells, but insulins different from porcine insulin only at these three amino acid residues induce poor or reduced proliferative responses. In contrast, (2 × 13) F1 animals respond well to all A chain insulins. To determine the site of the *Ir* gene defect, T-cells from (2 × 13) F1 were tested for their ability to respond to parental strain macrophages (Figure 7–6). If the defect were at the level of the responding T-cells, then F1 T-cells should have responded independent of which parental macrophage "presents" the antigen. This was not the case, which suggests that the determinant recognized by the T-cells depends on the *I* region of the antigen-presenting cell. Similar studies have been done in mice, and the implication is that the *Ir* genes act at the level of the macrophage by selecting discrete regions within the antigen for recognition by the T-cell.

In mice, delayed type hypersensitivity to GAT is under *Ir* gene control expressed at the macrophage level as is the response to the GLΦ copolymer (discussed earlier) that is regulated by complementing *Ir* genes. Because of technical difficulties, unequivocal evidence for *Ir* gene regulation by T-cells in mice using such techniques as adoptive cell transfer or in vitro cell culture has yet to be reported. Considerable indirect evidence exists to suggest that T-cells are a site of expression of *Ir* genes, although it should be remembered that T-dependent *Ir* genes function at the apparent interface of macrophage-T_h-cell interaction. The discovery of soluble factors produced by helper and suppressor T-cells and bearing Ia determinants has been interpreted as an expression of *Ir* gene function in T-cells.

Ir gene expression in B-cells has been demonstrated by in vitro techniques similar to those applied to macrophage studies. In the GAT system, primed (responder × nonresponder) F1 helper T-cells do not provide help for nonre-

a) Amino acid sequence: Insulin A-chain loop

	6	7	8	9	10	11
Pigs	Cys	Cys	Thr	Ser	Ile	Cys
Cattle	——	——	Ala	——	Val	——
Sheep	——	——	Ala	Gly	Val	——

b) DNA synthesis by (2 × 13) F_1 T cells

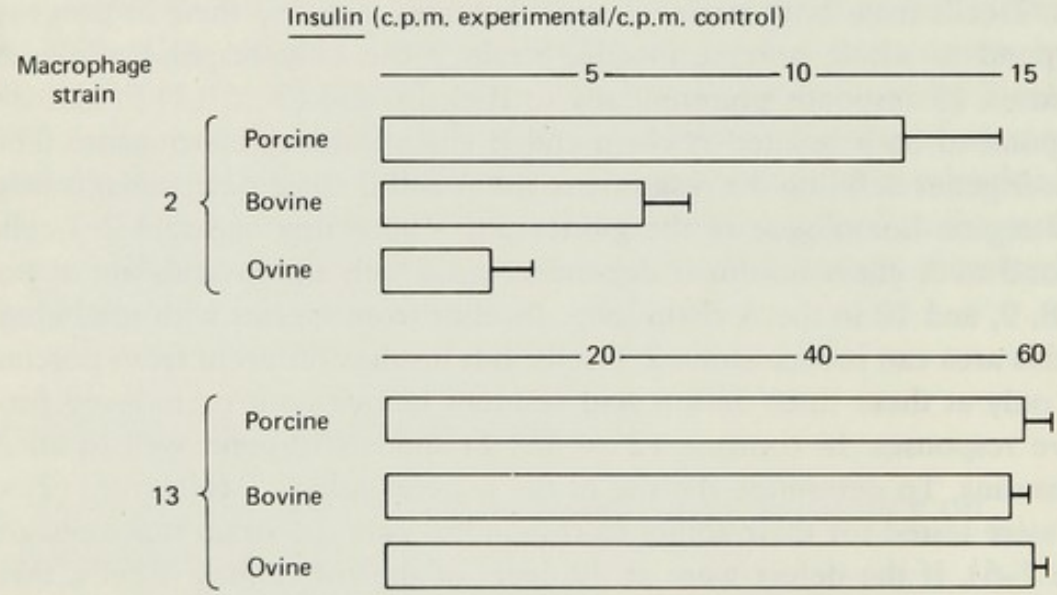


FIGURE 7–6. The amino acid sequences of porcine, bovine, and ovine insulins only differ at positions 8 to 10 in the A chain loop, yet the immune response to these insulins varies in mice and guinea pigs. (b) "*I*-region" disparate strain 2 and strain 13 macrophages differ in their ability to evoke anti-insulin responses by (2 × 13)F1 T-cells. Porcine insulin presented by strain 2 macrophages evokes a good proliferative response, but ovine insulin evokes almost no response. In contrast, all insulins presented by strain 13 macrophages are strongly immunogenic.

Source: Adapted from Rosenthal, A. S., *Immunol. Rev.* 40:146, 1978. © Munksgaard International Publishers Ltd., Copenhagen, Denmark.

sponder strain B-cells, but cooperate with responder B-cells to yield excellent antibody production. Similar results have been found with DNP-GLT and the GLΦ complementing gene system. As discussed earlier, experiments indicate that the *Ir-GAT* gene is also expressed in macrophages, a result that may have great bearing on proposed mechanisms of action of *Ir* genes. Because the *H-2* linked *Ir* genes map in the *I* region, and this region also codes for the Ia antigens expressed on T-cells, B-cells, and macrophages, it has been tempting to speculate that Ia molecules may be *Ir* gene products. As already noted, several studies have indicated at least a close association of Ia specificities with *Ir* gene functions.

Let us now consider the role of *I* region products (i.e., both Ia molecules and *Ir* genes) in the interaction of cells in the immune response. *I* region controlled restriction in cellular interactions was first noted for the presentation of antigen to T-cells by macrophages in the guinea pig response to DNP-GL and DNP-GT. This phenomenon was shown to be related to the functions of *Ir* genes and Ia molecules. In mice, the stimulation of primed specific helper T-cells by antigen-presenting macrophages was shown to be restricted by the matching of *I-A* region genes. Primed T-cells provided helper function to syngeneic unprimed B-cells only when antigen was presented with macrophages bearing the same I-A region determinants as the helper T-cells. The generation of killer T-lymphocytes (Tk) is also an H-2 associated phenomenon. Antigen primed Tks will only lyse antigen bearing target cells that express syngeneic H-2 determinants. This well-established phenomenon has been described for several viral systems including influenza, vaccinia, and lymphocytic choriomeningitis virus, as well as the Tk response to H-Y antigens. *Ir* gene regulation has been reported in the response to H-Y or male-specific antigens and in the primary cytotoxic T-cell responses to virus-infected target cells. The H-Y Tk response is controlled by allelic complementing genes mapping in the *I* region of *H-2,* with responsiveness being dominant. Investigations in several virus systems have shown H-2 K or D related *Ir* gene control in the generation of H-2 K or D restricted Tk responses. In these instances, low or nonresponsiveness is dominant. The consequences of inherited nonresponsiveness to particular viral infections in individuals of any species may be disastrous.

Effects on Antibody Isotypes and Idiotypes

In numerous instances, *Ir* genes of the *MIC* have been found to regulate the switchover from IgM to IgG production, especially in secondary immune responses. The IgM response to antigens such as (T,G)-A-L and TNP-MSA in vivo is T-independent and not under the primary control of *I* region genes. However, the IgG response to these antigens is T-dependent, as indicated by thymectomy studies, and is governed by one or more *H-2I* genes. No lack of responsiveness exists at the B-cell level in these examples, as immunization with the antigen complexed to a xenogeneic carrier (e.g., TNP-MSA complexed with methylated bovine serum albumin) results in an excellent IgG response in all normal mouse strains. These findings are consistent with the conclusion that certain *MIC*-associated *Ir* genes regulate helper T-cell activity. The T-dependent IgE response to ragweed antigens in mice appears under the control of both *H-2K* and *Ir-IA* genes.

Direct control of antibody idiotype in mice is exerted by allotype-linked genes or immunoglobulin genes, many of which map close together on chromosomes 6 and 12 (see Figure 1–6). As already indicated in Chapter 2, heavy chain loci are now given the symbol *Igh* followed, in the case of variable-region loci, by the antigen symbol for the corresponding antibody or idiotype, for example, *Igh-Pc* or *Igh-Ars.* Variable-region loci for kappa-chain or lambda-chain variants may be correspondingly designated *Igk-Efl* and *Igl-Lo,* respective-

ly, with the marker trait shown to the right. In essence, the Ig part of the symbol is for immunoglobulin while the heavy, kappa, and lambda chains are identified by the letters *h, k,* and *l,* respectively. Allotype variants at the *Igh* constant-region loci become *Igh-1* to *Igh-6* in lieu of the six loci previously designated *Ig-1* to *Ig-6* if the new nomenclature gains general acceptance. Because the *H-2*-linked *Ir* genes are quite distinct from the immunoglobulin *V* region genes, they do not directly control idiotype. *MIC*-associated *Ir* genes can, however, affect the specificity of the antibody produced and the clonal nature of the response. An example of *MIC*-linked *Ir* genes controlling the recognition of simple determinants in a complex antigen has already been cited (i.e., the GAT system in guinea pigs). It has been shown, using staphylococcal nuclease as an antigen, that $H\text{-}2^a$ high responder strains produce different proportions of antibodies to different determinants on the antigen compared to low responders ($H\text{-}2^b$ strain). As these results were obtained using congenic strains that presumably differed only at *H-2,* the antibody differences should reflect *Ir* gene directed recognition of different determinants on the same macromolecule. This is a logical consequence of the response to a complex antigen being governed by a number of *Ir* genes, each recognizing separate determinants on the molecule.

Pleiotropy: Structural Versus Regulatory Effects

If a single gene codes for two or more distinctive functions it is said to be pleiotropic. Such a description applies to *Ir* genes to the extent that both structural and regulatory expression of the genes is evident. The regulatory manifestations of *Ir* gene expression have been extensively covered in preceding sections. We consider here the evidence for structural correlates of *Ir* gene expression.

As already noted, there is circumstantial evidence to link *Ir* genes and Ia molecules expressed at the surface of lymphocytes and macrophages. The experimental evidence for this association is twofold. First, in a number of instances, *Ir* gene control has been found to be associated with a given Ia specificity (Figure 7–5). This appears true for random-bred populations as well as for inbred strains of guinea pigs and mice. Second, monospecific anti-Ia serums have been used to inhibit in vitro lymphocyte responses of cells sensitized to antigens under known *Ir* gene control in vivo. By these methods, associations between Ia.1 and Ir-GT, Ia.2 and Ir-DNP-GL, and Ia.3 and Ir-DNP guinea pig albumin have been established in the guinea pig. In mice there is an association between Ia.7, specified in the *I-E/C* region, and the *Ir-GLΦ α* gene.

Another proposed class of structural correlates of *Ir* gene expression are the antigen-specific molecules or factors produced by mouse T-cells. These factors, possessing either helper or suppressor activities, bear antigenic determinants encoded by genes in the *I* region thus linking them with the *Ir* genes and Ia molecules. Helper molecules of this type have been demonstrated in

the murine responses to (T,G)-A-L, (T,G)-Pro-L, and GT. Obtained from primed lymphocyte extracts, helper factors specifically enhance the in vitro humoral response to the priming antigen. They bear determinants encoded by the *I-A* region. Suppressor factors inhibit humoral and cellular responses in vitro and in vivo. In contrast to helper factors, certain suppressor molecules bear I-J determinants. They have been reported in responses to several immunogens including hapten-carrier conjugates, GAT, and GT.

A number of characteristics are apparently shared by the two oppositely acting helper and suppressor T-lymphocyte products:

1. They are produced by T-cells as a result of antigen stimulation.
2. They possess antigen-binding ability and can accordingly be selectively absorbed.
3. Structural characterization indicates a molecular weight between 40,000 and 50,000 daltons, and an absence of immunoglobulin constant region determinants.
4. Both factors can be adsorbed on anti-I region immunoadsorbents. Some suppressor factors carry I-J region determinants, whereas helper factors possess I-A region determinants.

Direct evidence that antigen-specific factors are encoded by *Ir* genes has not been obtained. Nevertheless, the inability to evoke helper factors to stimulate B-cells has been proposed as the mechanism of *Ir* gene control in the (T,G)-A-L and (T,G)-Pro-L systems. There is mounting evidence to indicate an association between *Ir* genes and molecules such as Ia and antigen-specific factors that are encoded by genes mapping in the *I* region of the *MIC*.

Models for *Ir* Gene Function

Despite rapid advances in recent years in understanding *Ir* gene regulation of the immune response, no definitive mechanism for the action of these genes has been ascertained. However, at least two mechanisms differing in their sites of expression of *Ir* genes are suggested by the accumulated experimental evidence from a number of antigen systems in guinea pigs and mice. The macrophage/B-cell model postulates that *Ir* genes control the expression of Ia molecules on macrophages and B-cells. An alternative model puts forward the T-cell as the crucial site of *Ir* gene expression. The basic hypotheses set out below are shown in diagrammatic form in Figure 7–7.

Macrophage/B-Cell Model

The Ia molecules expressed on macrophages, and perhaps on B-cells as well, are capable of interacting with antigen. B-cells are certainly capable of binding appropriate antigenic determinants to their corresponding Ig receptors abundantly present on the cell surface. The T-cell, on the other hand, possesses receptors capable of recognizing or being stimulated by the Ia-antigen complex.

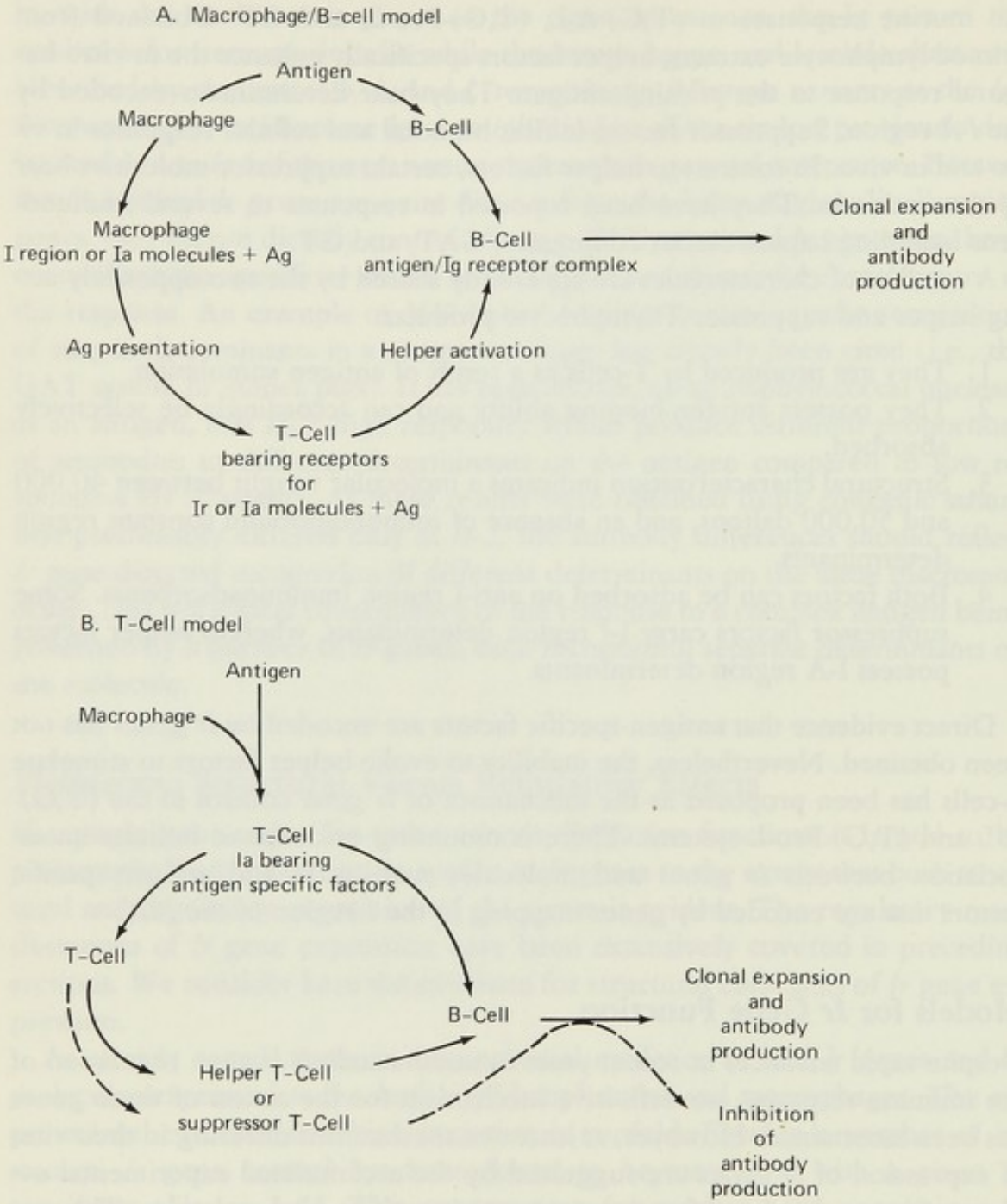


FIGURE 7–7. Hypothetical models for *Ir* gene expression and function. In model A, *Ir* gene function via I region or Ia molecules is postulated mainly at the macrophage and B-cell levels. The second model (B) assumes that Ia-associated antigen-specific factors produced by T-cells are the key effectors of *Ir* gene functions.

Thus in a T-dependent response, antigen is processed by macrophages leading to associated expression with Ia molecules on the macrophage surface. This stimulus is able to activate T-cells bearing receptors with specificity for both the Ia and antigen components. The Ia antigens, according to this model, act to orient and present antigens and/or act to trigger specific T-cell subsets. The activated T-cell is, in turn, able to recognize antigen/Ig receptor complex on

B-cells which leads to triggering of the B-cell. Perhaps the activated complex on the B-cell is formed by the association of Ia molecules with antigen bound via the immunoglobulin receptors. Whereas each macrophage probably expresses the full complement of Ia molecules encoded in the *I* region and is thus not clonally restricted, the question of Ia clonal restriction in T- and B-cells remains open. Surely the B-cell immunoglobulin receptor for antigen is restricted in this way.

T-Cell Model

Ir genes are expressed in T-cells and are associated with the production of specific helper or suppressor factors. These factors may directly stimulate appropriate B-cell populations or may promote the development of specific helper or suppressor T-cells, which in turn act upon potential effector cells. This model is more attuned to *Ir* gene regulation of high-versus-low antibody responsiveness than to all-or-none responsiveness.

In light of available evidence, neither of these hypotheses should be regarded as definitive; the reality may include components of both mechanisms. Moreover, *Ir* genes are located not only within the major immunogene complex suggested by the models, but at numerous other chromosomal locations as well. Certainly the triad of macrophages, T-cells, and B-cells all interact in diverse immune responses, although the molecular basis of the sequential interactions is unclear.

KEY REFERENCES

Books and Review Articles

Benacerraf, B., and Germain, R. N. The immune response genes of the major histocompatibility complex. *Immunol. Rev.* 38:70–119, 1978. Specific *Ir* genes in mice and guinea pigs are described giving details of present available data. Complementing *Ir* genes receive special attention as does *Ir* gene control at the cellular level. This extensive article concludes with two models for the function of *Ir* genes.

Hildemann, W. H. Genetics of immune responsiveness. *Ann. Rev. Genet.* 7:19–36, 1973. Phylogeny of immune responsiveness, immune responses to infectious agents, genetic control of immune responses in man, mice, and guinea pigs, and mechanisms of *Ir* gene control are covered.

Katz, D. H. *Lymphocyte Differentiation, Recognition and Regulation.* New York: Academic Press, 1977. An in-depth review of *Ir* genes in mice. Gives particular attention to "immune suppression genes" and to the role of *MIC* products in cellular interactions regulating the immune response.

McDevitt, H. O. (ed.). *Ir Genes and Ia Antigens.* New York: Academic Press, 1978. A wide-ranging series of articles reviewing immune response genes in guinea pigs, mice, and man.

Articles

Benacerraf, B., and Dorf, M. E. Genetic control of specific immune responses and immune suppressions by *I*-region genes. *Cold Spr. Harb. Symp. Quan. Biol.* 41:465–475,

1977. Complementing *Ir* gene control for responsiveness to GLPhe and for immune suppression by GT is reported.

Doherty, P. C., Biddison, W. E., Bennink, J. R., and Knowles, B. B. Cytotoxic T-cell responses in mice infected with influenza and vaccinia viruses vary in magnitude with *H-2* genotype. *J. Exp. Med.* 149:534–543, 1979. *H-2*-linked genes control the generation of cytotoxic T-cells.

Kipps, T. J., Benacerraf, B., and Dorf, M. E. Analysis of the restricted dinitrophenol (DNP) antibody response to the DNP conjugate of poly-(L-Glu^{56}, L-Lys^{35}, L-Phe^{39}). *J. Immunol.* 120:46–51, 1978. Isoelectric focusing revealed restriction in the number of DNP-specific antibody-producing clones arising in responders to DNP—(G,L, Phe).

Kolsch, E., and Falkenberg, F. W. Genetic control of the immune response to phage fd. IV. Complementation between *H-2*-linked *Ir* genes. *J. Immunol.* 119:2114–2119, 1977. Complementing *H-2*-linked genes, $Ir_{fd}\alpha$ and $Ir_{fd}\beta$, control responsiveness to phage fd in conjunction with modulating non-*H-2*-linked genes.

Merryman, C. F., Maurer, P. H., and Zeiger, A. R. Genetic control of two independently segregating loci in mice to the sequential polypeptide (Tyr-Ala-Glu-Gly)^n. *Immunogenetics* 4:373–380, 1977. Responsiveness to the polymer (T-A-G-Gly)^n is controlled by two genes, one *H-2*-linked and the other non-*H-2*-linked.

Meruelo, D., Deak, B., and McDevitt, H. O. Genetic control of cell-mediated responsiveness to an AKR tumor-associated antigen. *J. Exp. Med.* 146:1367–1379, 1977. The locus controlling the cell-mediated immune response to an AKR tumor cell antigenic determinant maps in the *I-J* region of *H-2*.

Mouton, D., Heumann, A. M., Bouthillier, Y., Mevel, J. C., and Biozzi, G. Interaction of *H-2* and non-*H-2* linked genes in the regulation of antibody response to a threshold dose of sheep erythrocytes. *Immunogenetics* 8:475–486, 1979. Antibody responses to an optimal dose of sheep cells reflect the additive effect of about ten independently segregating loci, one of which is *H-2* linked, whereas responsiveness to a threshold dose is determined mainly by two loci.

Passos, H. C., Sigueira, M., Reis, M. H., Ferreira, V. C. A., Ibanez, O. M., Sant'Anna, O. A., and Biozzi, G. Genetic control of immune response to protein antigens I. Two way selective breeding of mice for quantitative antibody responsiveness to bovine serum albumin and rabbit γ-globulin. *J. Immunol.* 119:1439–1444, 1977. The immune response to complex protein antigens is under multigenic control.

Pisetsky, D. S., Berzofsky, J. A., and Sachs, D. H. Genetic control of the immune response to staphylococcal nuclease VII. Role of non-*H-2*-linked genes in the control of the anti-nuclease antibody response. *J. Exp. Med.* 147:396–408, 1978. Although *H-2*-linked genes determine initial responsiveness to staphylococcal nuclease, non-*H-2*-linked genes determine the overall magnitude of the antibody response.

Rosenthal, A. S. Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* 40:136–152, 1978. *Ir* gene function in both guinea pig and mouse appears to operate by intramolecular selection of discrete regions within the antigen by macrophages for recognition by T-cells.

Urba, W. J., and Hildemann, W. H. *H-2*-linked recessive *Ir* gene regulation of high antibody responsiveness to TNP hapten conjugated to autogenous albumin. *Immunogenetics* 6:433–445, 1978. The gene locus, *Ir-6*, controlling responsiveness to TNP-MSA maps to the *I-A* region of *H-2*. Alleles of this gene locus regulate switchover from IgM to IgG antibody production in thymus-dependent high responders. See also follow-up article in *J. Immunol.* 123:209–215, 1979.

Wettstein, P. J., Haughton, G., and Frelinger, J. A. *H-2* effects on cell-cell interactions in the response to single non-H-2 alloantigens. I. Donor *H-2D* region control

of H-7.1 immunogenicity and lack of restriction in vivo. *J. Exp. Med.* 146:1346–1355, 1977. Self-H-2 molecules differ in their ability to affect the immunogenicity of other cell-surface molecules.

Zinkernagel, R. M., et al. *Ir*-genes in *H-2* regulate generation of anti-viral cytotoxic T cells. *J. Exp. Med.* 149:592–606, 1979. In *H-2*-linked *Ir* gene control of cytotoxic T-cell generation, unresponsiveness is dominant and the genes map to either the *K* or *D* regions in *H-2*.

8

IMMUNODEFICIENCY AND IMMUNOGENETIC DISEASES

HOST RESISTANCE TO INFECTIOUS DISEASE

The capacity to resist or withstand infection and to dispose of foreign substances that enter the tissues is essential to the survival of all species ranging from microorganisms to man. Even bacteria must cope with virulent phages just as man must be able to cope with virulent bacteria. The immune system must aid the individual in the defense against the constant onslaught of pathogens and nonreplicating agents that may produce infection and disease. Host resistance may depend on cell-mediated immunity and the phagocytic system as well as humoral immunity acting together or independently. It has long been recognized that diverse stocks or strains of domestic animals from honey bees to horses show profound differences in genetic resistance to particular pathogens. Given the complex interactions and pathways of the immune response (see Figures 2–1 and 9–1), it is not surprising that such resistance often has a multigenic basis. Resistance of the all-or-none type to a virulent pathogen is uncommon. A strain of animal highly resistant to a pathogen at a low dose will usually show an increasing susceptibility at much higher doses. To test for inherited susceptibility requires not only a uniform pathogen genotype and a uniform environment but also well defined host genotypes. Experimental use of inbred host strains and selectively cloned stocks of pathogens control for major variables most desirably. In the now classic studies of Gowen, selectively inbred strains of mice were shown to possess a characteristic degree of resistance to a cloned strain of *Salmonella typhimurium.* As shown in Figure 8–1, the range was from nearly complete resistance to total susceptibility measured as the percent of animals surviving infection. Moreover, the same host genotypes were capable in similar degrees of developing specific immunity induced by a killed vaccine of this *Salmonella* strain. Progeny tests revealed dominance of most of the multiple genes facilitating immunity. The immunologic superiority of females over males was also observed. More recent work has borne

out the general rule that females display stronger immune responses than males. The relative specificity of immunogenetic resistance to disease was also demonstrated in this early study. Thus, one of Gowen's strains most resistant to mouse typhoid proved highly susceptible to pseudorabies virus.

Specific immune response genes as detailed in Chapter 7 can affect recognition and processing of antigens as well as the cooperative interactions of responding lymphoid subsets. In this chapter deviations of *Ir* gene action leading to disease are considered. Resistance or susceptibility to infectious agents usually involves complex interactions between host and parasite. Indeed, under certain circumstances the host immune response may be only of secondary importance. In sickle-cell anemia, for example, substitution of a valine residue for a glutamic acid in the beta chain of hemoglobin is the molecular basis of the disease. Patients with sickle-cell anemia are unusually susceptible to *Salmonella* infections with concomitant osteomyelitis; however, heterozygous individuals with the sickle-cell trait are partially resistant to falciparum malaria. Thus, the otherwise deleterious sickle-cell gene represents a selective advantage in malarious regions such as tropical Africa. Resistance to viral infection is determined not only by host immune response genes but also by genes that modify the physiology of the target cell virus or affect the replication of virus or the expression of viral determinants. For instance, the *Fv-1* locus on chromosome 4 in the mouse determines if murine leukemia viruses can replicate efficiently or not. Several genes in the mouse, such as *W, S1,* and *f,* that produce anemia also determine susceptibility to Friend virus (*FV*)-induced leukemogenesis, presumably by affecting the metabolism of host target cells. The fungal disease disseminated coccidioidomycosis occurs markedly more frequently in Filipinos and American Negroes than in Caucasians. Here susceptibility is associated with dark pigmented skin, and the specific immune response is not a decisive factor. These examples underscore the importance of seemingly nonimmunologic gene products in resistance to infectious disease.

Another level of *Ir* gene control has been identified by studies of immunodeficiency disorders in man and experimental animals. These hereditary defects impair the development of the immune response at specific points of lymphoid differentiation. Major pathways of the immune response that govern resistance to various infections have been identified in patients with immunodeficiency diseases. Thus, T-cell deficiency allows susceptibility to opportunistic viruses, while B-cell defects weaken resistance to pyogenic bacteria. Much remains to be learned about cellular interactions constituting host resistance. For instance, we know T-cells together with macrophages can effectively eliminate virus from some solid tissues, but little is known about the role of components such as interferon and natural killer cells which may be involved in virus-induced inflammatory exudates. Another group of genes discussed in this chapter affects normal immunoregulatory mechanisms. Effects of these loci may lead to autoimmune disease and other immunologic disorders.

Single genes have been found to influence viral infections in the mouse, but

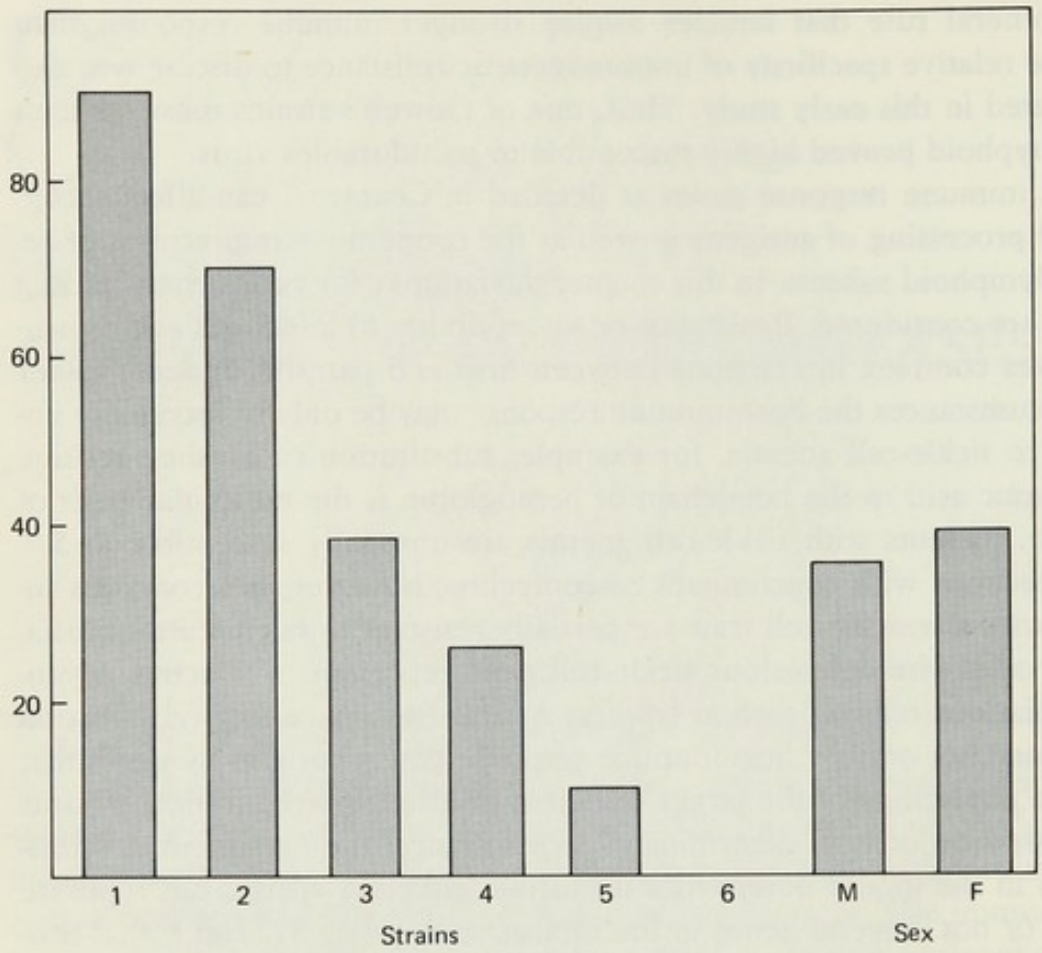


FIGURE 8–1. Resistance of different strains and sexes of inbred mice to a cloned line of *Salmonella typhimurium*: measured with respect to survival twenty-one days after infection with 2×10^5 bacteria.

Source: Adapted from Gowen, *Bacteriol. Rev.*, 24:192, 1960. © American Society for Microbiology, Washington, D.C.

in these genetically "simple situations" the gene probably acts at an early stage of the virus-host relationship. Predictably, polygenic control is evident under most circumstances (Table 8–1). Pioneering studies of the genetics of host resistance to group B togaviruses (formerly B arboviruses) were carried out by Lynch and Hughes and by Webster over forty years ago. Webster identified autosomal genes conferring resistance to St. Louis encephalitis virus which were unlinked to a gene controlling resistance to *Bacillus enteriditis.* Four mouse strains designated BSVR, BRVR, BSVS, and BRVS were developed by selection for bacteria-susceptibility (BS) or resistance (BR) and virus-resistance (VR) or susceptibility (VS). Using resistant BRVR and PRI-strain mice and susceptible C3H/He mice as prototype strains, Koprowski and co-workers have examined the genetics and mechanisms of resistance to togaviruses. Dominance of resistance was shown to be enforced by the virucidal action of histiocytes and macrophages, whereas the recessive allele in the phagocytes of susceptible mice allows virus multiplication and continuing invasion. Certain

inbred strains of mice are susceptible to yellow fever virus but wild mice are notably resistant. Studies of murine resistance to the mouse hepatitis virus have shown genetic patterns indicating a single dominant gene for susceptibility. The genetic susceptibility depends on the selective destruction of macrophages that normally prevent viremia. The genes affecting macrophage resistance to togaviruses and hepatitis virus are not allelic but both probably act on lysosomal enzymes involved at an early stage of viral infection.

The immune responses to the mouse pox virus (i.e., ectromelia) and to lymphocytic choriomeningitis virus (LCMV) have both been shown to be under dominant genetic control. However, in ectromelia the host immune response is protective; accordingly, the dominant gene for immune responsiveness confers resistance to the disease. In contrast, the antibody response to LCMV leads to appearance of immune complexes that are detrimental to the host. Overall susceptibility to the LCMV-associated disease is inherited as a polygenic trait. Whether "resistance" or "susceptibility" to a disease is dominant, recessive, or polygenic depends on the consequences of the immune response to the virus.

TABLE 8–1. Genetic Basis of Host Resistance Determined for Various Infectious Agents

		Genetic resistance (+)			
Pathogen	Host species	Single dominant	Recessive	Polygenic	MIC involvement
BACTERIA					
Salmonella	Chicken			+	
	Mouse				
Bacterial/viral Pneumonitis	Mouse			+	
NON-C-TYPE VIRUSES					
Togaviruses	Mouse	+			
Ectromelia	Mouse	+			
Mouse hepatitis	Mouse		+		
Herpes simplex	Mouse			+ (≥3 loci)	
Influenza A	Mouse	+			
Lymphocytic choriomeningitis	Mouse			+	+
Marek's disease	Chicken			+	+
C-TYPE VIRUSES					
Erythroblastosis	Chicken	+			
Friend leukemia	Mouse			+ (≥3 loci)	+
Gross leukemia	Mouse			+ (≥2 loci)	+
Mammary tumor	Mouse			+	+
Polyoma	Mouse			+ (≥3 loci)	
Rous sarcoma	Chicken			+	+

INHERITANCE OF SUSCEPTIBILITY TO TUMOR VIRUSES

From the very earliest studies of cancer by Murray and co-workers at the turn of the century, it was evident that the appearance of cancer was under genetic control. The development by Little, Strong, Furth, and others of inbred strains of mice with high and low tumor incidence, particularly strains such as C58 and AKR which have a high frequency of lymphoid tumors, provided the insight that tumor susceptibility could be decisively influenced by particular genes. The discovery that inbred strains differ in their susceptibility to oncogenic viruses triggered a search for genes that influenced viral oncogenesis.

Certain exogenous tumor viruses furnish a unique opportunity for concurrent study of the genetic basis of susceptibility to infection as well as to tumor development. Chang and Hildemann investigated the genetic control of susceptibility to neonatal injection of plaque-purified polyoma virus. Multiple criteria were used to measure susceptibility, including the latency period before the appearance of tumors, the spectrum and multiplicity of tumors, the incidence of runting/tumor development, and the capacity of a virus to multiply and produce cytopathic effects in cell cultures of various host genotypes. At least three independently segregating gene loci were shown to influence the susceptibility to polyoma virus in neonatal F_1 hybrids and reciprocal backcross progeny of highly susceptible AKR/J and relatively resistant C57BL/6J strains of mice. High susceptibility of albino AKR mice to the runting syndrome was recessively inherited (Table 8–2). Numerous runted mice (e.g., AKR) died before the tumors were detectable, but long-surviving runts always developed the tumors. In contrast, susceptibility to tumorogenesis appeared to be determined by a single autosomal gene with incomplete dominance. High but submaximal susceptibility of (AKR $\times$ C57BL/6) F_1 hybrids was found at both low and high virus doses. Moreover, the recessive resistance of the C57BL/6 strain was substantially overcome by infection with a high dosage of polyoma virus. Analysis of the $F_1 \times$ AKR backcross progeny showed a much increased susceptibility to runting of albino compared with black progeny at both virus dosages tested. Analysis of the segregation data showed that susceptibility to runting involved interaction of the albino gene or a closely linked gene and an independent recessive gene also of AKR origin. Moreover, the incomplete dominance ascribed to the two genes regulating susceptibility to tumorogenesis and resistance to runting may well mean that other host genes operate in the development of polyoma virus-induced disease.

Cell cultures were uniformly susceptible to the replication and cytopathic effects of polyoma virus irrespective of the host genotype; thus the host resistance in vivo did not depend on the absence of vulnerable target cells. Early maturation of immunologic capacity appears to be the decisive component in the genetic resistance to malignant infection associated with the C57BL/6 genotype. Increased vulnerability to malignancy reflected either in runting or tumors was also marked by substantial reduction in the average latent period for tumor development. Among the diverse criteria of susceptibility evaluated, far

TABLE 8–2. Incidence of Runting and Tumors in Mice Inoculated with 1.4×10^3 Plaque-Forming Units (PFU) of Polyoma Virus (Low Dosage) by Intracardiac Route Within Twenty-Four Hours after Birth

Mouse strains and progenies	Number of mice infected	Runted mice[a]		Mice with tumor[b]		Mice runted and/or developing tumor		Latent period for tumor induction (weeks)	
		No.	%	No.	%	No.	%	Average	Range
AKR	67	64	95.5	21	31.3	67	100.0	8.00	6–9
C57BL/6	52	4	7.7	13	25.0	16	30.8	16.15	7–26
(C57BL/6 ♀ × AKR ♂)F1	85	8	9.4	70	82.4	75	88.2	11.04	7–20
F1 ♀ ×AKR ♂ (backcross)									
Albino	89	35	39.3	64	71.9	87	97.8	8.27	6–13
Black	77	9	11.7	70	90.9	72	93.5	8.88	6–21
Total	166	44	26.5	134	80.7	159	95.8	8.59	6–21
C57BL/6 ♀ ×F1 ♂ (backcross)	50	8	16.0	27	54.0	34	68.0	13.67	10–25

After Chang and Hildemann, *J. Nat. Cancer Inst.*, 33:303–313, 1964.

[a]Incidence of naturally occurring runts was consistently less than 2 percent in control litters of all progenies tested.

[b]This indicates the total number of infected mice that subsequently developed tumors irrespective of presence of runting. Numerous runted mice died before tumors were detectable, but long-surviving runts always developed tumors.

more significant differences were revealed at the low virus dosage. At very high virus dosage, genetic resistance to tumorogenesis could be almost entirely overcome. The major "take-home lesson" from this study is the complexity of the immunogenetic basis of infectious disease susceptibility. The importance of using optimal experimental conditions is evident.

The identification of the genes controlling susceptibility to type C leukemia viruses is even more complex because these endogenous viruses not only are chromosomally integrated and vertically transmitted in the host genome, but also are extremely polymorphic and have a propensity to form new recombinant types. The murine leukemia viruses (MuLV) discussed here can be divided into three types according to their host range: *ecotropic* viruses replicate in cells of the species in which they arose but not in lines from other genera; murine *xenotropic* viruses replicate in cell lines from genera other than *Mus* but not in mouse cell lines; *amphotropic* viruses can replicate in cell lines derived from either *Mus* or other species (Table 8–3). There are many different strains of ecotropic MuLV. Some, such as the Friend, Moloney, and Rauscher lymphatic leukemia viruses, can replicate on their own and produce lymphoproliferative disease. Other viruses such as murine sarcoma viruses, the Abelson virus, and the spleen-focus-forming virus (SFFV) are defective. These replication defective viruses lack a portion of the MuLV genome which codes for the major envelope glycoproteins. They can replicate and produce their biological effects only in the presence of an accompanying helper virus, which replicates within the same cell. The Friend MuLV strain, for example, contains both the Friend helper leukemia virus and defective SFFV.

Several classes of genes are involved in the biology of MuLV leukemogenesis (Table 8–4). First, there are loci that code for the expression of MuLV or viral antigens. For example, AKR mice have high levels of infectious ecotropic MuLV in their tissues early in life. Expression of this virus is dominant in F_1 hybrids of AKR and certain virus-negative strains of mice. Analysis of F_2 and backcross progeny indicates that two independently segregating loci, designated *Akv-1* and *Akv-2,* govern viral expression. These dominant *Akv-1* and *Akv-2* genes have been shown to be integrated ecotropic MuLV genomes

TABLE 8–3. MuLV Expression in Selected Strains of Mice

		Virus replication		
Mouse strain	Tropism	Ecotropic	Xenotropic	Amphotropic
AKR	N	H	I	–
BALB/c	B	I	I	–
C57BL/6	B	L	I	–
NZB	N	–	H	–
129	N	–	–	–
Wild mice	N	+	–	+

N = permits replication of N-type viruses; B = permits replication of B-type viruses; H = high; I = intermediate; L = low; – = not expressed or incomplete expression; + = expression.

TABLE 8–4. Murine Genes Affecting Stages of Leukemia Virus Infection

Probable site of gene action	Locus (chromosome number)	Allele	Prototype strains
Virus induction	*Akv-1* (7)	—	AKR
	Akv-2 (?)	—	AKR
	Gv-1 (17 or 4 ?) and	1	C3H/An, DBA/2, SJL
	Gv-2 (7)	2	A, AKR, C3H/He, C58
		3	CE, 129
		—	BALB/c, CBA, C57BL/6, DBA/1
Virus replication and spread	*Fv-1* (4)	b	A, BALB/c, C57BL/6
		n	AKR, CBA, C3H, C58, DBA/2
		nr	NZB, RF, 129
	Rev-1 (5)	—	not polymorphic
Growth of transforming defective variants	*Fv-2* (9)	r	C57BL/6, C58
		s	A, AKR, BALB/c, CBA, DBA/2, NZB, SJL, 129
	Av-2 (?)	r	C57BL/6
		s	BALB/c
Physiology of target cell for transformation	W (5)	—	—
	Sl (10)	—	—
	f (13)	—	—
Resistance following viral transformation	*Rgv-1* (17)	r	*H-2K*^{b} strains
		s	*H-2K*^{k}, *H-2K*^{d} strains
	Rgv-2 (?)	r	C57BL/6
		s	C3H
	Rfv-1 (17)	r	*H-2D*^{b} strains
		s	*H-2D*^{d} strains
	Rfv-2 (17)	—	—
	Rfv-3 (?)	—	—
	br (14)	—	—
	Fv-3 (?)	r	C57BL/6, C58
		s	A, BALB/c, C3H, CBA, DBA/2, NZB, SJL, 129

by cross-hybridization studies using cDNA probes, but it is not clear if these viruses are identical. Chromosomally integrated ecotropic and xenotropic viral genomes have been identified in several other mouse strains. In addition, the *Gv-1* and *Gv-2* loci regulate the expression of G_{IX}, the gp70 envelope glycoprotein coded by the *env* MuLV gene.

A second group of genes affects the growth and spread of murine leukemia virus. Somatic cell genetic analysis has shown that the *Rev-1* gene on chromosome 5 codes for a cell-surface molecule that is the receptor for ecotropic MuLV. There are no known polymorphisms for mouse MuLV receptors, unlike the situation in chickens. The *Fv-1* locus on chromosome 4 is the best stud-

ied example of a gene that suppresses MuLV expression and also leukemogenesis. Three alleles at *Fv-1* have been identified, *Fv-1*^{n}, *Fv-1*^{b}, and *Fv-1*^{nr}; mouse strains are either N-type or B-type depending on their *Fv-1* genotype (Table 8–3). An ecotropic MuLV which grows much better in NIH Swiss (N) cells or cells from other N-type strains is an N-tropic virus, whereas a virus that replicates readily in BALB/c (B) cells is called B-tropic. Some viruses are NB-tropic, that is, they grow equally well in cells from mice of either type. The *Fv-1*^{nr} strains such as NZB are much more resistant to N-tropic viruses than are other N-type strains. As with the polyoma virus, the resistance to ecotropic MuLV conferred by *Fv-1* is relative and not absolute and can be overcome at high virus doses. Relative resistance is dominant for both N- and B-tropic viruses, since *Fv-1*$^{n/b}$F$_1$ heterozygotes are resistant to both N- and B-tropic viruses. The molecular events associated with *Fv-1* suppression of viral replication are not yet known, but it appears that *Fv-1* acts at a late stage in the viral replication cycle. Possibly the *Fv-1* gene product prevents viral integration into the host genome.

A third group of genes affects the resistance of differentiated cell types to transformation by defective MuLV variants such as SFFV and Abelson virus mentioned above. These genes have the property of being dominant for susceptibility to infection. The *Fv-2* gene (chromosome 9) exerts a pronounced effect on the response of mice to Friend virus. *Fv-2* does not affect the helper viral component of the FV complex, but rather acts primarily to restrict SFFV infection. Resistance to the defective Abelson virus is influenced by two genes designated *Av-1* and *Av-2.* Once again, both these genes exhibit dominance in conferring susceptibility to infection. The strain distribution pattern of the *Av-2*^{r} allele coincides with the inheritance of a differentiation antigen found on normal hematopoietic cells which is also expressed on cells transformed by the Abelson virus. Possibly *Av-2* controls the expression of this normal antigen which in some unknown way confers susceptibility to viral transformation. The events regulating the interaction between MuLV genes and normal differentiation genes are just beginning to be unraveled. Whether or not these genes also act to circumvent a protective immune response is unknown.

A fourth group of genes affecting MuLV-induced leukemogenesis includes those loci that affect normal hematopoiesis and, as a secondary manifestation, influence susceptibility or resistance to leukemia viruses. All three of the hereditary anemias thus far examined lead to a marked decrease in susceptibility to the Friend SFFV. The spleen-focus-forming virus preferentially infects erythroid targets in the spleen rather than lymphocytes and it is probably because of this target specificity that loci such as *W*(dominant spotting), *Sl* (steel), and *f* (flexed-tail) influence susceptibility to Friend virus. All these loci probably affect the quantity or quality of the SFFV target cells. The *W* locus affects progenitor stem cells themselves whereas mice with the *Sl* mutation have normal stem cells but have an inhibiting environment that prevents their normal differentiation. The *f* mutation induces a transitory anemia which is caused by a disturbance in normal hematopoiesis in the fetal liver.

Finally, we consider loci that control MuLV-induced tumorogenesis by affecting the expression of viral immunogens or by regulating the immune response to virus or virus-transformed cells. These loci do not affect initial viral replication but rather act at a later stage. In general, the resistance conferred by this class of genes is inherited as a dominant trait. In particular, genes within the *H-2* complex have a marked influence on the outcome of infection by various murine retroviruses including Gross, Friend, and Tennant viruses, the radiation leukemia virus and the mammary tumor virus. The mechanism of action of the *H-2*-linked loci is unknown but could well involve immunoregulation.

In one early study Lilly and co-workers examined the genetic control of susceptibility to Gross leukemia virus in mice. (C3H × C57BL/6) F_1 hybrid mice, like their C57BL/6 ($H\text{-}2^b$) parents, are resistant to Gross virus-induced leukemogenesis, whereas C3H ($H\text{-}2^k$) animals are uniformly susceptible. Segregation of resistant and susceptible mice in backcross and F_2 progeny indicated that at least two independent loci are determinative. The incidence of leukemia among $H\text{-}2^k/H\text{-}2^k$ homozygotes was greater than 90 percent in backcross and F_2 mice, whereas mice carrying the $H\text{-}2^b$ allele were more resistant. One locus designated *Rgv-1*, for *r*esistance to *G*ross *v*irus *1*, was shown to be closely linked to *H-2;* the location of the other locus *Rgv-2* is not known. Further mapping studies with *H-2* recombinant mice have mapped *Rgv-1* toward the *K* end of the *H-2* complex centromeric of the *H-2S* region. A perhaps related *H-2I* gene, *Rrv-1*, and another *K* end gene, *Rfv-2*, control resistance to radiation leukemia virus (RadLV) and Friend virus, respectively. The close association of these loci to the *H-2I* region raises the intriguing but as yet unproven possibility that these loci are *Ir* genes controlling the immune response to virus-specific or tumor-specific surface antigens. Support for this idea comes from studies showing that the development of antibodies to Gross virus-specific antigens is controlled by *H-2K* end genes. Furthermore, the cell-mediated cytotoxic response to spontaneous AKR strain leukemias is under *I* region control.

Resistance to Friend virus-induced leukemogenesis is regulated by a gene *Rfv-1* associated with the *H-2D* region. Although $H\text{-}2^b$ mice are resistant, $H\text{-}2^d$ mice are susceptible to FV-induced disease. Resistance to radiation-leukemia virus is also controlled by *H-2D* end genes, but in this instance different *H-2* alleles confer resistance and susceptibility. Perhaps this association is related to the *H-2* control of cytotoxic T-cell killing of virally infected cells (see Chapter 6). Viral and H-2 antigens may interact uniquely on the cell membrane; complexes of murine viral antigens and cell molecules coded by different *H-2* alleles may vary considerably in their ability to induce a protective immune response. This interpretation is consistent with the finding that immune responses to some MuLV antigens are controlled by *K* end genes whereas responses to other MuLV are controlled by *D* end genes. However, other mechanisms may be operative. For example, the amount of Friend virus expressed on the cell-surface of susceptible $H\text{-}2^d$ cells is less than that expressed

on resistant H-2^b cells. Furthermore, H-$2D^b$ molecules are preferentially incorporated in viral particles during the process of budding from the membranes but H-$2D^d$ molecules from susceptible hosts are not incorporated. These processes could lead to a greater immune response against the H-$2D^b$ infected cells and account for the greater resistance seen in these mice. Infection with RadLV induces a specific increase in the amount of H-2D antigens expressed on thymocytes of *H-2D*^{d} resistant mice. This effect is not seen in susceptible hosts. How an increase in H-2 expression may lead to increased resistance to leukemogenesis is unknown.

Non-*H-2* loci also influence susceptibility to MuLV-induced leukemogenesis. The *Rfv-3* locus controls FV viremia and also interacts with the *Rfv-1* locus to achieve recovery from the resulting leukemia. Non-*H-2* genetic influences on resistance to leukemia have been reported with other leukemia viruses such as radiation leukemia virus. Friend virus can act in susceptible hosts to suppress the ability of T- and B-cells to proliferate in response to specific mitogens. This effect apparently involves activation of suppressor T-cells. The *Fv-3* locus regulates this virus-induced immunosuppression. The *Fv-3*^{r} allele present in hosts that are resistant to FV leukemogenesis blocks the immunodepressive effect. This example illustrates that resistance to MuLV may be influenced by genes which counter the immunosuppressive effects of viral infection as well as by genes which promote immune response capacity.

The ability of a host to resist infection can be greatly influenced by changes in the phenotype of the pathogen. This is no better exemplified than by the major antigenic variations that influenza A viruses display and by the ability of certain parasites to modulate surface antigen expression (see Chapter 3). Influenza viruses show great genetic variability; their genomes consist of segments of nucleic acid that facilitate recombination by simple reassortment of segments into new sets. Genetic recombination between naturally occurring influenza strains yields new antigenic variants at frequent intervals. Murine leukemia virus genes may also recombine with each other to produce new variants with distinctive biological properties. For example, young and old AKR mice possess numerous cells which produce ecotropic MuLV but do not produce xenotropic virus. However, some thymocytes in old mice can produce both ecotropic and xenotropic viruses. Specific recombinants of the ecotropic and xenotropic viruses then can arise in these thymocytes. Many of the recombinants have crossovers in the small *env* gene coding for the viral envelope glycoprotein. It is not clear how these events relate to the high frequency of leukemia found in old AKR mice. In theory, new variants could facilitate bypassing of host resistance mechanisms.

In summary, genetic resistance to persistent viral infections, as illustrated here by endogenous leukemia viruses, can operate at the level of viral entry or replication, or influence host cell sensitivity to the effects of viral infection; the immune response to a pathogen can be protective or, alternatively, contribute to inflammatory changes associated with virally induced disease. Resistance can be mediated by genes increasing the protective immune response or counteracting immunosuppressive effects induced by infection.

IMMUNODEFICIENCY DISEASES IN MAN

Much of what we know about the development of the immune system and the factors necessary for resistance to infection has been aided by "experiments in nature" in which a mutation has led to a specific defect in the maturation of immune functions. As more has been learned about the multiple components of the immune system and how they interact in a normal immune response, more precise characterization of immune deficiency diseases has been possible. In particular, the characteristics of T- and B-cell subpopulations, the identification of cell-surface markers on T- and B-cells, and the development of assays in vitro to measure T- and B-cell functions have made possible sharper classification of immunodeficiencies. In turn, the study of immunodeficiency states has been instrumental in defining the pathways of normal lymphoid cell differentiation. Animal models with specific immunodeficiency disorders have become invaluable tools for the dissection of components of the immune response.

Five categories of hereditary defects associated with impaired immune responses to infectious agents in man are outlined in Table 8–5. Defects in stem cell development affect both cellular and humoral immunity. Thus, these patients early in life suffer recurrent infections by diverse microorganisms. Patients with T-cell developmental defects also suffer from severe, life-threatening infections, especially of viral origin. The purine nucleoside phosphorylase (PNP) deficiency appears to exert its principal effect on T-lymphocytes. These patients are particularly sensitive to viral infections. In contrast, patients with B-cell developmental defects frequently have pyogenic bacterial infections. A selective IgA deficiency in particular can lead to susceptibility to pulmonary infections. Defects in the phagocytic system and complement disorders can cause defective neutrophil chemotaxis and phagocytosis of microbes. Thus, these patients also display an increase in bacterial or fungal infections.

In other hereditary diseases, such as diabetes mellitus, the relationship between the primary abnormality and defective immune responsiveness is not known. The majority of people with diabetes mellitus show either autosomal recessive or polygenic inheritance of this disease. In addition, the well-known association between obesity and diabetes implicates other genetic-environmental interactions. Abnormal insulin metabolism leads to greater susceptibility to pyogenic bacterial infections and tuberculosis but does not impair the immune response to viruses.

Defects in Stem Cell Development

Multipotential hematopoietic stem cells that arise in the embryonic yolk sac are the precursors of the migratory cells in blood and lymphoreticular tissues. During development these pluripotential stem cells give rise to self-renewing progenitor cells such as the lymphoid stem cell. Within the fetal liver a subpopulation of these lymphoid stem cells begins to differentiate along the B-

TABLE 8–5. Some Primary or Congenital Immunodeficiencies in Man

Probable level of defect	Deficiency[a]	Cell types affected[b]	Inheritance	Associated infections
STEM CELL DEVELOPMENTAL DEFECTS				
Hematopoietic stem cell	SCID with reticular dysgenesis	↓T, B, and phagocytes	Autosomal recessive	Early onset of recurrent infections of all kinds of microbes in skin, gut and respiratory tract
Lymphocytic stem cell	"Swiss type" SCID	↓T- and B-cells	Autosomal recessive with some X-linked	
Adenosine deaminase (ADA)	SCID with ADA deficiency	↓T- and B-cells		
Pre-T-cell ± pre-B-cell	SCID with B-cells	↓T-cells, some B-cells	X-linked or autosomal recessive	
Unknown	Wiscott-Aldrich syndrome (thrombocytopenia and eczema)	↓T-cells, some B-cells	X-linked	Viruses
T-CELL DEVELOPMENTAL DEFECTS				
Thymus	DiGeorge syndrome (thymic hypoplasia)	↓T	Congenital but usually not familial	Chronic viral, bacterial, or fungal infections
Thymus development	Ataxia telangiectasia	↓T and plasma cells	Autosomal recessive	Recurrent pulmonary infections
Purine nucleoside phosphorylase (PNP) deficiency	PNP deficiency	↓T	Autosomal recessive	Viruses
DISORDERS IN T-CELL SUBSETS	Common variable immunodeficiencies	↑T_s-cells, ↓T_h-cells?	Unknown or familial	Viruses and bacteria
B-CELL DEVELOPMENTAL DEFECTS				
Arrested pre-B-cell	Infantile agammaglobulinemia	↓B-cell	X-linked	Pyogenic bacteria, some viruses

Failure to generate pre-B or B-cells	Some variable immunodeficiencies late onset hypogammaglobulinemia	↓Pre-B-cells or B-cells	Unknown or autosomal recessive	Bacteria
Defective terminal differentiation of B-cells	Selective IgA deficiency	↓IgA plasma cells ± ↓T-cells	Unknown	Pulmonary viruses and bacteria
	Selective deficiency of other Ig class	↓plasma cells ± ↓T-cells	Unknown	Some bacteria
	Ig deficiency with increased IgM	↓IgG and IgA plasma cells, ↑IgM plasma cells	Primarily X-linked	Pyogenic bacteria
	Common variable immunodeficiencies	± ↓B-cells	Unknown or familial	Bacteria
COMPLEMENT DISORDERS				
Structural genes	Clr, C4, C2, C3, C5, C6, C7, or C8	—	Autosomal recessive	C3-pyogenic bacteria C6, C7, C8, = *Neisseria*
PHAGOCYTIC DEFECTS				
Mobility and bactericidal activity?	Chediak-Higashi syndrome	Neutrophils macrophages	Autosomal recessive	Bacteria, slow viruses
Bactericidal activity	Chronic granulomatous disease	Neutrophils macrophages	X-linked some autosomal recessive	Bacteria, fungi
Bactericidal activity	Myeloperoxidase deficiency	Neutrophils monocytes	Autosomal recessive	—
Deficiency of granulocytes	Familial neutropenia	Neutrophils	Autosomal dominant	Bacteria

See WHO Report on Immunodeficiencies from *Clin. Immunol. Immunopath.* 13:296, 1979 for additional details.

[a]SCID = severe combined immunodeficiency disease affecting both T- and B-lymphocytes.

[b]↓ = cells greatly diminished in numbers or absent; ↑ = cells present in excessive or higher than normal numbers.

cell pathway, while another subpopulation migrates to the thymus where they proliferate and differentiate into thymocytes. In adult animals the bone marrow is the major source of lymphoid stem cells. The most profound immunodeficiency diseases, together termed severe combined immunodeficiency disease (SCID), affect these stem cell precursors, produce marked impairment of both T-cell and B-cell pathways, and thus are invariably fatal. The more common form of inheritance is autosomal recessive but X-linked SCID has also been reported. Patients with SCID have recurring infections very early in life in the skin, respiratory tract, and GI tract. They are lymphopenic, hypogammaglobulinemic, and display little or no response to mitogens, specific antigens, or alloantigens. SCID includes a broad spectrum of syndromes defined in terms of mode of inheritance and site of the cellular or enzymatic defect. The most severe form of SCID affects primitive hematopoietic stem cells. Patients with this disorder have virtually no T- and B-lymphocytes or granulocytes. The "Swiss" type of SCID originally termed hereditary thymic aplasia derives from defective lymphocytic stem cell development; consequently, patients with this disorder have much reduced numbers of T- and B-cells. Fortunately, the defect in these patients can sometimes be repaired with HLA-identical bone marrow or fetal liver transplants. About half of the autosomal recessively inherited SCID are associated with a deficiency in the enzyme adenosine-deaminase (ADA), which plays a key role in purine metabolism by degrading adenosine to inosine. Without ADA, adenosine and adenosine-monophosphate accumulate in cells at high concentrations and can be toxic. Lymphocytes and thymocytes are normally very rich in ADA and, therefore, may be particularly vulnerable to a lack of this enzyme. In man, ADA is controlled by a gene on chromosome 20. Another form of severe combined immunodeficiency disease is associated with the absence of T-lymphocytes and abnormally low levels of the T-cell dependent immunoglobulins IgG and IgA. These patients may have a defect in the stem cells required for T-cell maturation or in the thymic environment.

Fortunately, primary SCID are rare and compatible transplant or enzyme therapy is potentially available. A more common form of secondary SCID is associated with severe malnutrition, particularly protein deprivation. Severely malnourished children are frequently lymphopenic and have reduced numbers of T-cells. The metabolic basis of this immunodeficiency, a major problem in underdeveloped countries, invites further study.

T-Cell Developmental Defects

Upon migration to the thymus, T-cell precursors are induced to express a series of T-cell markers by thymic epithelial cells and hormones. The thymocytes undergo further differentiation in the thymus and several distinct T-cell subpopulations then migrate to the peripheral lymphoid tissues. T-cell-associated immunodeficiency disorders can be divided into two broad groups: the defects that impair the development and function of the thymus and thereby prevent

normal maturation of pre-T-cells into mature T-lymphocytes; and the diseases that produce abnormalities of regulatory cells such as helper or suppressor T-cells. Many T-cell deficiencies also influence B-cell activities to some degree, as might be expected.

Patients with the DiGeorge syndrome fail to develop structures derived from the third and fourth pharyngeal pouches during embryogenesis. This results in congenital heart disease, abnormalities of the parathyroids, and aplasia or hypoplasia of the thymus. The thymic hypoplasia leads to profound defects in T-cell functions, such as resistance to viral infections, but most patients have normal or increased numbers of B-cells and normal immunoglobulin levels. Because hematopoietic stem cells are not affected in the DiGeorge syndrome, this disease can be successfully treated with fetal thymus transplantation.

Patients with ataxia telangiectasia, an autosomal recessive disorder with characteristic pleiotropic effects, have an abnormal hypoplastic thymus usually embryonic in appearance. The immunologic abnormalities vary considerably from patient to patient. Serum and secretory IgA deficiency and IgE deficiency is common, whereas IgG and IgA levels are usually normal. Recent studies have shown that ataxia telangiectasia patients haye a defect in their DNA repair system and are extremely sensitive to x-rays. Lymphocytes from some patients have a high frequency of spontaneous chromosomal breaks. A DNA repair defect or cytogenetic abnormalities in this disease may explain the high incidence of cancer seen in these patients. T-cell development is also profoundly influenced by the absence of the enzyme purine nucleoside phosphorylase (PNP), which converts inosine to hypoxanthine. Patients lacking PNP have T-cell abnormalities but normal B-cells. This deficiency is inherited as an autosomal recessive disorder; the PNP structural gene is on chromosome 14. Why PNP deficiency leads selectively to T-cell dysfunction is not known. However, high concentrations of one of the substrates of PNP, 2'-deoxyguanosine is toxic to cultured T lymphoma cells. The efficient phosphorylation of this substrate by thymocytes may be related to its toxic effect.

Although most patients with hypogammaglobulinemia may have defects in the B-cell pathway (see below), recent studies suggest that some patients with variable hypogammaglobulinemia may have abnormalities in regulatory T-cells. An excessive number of suppressor T-cells have been found in certain patients with hypogammaglobulinemia and X-linked agammaglobulinemia, but it is not clear if the increase in suppressor cells is causative. In other disorders, the lack of helper T-cells may account for the hypogammaglobulinemic state. Indeed, certain defects associated with terminal B-cell differentiation may primarily involve T-cells.

B-Cell Developmental Defects

In adults the generation of pre-B-cells from stem cells occurs in the bone marrow where large and small pre-B-cells are found throughout life. The generation of antibody diversity probably occurs in the pre-B-cells; by the time each

pre-B-cell matures into a surface IgM+ B-lymphocyte, its antibody specificity is determined. All cells arising from a given clone of B-lymphocyte synthesize antibodies with the same specificity, although some cells appear genetically preprogrammed to shift from IgM producers to IgA or IgG antibody producers. For early B-lymphocyte differentiation, neither antigen nor T-cells appear to be required. However, actual completion of the isotype switch appears to be antigen and/or T-cell dependent. Patients with infantile X-linked agammaglobulinemia have a disorder affecting pre-B-cell differentiation. Because structural immunoglobulin genes are autosomally inherited, the regulatory genes must be mediating this defect. X-linked agammaglobulinemic patients have normal numbers of pre-B-cells which then fail to differentiate further.

A consistent deficiency of one or more immunoglobulin classes is termed a selective immunoglobulin deficiency. Patients with immune deficiencies that cannot be readily classified are grouped under the heading of "varied immunodeficiencies." These include a subgroup of immunoglobulin deficiencies classified as either "congenital" or "acquired" hypogammaglobulinemias. Specific sites of B-cell maturation are affected by some of these immunodeficiencies. Certain individuals with either acquired or congenital hypogammaglobulinemia may fail to generate sufficient numbers of pre-B-cells. Still other patients may have a specific deficiency in B-cell precursors of a specific isotype. For example, IgM production is normal in one form of Ig deficiency, but B-lymphocytes for IgA or IgG isotypes are absent. This may reflect faulty isotype diversification. Some families have been identified with deletions of heavy chain immunoglobulin structural genes. Homozygotes consequently have a selective immunodeficiency for that particular isotype and accordingly lack corresponding B-lymphocytes. These defects contrast with selective immunodeficiencies where B-cell levels are normal. An Ig deficiency with increased IgM levels has been observed as an X-linked inherited trait. Although γ- and α-chain producing B-lymphocytes are present, they do not differentiate into mature plasma cells. This disorder may be caused by a block in the differentiation of IgM-bearing cells into IgG- and IgA-bearing cells. These patients accordingly have elevated levels of serum IgM and are deficient in IgG and IgA immunoglobulins. Selective IgA deficiency, the most common immunodeficiency, may also be referable to a defect in the terminal differentiation of B-cells. In this case the lesion may affect the triggering of IgA B-cells into mature secreting plasma cells.

Several additional sites of defective terminal B-cell differentiation have been identified in patients with varied immunodeficiencies. One group has B-lymphocytes that fail to respond to polyclonal B-cell activators; such patients have no apparent regulatory T-cell defects, and therefore appear to have intrinsic B-cell defects. Another group of patients have normal numbers of circulating B-lymphocytes that can be activated to synthesize immunoglobulin but cannot secrete it. In vitro studies suggest that the secretory defect of the B-cells from these people results from failure in the glycosylation of the newly synthesized IgG. There is a great diversity of primary immunodeficiency diseases, many

of which affect stages in B-cell differentiation. Further studies of these defects should elucidate events in normal lymphocyte differentiation and hopefully provide solutions to their clinical treatment.

Complement Deficiencies

The mammalian complement system consists of a family of some twenty interacting plasma proteins, including nine numbered components and three inhibitors, which contribute selectively to antibody-mediated reactions. The first component of complement contains three subunits: Clq, Clr, and Cls. Upon interaction between Clq and aggregated IgG or IgM (i.e., cell-bound, antibody-antigen complexes) the classic complement pathway is activated. Protective inflammation and host resistance to infectious disease are often influenced by the complement system: lysis of bacterial or animal cells by antibody plus complement usually requires the activation of C3 to C9 components; phagocytosis is enhanced by the presence of C3 on the particles to be ingested; histamine release from mast cells and other activities associated with anaphylatoxin are mediated by two complement component cleavage products C3a and C5a. These fragments are also chemotactic for polymorphonuclear leukocytes. The pivotal C3 component can be activated by the classic antibody-antigen reaction involving initial fixation of C1, 4, and 2 or by an alternate pathway leading directly from C3 to C5, 6, 7, 8, and 9.

Polymorphism of the major immunogene complex has revealed certain associated complement genes identified with the C2 and C4 components (Chapter 6). Although C2 and C4 have been mapped within the *HLA* complex in man, other complement genes map elsewhere. The genes coding for C6 and C7 are linked but distinct from the *C3* locus. Genetic defects have been described for almost all the complement components in man including Clr, C4, C2, C3, C5, C6, C7, and C8. These deficiencies are inherited as autosomal recessive traits. In addition, deficiencies of inhibitors of certain complement components have also been described. For example, hereditary angioneurotic edema, an autosomal dominant defect, results from a genetically determined deficiency of Cl inhibitor. Patients with this disease or with primary C4 or C2 deficiency suffer from an increased incidence of immune complex diseases such as systemic lupus erythematosus (SLE) and glomerulonephritis (GN). Given the rarity of complement disorders, this association is unlikely to be fortuitous. One possibility is that complement components important in the early stages of the classical pathway are necessary for the elimination of antigenically aberrant cells. Without these factors, immune complexes are formed, the aberrant cells are not eliminated, and immune complex disease ensues. Deficiency of these complement components might also allow persistent infections. Alternatively, immune complexes may not be efficiently solubilized in Cl inhibitor, C4 or C2 deficient patients, thereby allowing more complexes to be deposited in the kidneys and elsewhere. Several animal examples of autosomal recessive complement deficiencies have been reported. These include C3 de-

ficiencies in rhesus and macaque monkeys and mice, C4 deficiency in guinea pigs, C5 deficiency in mice, and C6 deficiency in rabbits.

Leukocyte Abnormalities

Certain defects in the phagocytic system which affect polymorphonuclear or mononuclear leukocytes and sessile macrophages have been reported in man. People with the autosomal dominant syndrome designated familial neutropenia have a deficiency of granulocytes associated with bacterial infections, and often diminished chemotactic responsiveness. Chediak-Higashi syndrome (CHS) is an autosomal recessive disorder characterized by recurrent infections, partial albinism, and granulocytes with giant cytoplasmic granules. These CHS granulocytes exhibit defective chemotaxis, degranulation, and diminished intracellular bacterial killing, perhaps because their giant lysosomes fail to rupture. The peripheral blood monocytes of CHS patients also generate very large aberrant granules after they are stimulated in vitro. Homologues for the Chediak-Higashi syndrome have been found in mice, mink, and cattle. In mice, the corresponding autosomal recessive mutant is beige (*bg*, chromosome 13). Beige mice have defective granulocyte chemotaxis and defective lysosomal degranulation as found in CHS. Neutrophilic granulocytes from beige mice also display increased mobility of lectin-receptor complexes in the plasma membrane, perhaps connected with an abnormality in microtubular polymerization or function. Beige mice also have defective natural killer activity; although beige NK-cells can bind target cells, they are inefficient in lysing them. How this relates to the membrane-associated disorders in other leukocytes is not known.

Several syndromes in man, such as chronic granulomatous disease (CGD), affect the bactericidal function of phagocytes. Attachment and ingestion of bacteria occur normally in CGD granulocytes or monocytes, but the ingested bacteria are not killed. The basic defect of CGD is a deficiency in the activity of an enzyme responsible for the conversion of oxygen to a bactericidal form. Primary myeloperoxidase deficiency leads to impaired microbicidal activity because peroxidase activity is absent from neutrophils and monocytes.

Approaches to Therapy

Promising advances have been made in the treatment of immunodeficiency diseases. Once the basis of a patient's particular disorder is understood, suitable replacement therapy can be initiated. Bone marrow transplantation, preferably from an HLA-identical sibling donor, has been used with some success to correct severe combined immune deficiency disease, chronic granulomotous disease, and congenital neutropenia. This approach has also been used to cope with disorders such as aplastic anemia, Clq deficiency, and acute leukemias. Usually, an HLA-identical sibling donor is not available, so transplants of marrow from related histocompatible donors or histoincompatible marrow depleted of immunocompetent cells have been attempted. Unfortunately, se-

vere or fatal graft-versus-host disease is a frequent complication, even among HLA-identical sibs. SCID has also been corrected by fetal liver allotransplantation, but functional reconstitution remains uncertain, depending mainly on the immunogenetic disparity between donor and recipient. Thymus transplants have been tried to correct thymic hypoplasia and defects in patients with isolated T-cell deficiencies. Treatment of ataxia telangiectasia with thymus transplantation has proved unsuccessful, perhaps because this multiple disorder has so many pleiotropic effects. Primary humoral deficiencies are generally treated by replacement therapy with serum globulin or plasma infusions. The success of this approach may depend on the patient's rate of immunoglobulin catabolism and whether systemic reactions to allogeneic immunoglobulins occur. Treatment of X-linked agammaglobulinemia with bone marrow transplantation has been unsuccessful. ADA deficiency has been corrected by infusing patients with frozen erythrocytes as a source of adenosine deaminase.

Graft-versus-host reactions remain a major obstacle in the effective treatment of immunodeficiency diseases. Although several approaches have been taken to overcome this problem, none has yet succeeded. One such approach has been to attempt to deplete GVH reactive cells from bone marrow. Another area inviting further study is the identification of extrinsic factors which influence lymphocyte differentiation. Some B-cell defects, for example, may hinge on deficiencies in humoral factors that regulate the generation of pre-B-cells.

ANIMAL MODELS OF IMMUNODEFICIENCY

Specific mutations that impair the development of the immune system have been studied most extensively in mice (Table 8–6). At least six autosomal recessive mutations which lead to impaired T-cell functions have been identified in the mouse. Nude mice (*nu/nu*), as their name implies, lack hair and also do not develop a thymus or retain only a rudiment of thymic epithelium. These mice have been very useful in defining immune functions that are T-cell dependent. Nude mice do not make antibody responses to so-called T-dependent antigens such as sheep red blood cells (RBC) or respond to T-cell mitogens such as concanavalin A. They do not reject allografts or even xenogeneic tissue from chickens, snakes, or man. Thus, various human tumors can be grown in nude mice; indeed, the ability of a cell line to grow progressively in nude mice is used as a test for malignancy. Nude mice are often deficient in serum IgA and sometimes IgG, but have normal concentrations of IgM. The deficiency in nudes can be corrected by syngeneic thymus grafts: furthermore, nude mouse bone marrow is normal, and can be used to reconstitute deficient syngeneic hosts. In the presence of thymic hormones, a subpopulation of nude bone marrow cells can differentiate to express T-cell markers. A small percentage of nude spleen cells often express the Thy-1 antigen, which suggests that nude mice have some residual T-cells. However, it is not clear if these cells are mature T-cells or are thymocyte precursors, or natural killer cells.

TABLE 8–6. Mutations in Mice Affecting the Development of the Immune System

Gene name or source	Symbol (chromosome number)	Immunologic disorder	Associated phenotypes
Nude	*nu* (11)	Congenital hypothymia, severe T-cell deficiency, impaired T-cell functions, selective hypogammaglobulinemia	Hairless, lymphopenic, decreased life span
Hairless	*hr* (14)	Thymic cortical atrophy at 6 mo of age, reduced contact sensitivity and spleen cell GVH reactivity	Hairless, high incidence of spontaneous leukemia, very susceptible to carcinogen-induced tumorogenesis
Dwarf (Ames)	*df* (11)	Involution of thymus after weaning, impaired contact sensitivity, and T-cell dependent antigen responses	Primary anterior pituitary hormonal defect, no growth hormone, low body weight, fatal runting syndrome
Dwarf (Snell-Bagg)	*dw* (16)		
Lethargic	*lh* (2)	Involution of thymus after weaning, delayed alloreactivity	Sluggish behavior, high mortality by 45 days of age, lymphopenic
Obese	*ob* (6)	Decreased antibody response to sheep RBC, delayed allograft rejection, serum IgG2a deficiency	Obesity, hyperinsulinemia, mild or severe diabetes depending on genetic background
Dominant hemimelia	*Dh* (1)	Congenital absence of spleen, reduced number of B-cells, reduced antibody response to sheep RBC and LPS	Hind limb defects, urogenital and digestive system defects

X-linked immunodeficiency	*Xid* (X)	Subnormal number of B-cells, unresponsive to certain T-independent antigens, selective IgG3 and IgM deficiency	—
LPS response	*Lps* (4)	Defective mitogen and antibody response to LPS	Codominant inheritance, increased resistance to LPS toxicity
Beige	*bg* (13)	Defective granulocyte chemotaxis and bactericidal function, elevated serum IgM, defective NK-cells	Defective pigment granules, light eyes and fur, increased susceptibility to pneumonitis, *Candida albicans*, and *S. pneumoniae*; homologue of Chediak-Higashi syndrome in man
Motheaten	*me* (6)	Splenomegaly, polyclonal hypergammaglobulinemia, autoantibodies, immune complex disease, reduced number of B-cells, impaired T- and B-cell immunity	Epithelial abnormalities, patchy fur, high mortality by 9 weeks of age
Lymphoproliferation	*lpr* (5?)	Early onset lupuslike autoimmune disease, autoantibodies, nephritis, lymphadenopathy with proliferation of Lytl+ T-cells and Thy-1+ null cells, hypergammaglobulinemia	90% mortality by 7 months in females, 9 months in males, MRL/n wildtype have less severe autoimmune disorders
BXSB mice	Polygenic	Spontaneous autoimmune disease with Coombs positive hemolytic anemia, nephritis, hypergammaglobulinemia; B-cell deficiency	Dominant inheritance, Y-linked effect accelerates disease onset in males
NZB mice	Polygenic	Lupuslike autoimmune disease; polyclonally activated B-cells, T_s-cell deficiency, natural thymocytotoxic autoantibodies, refractory to T-cell tolerance induction	Chromosomal abnormalities with age, trisomy 15, high incidence of lymphomas and reticular cell sarcomas, high levels of xenotropic virus

Nude mice have been used to examine the role T-cells may play in host immune surveillance and destruction of arising tumors. Certain tumors do not grow well or at all in nude mice. Moreover, nude mice are as resistant or more resistant to chemically induced carcinogenesis than normal mice. These findings called into question the importance of T-cell immune surveillance in host resistance to oncogenesis. However, nude mice do have normal or elevated levels of NK-cells, which can participate in tumor rejection and could account for the observed tumor resistance. Thus, the existence of immune surveillance remains a possibility and may be attributable to both T-cells and NK-cells.

Hairless mice (*hr/hr*) also have impaired T-cell functions but, unlike nude mice, they are highly susceptible to tumor induction with chemical carcinogens. About 50 percent of hairless mice develop spontaneous leukemia by ten months of age compared to a 1 percent incidence in heterozygote controls. Hairless animals also have marked atrophy of cortical regions in the thymus by six months of age. T-cell dependent responses such as contact sensitivity to picryl chloride are very much reduced. Three-month-old *hr/hr* mice have an abnormal proportion of T-cell subsets; although the total number of T- and B-cells in young hairless animals is normal, there is an excess of Lyt $1^+2^+3^+$ T-cells in their spleens compared to other T-cell subsets. Hairless mice also have very high levels of C-type MuLV in their tissues compared to normals. It is not clear if this enhanced viral expression is a result of the loss of some normal negative regulatory mechanisms or if diminished immunocompetence in hairless mice is somehow related to their high leukemia incidence.

Several genes that have a primary effect on the endocrine system have a profound influence on T-cell function. Two autosomal recessive genes for dwarfism, Ames dwarf (*df*), and Snell-Bagg dwarf (*dw*) have primary lesions in the anterior pituitary and thus produce no growth hormones. The thymus of dwarf mice involutes early after weaning, and lymphopenia and a fatal runting syndrome normally ensue. T-cell functions are impaired in dwarf mice as shown by decreased responses to allografts, T-cell mitogens, and sheep erythrocytes. These defects appear to affect only a subpopulation of T-cells since serum levels of T-cell dependent IgA and IgG immunoglobulins are normal. The Ames and Snell-Bagg mutant mice differ in their response to sheep erythrocytes. Ames dwarf mice do not respond to sheep red blood cells even at high doses, whereas this deficiency as seen in Snell-Bagg dwarfs is dose dependent and can be overcome by high antigen dosage. Lethargic (*lh/lh*) mice also have impaired T-cell functions. Their deficiency may be related to hypersecretion of adrenocortical hormones. Obese (*ob/ob*) mice display decreased or delayed T-cell reactivity probably related to their diabetic condition.

Several other murine mutations affect principally the B-cell pathway of the immune response. Heterozygous mice with the semidominant mutation dominant hemimelia (*Dh/+*) have no spleen and in addition have hind limb, urogenital, and digestive system defects. The lack of a spleen leads to a compensating hypertrophy of the lymph nodes and leukocytosis. Asplenic mice have a moderate B-cell deficiency and display reduced antibody respons-

es to sheep erythrocytes as well as diminished responses to lipopolysaccharide (LPS). NK-cell activity and T-cell functions are not altered dramatically. An X-linked immunodeficiency has been characterized in CBA/N male mice. These mice respond normally to T-cell dependent antigens and certain T-independent antigens (e.g., TNP-LPS), but respond poorly to other T-cell independent antigens including type III pneumococcal polysaccharide and TNP-Ficoll; they appear to be unresponsive to bacterial carbohydrate determinants in particular. CBA/N mice have a selective deficiency in serum IgG3 and IgM, which are the principal immunoglobulins made in response to carbohydrate antigens. It is possible that an X-linked regulatory gene causes a dysfunction in the development of IgG3 precursors. The subpopulation of B-cells lacking in CBA/N mice is thought to express Lyb-3 and Lyb-5 gene products. Another locus, suitably termed *Lps*, regulates the ability of B-cells to respond to the polyclonal B-cell activator lipopolysaccharide. Defective C3H/HeJ mice neither respond to the LPS mitogen nor produce antibodies to the somatic-antigens of LPS. The *Lps* gene exerts its effect on many different cell types and confers resistance against the endotoxic effects induced during infection with Gram-negative bacteria. This locus may code for a receptor for LPS or an enzyme affecting the receptor. Certain other loci in mice lead to both immunodeficiency and autoimmune disorders, and these mutants will be discussed in a later section. Severe combined immunodeficiency has been observed in Arabian horses as an autosomal recessive disorder. Inherited varied immunodeficiency has also been reported in the chicken.

IMMUNODEFICIENCY AND MALIGNANCY

The mortality rates for cancer in primary immunodeficiency are 100 to 200 times the expected rates for the general population. Most tumors seen in immunodeficiencies are malignant lymphomas, reticulum cell sarcomas, or leukemias, although tumors of other frequently dividing cell types such as epithelial cells are also evident. Many of the immunodeficiency disorders with high tumor incidence manifest pleiotropic effects outside the immune system. Approximately 10 percent of patients with ataxia telangiectasia, Wiscott-Aldrich syndrome (a complex X-linked defect affecting T- and B-cell pathways), Bloom's syndrome, and X-linked agammaglobulinemia contract cancer. This association between immunologic deficiency and malignancy could result from one of several mechanisms: the immune surveillance theory would predict that immunodeficient individuals are at high risk to develop cancer because they have an impaired defense against spontaneously arising tumors or perhaps oncongenic viruses. However, this fails to explain why only certain immunodeficiencies have high tumor incidence and why the majority of tumors seen are of the lymphoid system. Another possibility is that lymphoid cells chronically stimulated by antigen mutate and become transformed. The immunodeficiency and cancer could thus be independent effects with the same common cause. For example, ataxia telangiectasia patients have a high inci-

dence of cytogenetic abnormalities. In particular, translocations of the long arm (q) of chromosome 14 are common. Translocations of $14q^+$ are known to be associated with the development of other lymphoproliferative disorders such as Burkitt's lymphoma and may be related to the high frequency of lymphomas seen in ataxia telangiectasia patients.

IMMUNODEFICIENCY, AUTOIMMUNITY, AND AGING

The immunologic states of immunodeficiency and autoimmunity are somehow intrinsically related. Various immunodeficiency syndromes in man and animals have a high incidence of autoimmune disease. Individuals with an acquired or varied hypogammaglobulinemia, or deficiency of certain complement components or IgA, are far more likely to develop autoimmune diseases such as systemic lupus erythematosus. One study revealed that 37 percent of selective IgA deficient patients had related autoimmune disease or dysfunction. As we shall see, certain murine immunodeficiencies have characteristic autoimmune disorders. A spectrum of autoimmune diseases is manifested clinically. Organ-specific autoimmune diseases such as thyroiditis have characteristic organ-specific autoantibodies. At the other extreme of the spectrum are major systemic autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE). In SLE, numerous autoantibodies are found, including autoantibodies to single-stranded and double-stranded DNA, and widespread pathologic changes are observed. Although autoantibodies are found in these diseases, it is uncertain whether they are directly responsible for the disease. Tissue lesions may result from immune complex formation, complement dependent cytotoxicity, cell-mediated cytotoxic reactions, or other mechanisms. Often there is a tendency for more than one autoimmune disorder to occur in the same individual. For example, SLE and rheumatoid arthritis are clinically associated. Autoimmune diseases also tend to aggregate in certain families and, as we shall see, the development of autoimmunity in man is controlled by *HLA* genes and other loci.

Autoimmunity

The term "autoimmunity" has been traditionally used to refer to a deleterious pathologic condition, something harmful. This concept has to be qualified to the extent that self-recognition is a major component of a normal immune response. Lymphocytes such as T-cells recognize foreign antigens on the cell surface in association with cell structures whose function may be to provide signals that regulate cell interactions during immune responses. Furthermore, during the course of a normal immune response, autoantibodies are generated against idiotypic determinants on the newly produced antibodies. These idiotypic autoantibodies can stimulate or suppress antibody responses and may have a major regulatory role. In this context "autoimmunity" can be thought

of as a process during which normal immunoregulatory mechanisms break down or are bypassed and a deleterious anti-self response ensues.

How, then, does the immune response, in spite of its intrinsic anti-self component, normally operate to prevent destructive autoimmune reactions? The classic experiments of Owen and of Medawar and co-workers showed that tolerance to foreign antigens can be acquired. Thus it was suggested that tolerance of self-macromolecules may be acquired through a similar mechanism. The "clonal deletion" theory assumes that potentially autoreactive B-cells may be clonally eliminated by the corresponding antigen at a sensitive stage of ontogeny. Such elimination could result from cross-linking of their surface immunoglobulin receptors by antigen or by anti-idiotype. B-cells with receptors for autodeterminants might arise through somatic mutation or after immunization against antigens cross-reactive with autodeterminants. Any intrinsic defect leading to genetic instability in B-cells could also promote autoantibody formation.

This theory has become less popular as more has been learned about the immunoregulatory network and the role of T-cell subsets in augmenting or suppressing antibody or cell-mediated immune responses. Some B-cells possess receptors capable of reacting with self-determinants such as thyroglobulin; however, many of the bodily constituents that can act as autoantigens require the presence of helper T-cells to allow antibodies to be made. Perhaps tolerance is actively acquired during development by another mechanism; the presence of suppressor cells, low levels of autoantibodies, or other regulatory molecules may operate continually to prevent or modulate autoreactive responses. Several immunodeficiency diseases in man and experimental hypogammaglobulinemia in chickens suggest that a functional excess of suppressor cells may lead to immunodeficiency. Other studies suggest that a functional deficiency of T_s-cells can lead to autoimmunity. Apparent overstimulation of helper T-cells is seen in certain chronic graft-versus-host reactions. In short, this line of evidence ascribes autoimmunity to a failure in normal immunoregulatory mechanisms.

Acquired tolerance may be viewed as a form of immunoregulation which is essentially antigen-driven by self-macromolecules. Mitchison has suggested as an alternative that much of the lack of reactivity to self is preprogrammed and not dependent on stimulation of lymphocytes by self-molecules. The immune system may not recognize certain molecules on the cell surface which alone are immunologically "silent" or nonimmunogenic. Regulatory T-cells may recognize antigens only in association with self-Ia molecules or other regulatory determinants. For example, the Thy-1 membrane antigen presented to the host alone on the cell membrane is weakly or nonimmunogenic—in effect "helpless." Autoimmunity may develop when viral infection, chemical alterations, or other cell-surface changes lead to the expression of new membrane "helper" or "carrier" determinants that convert normally silent molecules into autoimmunogens. In the case of Thy-1, non-*H-2* alloantigens, viral determi-

nants, or haptens coupled to the membrane can all act as carriers to promote the linked recognition of Thy-1 and thus potent Thy-1 antibody responses. Instead of assuming that an imbalance or dysfunction in the immune system leads to autoimmunity, this conception focuses on exogenous viruses or chemicals as evokers of autoreactive responses.

Several animal models for autoimmunity have provided much insight into the genetic control and underlying mechanisms leading to disease. Animals suffering from chronic systemic allogeneic or GVH disease share several features with disorders such as SLE, acquired hemolytic anemia, and rheumatoid arthritis. Cutaneous lesions are common and autoantibodies for example, against erythrocytes, epidermal cells, and DNA have been found. Lymphocytic thyroiditis can be induced in mice by injection of crude mouse thyroid extracts or purified thyroglobulin. The response to thyroglobulin is controlled by *H-2* genes; good responders develop severe thyroid lesions whereas poor responders have little or no disease.

A group of disorders in mice that share clinical features with systemic autoimmune diseases in man have been characterized (Table 8–6). The motheaten (*me*) mutation produces a severe early onset autoimmune disease. Homozygous *me/me* mice develop autoantibodies to thymocyte and to double-stranded DNA as early as three weeks of age. These mice develop very high serum immunoglobulin levels, have widespread immunoglobulin deposits, and usually succumb to nephritis or pneumonitis by two months of age. The lesion occurs at the level of the bone marrow because the disease can be adoptively transferred by injecting normal lethally irradiated recipients with motheaten bone marrow. Both T- and B-cell function are greatly impaired in motheatens. Although the number of B-cells in motheaten mice is drastically reduced, the frequency of mature plasma cells is greatly elevated; the remaining B-cells resemble mature B-cells that have recently been activated by antigens or mitogens. It is possible that the *me* mutation produces an intrinsic B-cell defect which leads to polyclonal B-cell activation. It is not yet clear to what extent other cell types are affected and at what level the primary lesion occurs.

The homozygous mutation lymphoproliferation (*lpr*) also produces a lupus-like disease but at a somewhat later age than is seen in motheatens. This mutation arose during the development of the strain MRL/l. Homozygous *lpr/lpr* mice have a characteristic massive lymphoproliferation; by four months of age their lymph nodes are enlarged ten times greater than normal. More than 90 percent of the proliferating cells in the enlarged nodes are Thy-1 positive cells, which indicates that the lymphadenopathy is largely a result of T-cell proliferation. These cells do not appear to be malignant because they do not grow upon transferring to other animals. The majority of the Thy-1+ cells are Lyt1+ or Lyt−; Lyt23+ cells are virtually absent, and in older mice with progressive disease Lyt123+ cells are greatly reduced. The Lyt1+ cell subset may still function to augment suppressor T-cell activity, but may itself be refractory to T_s-cell feedback signals. Thus, a defect in the T-cell regulatory network could account for the autoimmune disease in MRL/l mice. However,

like other strains with lupuslike syndromes, MRL/l mice very early in life have an excess of polyclonally activated B-cells and serum autoantibodies. At this juncture it is not clear how the T-cell deficiency in *lpr/lpr* mice is correlated with the B-cell defect.

The recombinant inbred BXSB was developed by Murphy from a cross between C57BL/6 and SB mice. Male BXSB mice die much more quickly than females. Though both males and females develop spontaneous autoimmune disease, the disease is greatly accelerated in males. The BXSB disease resembles lupus and has an early onset and a uniform progression. The marked acceleration of autoimmunity is dominant in F_1 hybrid males from crosses of BXSB mice to other strains, but is under polygenic control as shown by intercross and backcross analysis. Interestingly, the acceleration of the disease is seen in (NZB ♀ × BXSB ♂) F_1 males but not in (BXSB ♀ × NZB ♂) F_1 male mice. Thus, a Y-linked gene from BXSB derived from SB mice acts to accelerate the autoimmune process. Male BXSB mice display a substantial increase in B-cells and slight decreases in peripheral T-cells during the progression of the disease. Mild lymphoproliferation occurs in these animals associated with a preponderance of activated B-lymphocytes.

The relationship between abnormal genes and autoimmunity has been most extensively studied in inbred New Zealand black (NZB) and white (NZW) mice. By two to three months of age, autoantibodies to DNA, thymocytes, and eventually to erythrocytes are present in NZB mice; they subsequently develop immune complex nephritis and hemolytic anemia, and by one year many NZB mice develop lymphomas and reticulum cell sarcomas. A large number of genetic studies with NZB mice have shown that the pathogenesis of their disease is under complex polygenic control. One difficulty in many of these analyses is that the expression of a complex phenotype such as autoimmune hemolytic anemia is often not an all-or-none phenomenon. However, several general conclusions can be drawn. Uniformly, 100 percent of NZB mice develop hemolytic anemia whereas virtually no NZC mice develop this syndrome. F_2 and backcross analysis has shown that the expression of hemolytic anemia is controlled by one dominant gene in these strains. In other crosses, for example, between NZB and BALB/c mice, the expression of hemolytic anemia is inherited as a recessive trait. Thus, the dominant gene action in autoantibody expression can be modified by the presence of other genes.

Steinberg and co-workers have compared the genetic control of natural thymocytotoxic autoantibodies (NTA) and single-stranded DNA (ssDNA) autoantibodies in NZB and DBA/2 mice and their F_1 and backcross progeny. The production of ssDNA antibodies was shown to be regulated by a single dominant gene, whereas NTA expression was regulated by an unlinked codominant gene that displayed clear gene dosage effects. NZB mice have very high levels of xenotropic murine leukemia virus, and it has been suggested that xenotropic viruses may be involved in the etiology of their autoimmune disease. In an elegant study, Datta and co-workers have used genetic analyses to address this question. NZB have very high xenotropic viral titers and 100

percent incidence of autoimmune disease; however, another strain, SWR, is radically different from NZB in that it expresses no xenotropic virus and has no autoimmune manifestations. (NZB × SWR)F1 mice have very high titers of xenotropic virus. Approximately 75 percent of the F1 × SWR backcross progeny are virus positive, which suggests that either of two independently segregating loci permit virus expression. Further analysis of backcross and F2 progeny has shown that one of these autosomal dominant genes controls high-grade (*Nzv-1*) virus expression and the other permits low-grade (*Nzv-2*) expression. Subsequent analysis has shown that the presence of virus does not correlate with the presence of autoantibodies or nephritis. Some virus negative mice get autoimmune disease and some mice with high xenotropic virus titers are autoimmune-disease-free. The expression of the murine leukemia virus envelope glycoprotein gp 70 and the presence of autoimmune disease also do not segregate together, but the development of lymphomas was strongly correlated with the expression of serum gp 70. Thus xenotropic viruses alone do not evoke autoimmunity in NZB mice; however, they may participate secondarily.

What then is the site of the primary lesion leading to autoimmunity in NZB mice? The genetic defect is expressed in the bone marrow because normal irradiated hosts receiving adoptively transferred bone marrow from NZB donors develop autoimmune disease. However, it is not clear if the primary defect is expressed in T-cells, B-cells, or more than one cell type. NZB mice apparently have a deficiency of suppressor T-cells very early in life and are resistant to induction of T-cell tolerance. Six-month-old NZB mice have abnormal proportions of T-cell subsets; they have an excess of Lytl+ T-cells and a deficiency of Lyt123+ T-cells. Their major T-cell deficiency appears correlated with an absence or malfunction of an Lyt123+ T-cell subset responsible for feedback suppression. Possibly thymocytotoxic antibodies in NZB mice, which are more efficient in killing T_S-cells than T_H-cells, could be responsible for this deficiency. However, certain normal strains of mice have high titers of NTA, and the presence of autoimmunity in NZB as well as MRL/1 mice does not correlate with the presence of natural thymocyte autoantibodies.

Examination of NZB mice with additional immunodeficiencies has helped to clarify the role of B-cell defects in autoimmune disease. (NZB × CBA-*Xid*) F1 male mice have a deficiency in a subset B-cell which leads to defective T-cell independent responses. Unlike their normal female counterparts, these mice do not produce excessive IgM and have reduced levels of erythrocyte antibodies. Further study is required with homozygous NZB-*Xid* males, but the implication is that the absence of a specific T-cell-independent response may reduce the incidence of autoimmune disease. NZB-*Dh*/+ are asplenic but do develop erythrocyte antibodies and autoimmune disease. However, the level of natural thymocyte antibodies is greatly reduced with age in these mice. Although helper T-cell activity is reduced in asplenic NZB mice, unlike normal NZBs, NZB-*Dh*/+ thymocytes show no diminished ability with age to suppress GVH responses. In this instance, the suppressor cell function may

remain intact during the development of autoimmune disease. The B-cells of both NZB and hypothymic NZB *nu/nu* mice are polyclonally activated early in life, presumably because of a thymus-independent defect. Taken together, the results with *nu/nu, Xid/Y,* and *Db/+* NZB mice suggest that an intrinsic B-cell dysfunction may play a major role in the development of lupuslike autoimmune disease. Motheaten, MRL/1, and BXSB mice also appear to have hyperactive B-cells. However, defects in other lymphoid subsets such as T-cells may also develop and contribute to the disease process. Further study of specific immunodeficiency mutants should help to clarify how genes affecting target cell sensitivity, immunoregulation, and susceptibility to exogenous infections interact during the development of autoimmune disease.

By the age of three months, NZB mice display a high incidence of chromosomal aberrations. Spleen cells display a mosaicism of euploidy and aneuploidy not seen in other strains of mice. It is not clear if this chromosomal instability is a cause or a manifestation of the autoimmune process. However, the chromosomal abnormalities do increase with age and appear to have a clonal origin. The karyotypic changes which develop in NZB mice are highly nonrandom and often involve the 2, 15, 16, 17, and sex chromosomes. The fact that chromosome 17, which contains the *H-2* immunogene complex, is preferentially involved in NZB hyperploidy is of particular interest. An alteration in *H-2* function could potentially affect viral integration, expression, or production. The presence of trisomy 15 at a high frequency in NZB spleens is striking because this trisomy is found in many spontaneous, radiation-induced, and virally-induced leukemias and T-cell lymphomas. Longitudinal studies suggest that the presence of aneuploidy in NZBs and the subsequent development of malignancy are closely correlated. Aneuploid clones appear to be at least potentially neoplastic; furthermore, trisomy 15 is also found in NZB reticulum cell sarcomas. The associations between chromosomal instability, immune dysfunction, and malignancy seen in NZB mice remain to be clarified.

Aging

Although aging and death are essential features of the evolutionary process, little is known about the genetic factors that influence the life span of various species. A number of theories have been proposed to explain the aging process: somatic mutation, the accumulation of random errors, biological clocks, or underlying preprogrammed events limiting the life span of cells have all been implicated. Immunologic theories of aging emphasize pathogenetic or secondary processes that lead to progressive deterioration of dysfunction. Walford's pioneering immunologic theory avers that aging results from prolonged histoincompatibility reactions among immunologically diversifying cells within an individual. Accumulation of errors in regulatory immunocytes or other homeostatic defects may allow autoimmune responses, which in turn contribute to deleterious changes associated with aging.

To the extent that normal immune functions decline with age, the incidence of infections, certain kinds of cancer, and autoimmune manifestations increase. In particular, T-cell functions such as delayed-type hypersensitivity reactions or responsiveness to T-cell dependent antigens wane with age. However, immunologic stem cells of aged animals appear to be normal. This suggests that the age-related immunologic defects are not intrinsically preprogrammed in the precursor cells. Experimental models of aging involving graft-versus-host disease or parabiosis have shown that chronic histoincompatibility reactions can accelerate aging. Several studies have suggested that loci in the major immunogene complex and moderate histocompatibility genes may influence aging. Walford and co-workers have found that both *H-2* and non-*H-2* genes can significantly influence life span, the rate of aging, and the overall frequency of a wide variety of spontaneous cancers (Table 8–7). For example, male C57BL/10Sn mice have a mean survival time of 134 weeks, whereas B10.AKM *H-2* congenic mice have a lower survival time of 99 weeks. The $H\text{-}2^{m}$ haplotype in the latter strain also is associated with an increased incidence of spontaneous lymphomas at death. Similarly, C3H.K mice have a shorter life span and also display a higher tumor incidence than their *H-1* congenic C3H/He partners. The effect of a particular *H-2* haplotype on the life span varies according to the strain background. On C57BL/10 and C3H backgrounds, the $H\text{-}2^{b}$ haplotype tends to promote long survival but when on the A strain background leads to shorter survival. Thus, as might be expected, certain combinations of alleles favor increasing life span. In aged women, the frequency of the *HLA A1-B8* haplotype is decreased. Furthermore, the responsiveness to T-cell mitogens in elderly B8 females is decreased compared

TABLE 8–7. Genetic Control of Life Span and Tumor Development in Congenic Male Mice

Strain	*H-2*	*H-1*	Background	Mean survival time (weeks)	% At death of		
					Lymphoma	Hepatomas	Lung tumors
C57BL/10Sn	*b*	*c*	B10	134	29	2	3
B10.AKM	[*m*]	*c*	B10	99	53	5	0
B10.A	[*a*]	*c*	B10	128	20	3	18
B10.RIII (7INS)	[*r*]	*c*	B10	141	23	0	10
B10.D2 (58N)	*b*	[*a*]	B10	121	8	5	5
B10.129 (5M)	*b*	[*b*]	B10	129	43	3	2
A.BY	*b*	[?]	[A]	85	10	2	10
C3H.SW	*b*	[*a*]	[C3H]	108	3	38	0
C3H/He	*k*	*a*	C3H	98	5	51	10
C3H.SW	[*b*]	*a*	C3H	108	3	38	0
C3H.K	*k*	[*b*]	C3H	73	14	50	12
B10.BR	*k*	[*c*]	[B10]	113	34	2	14

Based on Smith and Walford in *Genetic Effects of Aging*, D. Bergsma and D. E. Harrison, (eds.). New York: Alan R. Liss, Inc., 1978, pp. 281–311.

to otherwise HLA-matched controls. How loci in the major immunogene complex may influence susceptibility to disease and as a result, life span, is discussed in the following section. Whether life ends because of degenerative disease or a fatal infectious disease, overall longevity clearly may depend on immunogenetic regulation. All major groups of multicellular animals, both invertebrates and vertebrates, may possess immunorecognition systems that defend somatic integrity but gradually allow immune dysfunction, a major cause of aging. This immunophylogenetic theory of aging is compatible with the specific immunologic competence seen at all levels of phylogeny (Chapter 9). Although little is known about pathogenetic changes and immunologic-type disease in invertebrates, proliferative disorders and true neoplastic disease in certain invertebrates has been convincingly confirmed. As more is learned about the immune systems of invertebrates, it should be possible to test the hypothesis that immune dysfunction is associated with aging throughout phylogeny.

HLA AND DISEASE

The discovery of blood group polymorphisms in man inspired much research into possible associations with disease. Efforts involving blood group typing of over a half million patients have revealed numerous correlations (Chapter 4). For example, stomach cancers are clearly higher in blood group A individuals, and duodenal ulcers are more frequent in people with blood group O. The demonstration by Lilly and others that resistance to virally-induced leukemogenesis in the mouse is controlled by *H-2*-linked genes and the discovery of *H-2*-linked immune response genes stimulated a search for associations between *HLA* genotypes and specific diseases. It has already become clear that genes linked to the *HLA* complex regulate susceptibility to several diseases. An understanding of the mechanisms leading to these associations should provide valuable information about the etiology and eventual prevention of these diseases.

There are two basic approaches to test for genetically controlled disease in man: population studies and family studies. If population studies reveal a significant statistical association between a disease and antigenic markers such as HLA, this can indicate correlations between particular genes and diseases. Family studies are invaluable for providing evidence of linkage; however, familial diseases often do not show simple Mendelian patterns. Even if linkage with a marker locus is established, other factors such as linkage disequilibrium with critical genes must be considered. The basic approach used to look for disease association with a particular HLA type is to compare the HLA phenotypic frequency of unrelated disease patients to unrelated healthy control individuals of the same age and ethnic background. The results are analyzed, for example, by Fisher's exact test or the Chi-square test, to determine if given HLA antigen frequencies in patients deviate significantly from controls. If a large number of antigens are examined, it is statistically probable that a "significant" association will be found for one of the antigens. Thus, the p value

obtained for a given antigen must be multiplied by the number of antigens studied to obtain a "corrected *p* value." A useful indicator termed *relative risk* measures the risk of getting a disease when an antigen is present versus when it is absent (see Table 8–8). This can be a good indicator of the strength of the HLA antigen–disease association and its potential biological significance.

Early studies comparing the frequency of HLA-A and HLA-B antigens in diseased and healthy individuals led to a number of suggestive correlations. With the development of HLA-D typing and the definition of HLA-D and HLA-DR specificities, it became possible to assess associations with the *D* and *DR* loci as well (Table 8–8). Several diseases show strong associations with alleles of more than one *HLA* locus. For example, patients with coeliac disease have a higher than expected frequency of A1, B8, and Dw3. Multiple sclerosis (MS) patients have a high incidence of B7 and Dw2. These population associations are observed because the alleles of certain closely linked *HLA* genes are in linkage disequilibrium, that is, they are inherited together on the same chromosome more frequently than would be expected (see page 207). For example, the association of A1 and B8 or B8 and Dw3 in Caucasoids is very high. This finding emphasizes that any HLA and disease association need not

TABLE 8–8. Some Positive Correlations between HLA and Disease[a]

HLA	Disease	Frequency (%)		Average relative risk[b]
		Patients	Controls	
A3	Hemochromatosis	72	21	9.7
B27	Ankylosing spondylitis	90	8	103.5
	Reiter's disease	80	9	40.4
	Yersenia arthritis	69	11	18.0
	Acute anterior uveitis	48	9	9.3
	Juvenile rheumatoid arthritis	35	11	4.4
B13	Psoriasis: Caucasian	23	5	5.7
	Japanese	18	1	24.8
Cw6	Psoriasis: Caucasian	50	23	3.3
	Japanese	53	7	15.0
Dw2 (B7)	Multiple sclerosis	55	23	4.1
Dw3 (B8)	Gluten-sensitive enteropathy	96	27	64.5
	Dermatitis herpetiformis	77	20	13.4
	Sjogren's disease	75	21	11.3
	Idiopathic Addison's disease	70	21	8.8
	Chronic active hepatitis	68	24	6.7
	Graves' disease	53	18	5.1
	Insulin-dependent diabetes	50	21	3.8
	Myasthenia gravis	30	17	2.1
Dw4	Rheumatoid arthritis	56	15	7.2
	Insulin-dependent diabetes	38	13	4.1

[a]Based on data in key book references edited by Dausset and Svejgaard, 1977 and Bodmer et al., 1978. All subjects were Caucasoids unless otherwise indicated.

[b]Woolf's relative risk = Pd (1–Pc)/Pc (1–Pd), where Pd = frequency in patients and Pc = frequency in controls.

derive from the *HLA, B, C,* or *D* marker locus but could be a result of a closely linked "disease-susceptibility" locus in linkage disequilibrium. The difficult task remains to determine which loci in the *HLA* region contribute to given susceptibilities. In the case of coeliac disease and multiple sclerosis, higher relative risks have been observed for *Dw3* and *Dw2* than for their associated *HLA-B* alleles. This suggests that loci close to or within *HLA-D* rather than *B*-associated genes are operative. In other instances, such as chronic active hepatitis, stronger relative risks are seen with *B8* than with *Dw3* or *DRw3.* A more precise definition of these susceptibility loci should be possible as better defined antiserums for *B* and *DR* region specificities and the gene products of loci between these two regions become available.

Numerous additional diseases not shown in Table 8–8 have been examined for HLA association, and only weak or nonsignificant correlations have been found. Since the *H-2* complex in the mouse has been implicated in resistance to virally-induced oncogenesis and also plays a principal role in immunoregulation, it has been suggested that abnormal or dysfunctioning genes in the *HLA* complex may predispose people to malignancy or immunodeficiency. However, for the most part, HLA associations with most disorders have not proved striking. Weak associations of HLA specificities with, for example, Hodgkin's disease (B5) and acute lymphocytic leukemia (A2,B12) have been reported. Although many autoimmune-associated diseases show strong associations with certain HLA types (see below), overt immune diseases such as systemic lupus erythematosus (B8) and glomerulonephritis (A2) are correlated with only moderately increased frequency in the HLA types indicated. Weak associations, which can be caused by any of several factors including statistical artifacts, should not be readily discounted. A disease under study may represent a heterogeneous group of closely related disorders with only a subset displaying high frequency of a particular HLA specificity. This occurs in myasthenia gravis where an association with Dw3 and B8 is predictable only with patients who do not have concomitant thymoma. The expression of a disease in an individual possessing a susceptible allele can be obscured by other genetic and environmental factors contributing to the etiology. A weak association with one *HLA* locus can frequently reflect the presence of a stronger association at a linked locus. For example, patients with the autoimmune disorder Sjogren's disease exhibit only a slight increase in HLA-B8, yet more recent comparisons of Dw specificities in Sjogren's disease have revealed a strong association with Dw3. Thus, weak correlations of certain HLA specificities with disease may reveal stronger associations upon subsequent typing for newly defined specificities.

Ankylosing Spondylitis and Arthropathies

The strongest association of HLA and a disease is seen between HLA-B27 and ankylosing spondylitis (AS), a disorder leading to inflammation and eventual fusion of vertebrae (Table 8–8). Ninety percent or more of AS patients have B27, although many carriers of the *B27* allele do not develop the disease.

Family studies of AS probands have shown susceptibility to AS is inherited as an autosomal dominant trait, yet not all B27 first-degree relatives are afflicted. Occasionally, but rarely, ankylosing spondylitis is seen in a B27-negative relative. This suggests the gene involved is not B27 but another immunogene tightly linked to it. Clinically, nearly five-fold more males than females are diagnosed with AS, even though this male preponderance does not emerge in family studies. Thus, the higher frequency of males observed with this disease is probably attributable to a nongenetic influence. The racial and geographic distribution of B27 closely parallels the incidence of AS disease. Both AS and the B27 antigen are absent in black Africans, whereas North American Indians have a high frequency of B27 and AS-like symptoms such as sacroiliitis. The *B27* allele is rare in Japan; nevertheless, over 90 percent of Japanese AS patients are B27 positive. Presumably the gene controlling susceptibility to ankylosing spondylitis and *B27* are in strong linkage disequilibrium in diverse ethnic groups.

The *B27* allele is also seen at a high frequency in patients of related disorders such as Reiter's disease. Of all the diseases showing striking correlations with HLA, Reiter's disease is most likely to have an infectious etiology. It usually is seen following the development of sexually transmitted urethritis. Patients with nonspecific urethritis have a normal B27 incidence. An elevated frequency of B27 is associated with those who subsequently suffer arthritic symptoms. Indeed, individuals with B27 who have urethritis, or gut infections with *Shigella, Salmonella,* or *Yersinia,* are forty times more likely to develop acute arthritis than the rest of the population. Psoriasis without rheumatoid disease shows no association with B27; however, psoriasis patients with arthritis, particularly with obvious spondylitis, have a high B27 incidence. It would be most interesting to know if B27 or any closely linked gene products are similar or identical to antigens of microorganisms. Possibly the causative agents of these disorders share determinants with B27-associated products and thus are not recognized as foreign and eliminated. The dominant inheritance of AS is compatible with such a "molecular mimicry" explanation. In summary, a group of separable conditions, all with articular involvement, appear to be under common genetic control of an *HLA-B27*-linked gene.

Immunologic and Endocrine Diseases

A number of systemic diseases, especially autoimmune diseases, are associated with an elevated frequency of A1, B8, and Dw3. Greater relative risks in general have been observed to date for Dw3 or DRw3 associations than for B8 or A1, which suggests that *D*-linked genes may be more influential. The incidence of many of these disorders, such as gluten-sensitive enteropathy (GSE) and Sjogren's disease, is higher in females than in males even though the frequency of Dw3 is the same in both sexes. The striking common feature of Dw3-associated diseases is the presence of some kind of immune dysfunction frequently leading to autoantibody or immune complex formation.

Several of the Dw3-associated diseases are gastrointestinal disorders. The most striking example is GSE, or coeliac disease. GSE results from an abnormal sensitivity to the wheat protein gluten; mature epithelial cells lining the small intestine which ingest gluten are destroyed, most likely as a consequence of Arthus-like immune reaction. Immunologic disturbances such as fluctuations of serum IgA levels and IgM antigluten antibodies are frequently present. GSE is known to be a familial disorder and HLA typing of afflicted families provides additional evidence for an autosomal dominant genetic component. In families manifesting the *A1-B8-Dw3* haplotype, nearly all members with coeliac disease have this haplotype, but there are exceptions. Not all relatives with the haplotype have the disease, therefore "incomplete penetrance" must be invoked. Environmental factors such as diet modified by sex, age, or genetic factors may be influencing the expression of the disease. The clinically related skin disorder dermatitis herpetiformis is also associated with a high incidence of Dw3, gluten sensitivity, and gastrointestinal lesions. This suggests an underlying connection with GSE although different consequences ensue. Autoimmune chronic active hepatitis, unlike chronic hepatitis resulting from hepatitis B virus infection, also correlates with an increased incidence of B8 and Dw3. Here again, a preponderance of females have this disorder and family studies suggest incomplete penetrance.

Several organ-specific autoimmune endocrine diseases also display a high incidence of Dw3, including insulin-dependent diabetes (IDD), idiopathic Addison's disease, and Graves' disease (GD). About 70 percent of Addison's disease patients have autoantibodies against the adrenal cortex. It is not clear what role these antibodies play in the disease; in one study 96 percent of the Dw3 positive patients with Addison's had autoantibodies compared to 40 percent of the Dw3 negative patients. This suggests that a Dw3-associated gene may be influencing autoantibody production. In Graves' disease, autoantibodies directed against thyroid stimulating hormone (TSH) receptors are probably important in mimicking the effect of TSH and thus provoking the hyperthyroidism seen in this disease. Patients who respond poorly to antithyroid drugs and go into relapse have a higher incidence of B8 and Dw3 than patients who go into remission. In this instance, HLA typing may be useful as a prognostic indicator. In the Japanese population Graves' disease is not associated with B8 but rather individuals with Bw35 are at risk. Both Graves' and Addison's diseases occur in association with other organ-specific autoimmune disorders including IDD, Hashimoto's thyroiditis, and pernicious anemia. Although an elevated incidence of Dw3 is also seen in IDD, this is not observed in either Hashimoto's thyroiditis or pernicious anemia. Hashimoto's with goiter is a classic example of an organ-specific autoimmune disease; it has common clinical, pathological, and immunological features as well as a clear genetic relationship with Graves' disease. Both aggregate in specific families, yet no Dw3 association is seen with Hasmimoto's. This finding emphasizes the complex polygenic control of these diseases.

Diabetes mellitus is a complex group of diseases characterized by hypergly-

cemia and glycosuria. Immunologic studies have helped to pioneer a new classification of diabetes based on dependence on insulin rather than on the age of onset. Two basic types are now recognized: insulin-dependent diabetes (Type I) and insulin-independent diabetes (Type II). As with other organ-specific autoimmune diseases, IDD is characterized by lymphocytic infiltration of the endocrine gland and selective destruction or perturbation of the hormone-producing epithelial cells, in this case the β-cells of the islets of Langerhans. HLA associations are seen almost entirely with IDD and not with insulin-independent (formerly maturity onset) diabetes. Three HLA-associated haplotypes are known to affect the development of Type I diabetes (Figure 8–2). Two susceptibility genes are strongly associated with Dw3 and Dw4, respectively. Initial studies suggested HLA control of insulin-dependent diabetes is inherited as an autosomal recessive trait. However, the incidence of diabetes in siblings of IDD patients is only 5 percent, therefore other factors clearly must be operative. Svejgaard has compared the relative risks associated with various *HLA* genotypes in distinctive groups of patients with insulin-dependent diabetes (Figure 8–2). Individuals homozygous for *Dw3* or *Dw4* were found to be at higher risk to develop IDD than corresponding *Dw3/X* or *Dw4/X* heterozygotes. Surprisingly, *Dw3/Dw4* heterozygotes have an even higher risk than *Dw4/4* or *Dw3/3* homozygotes. It is not clear if the *Dw3* and *Dw4*-linked susceptibility genes are alleles of the same locus and the increased susceptibility in heterozygotes results from overdominance, or if epistatic interaction between two closely linked loci is occurring. The effect of *Dw4* homozygosity is most pronounced in younger patients because IDD has two clinical peaks, one at age ten and the other in the twenties. Svejgaard has sug-

FIGURE 8–2. Three *HLA* haplotypes, each displaying linkage disequilibrium, which influence Type I diabetes.

Source: Based on articles by W. J. Irvine and A. Svejgaard in *Genetic Control of Autoimmune Disease*, New York: Elsevier North-Holland, 1978.

DRw3/Dw3	B8		A1	Susceptibility
DRw4/Dw4	B15	CW3	A2	Susceptibility
DRw2/Dw2	B7		A3	Resistance

HLA-D

Genotype	Relative Risk
Dw3/Dw3	++
Dw3/X	+
Dw3/Dw4	++++
Dw4/Dw4	+++
Dw4/X	++

gested that *Dw4*-associated genes may act to affect early onset diabetes and the *Dw3* gene may act later. It is tempting to speculate that the *Dw3* and *Dw4* genes act by independent mechanisms. The *Dw3* is strongly associated with autoimmune aspects of the Type I diabetes. There is a low prevalence of *Dw3* in patients without islet cell antibodies, a higher prevalence in patients with autoantibodies, and the greatest prevalence of *Dw3* in IDD patients with other associated "primary" autoimmune disorders. *Dw4* positive diabetics exhibit higher antibody responses to xenogeneic insulin than *Dw3* positive patients.

The very low frequency or absence of *Dw2* in IDD patients cannot be attributed to the increased occurrence of *Dw3* and *Dw4*. Thus, a *Dw2*-associated locus may be conferring strong resistance to diabetes. This gene may confer resistance to pancreatotropic viral infections or otherwise impede autoimmune reactions against islet cells. Insulin-dependent diabetes is uncommon in populations where the frequency of B8 is low, such as in the Japanese. In the Japanese, IDD occurs in association with Bw22. *Dw3* is also elevated in several diseases where autoantibodies are evident, including myasthenia gravis, SLE, and Sjogren's disease. Although there are interesting exceptions, the prevalence of *Dw3* is so strong in autoimmune diseases that autoimmunity should be considered as a possible component of any *Dw3*-associated disorder.

Other Disease Associations

Among numerous diseases for which HLA associations have been sought, several illustrate interesting principles. Rheumatoid arthritis shows no positive correlation with any of the HLA A, B, or C antigens but has a strong associtzion with the *Dw4* and *DRw4* alleles. Bone erosions indicative of the severity of arthritis are seen to a greater extent in DRw4 positive patients than in DRw4 negative patients. DRw4 typing may accordingly distinguish rheumatoid groups to predict milder versus more severe expression of the disease. Multiple sclerosis (MS) patients possess the *HLA-Dw2* or *DRw2* gene more often than unaffected individuals. The relative risk for *Dw2* positive individuals is not great in Europeans but it is significant. Although MS has its highest incidence in the Shetland and Orkney Islands, there is no clear association of MS with *Dw2* in this population. Similarly, *Dw2* positive Israelis and Japanese are also not at risk. The association between HLA and MS is of particular interest because of the possible role of measles virus or a similar agent in the etiology. In the populations where *Dw2* is not associated with MS, other alleles or distinct loci may confer resistance.

Both hemochromatosis (A3) and psoriasis vulgaris (B13, B17, B37, Cw6) reveal an increased incidence of certain HLA antigens as indicated, but these disorders are not known to involve immune mechanisms. Psoriasis is a widespread skin disease with a clear familial association. Its complexity is reflected in its association with several HLA specificities in both Caucasians and Japanese. Japanese with A1, Cw6, B13, or B37 are at high risk to develop psoriasis. These multiple associations could be attributable to a common locus in

linkage disequilibrium with the HLA marker genes. The lack of any obvious immunologic basis for the diseases suggests that a linked locus, for example, coding for an enzyme, may be involved. Alternatively, distinct subsets of psoriasis disorders may be associated with different HLA antigens.

It has long been known that susceptibility to allergic diseases is familial and under polygenic control. Moreover, IgE and IgG antibody responses to allergens in mice are controlled by *H-2* associated immune response genes. In humans, both *HLA* and non-*HLA* genes influence immune responses to allergens. Family studies have shown that elevated IgE levels in allergic patients are controlled by a single recessive gene not linked to either *HLA* or *Gm* allotype loci. *HLA*-linked genes do, however, influence responses to specific allergens such as ragweed allergen Ra3 and rye grass allergen Rye 1. An increased frequency of A2 is seen in subjects sensitive to Ra3, whereas B8 is more common among those sensitive to Rye 1. An increased frequency of the HLA specificities indicated is evident in atypical allergics with low serum IgE levels. In contrast, the A3 specificity is significantly reduced in individuals with Ra3 sensitivity. Thus, both responsiveness to Ra3 (A2) and a failure thereof (A3) appear to be under *HLA*-linked gene control. Responses to other allergens may be controlled by other *HLA* alleles. In one large family, hypersensitivity to antigen E was associated with HLA-B12, but among other families this response was associated with elevated frequencies of different HLA markers.

Analyses of *HLA* and other immunogene correlations with non-neoplastic diseases show much promise. The nature of the mechanisms accounting for the associations are just beginning to be elucidated. Many of the strongest relative risks appear associated with the *HLA-D* region, and it has become important to distinguish between *D* or *DR* control, or control by closely linked genes. Because this region is the homologue of the *H-2I* region in the mouse, it is quite possible that immunoregulatory (*Ir*) genes are operative. The *Dw3* associations in particular suggest the involvement of an underlying mechanism controlling self-recognition. The regulatory network and interactions among antigens, antibodies, and idiotypes are possible sites of HLA-controlled dysfunctions. Several diseases such as Graves' disease, myasthenia gravis, and a subset of diabetes are associated with the presence of receptor antibodies. These could arise in response to the hormone receptor or to antibodies specific to the hormone. Molecular mimicry of *HLA* gene products by pathogens could allow circumvention of host resistance and thereby facilitate persistent infection leading to progressive disease.

Whatever the mechanisms, the genetic control of HLA-associated diseases is clearly polygenic. Although familial transmission is found in nearly all disorders with an HLA association, as would be expected, inheritance of susceptibility is not simple. Models invoking low or incomplete penetrance often appear necessary. Other loci controlling target sensitivity, disease onset and severity are clearly operative. The role of sex-linked genes in diseases with a preponderance of one sex and the role of Ig allotype loci invite examination.

HLA-linked complement genes such as the *Bf, C2,* and *C4* loci may also influence disease susceptibility. For example, a rare *Bf* allele has been found to occur in relatively high frequency among French people with insulin-dependent diabetes. The disorder congenital adrenal hyperplasia is caused by a deficiency in the enzyme C-21-hydroxylase. This disease is strongly associated with HLA-B5 (relative risk 5.8), probably because the enzyme structural gene is linked to *HLA*. This finding underscores the value of typing enzyme marker loci as well as *HLA* loci on chromosome 6. We can anticipate new disease associations with the loci in the *HLA* region on chromosome 6. Further clarification of autoimmune disorders should be achieved by HLA typing. HLA typing should find increasing use as a prognostic indicator and as a tool for suggesting approaches to therapy. As monoclonal and presumably monospecific HLA antibody reagents become widely distributed, HLA phenotypes may begin to provide a basis for genetic counseling.

KEY REFERENCES

Books

Bodmer, W. F., Batchelor, J. R., Bodmer, J. G., Festenstein, H., and Morris, P. J. (eds.). *Histocompatibility Testing 1977.* Copenhagen: Munksgaard, 1978. Contains extensive discussion of HLA and disease and HLA-DR-coded lymphocyte antigens.

Dausset, J., and Svegaard, A. (eds.). *HLA and Disease.* Copenhagen: Munksgaard, 1977. HLA associations with distinct groups of disease, implications, and mechanisms are evaluated.

Makinodan, T., and Yunis, E. (eds.). *Immunology and Aging.* New York: Plenum Press, 1977. The aging process, its relationship to hormonal changes, immunodeficiency of the immune system, and autoimmunity are considered.

Rose, N. R., Bigazzi, P. E., and Warner, N. L. (eds.). *Genetic Control of Autoimmune Disease.* New York: Elsevier North-Holland, 1978. Excellent discussions of HLA and disease, autoimmunity, and experimental models of autoimmunity.

Rygaard, J. *Thymus and Self: Immunobiology of the Mouse Mutant Nude.* London: John Wiley, 1973. Comprehensive monograph on the hypothymic nude mouse.

Articles

Bubbers, J. E., and Lilly, F. Selective incorporation of *H-2* antigenic determinants into Friend virus particles. *Nature* 266:458–459, 1977. Virus particles preferentially incorporate certain H-2 determinants into their envelope when budding.

Chang, S. S., and Hildemann, W. H. Inheritance of susceptibility to polyoma virus in mice. *J. Nat. Cancer Inst.* 33:303–313, 1964. At least three independently segregating loci account for overall control of susceptibility to polyoma virus. Also see later paper in *J. Nat. Cancer Inst.* 40:363–375, 1968.

Clark, E., Lake, P., Mitchison, N. A., and Winchester, G. New ideas about self tolerance and autoimmunity. In *Immunology 1978* J. Gergely, G. A. Medgyesi, and S. R. Hollan, (eds.). Budapest: Akademiai Kiado, 1978, pp. 3–16. Certain cell-surface molecules may be immunologically silent and accordingly not seen by T-cells. The addition of new structures on the membrane, such as viral antigens or Ia molecules, may trigger an autoimmune response.

Cooper, M., and Lawton, A. R. (eds.). *Immune Deficiency I and II. Springer Seminars Immunopathology.* Vol. 1, Nos. 3 and 4, 1978. Series of cogent reviews on human immunodeficiency disorders.

Datta, S. K., Manny, N., Andrzejewski, C., Andre-Schwartz, J., and Schwartz, R. S. Genetic studies of autoimmunity and retrovirus expression in crosses of New Zealand black mice. I. Xenotropic virus. *J. Exp. Med.* 147:854–871, 1978. Genes controlling expression of xenotropic murine leukemia virus clearly disassociated from regulation of autoimmune disease. Follow-up article (*J. Exp. Med.* 147:872–881, 1978) shows that the development of autoimmunity versus gp 70 expression or lymphomas in NZB mice is regulated by distinct genes.

Duquesnoy, R. J., Christensen, K., Pederson, G. M., and Kemp, R. G. Development of immunodeficiency of pituitary dwarf mice. *Amer. Zool.* 15:167–174, 1975. The Snell-Bagg and Ames dwarf mutants display similar T-cell deficiencies except that unlike Ames dwarfs, the Snell-Bagg mice can produce antibodies to high doses of T-cell dependent antigens.

Fialkow, P. J., Bryant, J. I., and Friedman, J. M. Specificity of acquired clonal chromosome abnormalities in New Zealand black mice. *Int. J. Cancer* 21:505–510, 1978. The chromosomal abnormalities seen in spleen cells of NZB mice are of monoclonal origin. Aneuploidy involving the 2,15,16,17, or sex chromosomes is frequently seen.

Fletcher, M. P., Ikeda, R. M., and Gershwin, M. E. Splenic influence of T cell function: the immunobiology of hereditarily asplenic mice. *J. Immunol.* 119:110–115, 1977. The immunocompetence of asplenic *Dh/+* mice is examined.

Goodman, G. T., and Koprowski, H. Study on the mechanism of innate resistance to virus infection. *J. Cell Compar. Physiol.* 59:333–373, 1962. Resistance to flavovirus infection in mice is dependent on a dominant gene affecting the ability of macrophages to destroy virus.

Gowen, J. W. Genetic effects in nonspecific resistance to infectious disease. *Bact. Rev.* 24:192–200, 1960. Summarizes many years of author's work revealing polygenic determination of degrees of resistance of mice to *Salmonella typhimurium* and the importance of specific immunity.

Hartley, J. W., Walford, N. K., Old, L. J., and Rowe, W. P. A new class of murine leukemia virus associated with development of spontaneous leukemia. *Proc. Nat. Acad. Sci. USA* 74:789–792, 1977. Concurrent with the development of leukemia in AKR mice, new recombinants between ecotropic and xenotropic viral genomes emerge.

Hildemann, W. H. Phylogenetic and immunogenetic aspects of aging. In *Genetic Effects on Aging, Birth Defects: Original Article Series, Vol. XIV, 1.* D. Bergsma, and D. E. Harrison, (eds.). New York: The National Foundation, 1978, pp. 97–107. The immunophylogenetic theory states that all major groups of multicellular animals possess immunorecognition systems that defend somatic integrity. Gradual immune dysfunction may be a major cause of aging. See related articles by Smith and Walford and by Greenberg and Yunis in same volume.

Hilkens, J., Colombatti, A., Strand, M., Nichols, E., Ruddle, F. H., and Hilgers, J. Identification of mouse gene required for binding MuLV envelope gp 70. *Somatic Cell Genetics* 5:39–49, 1979. Analysis using mouse-hamster somatic cell hybrids shows a receptor for MuLV is coded for by the *Rev-1* locus on chromosome 5.

Klein, G. Lymphoma development in mice and humans: diversity of initiation is followed by convergent cytogenetic evolution. *Proc. Nat. Acad. Sci. USA* 76:2442–2446, 1979. Provocative discussion of possible sequential events leading to malignancy. After cell transformation, the development of tumors is dependent on additional changes at the genetic level, perhaps specific translocations.

Louis, S., and Schwartz, R. S. Immunodeficiency and the pathogenesis of lymphoma and leukemia. *Sem. Hematol.* 15:117–138, 1978. A number of congenital immunodeficiency disorders and other immunogenetic diseases are associated with high incidence of malignancy.

Notkins, A. L. The causes of diabetes. *Sci. American* 241:62–73, 1979. A concise discussion of Type I and Type II diabetes.

Pincus, T., and Snyder, H. W., Jr. Genetic control of resistance to viral infection in mice. In *Viral Immunology and Immunopathology.* A. L. Notkins, (ed.). New York: Academic Press, 1975, pp. 167–187. A discussion of response to flavoviruses, other non-C-type viruses, and C-type murine leukemia viruses. Other articles of interest discuss the immune response to leukemia viruses, immune-complex disease and autoimmunity.

Raveche, E. S., Steinberg, A. D., Klassen, L. W., and Tjio, J. M. Genetic studies in NZB mice. I. Spontaneous autoantibody production. *J. Exp. Med.* 147:1487–1502, 1978. Gene controlling the development of thymocytotoxic autoantibodies dissociated from the regulation of the development of DNA autoantibodies.

Risser, R., Potter, M., and Rowe, W. P. Abelson virus-induced lymphomagenesis in mice. *J. Exp. Med.* 148:714–726, 1978. The development of B-cell lymphomas induced by Abelson virus is regulated by two genes *Av-1* and *Av-2.*

Rowe, W. P. Genetic factors in the natural history of murine leukemia virus infection. *Cancer Res.* 33:3061–3068, 1973. Good summary of genetic studies leading to identification of *Akv-1, Akv-2,* and other loci influencing MuLV infections.

Steeves, R., and Lilly, F. Interactions between host and viral genomes in mouse leukemia. *Annu. Rev. Genet.* 11:277–296, 1977. Cogent summary of genes that affect the development of murine viral-induced leukemogenesis.

Theofilopoulos, A. N., Eisenberg, R. A., Bourdon, M., Crowell, J. S., Jr., and Dixon, F. J. Distribution of lymphocytes identified by surface markers in murine strains with systemic lupus erythematous-like syndromes. *J. Exp. Med.* 149:516–534, 1979. B- and T-cell subset deficiencies and excesses compared on NZB, BXSB, and MRL-1*pr* strains with autoimmune disorders.

Waldmann, T. A. (moderator). Disorders of suppressor immunoregulatory cells in the pathogenesis of immunodeficiency and autoimmunity. *Ann. Inter. Med.* 88:226–238, 1978. Excessive activity of suppressor cells may underlie certain immunodeficiencies in man whereas a deficiency of suppressor regulation may lead to autoimmunity.

Warner, N. L. Genetic aspects of autoimmune disease in animals. In *Autoimmunity* N. Talal (ed.). New York: Academic Press, 1977, pp. 33–62. Genetic control of autoimmune disease in NZB mice is discussed for erythrocyte autoantibodies and nephritis symptoms.

Watson, J., Kelly, K., and Largen, M. The genetic mapping of a locus in mice that controls immune and nonimmune responses to bacterial lipopolysaccharides. In *Infection, Immunity and Genetics* H. Friedman, T. J. Linna, and J. E. Prier (eds.). Baltimore: University Park Press, 1978, pp. 24–38. The *Lps* locus controlling the response to LPS is mapped in mice. Several lines of evidence suggest it may code for the lipopolysaccharide receptor.

Webster, L. T. Inheritance of resistance of mice to enteric bacterial and neurotropic virus infections. *J. Exp. Med.* 65:261–286, 1937. A classic contribution in which all four possible combinations of susceptibility and resistance to *Salmonella* species and flavoviruses were produced by selection and inbreeding mice.

WHO Scientific Group on Immunodeficiency. *Clin. Immunol. Immunopath.* 13:296–359, 1979. Classification of primary immunodeficiencies, approaches for early diagnosis, and discussion of secondary immunodeficiency resulting from parasitic, bacterial, or viral infections, malnutrition, or other disorders.

Willcox, H. N. A., and Marsh, D. G. Genetic regulation of antibody heterogeneity: its possible significance in human allergy. *Immunogenetics* 6:209–225, 1978. The immune response to specific allergens is under polygenic control by *HLA*-linked genes as well as other loci.

9

PHYLOGENY OF IMMUNOCOMPETENCE

PATHWAYS OF MAMMALIAN IMMUNE RESPONSES

Macrophage–Lymphocyte Functions

Even in much-studied mammals, such as mice and men, our understanding of immune pathways is still rudimentary and partly conjectural. Immunologists speak often of immune surveillance, antigen recognition, and antigen processing, but these are concepts rather than understood phenomena. We know that macrophages and their products may either stimulate or inhibit immune responses of T- and B-lymphocytes depending on the circumstances. Many *Ir* genes are believed to control high-versus-low responsiveness at the macrophage level (Chapter 7). Separate pathways of cell-mediated immunity and humoral immunity operate in mammals with sequential events as depicted in Figure 9–1. Although much is known about the Ig system of antibodies derived from B-lymphocytes, the activation of T-lymphocytes and cell-mediated immunity (CMI) remain mysterious at the molecular level. This is notably evident in dynamic immunoregulation or integration of T- and B-cell functions. The existence of subsets of T-cells, commonly designated killer, helper, and suppressor, emphasizes the multifunctional and perhaps overriding importance of this system. As if all this were not sufficiently complicated, an additional monocyte/lymphocyte class including natural killer (NK) or K-cells in mammals has been identified that is distinct from both T-cells and B-cells. K-cells can kill antibody-coated target cells presumably by recognition of the Fc region of cell-bound antibody by an Fc receptor on the killer cells. This antibody-dependent cellular cytotoxicity may also operate the other way around by first "arming" the K- or NK-cells with appropriate antibodies. Mammals obviously have numerous alternative mechanisms to maintain the integrity of the body. However, the critical functions of the Ig system may reside in fine-tuning regulation of the cell-mediated immune responses of higher verte-

brates. The elaborate interdependence of multiple types of immunocytes and their products is the most striking feature of mammalian responsiveness.

Immunoregulatory Networks

Serum antibodies function as essential effectors of protective immunity only in a few reactions of toxin neutralization. Virtually all antibodies, even those serving as opsonins or lysins, may be regarded as ancillary to CMI functions rather than absolutely essential. Regulatory roles of specific IgG-blocking antibodies have been convincingly demonstrated in prevention of maternal Rh immunization and allergic desensitization in man. Such immunosuppressive antibodies can predictably modulate or turn off CMI directed against organ allografts or immunogenic tumors in experimental animals. However, underlying mechanisms of such immunoregulation, including effects of antigen–antibody complexes, remain poorly understood. Most of the *Ir* genes linked to the major immunogene complex (*MIC*) of laboratory mammals and chickens appear to be regulatory in governing high-versus-low rather than all-or-none antibody responses. A large number of dominant *Ir* genes for high responsiveness to thymus-dependent antigens have been mapped by genetic recombination to the *I* region of the *H-2* complex in mice. Few or none of these *Ir* loci appear to affect the specificity (idiotype) of the antibodies evoked, but most affect the capacity to switch over from early IgM to later IgG production.

Complex regulation under immunogene control as described in Chapter 7 is clearly evident in the reactions of *MIC*-disparate guinea pigs to dinitrophenyl (DNP)-poly-L-lysine (PLL) conjugates. Inbred strain 2 animals are fully responsive to this immunogen by both major pathways (Figure 7–3) as a dominant trait. By contrast, neither DNP-PLL antibody nor concomitant Arthus reactivity is induced in strain 13 carrying the recessive *PLL* allele unless the antigen is conjugated to a protein carrier that then triggers antibody production as found in strain 2 animals. Only the CMI pathway of delayed-type hypersensitivity is retained under all-(strain 2)-or-none (strain 13) *MIC/Ir* gene control in this situation. Possibly an integrated, polyfunctional *MIC* first emerged in higher vertebrates as a requisite for dynamic immunoregulation by T-cell subsets and multiple classes of Ig. A focus on regulatory circuits is inherent in current network theories involving isotypic, allotypic, and idiotypic specificities of antibodies (see Chapter 2). We shall return later to the probable existence of two immunorecognition systems, an *H* system characteristic of all multicellular animals and the *Ig* system that has progressively evolved among vertebrates.

In addition to specificity, selective memory is another major attribute of immunologic responsiveness. Memory may be short-lived or more often long-lived in mammals, persisting for at least several years after immunization for tetanus or smallpox for example. Immune memory may also have either a positive or negative quality. Thus, a secondary exposure of a normal adult to an antigen usually evokes a heightened and accelerated response, but a dimin-

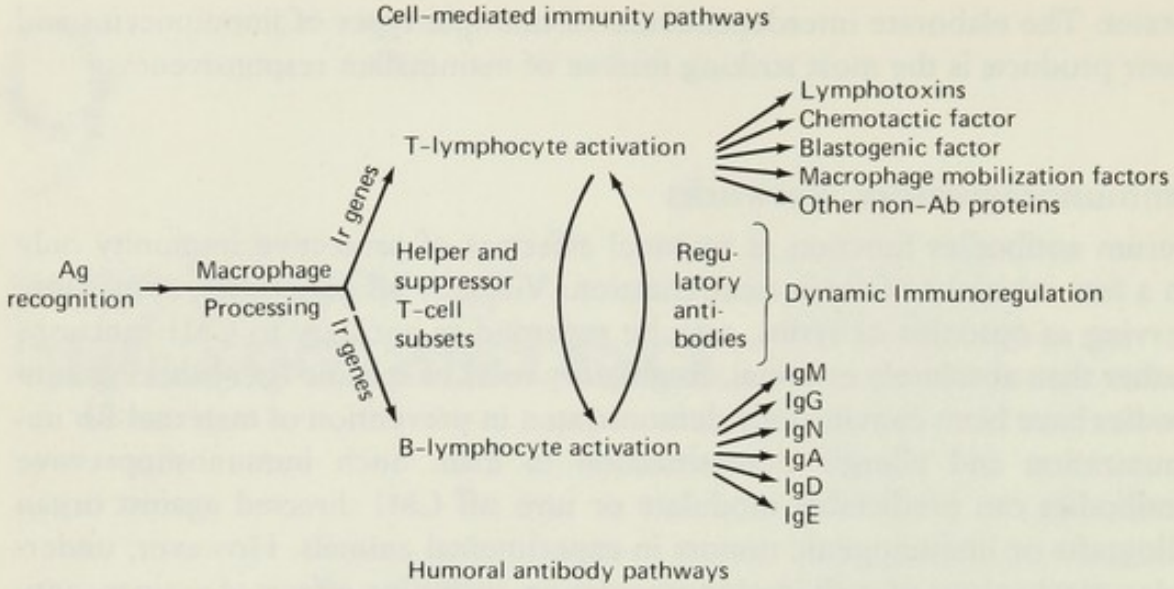


FIGURE 9–1. Pathways of immune responsiveness in mammals. The cell-mediated immunity pathways probably characteristic of all multicellular animals have remained preeminent in phylogenetic progression. B-lymphocyte pathways of humoral antibody production have become well defined in many mammals, but mechanisms of dynamic immunoregulation and *Ir* gene action remain poorly understood.

Source: Reprinted from Hildemann, *Fed. Proc.* 37:2103, 1978.

ished or specifically suppressed response (i.e., tolerance or negative memory) may occur under certain circumstances. In any case, immune memory has been shown to reside in both T- and B-cell lineages. The actual memory cells are long-lived, circulating small lymphocytes that remain quiescent or nondividing until reactivated by an encounter with the appropriate antigen. According to clonal selection theory, each memory lymphocyte is preselected or exclusively committed by synthesis of specific cell-surface receptors to a particular immune response. Fewer than 0.1 percent of an individual's lymphocytes will normally respond to a secondary challenge with an antigen by mitosis leading to rapid expansion of a clone. Since most antibody responses by B-lymphocytes require the cooperation of "helper" T-lymphocytes in a specific interaction, these two cell types presumably recognize or react to different determinants of the same immunogen. Another subset of T-cells, commonly called suppressor cells, often appear late in an immune response and have the capacity to turn off further production of the corresponding antibody. Their relationship to "killer" T-cells and to cytotoxic versus immunoblocking antibodies is unclear. Some skeptics have suggested that suppressor T-cells are just helper T-cells incognito. The types of cells, effector macromolecules, and sequence of events even in the most studied mammalian reactions remain controversial. The fascinating lore of mammalian, though actually mostly mouse, immunology is reintroduced here to remind the reader that mammals represent complicated "jet airplane" or "motor car" models of immunocompetence. This of course implies an equally imposing array of structural and regulatory *Ir* genes. We should now be receptive to strikingly successful "motorcycle" or "bicy-

cle" models of immune responsiveness as found among less elaborate animals, including invertebrates. We proceed then back to the beginnings of immunity in evolution.

IMMUNORECOGNITION AND IMMUNOCOMPETENCE IN INVERTEBRATES

Occurrence of specific immune systems, including selectively inducible responses with a memory component, was until recently considered to be restricted to vertebrates. The prevailing paradigm considered invertebrate defenses, often equated with phagocytosis and encapsulation of foreign entities, as lacking in sharp discrimination. The idea that specific recognition in invertebrates is restricted to self-constituents and that only vertebrates recognize foreignness in a discriminating manner persists to the present time. Reluctance to accept the increasing evidence of specific adaptive immunity displayed by diverse invertebrates usually hinges on application of restrictive criteria appropriate to higher vertebrates. Thus, participation of a family of lymphocytic cells and an immunoglobulin system has been included by some mammalian-oriented workers in the essential definition of bona fide immunity. Actually, essential immunity is better defined in phylogenetic perspective in terms of any cells or macromolecules that contribute decisively to adaptive defense of the integrity of the body. Immunological competence need only involve three *functional* components: (a) selective or specific recognition, (b) cytotoxic or antagonistic reaction following sensitization, and (c) inducible memory or selectively altered reactivity on secondary contact. This approach, as we shall soon see, has the virtue of leaving the observer unbiased as to underlying mechanisms. These three criteria are minimal in the sense that specificity and incompatibility alone include cell recognition reactions characteristic of protozoan and plant reproduction. A botanically-oriented reader may pause to note the curious reversal of incompatibility in angiosperms, namely, that self-pollen is actively rejected whereas nonself-pollen with appropriate cell-surface markers is accepted by the stigma. Specificity and memory alone are insufficient benchmarks of immune reactivity because of their obvious pertinence to the central nervous system. Indeed, the additional feature of antagonistic or cytotoxic reactivity following a process of sensitization to foreign molecules may be regarded as a unique property of immune systems.

Although tissue transplantation immunity may be regarded as one of many possible indicators of immunocompetence, it is probably the only indicator that can be readily measured across the whole phylogenetic spectrum. Tests of specially adapted animals such as marine invertebrates with exotic microorganisms or xenogeneic proteins or polysaccharides can easily be misleading in the face of negative results. This is because the test animals may simply lack appropriate enzymes or catabolic pathways to process potential immunogens not encountered in their natural environment. Certain other reservations can be applied to our minimal criteria for immunocompetence. First, phagocytosis

and destruction of natural pathogens need not necessarily lead to specific immunity. Second, recognition of foreignness and even specific reaction to foreignness need not necessarily lead to subsequent development of either long- or short-term immune memory.

Lower Invertebrates: Protozoa, Sponges, Coelenterates

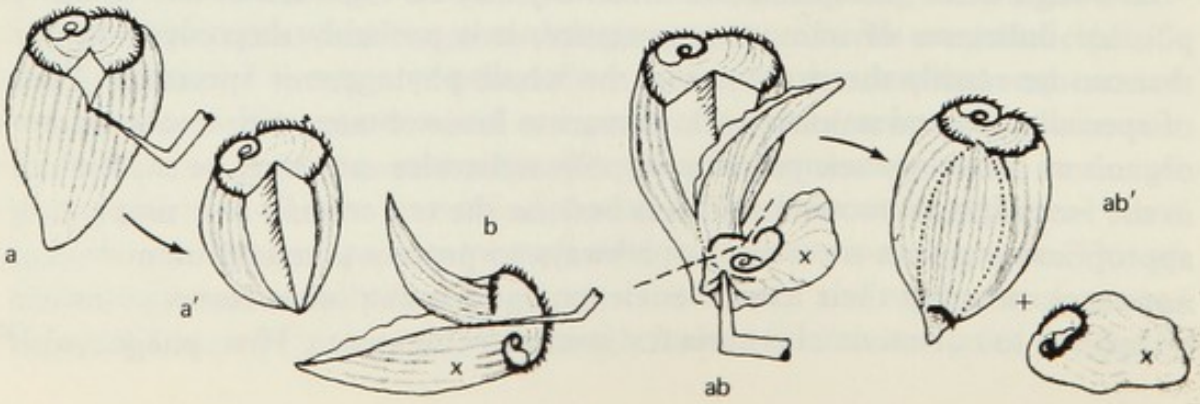
Interactions in Protozoans

The uninitiated reader may be surprised to learn that microscopic, unicellular animals, or protozoans, are amenable to precise transplantation studies. By use of microprobes or glass needles, membranes of separate cells may be accurately cut and joined or nuclei may be transplanted from one cell to another. With *Paramecium,* in which cells of different mating types may engage in sexual conjugation, the two fused cells may also be pulled apart at an early stage with resultant membrane transplantation. Extensive transplantation studies with protozoans have been reviewed by Tartar (1970), who has pioneered investigation especially in the ciliate *Stentor.* Parallel studies of nucleocytoplasmic incompatibilities in free-living amoebae have also been performed.

In *Stentor coeruleus,* the cytoplasmic cortical membrane pattern is outlined by cerulean blue pigmented stripes that surround the cell in graded widths. Given these markers, repeatable fusions of cells or transplantation of specific parts can be made in definite arrangements. The technique of cytoplasmic transplantation in this species is illustrated in Figure 9–2. Thus, *Stentor* of different races or species can be joined as chimeras with subsequent determination of possible

FIGURE 9–2. Technique of cytoplasmic transplantation in the ciliate *Stentor coeruleus.* (a) The cell with its ectoplasmic pigment stripes is cut open on one side with a glass needle. (a') Extension of cut from pole to pole lays open the cell and exposes the endoplasm. (b) Another specimen is sliced from tail to head, nearly separating a meridional sector of cytoplasm. (ab) Sector is implanted into host cell, using the cell remainder (x) as a handle and thrusting the two exposed endoplasms together with blunt needle to achieve adhesion. (ab) Adhesion has spread and ectoplasms healed. Polarity of implant is reverse that of the host. Superfluous part (x) is excised and discarded.

Source: Figure courtesy of Vance Tartar; After Hildemann, *Transplantation Antigens,* New York: Academic Press, 1972, p. 6. © Academic Press, Inc., New York, NY.



incompatibilities. Intraspecific transplants in *S. coeruleus* are compatible in the sense that destruction or disappearance of the grafted part is not observed. Allografted *Stentors* with reversed mouthparts and wide stripes on the wrong side that are mirror images of the normal may be produced, but they maintain their unusual integrity despite the continued presence of a "normal" nucleus. Although fused cells may be in different states of division or development at the time of allogeneic transplantation, they soon become synchronized. For example, two cells forming a chimera with one early and the other late in the division cycle will complete the process at the same time. Meridional patches from dividing cells grafted anywhere on other cells in division undergo fission at the same time that the host *Stentor* divides. Allogeneic histoincompatibilities as such have not been observed in *Stentor.*

In xenogeneic *Stentor* transplantations, by contrast, definite incompatibility has been observed. Portions of *S. polymorphus* transplanted to whole *S. coeruleus* led to a shedding of the symbiotic algae of *S. polymorphus* and fading or disappearance of pigment of *S. coeruleus* within a few days. Such chimeras may survive with little growth up to twenty days without cell division, or death may occur earlier after cell division. The duration of survival of interspecific chimeras may depend on intracellular (i.e., genetic and enzymatic) incompatibility of the two species rather than intercellular or cell-surface incompatibility.

Many studies of amoebae have involved transplantation of a nucleus from one cell to another. Although the cell membranes of two *Amoeba proteus* punctured in close contact for nuclear transplantation show excellent self-sealing, fusion of the two cells has never been observed. Since such membrane incompatibility exists even for two sister cells that have recently divided, an immunogenetic basis appears to be ruled out. However, the cytoplasm of an *A. proteus* strain will not tolerate the nucleus of any other strain. Reciprocal nuclear transplantation between the similar species *A. proteus* and *A. discoides* yield "hybrids" that die within two weeks. By contrast, normal amoebae survive indefinitely and may even be considered immortal. Although the hybrid combination of a proteus nucleus in discoides cytoplasm maintains normal cell functions, including movement, phagocytosis, digestion of food, and growth for a maximum of four cell divisions, gradual loss of all cell functions reveals chronic incompatibility.

To determine whether nuclei or cytoplasm of hybrid cells reaching the dying stage could be restored, second transplants with either new cytoplasm or nuclei were made. Cytoplasm of dying cells was not saved by nuclei from any strain, whereas nuclei of dying cells could be revitalized in fresh cytoplasm of either parent "strain," as shown by their capacity to utilize labeled thymidine and uridine and to initiate mitosis. Such rejuvenation of nuclei could be repeated serially, but the essential incompatibility of a proteus nucleus in discoides cytoplasm persisted. Possibly the hybrid amoebae succumb because the cytoplasm fails to use the nuclear messages. However, nucleocytoplasmic incompatibility can develop among members of the same clone after culture in different environments in only 200 cell generations.

More direct evidence for the existence of transplantation incompatibility in

large amoebae comes from comparative studies of cytoplasmic transfers in intraclonal, intraspecific, and interspecific combinations. If donor and recipient are autogeneic (self), isogeneic (from the same clone), or even allogeneic (from separated geographic areas), prolonged survival or indefinite tolerance is the rule. Although intrastrain and intraspecies transplants were compatible in Pelomyxae, interspecies transplants between any two of three species of amoebae were not. Allogeneic transplants among three strains of *Pelomyxa carolinensis* remained viable, but intergenus transplants resulted in eventual cell death in all instances. Some reaction to foreignness then is apparent in certain protozoan chimeras. Existence of an immunogenetic component in these incompatibilities is doubtful. Unlike metazoan transplantation reactions evoked at cell surfaces, protozoan incompatibility may be characterized as intracellular/enzymatic rather than immunological. This is not to aver that protozoans lack specific binding sites on their cell surfaces. The impressive polymorphism of ciliary antigens and the multiplicity of mating types in *Paramecium* and in *Tetrahymena* (Chapter 3) clearly reveal an extensive assortment of cell-surface specificities. Whether such potential receptors and others governing specific uptake of different amino acids may have evolved into those responsible for immunorecognition in metazoans is open to speculation.

Highly Discriminating Sponges

Sponges or Porifera represent the simplest metazoans or organized colonies of differentiated cells. Because continuing association of such cell aggregates requires some molecular specificity at cell surfaces, the nuances of sponge cell aggregation have long fascinated biologists. As early as 1907, Wilson found that cell suspensions of sponges following dissociation could reassemble spontaneously into a functional sponge. Because cells of different species do not combine to form functional colonies, sponge aggregation has been viewed as essentially species-specific. However, temporary aggregation of cells from different species may occur. A mixture of *Microciona prolifera* and *Halichondria panicea* cells, for example, will initially form completely mixed aggregates but then sort out within a few hours into separate aggregates containing cells mainly of one species or the other. In several species this aggregation is attributable to specific glycopeptides or glycoproteins present on cell surfaces. In species combinations exhibiting less aggregation specificity, the glycoprotein of one species may promote fusion of cells of the other, as might be expected. We should note here that complementary, cell-surface glycoproteins have also been isolated from yeasts of opposite mating type. Such interaction of complementary macromolecules may be responsible for specific cell associations in general.

Recent work indicates that neither cell adhesion nor cell aggregation is limited to species-specific recognition in sponges. Although incompatibilities in mixed cell aggregation experiments have not led to cell death, at least in experiments of only a few days duration, a quite different outcome may follow

parabioses of intact sponge tissues from either the same or different species. Early interspecific rejection reactions were observed in contact zones between intact branches of *Microciona* and *Haliclona,* but no incompatibility was discerned between allogeneic *Microciona* grafts in experiments of three weeks duration at low temperatures. Allogeneic incompatibility was first recorded about 1970 among separate strains of individual sponges of the freshwater species, *Ephydatia fluviatilis.* Whereas control intrastrain grafts placed in contact fused or merged compatibly, allogeneic sponges consistently failed to fuse. However, no cytotoxic sequelae were reported in conjunction with incompatibility reactions.

Convincing evidence of tissue transplantation immunity with a surprising memory component is now at hand for the tropical Indo-Pacific sponge, *Callyspongia diffusa* as studied in Hawaii. *Callyspongia* exhibit a uniform purple pigmentation throughout their soft tissues; their skeletal framework, consisting of brown horny fibers, is resistant to decomposition in sea water. Thus, any soft tissue death is promptly quantifiable by disappearance of purple-colored tissue from the skeletal framework. Compatible intracolony fusions in this ramose species are abundant in nature, but intercolony of allogeneic fusion is not observed, even where separate colonies are in close proximity. Under laboratory test conditions, intracolony parabionts become firmly fused at all interfaces after two to three days at 25° to 27° C and persist indefinitely in compatible confluence (Figure 9–3). By contrast, alloparabionts not only fail to fuse despite intimate contact, but also exhibit interfacial soft tissue death beginning as early as four to five days in certain interclonal combinations. Allogeneic cytotoxicity was objectively scorable as definitive when soft tissue destruction extended 1 mm or more to either side of an interface (Figure 9–3). Generally, acute primary reactions with median reaction times (MRT) of 7.2 to 9.0 days were followed by accelerated secondary reactions (MRT = 3.8 to 4.2 days) in interclonal combinations tested from three different geographic locations at 27° C. Third-party parabioses in lieu of specific second-set grafting yielded MRTs intermediate between those of first-sets and second-sets. These significant differences suggested both specificity in alloimmune memory and considerable sharing of H antigens among three sources of *Callyspongia.* Since more than 900 different alloparabionts have proved incompatible without exception, extensive polymorphism of H molecules occurs within this species.

Depending on the interclonal combination, primary reactions ranged from strong to weak, suggesting well-modulated responsiveness to different degrees of foreignness. However, acute rejection with conspicuous cytotoxic sequelae was typical, a surprising finding at this lower level of phylogeny, especially under the circumstances of highly discriminating immunity with specific memory. The genetic constitution of a clone is the most decisive variable governing the degree of alloincompatibility. Both primary and secondary responses are closely temperature-dependent over 22° to 28° C, the normal range of this species. The direction and intensity of allogeneic incompatibility are independent of graft dosage over a very wide range. Induction of maximal allosensitization

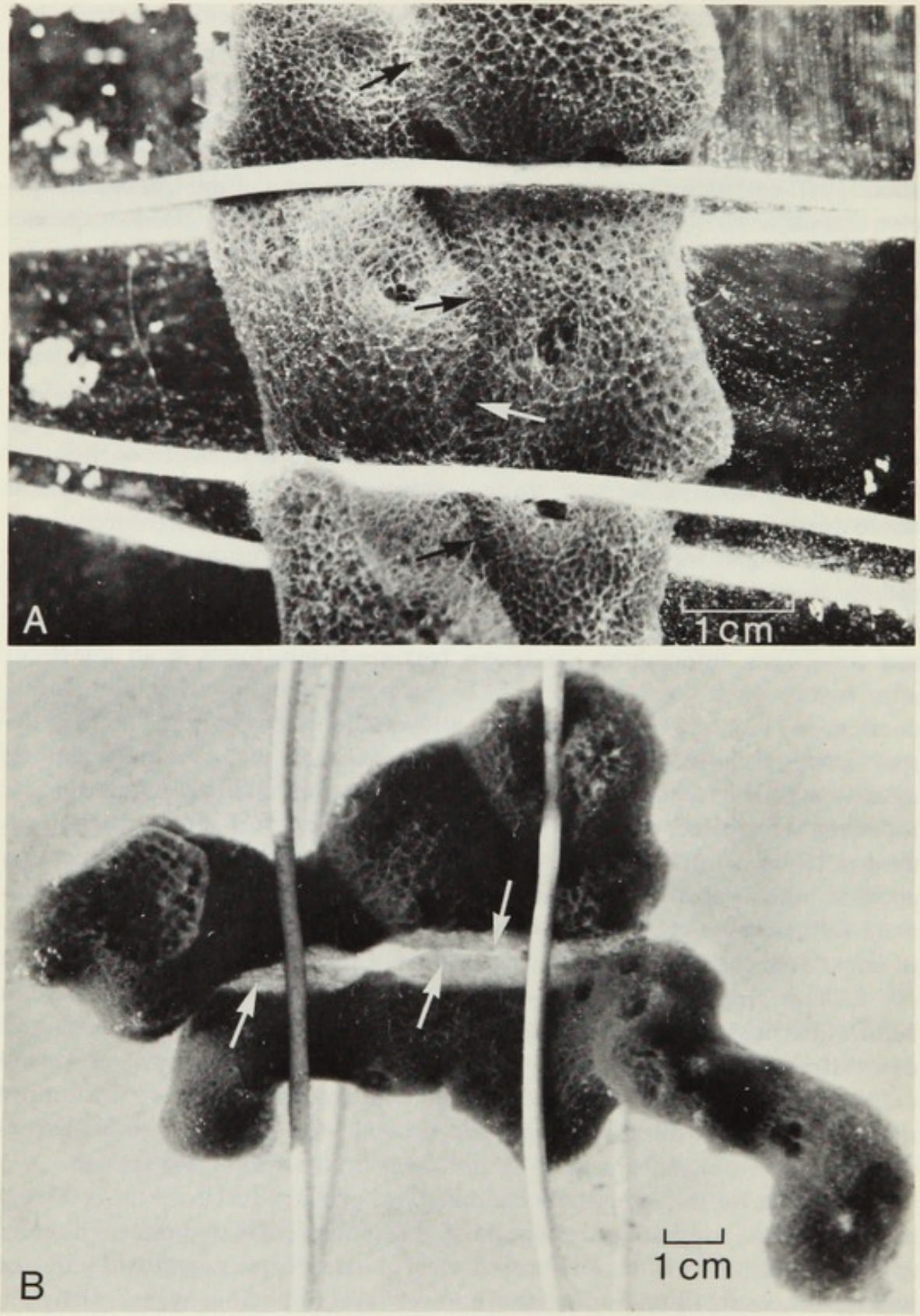


FIGURE 9–3. Parabiotic reactions between intact fingers of *Callyspongia* held together by vinyl-covered wire tie-downs on plexiglas plates. (A) Compatible interfacial fusion (arrows) of syngeneic or isogeneic parabionts after two to three days in contact. (B) Incompatible bilateral cytotoxicity between allogeneic parabionts showing skeletal framework (arrows) after local soft tissue necrosis at seven to nine days.

Source: After Hildemann et al., *Science* 204:422, 1979. © 1979 American Association for the Advancement of Science.

reflected in accelerated second-set reactivity requires only about four days at 26° to 27° C. The cellular and molecular sources of these tissue destructive, allogeneic interactions are still unknown. Several morphologically distinguishable leukocyte-type cells called archeocytes are found in sponges. Some of these cells are known to be capable of efficiently phagocytosing foreign particles and cells, but there appears to be no organization of defensive cells or immunocytes into separate tissues. *Callyspongia* are capable of both asexual and sexual reproduction in their shallow water, coral reef habitat. If deliberate reproduction and rearing of larvae becomes achievable, progeny testing could follow. Allogeneic incompatibilities reflected in consistent nonfusion reactions in controlled pilot experiments at 15° C have also been demonstrated in two species of California sponges, *Axinella mexicana* and *Cyamon argon.*

Diversity in Coelenterates

The phylum Coelenterata includes an amazing diversity of animal forms—hydroids, sea anemones, sea fans, corals, and jellyfishes. These animals lack organ systems or true vascular systems, but they do possess leukocyte-type cells or amebocytes similar to those found in sponges. Convincing evidence of allogeneic incompatibility under controlled testing has been reported for representatives of two of the three classes—Hydrozoa and Anthozoa—but not Scyphozoa (jellyfishes). In hydra, the much studied freshwater hydrozoan, autografts and intraclonal isografts of tissue heal rapidly and usually persist indefinitely, as would be expected on the basis of genetic identity. Allografts between hydras appear to retain their integrity for prolonged periods, yet some strains independently isolated from different ponds have proved incompatible as evidenced by eventual graft separation or rejection. In the hydroid, *Hydractinia echinata,* allogeneic colonies regularly fail to fuse when grown in contact, whereas clones obtained asexually from single colonies fuse compatibly. Breeding experiments revealed that allogeneic incompatibility is governed mainly by multiple histocompatibility alleles of one locus in this species. Moreover, there is a hierarchy of histoincompatibilities reflected in the intensity of hyperplastic overgrowth reactions. Although autografts are indefinitely accepted in gorgonians (sea fans), xenografts and allografts are rejected. Both ectodermal and combination ectodermal-mesogleal grafts behave in this way. At 10° to 15° C, Theodor observed disintegration of foreign grafts beginning at four to five days. The severity of cytotoxic reactions was greater with xenografts (*Eunicella stricta* $\rightleftharpoons$ *Lophogorgia sarmentosa*) than with allografts. No repeat graftings to test for secondary responsiveness have been reported. Primary allogeneic interactions in gorgonians are reported to range from mild nonfusion to acute cytotoxicity. Underlying mechanisms remain to be dissected.

Most newer insights derive from experiments with sea anemones and corals. In the anemones *Anthopleura elegantissima* and *A. krebsi,* isogenic individuals comprising separate clones derived by asexual reproduction live in compatible, intimate contact. However, when allogeneic nonclonemates come into

contact, an antagonistic behavior pattern is initiated that usually results in tissue damage to one or both anemones. Physical contact between tentacles or acrorhagi of genetically different anemones, not mere proximity, is required to elicit a cytotoxic response. The acrorhagi or marginal spherules at the base of the tentacles are the source of the allocytotoxic molecules. Localized necrosis is caused by nematocyst discharge, but ectodermal products from stimulated acrorhagi may also be involved. This response is specific or highly selective in the sense that only anemones of the same or closely related species evoke strong reactions. Because this type of incompatibility is manifest within minutes or a few hours in presumably naive animals, it has been viewed as aggressive rather than immunological in character. The required contact recognition of subtle differences in cell-surface molecules is suggestive of immune sensitization, albeit no memory component has been discerned. Quite possibly these intertidal animals, being exposed to each other's macromolecular products, are rarely naive. When two different clones or another anemone (*Metridium senile*) were confined in close quarters for nine weeks, individual animals developed specialized "catch tentacles." Secretion of toxins or nematocyst discharge from these tentacle tips then occurred upon direct contact with nonclonemates, which resulted in local necrosis. Although this sequence of events is analogous to transplantation immunity in higher animals, aggressive behavior is ultimately involved. As with other sea anemone species in the wild, genetically different clones usually remain separated by open corridors free of anemones. Acute or hyperacute allogeneic incompatibility appears to be the rule in these anthozoans. The effector phase, once triggered, may well be nonspecific, but the early recognition phase suggests impressive specificity.

Tissue transplantation immunity, without the complication of aggressive behavior found in anemones, has been widely investigated in corals by Hildemann and co-workers. Intracolony isogenic transplants of parabionts of living coral are consistently compatible, but intercolony allografts and xenografts are invariably incompatible. Far from being a laboratory artifact, transplantation-type immunity in corals emerges as a characteristic feature of tropical reefs in general. In parabiosis experiments between nonclonal plates of *Montipora verrucosa* ranging in size from 9 to 72 cm^2 (Figure 9–4), contact avoidance reactions in zones of soft tissue proximity were seen early, long before any allogeneic cytotoxicity appeared in areas of forced contact. Thus, cell-surface recognition of foreignness precedes tissue destruction which requires a sensitization period of a week or more. Allograft reactions in a typical Hawaiian series led to localized bilateral necrosis with a primary MRT of 22.0 (19.2 to 25.3) days at 25° C. After a forty-day interval, parabioses between the same pairs of *Montipora* at opposite, fresh interfaces yielded accelerated rejection with an MRT of 11.6 (9.4 to 14.4) days. Similarly, vigorous alloimmune memory persisted in other experiments for at least four weeks after separation from primary parabiosis at four weeks. Third-party allografting from distant populations, following completion of first-set interfacial reactions, showed definitive reactions either at seven to sixteen days, characteristic of heightened

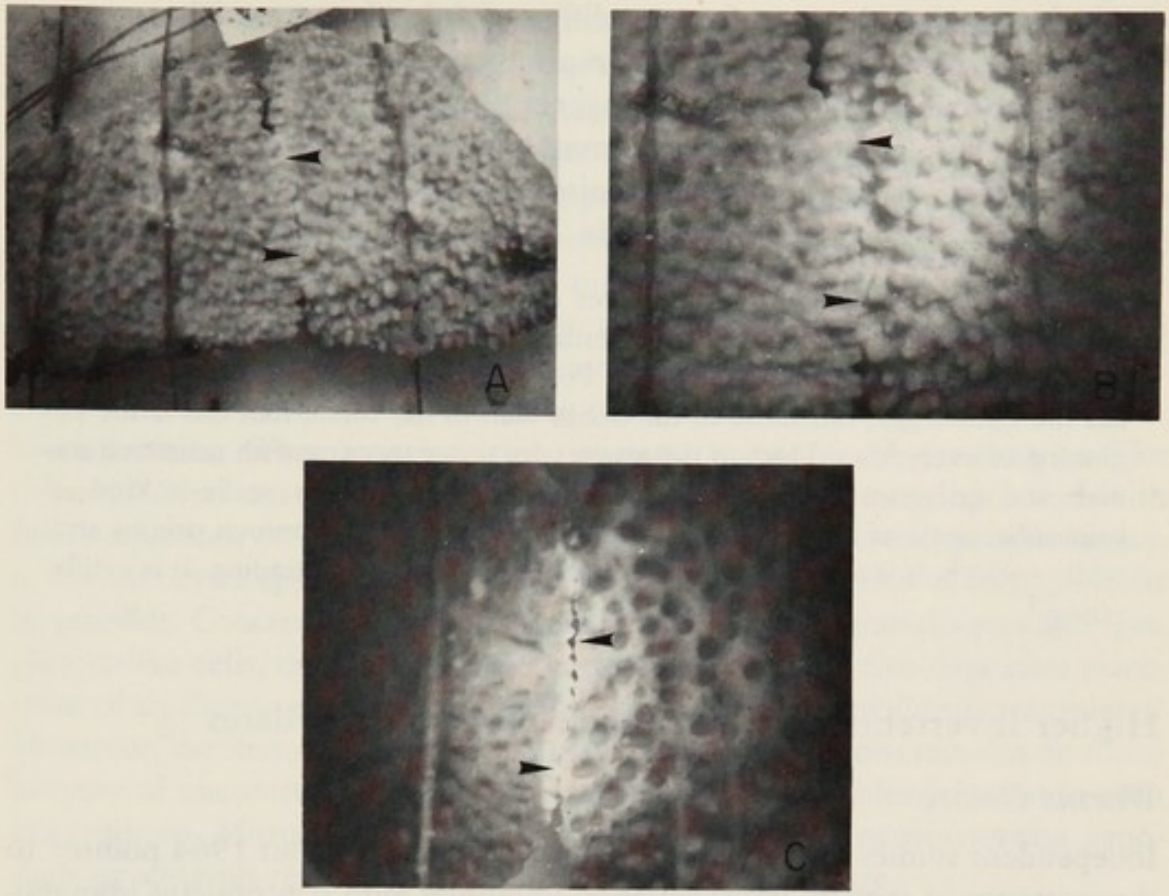


FIGURE 9–4. Intracolony isograft and intercolony allograft reactions in the coral *Montipora verrucosa*. (A) Compatible isograft fusion at interface (arrows) about a month after parabiosis ($\times 3/4$). (B) Same as (A) at higher magnification. (C) Bilateral allogeneic cytotoxic incompatibility showing blanching and soft tissue death at interface (arrows); this allograft incompatibility develops after two to four weeks and persists indefinitely. Approximately $\times 1\frac{1}{2}$.

secondary responses, or at nineteen to thirty-five days, typical of primary reactions. The dichotomy between accelerated and nonaccelerated reactions supports the assumption of immunogenetic specificity underlying this memory. As in *Callyspongia* sponges, degrees of primary incompatibility varied from strong to moderate or weak in these corals as a function of their genetic constitution. Chronic primary allograft reactions are the rule in *Montipora* (Table 9–1), although definitive reaction times are intermediate in comparison to the usual strong-versus-weak range observed among vertebrate classes. Extensive polymorphism of histocompatibility antigens is evident from the incompatibility of more than 980 allogeneic combinations of *Montipora* involving six separate populations tested. The nicely modulated alloimmune reactions at this lower level of phylogeny may eventually reveal the origins of both the major immunogene complex and of cell-mediated immunity. Availability of separate clones of large size in the form of foliaceous "tabletop" colonies in such genera as *Montipora* and *Agaricia* provides the equivalent of numerous identical in-

dividuals, as if each were from a different inbred source. Thus, current inability to achieve controlled sexual reproduction at will in scleractinian corals does not preclude further immunogenetic analyses. This area of marine invertebrate immunogenetics now invites pursuance from the level of phenomenology to underlying mechanisms. The wonder of it all was well expressed long ago by Eugene Burdick in *The Blue of Capricorn:*

> Now every person can, and should, peer into the hauntingly beautiful world of the coral reef. It is more than just an aesthetic experience, more than just the seductiveness of exotic colors and shapes. Peering into the life of the coral reef one has the knowledge, felt more in the bones than in the brain, that this is the beginning of everything. Here in the warm salty water teeming with primitive animals and gorgeous color one senses that life began. It is really a kind of primordial sense of kinship, of looking backwards into the common origins and, oddly enough, it is not an experience that is depressing or degrading. It is exhilarating.[1]

Higher Invertebrates: From Worms to Protochordates

Worms Galore

Independent studies of Cooper and Duprat beginning about 1964 pointed to the existence of specific transplantation immunity with concomitant immunologic memory in earthworms. Earlier, autogenous and allogeneic transplants of tentacles in sipunculid worms (*Dendrostomum zostericolum*) exhibited no distinctive reactions. Encapsulation of these heterotopic grafts by host leukocytes in the coelom occurred at the same rate on rechallenge, but such undiscriminating foreign body reaction is not surprising in retrospect under these abnormal circumstances. More recent work reveals that leukocytes from sipunculid worms, but not their cell-free coelomic fluid, can destroy both xenogeneic and allogeneic target erythrocytes in vitro. A broad range of allogeneic incompatibilities as a function of geographic origins suggests a substantial diversity of *H* genes in these marine worms. Chronic reactions suggestive of weak histoincompatibility often occurred in persistent chimeras resulting from xenografts between planarian flatworms from the same genus (*Euplanaria polychroa* and *Euplanaria lugubris*). Orthotopic integumentary allografts and control autografts of modest size were studied in a large series of earthworms (*Lumbricus terrestris*) maintained at an optimal 15° C by Cooper. Autografts survived permanently whereas allografts exhibited varying degrees of incompatibility. Prolonged rejection of cutaneous allografts from 38 to 153 days obtained in tests among Canadian worms or Oregon worms and in tests between these populations. Yet most primary allografts in all four combinations were partly viable or intact even after more than eight months. Second-set transplants made about a week after first-set grafting yielded every conceivable immune reaction—some accelerated, some equal, and some prolonged responses

[1] Eugene Burdick, *The Blue of Capricorn.* Boston: Houghton Mifflin Co., 1961, p. 124.

in comparison to first-set survival times. This erratic manifestation of incompatibility and "memory" has led some investigators to question the existence of adaptive immunity in annelids. Alloimmune memory engendered across weak H barriers in various amphibians and reptiles and in certain strain combinations of hamsters and mice also appears delicately poised between positive and negative effects. Accelerated rejection of repeat grafts is not an invariable consequence of prior sensitization in animals with or without backbones.

Xenograft incompatibilities between species of earthworms predictably tend to be stronger and more rapidly evident. First-set grafts between *Eisenia foetida* and *Lumbricus terrestris* showed a mean survival time of about thirty days in either direction. The transplantation immune responses of earthworms like those of other ectothermic animals are closely temperature-dependent with faster rejections occurring at higher temperatures. Leukocyte-type cells known as coelomocytes appear to be chiefly responsible for rejection of foreign grafts in annelids. Concentrated coelomocytes, including both granulocytes and lymphocytelike cells, on adoptive transfer to naive *Lumbricus* five days after placement of an *Eisenia* graft caused accelerated rejection of new *Eisenia* transplants. However, the immunologic specificity of xenograft reactions remains doubtful because of uncontrolled biochemical and metabolic variables inherent in such experiments. Moreover, the sequence of cellular events accompanying xenograft or allograft rejection in annelids is open to conjecture.

Attempts to detect production of humoral antibodies in annelids have given uniformly negative results, although a bactericidin response is inducible in sipunculids. Although hemagglutinins, a hemolysin, a ciliate lysin, and a ciliate-immobilizing factor of separate specificities are demonstrable in sipunculid coelomic fluid, these reactivities do not reside in vertebrate-type immunoglobulins, nor do they appear to be specifically inducible. Cellular immunity inherent in circulating leukocytes appears responsible for allograft rejection in annelids and is accompanied by short-term, though erratic, immunologic-type memory. That such cell-mediated immunity functions in the complete absence of immunoglobulin-type antibodies is noteworthy. As we survey invertebrate phylums, the assumption emerges that cellular immunocompetence evolved long before the capacity to elaborate specific humoral antibodies.

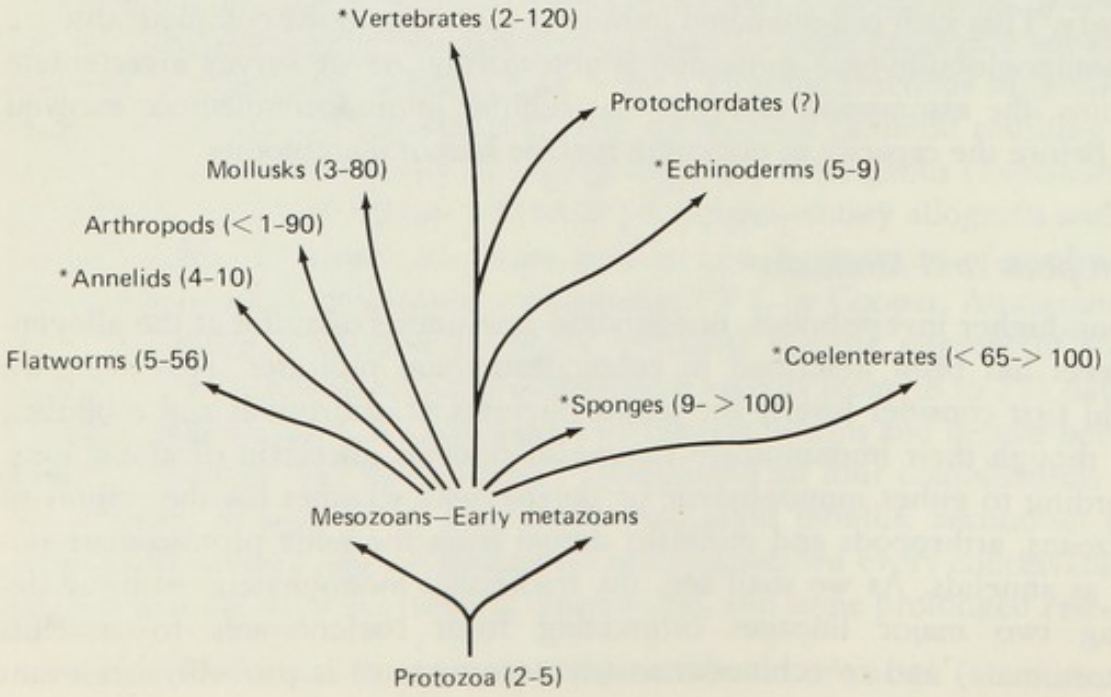
Arthropods and Mollusks

Among higher invertebrates, unequivocal immunorecognition at the allogeneic level has been described in echinoderms and tunicates. However, we should first consider briefly the major phylums of arthropods and mollusks, even though their immunologic status still remains uncertain or ambiguous. According to either monophyletic or polyphyletic schemes for the origins of metazoans, arthropods and mollusks derive from the same protostomate lineage as annelids. As we shall see, the traditional monophyletic pathway depicting two major lineages bifurcating from coelenterates to annelids (protostomate) and to echinoderms (deuterostomate) is probably irrelevant

from an immunologic standpoint. Newer polyphyletic schemes of phylogeny (Figure 9–5) are based on increasing evidence that fossils of diverse multicellular invertebrates suddenly appear in rocks of Cambrian age, but not earlier. Invertebrate immunopotentialities, as we shall further elaborate, now appear more concordant with the polyphyletic view that major groups of metazoans arose independently from protozoans or at early stages of multicellularity.

Much emphasis has been given to the lack of specific inducibility of immunoglobulin-type antibodies in arthropods. Naturally occurring agglutinins for vertebrate erythrocytes are found in the hemolymph of many arthropods, but attempts to increase titers by immunization have yielded negative results. Arthropods possess humoral macromolecules that inactivate viruses, bacteria, and protozoans, but often with little specificity. However, arthropods such as crayfish, lobsters, and crabs reveal well-developed nonself-recognition at the level of clearing radiolabeled viruses and xenogeneic proteins from the circulation. The large milkweed bug (order Hemiptera) responded to *Pseudomonas aeruginosa* vaccine by lysin production shown to increase specific resistance to bacterial infection. Both lysins and acquired resistance became maximal by twenty-four hours after vaccination and disappeared by five days. Normal bugs given immune serum became as resistant as vaccinated individuals. Moreover, injection of unrelated antigens did not evoke resistance to challenge with *Pseudomonas.* Similarly, protective immunity and bactericidal activity against this

FIGURE 9–5. Polyphyletic phylogeny indicating major groups of metazoans arising independently from early multicellular animals. Essential organizational patterns of different invertebrate groups arose separately during Cambrian age rather than sequentially during later ages. Consistent with this view is the finding of specific immunocompetence with a memory component (asterisks) even in sponges and coelenterates. Recorded longevities in years of representatives of each phylum are shown in parentheses.



same organism show parallel development in actively immunized wax moth larvae of the order Lepidoptera. The promptness and short duration of these immune responses are noteworthy, since selective reactivity appears within hours and then disappears after several days. Indeed, this timing alone could be viewed as evidence against vertebrate-type adaptive immunity in arthropods. However, other examples tend to counteract the view that arthropods lack immunologic potential altogether. Spiny lobsters immunized with a Gram-negative bacterium isolated from their own normal intestinal flora soon produce a typical nondialyzable bactericidin. Cross-reactions are found with other Gram-negative organisms, but not with Gram-positive bacteria. Higher bactericidin titers are elicited by secondary injections of the same antigen, whereas various unrelated substances do not provoke bactericidin responses. In American cockroaches, injection of *Tetrahymena* yielded a specifically immune hemolymph after three days, capable of immobilizing suspensions of these ciliates. This hemolymph also passively protected naive cockroaches from otherwise lethal doses of *Tetrahymena.* Immobilizing activity resided in a particular protein band isolated by disc-gel electrophoresis.

Regular acceptance of imaginal disc and ovarian allografts in larval *Drosophila* has been known since the classic studies of Beadle and Ephrussi in 1935. Among cockroaches, skin allografted between nymphs or from adults to nymphs retained its integrity through successive molts. Even supernumerary legs allografted between larval house crickets have persisted through molting to the adult stage. Given the immature larval state of the recipients in these experiments, acquired tolerance could be implicated. However, orthotopic allografts of legs between adult crayfish have regularly been found to remain viable for many weeks at 25° C, even after molting. Absence of allograft encapsulation in several species of insects where xenografts do elicit this response to foreignness could be interpreted as a lack of subtle immunorecognition by participating leukocytes or hemocytes. Yet other studies reveal a reproducible distinction between allograft and isograft reactions leading to the opposite conclusion, at least with Hymenoptera. Definitive transplantation studies in adult arthropods, including tests for selective memory, are still lacking. If weak histoincompatibilities are the rule in arthropods, their detection could require a species with a long life span—months rather than just weeks. At this juncture, the very existence of discriminating adaptive immunity in arthropods is in doubt.

Orthotopic tissue grafting has proved quite difficult in many mollusks for technical reasons. Certain species of snails can effectively distinguish among isografts, allografts, and xenografts, but quantitative studies including tests of memory by repeat grafting have yet to be reported. A preliminary study of tissue transplantation in scallops suggested better survival of autografts than of similar heterotopic allografts, but no conclusions were drawn because of technical shortcomings. Some cellular immunocompetence in oysters is suggested by studies revealing immune elimination of bacteriophage. As in other invertebrates, the capacity of mollusks to synthesize immunoglobulin anti-

bodies has never been demonstrated. Naturally occurring agglutinins with some specificity for blood cells of diverse invertebrates have been found in the body fluids of numerous gastropod and pelecypod species of mollusks, but these agglutinins were not specifically inducible by immunization. Incidentally, most natural agglutinins of invertebrates have physicochemical properties unlike any known vertebrate immunoglobulin. When snails (*Helisoma duryi*) were separately challenged with three species of bacteria, no agglutinins or lysins were detectable. These negative results could hinge on insufficient time, since the animals were tested for only ten days postinjection at 21° to 22° C. In essence, the nature and scope of potential immunocompetence in mollusks remains conjectural.

Chordate Ancestors: Echinoderms and Tunicates

Phylogenetic characteristics of echinoderms and protochordates put them just below the vertebrates. In terms of structural-developmental affinities, many zoologists assume that the subphylum Vertebrata evolved directly from the subphylum Tunicata. Of course, we are interested in knowing whether immune responsiveness showed a parallel evolution. At this juncture we need not belabor experimental shortcomings leading to negative findings in early studies. Compelling evidence of specific allograft immunity with at least short-term memory was first obtained with a sea cucumber (*Cucumaria tricolor*) and a sea star (*Protoreaster nodosus*), both from the tropical Pacific. All initial integumentary allografts in *Cucumaria* exhibited slow rejection at 22° to 26° C with survival times of 129 to 185 days, whereas early repeat grafts survived only sixty days or less. Chronic rejection of initial allografts was also found to be typical of *Protoreaster.* Similar findings of slow rejection of integumentary allografts with impressive specificity were recently obtained with the California sea star *Dermasterias* at a maximum but near optimal temperature of 15° to 16° C. Median allograft survival times were 213(170 to 266) days for the first-sets and 44.2(17.7 to 110.5) days for second-sets. In all instances, allograft rejection was marked by infiltration with lymphocytelike cells and granular leukocytes. At 15° to 16° C, unrelated sea urchins (*Lytechinus*) reject primary integumentary allografts in about thirty days and secondary grafts in about twelve days. This surprisingly early responsiveness at low temperature is suggestive of strong histoincompatibility in contrast to the results with other echinoderms. On the basis of survival times of third-party grafts from presumably unrelated donors, accelerated memory reactions appeared to be more specific in the sea stars than in the sea urchins. Because the extent of polymorphism of H molecules in these populations is still unknown, the issue of specific versus wide-ranging memory is unsettled.

Limited attempts to detect other types of immune reactions in echinoderms have been less rewarding. Foreign macromolecules injected into sea urchins (*Strongylocentrotus*) were removed more rapidly from the coelomic fluid than native molecules. Leukocytes (coelomocytes) responded selectively in relative

uptake of ^{14}C-labeled bovine and human albumins or globulins. Yet attempted immunization with these xenogeneic proteins revealed neither accelerated uptake by leukocytes nor clearance. In another approach, injection of sea stars (*Asterias*) with cells from sea urchins (*Arbacia*) led to phagocytosis of the donor cells with rapid clearance and clumping of recipient amebocytes, but sequential challenge over an eighteen-day period yielded no heightened responsiveness. We may conclude that discriminating cell-mediated immunity exists in echinoderms, but underlying mechanisms and humoral components remain as unknowns.

Both solitary tunicates and colonial tunicates display allograft incompatibility leading to chronic rejection reactions. Progressive primary rejection of tunic allografts in the solitary species *Ciona* is associated with invasion by lymphocytes, granulocytes, and phagocytes. In three species of the family Perophoridae, isogeneic pieces from the same colony always fused to share a common vascular system, whereas random colonies were rarely compatible in parabiosis. All colonies obtained by sexual reproduction were reported to fuse with either parent colony. These tunicates possess numerous distinct types of blood cells, including lymphocytes. Allogeneic fusion (i.e., parabiosis) experiments in various colonial ascidians have revealed extensive H polymorphism under complex genetic control. Colony specificity in *Botryllus primigenus,* for example, has been ascribed to a single gene complex with multiple alleles. Colonies postulated to have at least one allele in common, for example, AB and AD, fused compatibly when placed in contact. Mating of two incompatible colonies exhibiting maximal allelic diversity (e.g., AB $\times$ CD) produced four classes of progeny (i.e., AC, AD, BC, BD), each able to fuse compatibly with both parents. Compatible parabiotic fusion between F_1 and F_2 progeny colonies was observed only when at least one postulated allele was common. Thus, AC could fuse with AD but not with BD colonies.

These surprising findings clearly conflict with the immunogenetic rules of transplantation applicable to vertebrates. If each postulated allele yielded an antigenic product in the usual codominant manner, $F_1 \rightleftarrows$ Parent combinations in the example just given should all be mutually incompatible. This atypical histocompatibility system invites more extensive testing, especially of progenies derived from interpopulation matings. Compatible zooid chimeras have been obtained by fusing and subsequently separating two colonies sharing at least one *H* allele. Such chimeras behaved like heterozygotes of their constituent genotypes in subsequent reactions with other colonies. Peculiarities of this intriguing system include dependence of the strength of incompatibility on the degree of chimerism achieved and the initial size of the parabionts. In *Amaroecium* species, experimental fusion of tunic-free zooids of two potentially histoincompatible, larval colonies is claimed to produce reciprocal tolerance. Two separate zooids can be cut into pieces and grafted together, resulting in viable stable zooid chimeras with organs composed of cells of each genotype as shown by ^{3}H-thymidine labeling. This repeated finding that mutual tolerance is readily achievable by allogeneic parabiosis of young tuni-

cates could explain the inconsistency of allograft rejection reported in colonial species. Neither leukocyte involvement nor a possible memory component has been reported in the alloimmune reactions of colonial tunicates. These information gaps are attributable to lack of investigative effort under difficult experimental conditions. None of several potential mitogens added to cultured blood of the Australian tunicate *Pyura* led to uptake of ^{125}Iododeoxyuridine in a recent study. Nor was there a detectable response in mixed leukocyte cultures of cells from animals taken at widely separated localities. Such negative reactions with unfractionated blood cells are hardly compelling because both culture and test conditions could have been inappropriate. The functions of specialized leukocytes in invertebrates remain unsettled.

From the genetic standpoint, there is an obvious paucity of data from controlled matings and progeny testing of immunopotentialities in invertebrates generally. The big picture summary in Table 9–1 tends to minimize the thinness of the evidence actually available. Extensive allogeneic polymorphism of cell-surface macromolecules is certainly a characteristic shared by all animals, beginning with protozoans. Immunorecognition of subtle differences among allogeneic markers appears characteristic of most major groups of multicellular animals, although arthropods may represent a striking exception. The highly discriminating adaptive immunity found in sponges and coelenterates becomes less astonishing when one realizes that this critical capacity persists throughout metazoan phylogeny. Because information voids or gaps at the whole animal, cellular, and molecular levels are conspicuous in every phylum of invertebrates, some immunogeneticists remain skeptical about the develop-

TABLE 9–1. Immunopotentialities Known in Invertebrates[a]

Phylum or subphylum	Immunocytes or defensive cells demonstrable	Allogeneic cell-surface polymorphism evident	Specific allograft rejection[b]	Immunologic memory demonstrable[c]	
				Short-term	Long-term
Protozoans	No	Yes	No	No	No
Sponges	Yes	Yes	Yes	Yes	No
Coelenterates	Yes	Yes	Yes	Yes	No?
Annelids	Yes	Yes	Yes	Yes	?
Arthropods	Yes	Yes	Yes?	?	?
Mollusks	Yes	Yes	Yes?	?	?
Echinoderms	Yes	Yes	Yes	Yes	?
Tunicates (Protochordates)	Yes	Yes	Yes	?	?

[a]From Hildemann et a., in *Phylogeny of Immunological Memory*, New York: Elsevier North-Holland, p. 12, 1980. Vertebrate-type immunoglobulin antibodies have *not* been demonstrated in any invertebrate species; inducible circulating "antibodies" found in annelids and arthropods are mainly bactericidins and are not strictly specific.

[b]Xenograft rejection is characteristic of *all* invertebrates, including protozoans, but this probably involves biochemical or enzyme-substrate incompatibilities.

[c]Short-term memory is meant to indicate specifically heightened reactivity for up to eight weeks after a primary reaction; long-term memory implies heightened allogeneic reactivity persisting for > eight weeks.

ing lore of invertebrate immunocompetence. At least the beginnings of both cell-mediated immunity and of the *MIC* of higher vertebrates are discernible among lower invertebrates.

IMMUNOEVOLUTION AMONG VERTEBRATES

Lower Vertebrates: From Primitive Fishes to Advanced Fishes

Immunocompetence in all vertebrates is reflected in the capacity to produce circulating immunoglobulin antibodies, at least of the IgM type. This is the single most striking feature in the transition from invertebrate to vertebrate responsiveness. Cell-mediated immunity, already well-developed in metazoan invertebrates ultimately evolves into T-lymphocyte subsets and Ig-producing B-lymphocytes among the classes of vertebrates. This diversification of immunocyte functions, first without apparent separation of T- and B-cell lineages, begins with primitive fishes.

Agnathans or Cyclostomes

Hagfish and lampreys with rudimentary eyes and multiple hearts are the most primitive living vertebrates. They lack jaws, paired fins, stomach, thymus, and bone marrow. However, their extensive gut is richly endowed with lymphoid tissue and their blood carries an array of lymphocytes and granulocytes. These fish probably evolved from the *Ostracoderm* group of jawless Devonian fishes which are thought to have been the first chordates. Their "most primitive vertebrate" status notwithstanding, Pacific hagfish (*Eptatretus stoutii*) invariably recognize and reject skin allografts (Figure 9–6) even at a chilly 18° to 19° C, the highest temperature this bottom-dwelling marine species can tolerate indefinitely and remain healthy. The sequence of immune events—lymphocyte/granulocyte infiltration, capillary hemorrhage, and pigment cell destruction—is typical of reactions toward skin allografts observed in other vertebrates. Chronic reactions are typical with MSTs for first-set grafts about seventy-two days and second-sets a curtailed twenty-eight days. Survival times of third-party grafts on sensitized hagfish in lieu of second-set grafts ranged widely from nineteen to eighty-five days. This result in one large population points to the existence of diverse transplantation antigens with many of frequent occurrence in this species. Progeny analyses of *H* gene-antigen relationships have been stymied because only large sterile eggs have been forthcoming in captivity. Moderate histocompatibility barriers are also typical of lampreys as evidenced by prolonged allograft rejection times even at 20° C. Positive MLC reactions have been obtained with difficulty in both hagfish and lampreys.

Prompt bactericidin responses to various Gram-negative bacteria are given by hagfish, as well as by certain annelids, arthropods, and higher vertebrates including mammals. This nonimmunoglobulin reactivity yields short-lived

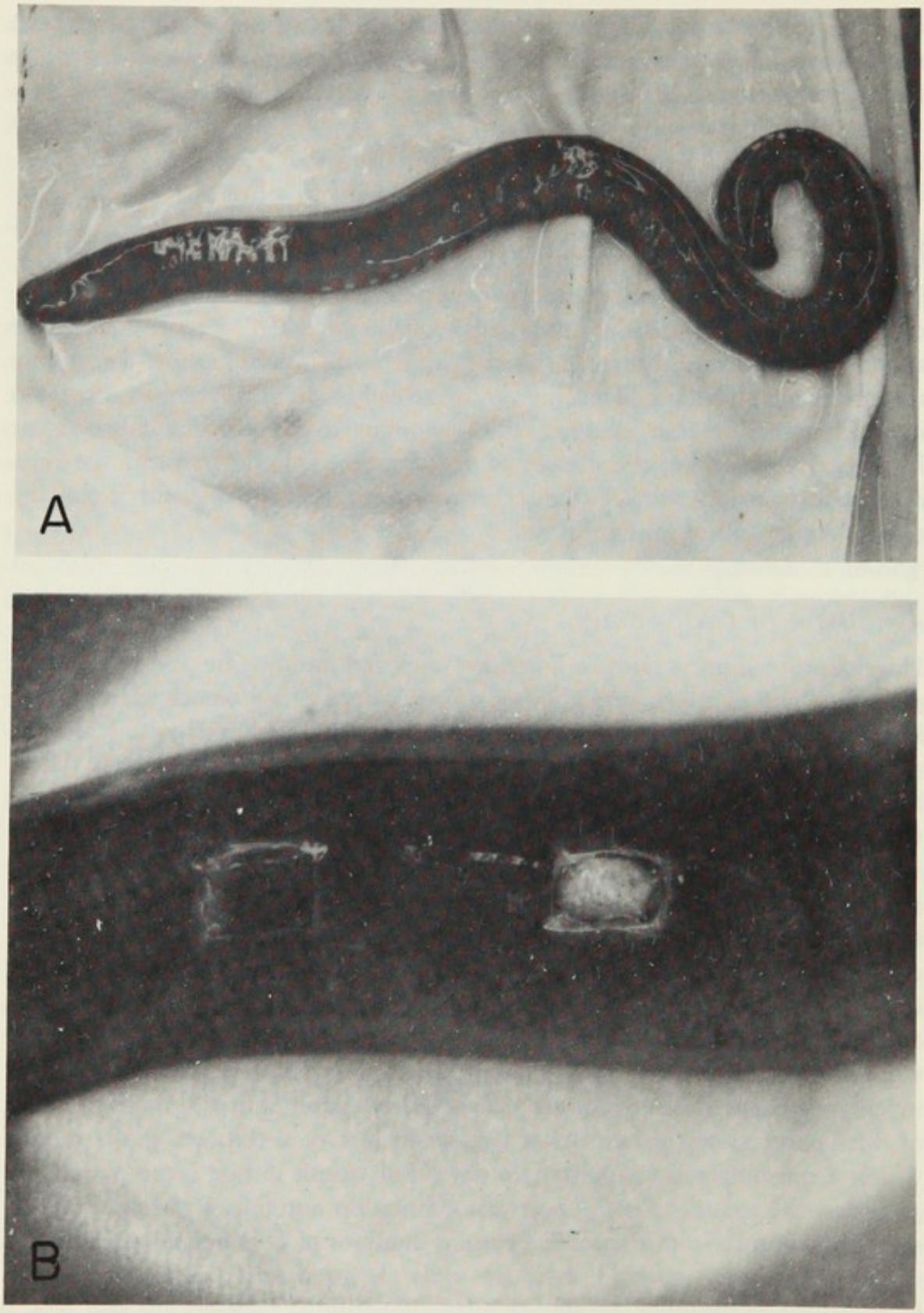


FIGURE 9–6. Transplantation immunity in agnathan Pacific hagfish. (A) Entire hagfish showing skin grafts in anterior-dorsal position several centimeters behind the head. (B) Close-up view of fully viable autograft (left) and blanched allograft (right) at survival end point showing complete pigment cell destruction.

Source: From Hildemann and Thoenes, *Transplantation* 7:510, 1969. © 1969 The Williams and Wilkins Co., Baltimore, MD.

memory, but its specificity is rather broad. This primeval, humoral immunoresponsiveness, as suggested by the limited selectivity and kinetics of bactericidin production, evidently evolved in invertebrates and persisted despite subsequent evolution of more discriminating immunoglobulins. No data have been reported on the genetic control of bactericidin responses in any species. The serum complement system typical of higher vertebrates is apparently not required for bactericidin activity. Whether primitive fishes or invertebrates even possess a complement system is unclear.

Hagfish and lampreys are both capable of producing specific serum antibodies to diverse xenogeneic antigens. IgM-type antibodies have been evoked in high titer in hagfish after repeated immunization with sheep erythrocytes, keyhole limpet hemocyanin, or streptococcal carbohydrate. Certain molecular properties of hagfish immunoglobulins, including carbohydrate content, are similar to those described for lampreys and elasmobranchs. Purified hagfish antibodies have an intact molecule weight of about 160,000 daltons and are noncovalently associated in a polymer of high molecular weight. Heavy chains of mobility identical to that of murine μ chains and light chains of mobility significantly slower than murine λ chains are obtained after sodium dodecyl sulfate gel electrophoresis under reducing conditions. Instability of the tertiary structure of this antibody is indicated by dissociation at low concentrations of reducing agents. The most surprising finding from a genetic standpoint is that this most elaborate of antibody classes should occur at the apparent beginning of immunoglobulin phylogeny. If the ancestral immunoglobulin gene coded for a basic homology unit of about 110 amino acids (Chapter 2), repeated gene duplication concomitant with diversification and adaptive selection were required to achieve the first functional IgM characteristic of all vertebrates (Figure 9–7). This identification of the "original antibody" is surely doubtful: functional precursors are therefore being sought among the advanced invertebrates. In contrast to an impressive system of cell-mediated immunity, hagfish exhibit only a minimal system of circulating antibody production. Efforts have thus far failed to detect induced serum alloantibodies in any species of primitive fishes below teleosts. No evidence for divergence of cyclostome lymphocytes into separate T- and B-cell systems has yet been discerned.

Elasmobranchs

The success of aquatic vertebrates was already evident by the diversity and abundance of fishes—cartilaginous and bony—in the Devonian period. About 550 species of sharks, skates, and rays, constituting the cartilaginous Elasmobranchii, persist to the present time. In terms of immunologic potentialities, elasmobranchs reveal modest advancement in complexity over agnathans. Sharks do possess a definitive thymus and a discrete spleen; advanced elasmobranchs also display some plasma cells. A divergence of T- and B-cell systems is therefore suggested even though bone marrow equivalents must reside in other tissues. Very much the same picture of slowly mobilized transplantation

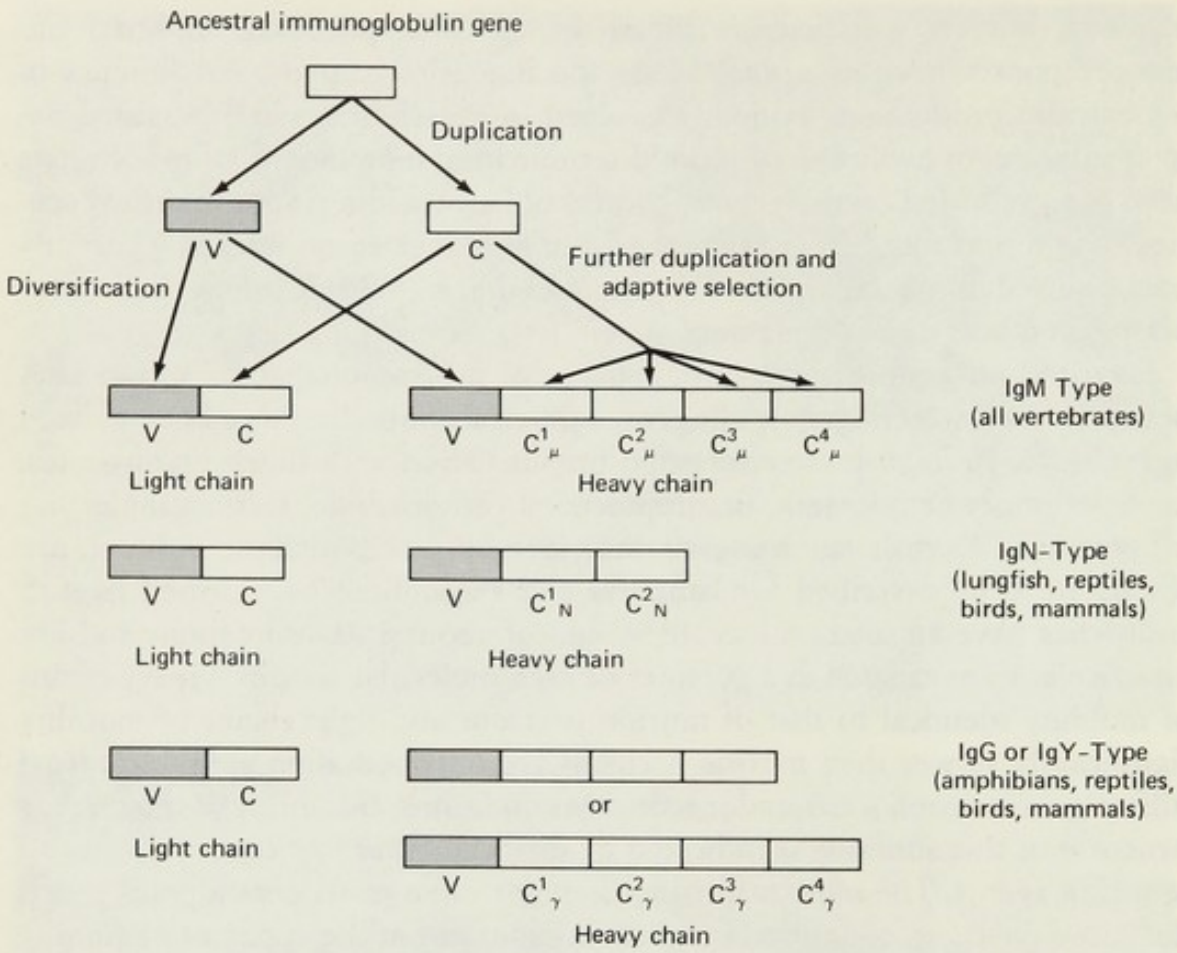


FIGURE 9–7. Immunogenetic scheme for origin of vertebrate immunoglobulins and molecular representation of heavy and light polypeptide chains. The lengths of the chains are proportional to the carbohydrate-free molecular weight. The basic unit of homology is approximately 110 amino acids for which the ancestral gene presumably coded. The letters V and C identify the variable and constant regions of the chains. The particular class of C region homology units is given by the subscript, whereas the superscript numeral gives the position.

Source: Modified from Hildemann, *Annu. Rev. Genet.* 7:21, 1973. Reproduced, with permission, by *Annual Review of Genetics*, © Annual Reviews, Inc.

immunity found in agnathans appears typical of sharks and stingrays, even at elevated water temperatures of around 22° C. In Pacific horn sharks (*Heterodontis francisci*), four successive sets of skin allografts yielded MSTs of 41.1, 16.7, 9.3, and 7.4 days, respectively. A gradually increasing immunity was apparent, culminating in acute destruction of the third- and fourth-sets. Stingrays (*Dasyatus americana*) rejected initial grafts around forty to fifty days, but second-sets provoked more severe inflammatory reactions, which led to accelerated rejection. Characteristically moderate or weak incompatibilities in these animals may be made much stronger by preimmunization. This is an intriguing immunogenetic rule of transplantation, applicable in effect across the whole phylogenetic spectrum. Much remains to be learned about the development or persistence of immunologic memory at successive levels of phylogeny. Long-term memory may not be restricted to higher vertebrates because all phylums of animals include species with long life spans (Figure 9–5). In

other words, persistence of specific memory becomes a potential advantage to any species with a characteristic life expectancy of several to many years.

Sharks and rays are capable of both early 17-19S and later 7S IgM responses to diverse xenogeneic antigens, including influenza virus, bovine serum albumin, and the dinitrophenyl hapten. Heavy polypeptide chains of both high and low molecular weight immunoglobulins were found to be identical ($\sim$70,000 daltons) and quite similar to mammalian μ chains. Light chains of about 20,000 daltons roughly corresponding to mammalian light chains were also found in both pentameric and 7S monomeric forms of elasmobranch antibodies. Amino-terminal sequence analysis of heavy and light chains of sharks reveals variable region heterogeneity comparable to that described in mammalian Igs. Molecules homologous to the J chain of IgM polymers of higher vertebrates join the monomers of elasmobranch Ig together. Although a J chain requirement for polymeric Igs is to be expected, this necessitates one or more additional structural genes. We must also assume one or more genes for glycosyl transferases to attach the requisite carbohydrate to specific positions on the H and L chains. Thus, an absolute minimum of seven structural genes—two each for the V and C regions of the H and L chains, one for the H chain hinge region, one for the J chain, and one for the carbohydrate—must be invoked to account for the original, IgM antibody! Shark light chains are not identifiable by amino acid sequence analysis as either kappa or lambda chains similar to those found in higher species. However, presence of a hypervariable region in horned shark light chains is evident. The known properties of advanced shark and ray antibodies, more than those of agnathans, resemble those of the IgM class of mammals. However, H chain structural differences recently detected in numerous species of sharks indicate either multiple subclasses of IgM or multiple classes of shark immunoglobulin. From two to four serum immunoglobulins, each with different antigenic determinants on their heavy chains, have been found among 16 species of sharks. The spiny dogfish (*Squalus acanthius*) exhibited the greatest heterogeneity with four serum Igs, three found in both 19S and 7S molecules and the fourth only in the 19S form. This raises the possibility that multiple Ig classes of higher vertebrates arose from different immediate molecular ancestors. To add to the genetic interest, IgM polymers of different sizes are characteristic of different species of rays. Polymorphism of J chain properties probably determines the extent of polymerization of IgM subunits. Depending on the species and class of vertebrates, IgM antibodies may occur as dimers (ray), tetramers (bony fish), pentamers (sharks and mammals), and hexamers (anuran amphibians). The adaptive significance of such variation is unclear.

Teleost or Bony Fishes

Some 97 percent of all living fishes, comprising more than 21,000 different species, are advanced bony fishes. Even now, 75 to 100 new species of teleosts are described or discovered each year. This evolutionary success as the predominant group of aquatic vertebrates is at least partly attributable to prompt

and vigorous immunoresponsiveness. Compared to primitive fishes, bony fishes exhibit an evolutionary transition or progression in both transplantation immunity and serum antibody responses. A primitive teleost, the tropical arowana, reacted moderately toward initial allografts, but promptly rejected second-set grafts. Consistently accelerated secondary reactions in arowanas may be contrasted with a wide range of third-party survival times, indicating that the usual polymorphism of *H* genes and antigens occurs in this species also. Slow rejection of initial allografts is also characteristic of paddlefish (*Polyodon spathula*), a member of the most primitive group of ray-finned bony fishes, the Chondrostei. Fishes more advanced in the phylogenetic hierarchy of the Osteichthyes sampled from natural populations regularly show acute allogencic incompatibility (Table 9–2). Quantitative studies of transplantation reactions in fifteen species of higher bony fishes have invariably revealed strong alloimmune reactions among genetically disparate individuals. Inflammatory reactions are as vigorous and complex as any observed among avian or mammalian species. This highly developed CMI is demonstrable in fishes from fresh and marine water as well as species from temperate, semi-tropical, and tropical climates. Teleost lymphocytes display distinctive proliferative responses to the mitogens ConA, PHA, and LPS suggestive of a dichotomy in their T- and B-cell lineages. MLC reactivity has been demonstrated in trout but not in gray snappers. Improved cell culture techniques will no doubt have to be adapted to achieve reproducible results in lower animals.

Whereas repeated attempts to elicit alloantibodies in hagfish and horn sharks have yielded negative results, serum alloantibodies in high titer are easily induced in bony fishes typified by carp and goldfish. This has allowed extensive characterization of erythrocyte alloantigen systems in species of special interest such as trout and salmon. Bony fishes at their "normal" temperatures ranging from 18° to 28° C give excellent IgM-type antibody responses to diverse cellular, viral, and protein antigens. The immune macroglobulin of several species circulates mainly as a 14-16S tetramer, but may also occur as monomeric antibody. The tetrameric conformations of IgMs in primitive teleosts (gar and paddlefish) and advanced teleosts (carp) have been determined directly by electron microscopy. Although many teleosts, like agnathans and elasmobranchs, seemingly produce only two populations of high and low-molecular weight IgM, others, exemplified by the giant grouper and lungfishes of both African and Australian species, produce a second major class of Ig, defined in terms of distinctive heavy chains or isotypes. Thus, bony fishes represent a transitional class in vertebrate evolution of immunoglobulin complexity (Figure 9–7). This second molecular class of Ig is frequently designated IgN (N for new) because of its distinctive heavy chain structure. General divergence of nonmammalian Ig classes other than the conservative IgM strongly suggests adaptive specialization among higher vertebrate classes. This implies selection of duplicated mutant genes encoding heavy chain domains during evolution of separate vertebrate classes. There is evidence for a lytic antibody-complement system in various bony fishes and in nurse sharks, under

TABLE 9–2. Comparative Skin Allograft Survival Times Among Diverse Fishes

Class and (order)	Species	Median survival times		Interval between first-set and second-set grafting (days)	Water temperature (°C)
		First-set	Second-set		
Agnatha		71.9	28.0	30	18.5 ± 0.5
(Myxiniformes)	Hagfish (*Eptatretus stoutii*)	~38	~18	39	18–21
(Petromyzontiformes)	Lamprey (*Petromyzon marinus*)	(21–>291)	(7–>252)		
Chondrichthyes		>31	<12	53	18–28
(Rajiformes)	Stingray (*Dasyatus americana*)	(<31–53)	(?)		
(Squaliformes)	Horn shark (*Heterodontis francisci*)	41.1	16.7	60	22.0 ± 1.0
		(27–48)	(15–22)		
Osteichthyes		42–68	~12	68	18–26 (1st set)
(Acipenseriformes)	Paddlefish (*Polydon spathula*)	(21–>76)	(?)		6–13 (2nd set)
(Clupeiformes)	Arowana (*Osteoglossum bicirrhosum*)	17.9	5.1	30	25.0 ± 0.5
		(13–25)	(3–7)		
(Cypriniformes)	Goldfish (*Carrasius auratus*)	7.2	4.7	25	25.0 ± 0.5
		(6–11)	(4–6)		
(Perciformes)	Blue acara (*Aequidens latifrons*)	7.2	4.5	25	25.0 ± 0.5
		(6–11)	(3–6)		
	Hawaiian mullet (*Mugil cephalis*)	13.2	6.2	35	23–25
		(9–16)	(4–8)		

the appropriate conditions of employing antiserum and complement from the same species. We defer further consideration of the phylogeny of the complex complement system until the end of this chapter.

Bony fishes occupy the lowest level of phylogeny for which progeny analyses concerning the genetics of tissue transplantation exist. In the early 1950s, Hildemann and Owen undertook the first investigation of histocompatibility genetics in fish by performing extensive cross-grafting of skin (scales) among F_1 and F_2 sibships derived from outbred parental goldfish. Given a limiting assumption of maximal allelic diversity in the parents (i.e., $A_1A_2 \ldots \times A_3A_4 \ldots$), at least seven independent *H* loci were invoked to account for the uniform allograft rejection found in 506 combinations each of F_1 and F_2 sibs. Note that assumption of a single, polymorphic *MIC* hardly begins to account for the invariably acute incompatibility demonstrable in this extensive array of siblings. Noninbred fish of nearly all species studied uniformly reject tissue allografts, thereby revealing impressive polymorphism of *H* genes and their antigenic products. In other words, any two individuals chosen at random from a large interbreeding population are virtually certain to be histoincompatible.

The pioneering studies of Kallman and Gordon revealed the operation of at least ten to fifteen independent *H* loci in inbred strains of xiphophorin fishes. Interstrain and F_1 hybrid to parent fin grafts are rejected in inbred strains of platyfish (*Xiphophorus maculatus*), whereas intrastrain and parent to F_1 grafts are compatible. These findings are entirely consistent with the genetic rules of transplantation confirmed for mammalian species (Chapter 5). The low frequency of P_1 grafts accepted by F_2 and backcross recipients is similar among inbred strains of platyfish, mice, and rats. A mammalian level of immunogenetic complexity is already evident in teleost fishes. *H* genes exhibit both additive and dosage effects in platyfish. $F_1 \rightarrow P_1$ fin grafts survive much longer than interstrain grafts, and twice as many F_1 grafts as P_1 grafts display prolonged survival in backcross recipients. P_1 and F_1 grafts made concurrently to backcross recipients always resulted in earlier rejection of the P_1 graft. If one assumes only half as much alloantigen on the cell surfaces of F_1 grafts as P_1 grafts, the F_1 cells could be either less immunogenic or less vulnerable to destruction. This dosage effect was low-temperature dependent; the longer persistence of heterozygous grafts progressively disappeared at high temperatures (26° to 32° C).

When heart and fin were simultaneously grafted from the same F_1 donor into a P_1 recipient, the fin was always rejected first. The long survival of these heterotopic F_1 heart allografts, some for more than a year, is similar to that found with outbred salamanders and *Rt-1* compatible combinations of rats. The early rejection of homozygous, interstrain heart grafts in platyfish at twelve to twenty-one days also parallels the stronger reactivity directed toward *Rt-1* incompatible rat heart allografts. Heart, kidney, and gonadal tissues are less vulnerable to alloimmune reactions than cutaneous (i.e., skin, scale, or fin) tissue.

Male-specific (*H-Y*?) antigens have been detected in platyfish with scale

grafts, but not with larger intrastrain fin transplants from males to females. Female acceptance of cutaneous grafts from males within certain strains of platyfish as well as higher animals emphasizes the unusual and partly unpredictable "weakness" of sex-associated histoincompatibility. Existence of X-linked and Y-linked *H* loci in xiphophorin or other fishes remains to be tested by parental male → F_1 hybrid male grafting. Certain allelic combinations of autosomal *H* loci yield weak immunogens, sometimes too weak to cause graft rejection. Acceptance of F_1 grafts by backcross platyfish recipients that reject P_1 grafts illustrates this deviation from the "one gene → one antigen" rule. Such allelic interaction has become abundantly evident with both *H-2* and non-*H-2* loci in congenic strains of mice. Viewed from another perspective, the interaction of donor specificities and recipient immune responsiveness (i.e., *Ir* genes) together govern the degree of incompatibility. Small wild populations of certain xiphophorin fishes often show prolonged acceptance of intrapopulation allografts, a result suggestive of many *H* alleles and antigens held in common. Fully histocompatible clones of gynogenetic (*Poecilia formosa*) and hermaphroditic (*Rivulus marmoratus*) teleosts constitute extreme forms of genetic fixation in natural vertebrate populations.

Higher Vertebrates: Amphibians, Reptiles, Birds

A distinction between higher and lower vertebrates is a matter of rather arbitrary convenience. There is surely no disparity in the sense of superior versus inferior immune responsiveness when we compare the bony fishes to the land or airborne tetrapods. However, emergence onto land implies substantial adaptive changes, and thus we proceed with the amphibians whose ancestors first came upon the scene some 350 million years ago.

Amphibians: Anurans, Apodans, and Urodeles

The occurrence and immunogenetic basis of allograft rejection in amphibians long remained obscure despite the developmental studies of experimental embryologists and neurophysiologists. In 1958, Hildemann and Haas first showed that bullfrogs (*Rana catesbeiana*) as larvae and as adults regularly mounted vigorous immune responses to skin allografts. MSTs of eleven to fourteen days for initial allografts were obtained at 25° C on animals ranging from tadpoles of two months to adults of two years. Accelerated second-set reactions were regularly demonstrable, with underlying short- and long-term memory. Despite the profound changes of larval metamorphosis, this immune response capacity once developed remained unchanged whatever the age or stage of development. However, newly hatched bullfrog larvae at standard stage twenty-four and up to thirty-six days of age are sufficiently immature to become partially or completely tolerant toward allografts. Acute allogeneic incompatibility in bullfrogs and other adult anurans (i.e., frogs and toads) has been repeatedly confirmed. Strength of MLC reactivity in anurans is largely

under the control of a single genetic region, perhaps equivalent to the mammalian *MIC*. There is also a gene dose effect in this form of immunorecognition. One-haplotype differences generally result in weaker MLC stimulation than do two-haplotype differences. MLC reactivity and acute allograft rejection appear functionally linked in *Xenopus*, since both are similarly depressed at metamorphosis.

In the less advanced orders Apoda (caecilians) and Urodela (newts and salamanders), transplantation immunity is less vigorous, but no less discriminating. In an aquatic apodan (*Typhlonectes compressicauda*), first-set skin allografts exchanged between adults at 25° C had an MST of twenty-nine days within a range of ten to 161 days. Although the whole gamut of fast to slow reactions occurred, over three-fourths of these wild-caught animals exhibited weak histoincompatibility. Depending partly on the interval between first- and second-set grafting, repeat grafts showed either curtailed or prolonged survival. This distinction between positive and negative memory has been evident in all groups of vertebrates, especially in connection with moderate to weak histocompatibility barriers. Persistent allogeneic tolerance, possibly the ultimate form of negative memory, appears to depend far more on antigen dosage in larval anurans than on stage of development of the recipient. Yet the "strength" of the H gap may really be the most important variable in all comparisons of tissue tolerogenesis. Tolerance across moderate H barriers is more easily achieved at all phylogenetic levels.

Extensive immunogenetic studies of newts and salamanders, especially by N. Cohen and co-workers, have involved some sixteen species representing four families and nine genera. A typically slow pattern of transplantation immunity prevails in urodele species. At temperatures of 20° to 25° C, first-set skin allografts yield MSTs of thirty-two to fifty days in adults, although individual survival end points have ranged from eight to four hundred days. Skin xenograft reactions in many, though not all, interspecific combinations are also weak. Third-party test allografts among newts (*Diemictylus*) from several geographic locations demonstrated widespread sharing of H antigens. Yet a substantial diversity of *H* genes distinguishing individuals within each population was also found. Allograft rejection in newts, even when fairly rapid, is referable to multiple *MoH* loci that can have additive effects. No *MIC* of integrated loci appears to be operative in newts from any of the populations analyzed. Two further insights emerged from these urodele studies. First, newts do have potentially vigorous alloimmune responsiveness, as evidenced by acute incompatibility in certain combinations. Second, the recipient-dependent variability in the intensity of immune responses is itself under *Ir* gene control. Remarkably prolonged survival of foreign whole organs or tissues other than skin is obtainable in adult urodeles. Allotransplants of supernumerary limbs, muscle, hearts, eyes, gonads, and even whole heads are reported to survive surprisingly long. Extended survival of orthotopic eye implants in adult *Triturus viridescens* from several donor species of newts has been recorded. Auxiliary allogeneic hearts implanted in *Diemictylus viridescens* continued to beat for many months—more than sixteen months in some recipients.

We pause again to focus on the fascinating phylogeny of immunologic memory. Even newts preimmunized by skin grafts subsequently exhibited prolonged survival of heart allografts from the same donors. Although active immunoblocking (i.e., negative memory) or differential susceptibility to immunologic attack may be invoked in this situation, the actual mechanisms are unknown. Positive memory was regularly found after a long time interval (> thirty days) between first and second skin allografts in the Japanese newt (*Cynops pyrrhogaster*). Thus, primary and secondary grafts showed MSTs of about forty-three and nineteen days, respectively, at 20° C. All repeat grafts succumbed to accelerated rejection and there was no overlap in the survival times of the two sets. In other experiments, positive memory was generally found to be long-lived (> three months) in the American newt *D. viridescens.* Instances of negative memory appeared unrelated to the interval between first-set rejection and second-set transplantation. Besides second-set reactions resulting in minimal survival times, Cohen found (a) accelerated rejection of third- but not second-sets and (b) both second- and third-set, but not fourth- and fifth-set rejection accelerated relative to the previous set. Two further points of heuristic value are worthy of note. First, the weak histocompatibility interactions of urodeles may yield a delicate balance between positive and negative components of memory. Second, long-lived memory is clearly not restricted to endothermic vertebrates or to strong bioincompatibilities. Unfortunately, practically nothing is known about the genetic control of immune memory. The chronology of allograft rejection and the recipient leukocyte types involved are essentially the same in amphibians as in all other vertebrates. Greater severity of inflammation typically corresponds with faster rates of rejection. If (a) acute allograft rejection, (b) lethal GVHR, and (c) strong MLC reactivity are taken as functional markers of an *MIC,* then an *MIC* is characteristic of advanced anurans, but not urodele or apodan amphibians.

Lymph glands or nodes first appear in phylogenetic progression in anuran amphibians. This peripheral lymphoid tissue is a major source of serum antibodies, whereas the thymus, as in higher vertebrates, is critical for cell-mediated immunities. Although urodele amphibians show well-developed thymic function, they possess neither lymphopoietic bone marrow or lymph nodes. The increasing cytoarchitectural complexity of the lymphoid system is paralleled by increasing complexity of the immunoglobulin system in the transition from newts to frogs (Table 9–3). Urodeles, like most fishes, produce only IgM-type antibodies, whereas anurans can synthesize both IgM and IgG-like antibodies. Urodeles such as axolotls and Spanish newts produce abundant antibodies to bacteria, phages, and foreign erythrocytes, but even after a year of immunization only 19S IgM-type antibody is evident. By contrast, bullfrogs regularly exhibit a transition from IgM to low molecular weight (7S) non-IgM antibodies following such immunization. Although anuran low-molecular-weight Ig resembles mammalian IgG, it has a higher molecular weight and higher electrophoretic mobility than human IgG. Isolated heavy chains of anuran 7S immunoglobulin also display physicochemical properties different from those of mammalian γ chains. Hence, the use of the designation IgY

TABLE 9–3. Phylogeny of Immunological Characteristics Among Vertebrates: Phylogenetic Progression Is Evidenced by Increasing Diversification of Immunocytes and of Immunoglobulin Classes/Subclasses

Class or Group	Typical reactions to primary tissue allografts (cell-mediated immunity)		Blood granulocytes and lymphocytes	Thymus	Bone marrow	Lymph glands or nodes	Types of immunoglobulin antibodies					
	Moderate	Strong					IgM	IgN	IgG or IgY	IgA	IgD	IgE
Hagfish and lampreys	+	○	+	?	○	○	+	○	○	○	○	○
Sharks and rays	+	○	+	+	○	○	+[c]	○	○	○	○	○
Bony fishes	+	+	+	+	+	○	+	○ or +	○	○	○	○
Amphibians	+	+[c]	+	+	+	+[c]	+	○	○ or +	○	?	○
Reptiles	+	○[c]	+	+	+	+	+	+	+	○	+?	○
Birds[a]	+	+	+	+	+	+	+	+	+	+	+	○?
Mammals	○[b]	+	+	+	+	+	+	+	+	+	+	+

[a]Data mostly limited to chickens, ducks, and turkeys, representing only two of the thirty-two known orders of birds.

[b]Chronic or slow reactivity appears characteristic only of selected strains or colonies of mice, rats, and Syrian hamsters thus far.

[c]See text for qualifications or exceptions.

rather than IgG for this nonmammalian Ig by many investigators. Comparative amino acid sequence studies will have to answer the question of direct homology between the γ-like chain of advanced amphibians and the γ chain of mammals. Since genes for the γ-like chain of anurans are apparently lacking in urodeles, these genes presumably emerged during the adaptive evolution of the less aquatic anurans. Similarly convergent evolution may be suspected in the transition from primitive to advanced air-breathing fishes. We have already noted striking differences in IgM polymerization among vertebrate classes, including the hexameric form in the toad *Xenopus*, so it would not be surprising to find adaptive specialization in other immunoglobulins.

Reptiles: Turtles, Lizards, and Crocodilians

We may proceed with the generalization that reptiles possess three distinct classes of immunoglobulins: the high molecular weight (17S-19S) form qualifies as IgM; a 7.5S immunoglobulin analogous to that found in birds is called IgY or IgG; the smallest 5-6S class is often designated IgN because of its similarity to lungfish IgN (Table 9–3). The high molecular weight immunoglobulins of turtles, tortoises, lizards, and alligators are all similar to human and mouse IgM in size and heavy chain structure. As in lower vertebrates, the IgM predominates in all antibody responses. The primitive New Zealand tuatara, apparently unchanged for 140 million years, produced only macroglobulin antibody after prolonged immunization with *Salmonella adelaide* flagellin. Yet the serum of tuataras contained both 18 and 7S immunoglobulins with distinctive heavy chains. As in higher vertebrates, production of IgG (7S) antibody may depend on the nature of the antigen and the genotype of the recipient. Turtles and tortoises regularly synthesize two molecular classes of antibodies to a broad spectrum of xenogeneic antigens. Their IgM-type response is prolonged, but IgG- or IgN-type antibodies are detectable about thirty days after immunization. Tortoises respond to immunization with pig serum by sequential production of IgM, IgG, and finally IgN antibodies. The Florida alligator possesses IgM and IgG (also IgN?) antibodies of separate classes based on sedimentation coefficients and distinctive heavy chains. This representative of the most advanced order of crocodilian reptiles readily produces serum antibodies. Distinct kappa and lambda types of light chains also characterize alligator immunoglobulins. This differentiation of light chains is otherwise known only in birds and mammals.

Although the molecular heterogeneity of antibodies in modern reptiles appears greater than that found at lower levels of phylogeny, the vigor or scope of their antibody responses is in no way superior. Secondary antibody responses suggestive of T-cell and B-cell memory have been recorded for tortoises, lizards, and desert iguanas. Shorter latent periods and markedly increased antibody titers following secondary stimulation with either particulate or soluble xenoantigens are found, but this is not necessarily associated with switchover to production of non-IgM antibodies. Whereas desert iguanas and chuckawal-

las develop high antibody titers after immunization with killed suspensions of *Salmonella typhosa* at 35° C, only low titers develop at 25° C. Diverse antibody responses of both tortoises and turtles were generally slower over the normal range of 18° to 30° C than those of other classes of vertebrates. The full panoply of granulocytes, lymphocytes, and plasma cells is found in reptiles along with thymuses, spleens, and bone marrow. True lymph nodes, though no more advanced than in amphibians, are evident in reptiles (Table 9–3). The first mesenteric lymph nodes in the ascending scale of vertebrates may be found in crocodiles. However, reptilian lymphoid organs exhibit less cytologic complexity than their avian and mammalian equivalents. In immunologic potentialities, birds may be better qualified as "advanced reptiles."

Extensive polymorphism of cellular alloantigens detectable by normal and induced serum antibodies is typical of many reptiles. A multiplicity of erythrocyte alloantigens and naturally occurring allohemagglutinins are found in species of turtles. Molecular polymorphism also extends to H antigens as evidenced by regular rejection of skin allografts in species representing all three major orders of reptiles. All of eight sexually reproducing species studied quantitatively showed protracted rejection of skin allografts at 20° to 33° C with initial graft survival times ranging from twenty-one to 245 days. Within the order Squamata, species of lizards, iguanas, and a garter snake have all displayed moderate allotransplant reactions with concomitant immunologic memory. In the unisexual lizard *Cnemidophorus tesselatus,* transplants among individuals from the same populations are not rejected, a result consistent with the assumption that this unusual species is parthenogenetic. The bisexual caiman of the order Crocodilia slowly rejects primary skin allografts, even at elevated temperatures.

The most extensive studies involving both skin allografts and xenografts in snapping turtles (*Chelydra serpentina*) at several temperatures have been reported by M. Borysenko. Xenografts from the turtles *Chrysemys picta* and *Emys blandingii* were destroyed faster than allografts in all parallel studies. Consistently accelerated rejection of both second-set allografts and xenografts indicative of positive memory was found at 25° C. All initial foreign grafts were rejected faster at 33° than at 25° C, but none were vulnerable at 10° C even after 100 days. However, first-set allografts survived about twenty-four days even at 33° C—notably prolonged survival in contrast to typically acute reactions in avian and mammalian species. Newly hatched snapping turtles required more than ninety days to reject skin allografts and xenografts whereas one-month-old recipients destroy such grafts about twice as fast. Remarkably slow maturation of the immune system in this chelonian species is apparent from findings in vivo and in vitro that adult levels of reactivity are not attained until about six months of age. Thus, newly hatched turtles are quite vulnerable to allogeneic GVH reactions after injection of adult spleen cells. A wide spectrum from chronic to acute GVHR as a function of the donor-recipient combination was found among outbred *Chelydra serpentina.* The data suggest mostly weaker bioincompatibility attributable to multiple *MoH* loci in this turtle species.

However, the incidence of lethal GVHR and the rapidity of these reactions were much greater at elevated temperatures. As noted repeatedly in this chapter, distinctions between strong and moderate allogeneic incompatibility offer at best a debatable criterion of phylogenetic progression, even among the vertebrates (Table 9–3).

Although positive MLC reactions generally correlate well with occurrence of strong alloincompatibility in vivo in higher vertebrates, the data available are too thin to permit conclusive generalization. The capacities of reptiles have hardly been tested in this connection. Anuran amphibians generally yield positive MLC reactions, as already noted, whereas urodele lymphocytes have consistently failed to respond beyond a low level of blastogenic stimulation. Despite otherwise vigorous alloimmune reactions, teleost and lower fishes have thus far given inconsistent results in MLC experiments.

Birds

Birds as the penultimate class of higher vertebrates bring us to the realm of endothermic or warm-blooded animals. The extinct dinosaurs may also have been endothermic, but this improved physiological efficiency was obviously insufficient for their continued survival in a changing environment. Nevertheless, one may adopt the view that birds are a branch of dinosaurs that diverged early from the reptilian line leading to mammals. Other considerations being equal, prompt and vigorous immune responses are generally promoted by higher temperatures short of hyperthermia. Although much has been written about the immune reactivities of birds, most findings apply to domestic chickens and, to a much lesser extent, ducks and turkeys. However, serum antibodies have been readily induced in a few studies of owls, pheasants, and quail. Chickens of course have remained popular research subjects for numerous reasons: they are easy to handle and breed in captivity; they are important to the poultry industry; inbred strains and their progenies are readily available; T-cell and B-cell systems have nicely separate localizations in the thymus and bursa of Fabricius, respectively. Only in birds is there a separate bursal organ as the site of primary differentiation of B-lineage lymphocytes. Differentiation of B- or T-lymphocytes in newly hatched chicks leads to the appearance of distinctive Bu-1 and Th-1 alloantigens, respectively. The presence of these differentiation alloantigens can be distinguished by complement-dependent cytotoxicity tests.

The types of immunoglobulins detectable in birds extend the pattern already described for reptiles (Table 9–3). Chickens can produce high and low molecular weight antibodies to a large array of antigens. Chickens possess at least three major classes of Ig: the ubiquitous IgM, abundant IgG-like or IgY molecules, and IgA, more concentrated in secretions than in serum. Ducks and geese additionally possess a low molecular weight 5-6S IgN, possibly homologous to the IgN of teleost groupers, lungfish, and sea turtles. This Ig presumably evolved into three heavy chain domains, each with a mass of about

11,000 to 12,000 daltons yielding a functional molecule (H_2L_2) of about 120,000 daltons (Figure 9–7). Although the IgN-like class is a major serum component of ducks, it has not been found in the serum of chickens. One might suspect that IgN is adaptively advantageous in a muddy aquatic environment if it were not for the finding that marsupials and rabbits also have antibodies with properties similar to those of IgN. Recent evidence suggests that the IgD class first appears in certain reptiles (i.e., tortoises) and birds (i.e., chickens). IgD has been identified in many mammalian species ranging from rodents to man. Its unique tissue distribution in mammals indicates a specialized function. It is found as a cell-surface molecule on most adult B-lymphocytes, accounts for 70 to 90 percent of the total Ig in lymph nodes and Peyer's patches, but is nearly absent from serum. Although the precise role(s) of IgD remains to be defined, its occurrence only in higher vertebrates suggests a regulatory role in sequential or selective production of non-IgM antibodies.

The existence of five major classes of immunoglobulins in birds, though not all apparently in a single species, is evident. We ask then whether a sixth class homologous to mammalian IgE is also present at the avian level. Immediate hypersensitivity reactions of the anaphylactic type, similar to those of mammals, have been observed in chickens and pigeons following repeated immunization with xenogeneic proteins. However, the responsible antibody in birds exhibits more of the characteristics of an IgG subclass than of mammalian IgE. This avian antibody appears antigenically similar to chicken IgG and it is less cytotropic and less labile to heating than mammalian IgE. Thus IgE may have evolved only in mammals as a distinctive molecular class. A clue to the physiological importance of IgE is its implication in resistance to animal parasites. Otherwise this antibody suggests a kind of negative evolution, causing higher mammals more harm than good. Apart from heavy chain evolution leading to Ig class divergence, we note that chicken light chains are mostly of the kappa type and few lambda chains are detectable. Both of these light chain isotypes are already present in crocodilian reptiles.

Contrary to popular impression, birds in general do have a well-developed lymphatic system with a spleen, lymph nodes or nodules, and "Peyer's patches" in the wall of the intestine. Lymphoid tissue aggregates occur along avian lymphatic and blood vessels as well as within the dermis. The lymph nodes of birds are much simpler structurally than those of higher mammals. Delayed hypersensitivity, allograft immunity, and graft-versus-host reactions in birds are all thymus or T-cell dependent. Humoral antibody production, hinging on the development of lymphoid germinal centers and plasma cells, is dependent on the bursa of Fabricius. The structural peculiarities of the avian reticuloendothelial systems may be regarded as multiple adaptations of warm-blooded animals to a combined airborne, aquatic, and terrestrial modus vivendi.

Extensive blood group and protein polymorphisms are evident in domesticated birds. Numerous intraspecific differences in cellular antigens are found in chickens, ducks, turkeys, and doves. The "one dominant allele→ one anti-

gen" rule generally holds in birds, as in other vertebrates. However, gene interaction products or "hybrid substances," namely antigens found on erythrocytes of hybrid progeny that are absent from the parents, have been identified in doves, ducks, fowl, and pigeons. Such unusual gene-product interaction yielding additional antigenic specificities, including weak H antigens in mice, are also known in rabbits and man (see Chapter 4). The *B* system in chickens qualifies as the major immunogene complex with constituent loci governing blood groups, histocompatibility reactions, and *Ir* gene functions. As a blood group system, *B* exhibits extensive polymorphism with some twenty-five alleles detectable by serologic testing. Allogeneic incompatibility at the *B* complex usually leads to acute graft rejection, lethal GVHR, and strong MLC reactivity. *B*-linked *Ir* genes regulate high-versus-low antibody responsiveness to synthetic polypeptides in a manner similar to *MIC*-linked *Ir* genes in mice (Chapter 7).

Full-thickness skin grafts exchanged between *B*-incompatible chickens from either inbred or outbred sources are completely rejected in ten to fourteen days. A single gene difference in the *B* system occurring in F_1 hybrids of two inbred lines caused acute reactions with first- and second-set skin grafts surviving 6 to 7 and 3 to 4 days, respectively. This same incompatibility caused severe GVHR in chick embryos. The *B* system also affects fitness and longevity. Certain heterozygous *B* genotypes promote fitness as measured by juvenile and adult mortality or egg production. Similar *B* heterozygote superiority becomes evident during progressive inbreeding. The *A, D,* and *L* blood group loci also function as *MoH* loci. Skin allografts between chickens with different alleles at these loci survived for at least forty days, with slow rejection occurring thereafter. Certain *A* locus disparities even allowed indefinite allograft survival. The alloantibody response to these weaker antigens is increased if potent *B* locus antigens are present on the same donor cells. Such synergism may also occur in transplantation incompatibilities (Chapter 6). A sex-associated *H* locus analogous to *H-Y* in xiphophorin fishes and mammals exists in chickens. Since female birds are the heterogametic sex, slow skin graft rejection is demonstrable in female → male combinations within certain inbred lines.

Strong bioincompatibilities at the allogeneic level are typical of random bred populations of domestic turkeys, muscovy ducks (*Cairina moschata*), and Peking ducks (*Anas platyrhinchos*). As in certain reptiles already cited, tissue transplantation has confirmed the occurrence of true parthenogenesis in certain strains of turkeys. Permanent survival of wattle and skin grafts from parthenogens to their dams was found, whereas control allografts suffered acute rejection as expected. Strong histoincompatibilities coupled to extensive allogeneic polymorphisms are probably the rule in outbreeding populations of most avian species. However, this assertion rests mainly on the Galliformes (chickens and turkeys) and the Anatiformes (ducks). There are thirty additional orders of birds, representing at least 8,600 living species, that have yet to be investigated.

NEW CONCEPTS IN IMMUNOPHYLOGENY

Two Immunorecognition Systems

Transplantation-type immunity, initiated by cell-surface recognition with exquisite discrimination, can be regarded as characteristic of multicellular animals ranging from sponges to mammals. An underlying and very extensive polymorphism of H markers is evident at all levels of phylogeny. However, the familiar *Ig*-based immune system appears to be restricted to vertebrates. How then can one account for the adaptive immunocompetence of diverse invertebrates? Two immunorecognition systems appear to be required, an *H* system of cell-mediated immunity and an *Ig* system of antibody immunity. The *H* system is postulated to have originated in metazoan invertebrates, beginning with sponges. This original system of immunorecognition based on polymorphic H molecules may have been retained essentially intact during subsequent evolution. The phylogenic transition from invertebrates to vertebrates is characterized by addition of the Ig system. Yet the salient features of specificity, memory, and protective effector molecules had already evolved in connection with the original *H* system. The question then arises whether H molecules and Ig molecules share a common origin or common precursors. This question can also be put in a more restrictive way: do ligand-binding molecules found in invertebrates share structural features with Ig variable (V) regions? This is a tough question inasmuch as V (variable) and J (joining) DNA segments together encode the V region of antibody polypeptides comprising the Ig domain involved in antigen recognition. Moreover, each antibody gene family appears to contain multiple V and J segments (see Figure 2–6).

The structure of *MIC* cell-surface specificities of mammals does suggest a relationship to the Ig system. *MIC* and other H glycoproteins are highly polymorphic for potential recognition or receptor determinants. Heavy and light chain dimers or tetramers are characteristic of both mouse H-2 and human HLA structures. Amino acid sequence analysis has shown that the β_2-microglobulin (β_2m) light chains are partly homologous to Ig constant region domains; these components also possess a similar disulfide configuration. This common molecular denominator could be the ancestral gene product from which both H and Ig molecules evolved by gene duplication and adaptive selection. β_2m-like components have been detected by immunofluorescence on the leukocytes of several species of sharks and chickens, using anti-human β_2m. In chickens the β_2m is associated with B antigens of the *MIC*. However, β_2-microglobulin itself with a nearly constant structure could not provide for polymorphic recognition. The variable heavy chains of H molecules do show microheterogeneity in their amino terminal sequences projecting from leukocyte surfaces. The carbohydrate associated with variable H chains of H-2 glycoproteins could also affect differences in specificity. However, the equivalents of Ig hypervariable regions have yet to be found in *MIC* molecules and this is a cause for continuing skepticism about the existence of a separate *H* system of immunorecognition. Whether there is sufficient peptide variability

in the MIC molecules of a single individual to allow for a large array of Ig-type receptors is unclear. Highly variable amino acid sequences of the α and β polypeptides of Ia molecules in guinea pigs, humans, and mice suggest that the multigenic system of the *I* region could well serve as a source of recognition receptors. The ancestral homolog of the *MIC* possibly appeared early in invertebrate phylogeny (sponges). Yet immunorecognition functions may reside in numerous other immunogenes and preoccupation with the *MIC* alone could be counterproductive.

Why then did the additional Ig system evolve, beginning with primitive vertebrates, if an H system of immunorecognition is fully adequate for invertebrates. Almost certainly the concurrent transition to animals with backbones is irrelevant. Echinoderms, tunicates, and primitive fishes all continue to occupy the same aquatic, marine environment, exposed to the same pathogens. Differentiation of numerous tissue-specific molecules coupled to intricate blood-vascular systems is a more attractive possibility. This increased physiological complexity of vertebrates may have required more fine-tuned monitoring and regulation of self versus nonself constituents. Such regulation is admirably provided by the Ig system of circulating antibodies. Antibodies serving as opsonins, agglutinins, lysins, or even antitoxins may be regarded as helpful or ancillary to cell-mediated immunity, rather than essential. At the mammalian level, the congenital absence of T-cells or macrophages constitute fatal defects, whereas B-cell deficiencies alone are usually much less life-threatening (Chapter 8). This is not to denigrate the important roles of antibodies, but only to emphasize their subsidiary or subordinate function in relation to cell-mediated immunity. The decisive importance of thymus-derived lymphocytes is most clearly evident in virus infections. The finding that cytotoxic T-lymphocytes react only with virus-infected mouse target cells sharing *H-2K* or *H-2D* genes has led to altered-self or dual recognition hypotheses. In this respect, *MIC* genes seemingly function as self-markers promoting cell-mediated immunity to viruses. However, *MIC* molecules may also serve as receptors to combine with diverse foreign antigens. Other theories of immunorecognition, though not at odds with a preeminent role for T-type lymphocytes and macrophages, often give more weight to the role of *V* genes coding for receptor Igs on both T-cells and B-cells. Membrane immunoglobulin is detectable on nearly all lymphocytes, including thymocytes of elasmobranchs, bony fishes, and amphibians as shown by immunofluorescent techniques. This Ig, often of the monomeric IgM-type, is much less abundant than that found on mammalian B-cells. Whereas the function of structural Ig on the cell-surface of certain T-cells is unclear, the recognition role of H molecules on T-cells is beyond doubt.

Diversification of Immunocyte Functions

All vertebrates exhibit cell-mediated immunity with memory and are capable of IgM antibody production. Underlying this combined immunocompetence,

all vertebrates possess gut-associated lymphoid tissue as well as blood lymphocytes and granulocytes (Table 9–3). Immunophylogenetic progression among vertebrates is evidenced by increasing diversification of immunocytes and of immunoglobulin classes/subclasses. The stepwise appearance of specialized tissues as the thymus (elasmobranchs), bone marrow (teleost fishes), and lymph nodes (anuran amphibians) reflects increasing cytoarchitectural complexity. Strictly speaking, a lymphopoietic bone marrow and encapsulated lymph nodelike structures first appear in anuran amphibians. Yet the first appearance of three or more molecular classes of Igs in reptiles and birds has no obvious correlates at the level of immunocyte differentiation.

The combined presence of *H* and *Ig* systems of immunorecognition among vertebrates implies a need for cooperative interaction. Of course, production of antibody to most antigens does require recognition and response by both T- and B-cells. Whether these two cell types regularly react to different parts of the antigen molecule is not critical for present purposes. The salient point is that integration of cellular and humoral antibody immunity requires T-B lymphocyte cooperation (Figure 9–8). Such cooperation has been clearly demonstrated in amphibians and higher vertebrates. However, existence of subsets of helper, killer, and suppressor T-lymphocytes at the mammalian level may or may not apply to nonmammalian vertebrates. Two- or three-way T-cell, B-cell, and macrophage collaboration usually hinges on *MIC* compatibility in mammals. Although controversy still surrounds the molecular identity of the individual *MIC* products involved, there can be little doubt about the critical role of *H* system recognition in the network of immune responsiveness. Interconnecting networks of feedback regulation by both the Ig and *MIC* systems probably evolved during vertebrate phylogeny in concert with continuing differentiation of leukocyte functions. An integrated, polyfunctional *MIC* in higher vertebrates may be a requisite for complex immunoregulation by multiple molecular classes of Ig. If genes encoding hypervariable region sequences are inserted into framework gene structures, as evidence now suggests, then this gene interaction is the decisive mechanism by which antibody diversity is generated. Known structural *Ig* genes of course are not chromosomally linked to the *MIC*.

If leukocyte-type cells, ranging from the amebocytes of sponges and coelenterates to the granulocytes and lymphocytes of chordates, are the sources of CMI functions, the nature of the effector molecules should arouse our interest. Early in this chapter we noted that the CMI pathways of T-lymphocyte activation in mammals led to secretion of various nonantibody proteins collectively called lymphokines (Figure 9–1). These molecules function either by direct action on target cells (e.g., lymphotoxins) or indirectly by mobilizing phagocytic cells (e.g., chemotactic factor and macrophage mobilization factors) that then attack the target. In any event, these effector molecules are all nonspecific with respect to the target antigens. The specificity resides in the recognition or induction phase rather than the effector phase. The effector molecules with nonspecific cytotoxic or enzymatic properties should presumably display some

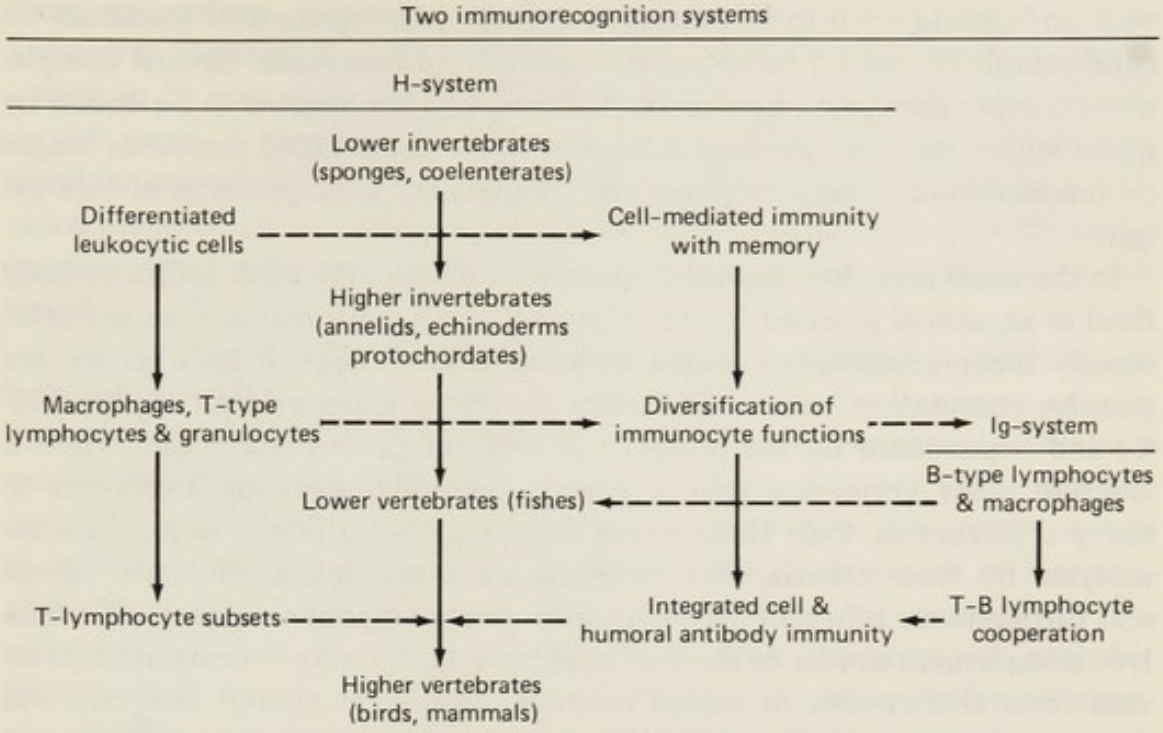


FIGURE 9–8. Phylogeny of immunologic reactivity based on the hypothesis of two immunorecognition systems, the *H* system of cell-mediated immunity and the *Ig* system of antibody immunity. The *H* system is postulated to have appeared in multicellular invertebrates, beginning with sponges. Subsequent differentiation and diversification of immunocytes is postulated to have led to the added appearance of the *Ig* system, beginning with primitive chordates or fishes. Progressive integration of the *H* and *Ig* systems then provided T-B-lymphocyte collaboration and the complex immunoregulation characteristic of higher vertebrates.

Source: After Hildemann, *Immunogenetics* 5:198, 1977. © Spinger Verlag, Inc., New York, NY.

evolutionary continuity. Little is yet known about the antecedents of mammalian lymphokines, but proteins such as lysozyme and complement components do occur in lower animals. Lysozymes of very similar structure and found generally in metazoans are quite effective in hydrolyzing polysaccharides of bacterial cell walls. There is a remarkable degree of sophistication in the obviously defensive activity of this group of enzymes. Both proteolytic enzymes and enterases are included in the array of some twenty proteins comprising the mammalian complement system with its two pathways of activation. If we restrict our consideration to the nine central components of the complement "cascade," the sequence is C1, 4, 2, 3, 5, 6, 7, 8, and 9, where C3 through C9 constitute the alternate pathway. The latter, as we shall see, is the more ancient and can be activated by numerous agents other than antigen–antibody complexes. This integrated system sustains the integrity of the body in diverse ways: production of inflammation, localization of infective agents, immune adherence, enhancement of phagocytosis, and even direct destruction of pathogens. The interaction of complement components at target cell surfaces and with the antibody portion of immune complexes is not yet

well understood even in laboratory mammals. Macrophages of mammals do exhibit both Fc and C3 receptors conducive to phagocytosis. Several complement components (e.g., human Bf, C2, and C4) are known to be coded by genes within the *MIC.* Perhaps activation of certain immune responses hinges on interaction of *H* gene, *Ir* gene, and complement gene products of *MIC* origin.

In the usual assay for classical complement activity, the fresh serum or body fluid of an animal is tested for hemolytic activity against mammalian red cells, usually sheep erythrocytes coated with rabbit antibodies. If lysis occurs, the putative complement activity is further tested for expected heat lability (56° C) and dependence on the presence of divalent cations (Ca^{2+} and Mg^{2+}). Because many vertebrate serums contain naturally occurring antibodies to sheep erythrocytes, their fresh serum alone may be sufficient to produce hemolysis. By these criteria, then, selected birds, reptiles, amphibians, teleost and cartilaginous fishes all appear to have multicomponent systems of hemolytic complement similar to those of mammals. In one recent study, normal serum from five species of snakes contained sufficient natural antibody and complement to be highly active in lysing sheep erythrocytes. However, no such hemolytic activity was found in the fresh serum of tortoises, lizards, or bullfrogs. In many xenogeneic test systems with rabbit antibodies, complement activities from different sources (e.g., leopard frog, marine toad, and salamander) are reported to differ markedly. Some antiserum plus complement incompatibilities derive from "anticomplementary effects" of extraneous molecules characteristic of the whole serum of each species. In real life, every vertebrate species must depend on its own serum complement components to act in concert with its own immunocytes and antibodies. Thus, intraspecific or even isogenic tests of effector functions should be the ultimate criterion. Artificially contrived tests in vitro can be quite misleading if interspecific mixtures of potential effector molecules are employed.

Hemolytic activity for mammalian red cells has not been detected in the serum of agnathans (hagfish and lampreys) or in the body fluids of invertebrates under the usual conditions of testing just described. However, hagfish, sipunculid worms, and horseshoe crabs do exhibit a lytic humoral system in the presence of cobra venom factor. In mammals, this factor activates C3 and the alternate pathway leading to hemolysis in the absence of either antibody or the initial components (C1, 4, and 2) of the "classical" pathway. Despite fragmentary evidence, the suggestion is strong that the late-acting components of complement were the first to emerge phylogenetically. The early-acting components preceding C3, depending as they do on specific antibody reaction, would be of no obvious use to invertebrates geared to cell-mediated immunity. The evolutionary addition of C1, C4, and C2 with their inhibitors and inactivators at the level of higher fishes presumably required emergence of genes for proteins capable of interacting with both immunoglobulins and the original C3-C9 components. If there were only nine components rather than twenty or more, one might be more comfortable with the prevailing lore of

complementology. However, even mammals appear unlikely to use so many proteins in sequence to achieve so few desired results, unless each protein fills a need in its own right. Perhaps the invertebrates will provide new insights on this score. A specific immunorecognition system characteristic of all multicellular animals should be well served by a nonspecific effector system, provided the latter offers a multiplicity of molecular choices. Proteins with similar immune effector functions should also display considerable evolutionary continuity.

Our still rudimentary understanding of immune pathways is conducive to humility if not awe. Although much is known about the Ig system of antibodies, the pathways of cell-mediated immunity and T-lymphocyte activation remain obscure. The decisive involvement of subsets of helper, killer, and suppressor T-cells reveals the multipurpose and central role of this system, at least in higher vertebrates. Progressive evolution of multiple classes of immunoglobulins among vertebrates suggests that the Ig system serves mainly to regulate cell-mediated immune responses. Controlling immunogenes probably fall into three categories: (1) histocompatibility or *H* genes govern immunorecognition reactions underlying cell-mediated immunity; (2) regulatory or *Ir* genes, including *MIC*- or *MHC*-associated genes, govern cell interactions and selection of pathways; (3) immunoglobulin or *Ig* genes govern antibody specificity and function by determining immunoglobulin structure (i.e., isotypes, allotypes, and idiotypes). We have only begun to unravel the complex interactions among the products of these three groups of genes.

KEY REFERENCES

Books

Cooper, E. L. *Comparative Immunology.* Englewood Cliffs, N.J.: Prentice-Hall, 1976. A moderately advanced treatise on the immune system of both invertebrates and vertebrates.

De Nettancourt, D. *Incompatibility in Angiosperms.* Berlin: Springer-Verlag, 1977. The various mechanisms of incompatibility known to operate in plants are presented in detail together with a critical examination of their importance for research in fundamental biology.

Hildemann, W. H., and Benedict, A. A. (eds.). *Immunologic Phylogeny.* New York: Plenum Press, 1975. A far-ranging collection of articles by leading immunologic phylogenists covering the whole gamut from lower invertebrates to mammals.

Manning, M. J., and Turner, R. J. *Comparative Immunobiology.* New York: John Wiley, 1976. An excellent book for the newcomer who has a background in zoology.

Marchalonis, J. J. *Immunity in Evolution.* London: Arnold, 1977. The most useful chapters deal with emergence of different immunoglobulin classes with emphasis on distinctive heavy and light chains.

Articles

Invertebrate Emphasis

Bigger, C. H. The acrorhagial response in *Anthopleura krebsi:* intraspecific and interspecific recognition. In *Coelenterate Ecology and Behavior,* G. O. Mackie (ed.). New York:

Plenum Press, 1976, pp. 127–136. Discriminating cell-surface immunorecognition is evident in sea anemones.

Boiledieu, D., and Valembois, P. Natural cytotoxic activity of sipunculid leukocytes on allogeneic and xenogeneic erythrocytes. *Develop. Compar. Immunol.* 1:207–216, 1977. A genetic diversity underlying allogeneic incompatibilities in sipunculid worms is now evident.

Cheng, T. C., and Galloway, P. C. Transplantation immunity in mollusks: the histoincompatibility of *Helisoma duryi normale* with allografts and xenografts. *J. Invert. Pathol.* 15:177–192, 1970. Snails effectively distinguish H markers among isografts, allografts, and xenografts.

Coffaro, K. A., and Hindgardner, R. T. Immune response in the sea urchin *Lytechinus pictus*. *Science* 197:1389–1390, 1977. Allograft rejection with memory under immunogenetic regulation is characteristic of this echinoderm.

Cooper, E. L. Transplantation immunity in helminths and annelids. *Transplant. Proc.* 2:216–221, 1970. Summarizes the definitive work on the immunogenetic basis of transplantation reactions in worms.

Hildemann, W. H. Phylogenetic and immunogenetic aspects of aging. In *Genetic Aspects of Aging*, vol. 14. D. Harrison (ed.). New York: The National Foundation, 1978, pp. 97–107. Presents immunophylogenetic hypothesis of aging; all major groups of multicellular animals, both invertebrate and vertebrate, possess immunorecognition systems that defend somatic integrity but gradually allow immune dysfunction, a major cause of aging.

Hildemann, W. H., Bigger, C. H., and Johnston, I. S. Histoincompatibility reactions and allogeneic polymorphism among invertebrates. *Transplant. Proc.* 11:1136–1142, 1979. Analyzes recent studies of immunorecognition and immunocompetence in diverse invertebrates.

Hildemann, W. H., Johnston, I. S., and Jokiel, P. L. Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponges. *Science* 204:420–422, 1979. Discovery of highly discriminating transplantation immunity with a specific memory component in Porifera.

Hildemann, W. H., Raison, R. L., Cheung, G., et al. Immunological specificity and memory in a scleractinian coral. *Nature* 270:214–223, 1977. Transplantation immunity with concomitant memory is analyzed in populations of *Montipora*.

Ivker, F. B. A hierarchy of histoincompatibility in *Hydractinia echinata*. *Biol. Bull.* 143:162–174, 1972. Graded degrees of allogeneic incompatibility as a function of genetic relationship are evaluated in this coelenterate in terms of possible multiallelic and multigenic determination.

Karp, R. D., and Hildemann, W. H. Specific allograft reactivity in the sea star *Dermasterias imbricata*. *Transplantation* 22:434–439, 1976. Chronic allogeneic incompatibility with selective memory appears characteristic of these echinoderms.

McCumber, L. J., and Clem, L. W. Recognition of viruses and xenogeneic proteins by the blue crab, *Callinectes sapidus*. *Develop. Compar. Immunol.* 1:5–14, 1977. Sophisticated recognition of foreignness is demonstrated, although the immunogenetic endowments of arthropods remain uncertain and invite further investigation. See also *Transplantation* 21:17–22, 1976, for evidence of cell-mediated immunity with a fine degree of specificity in the larvae of hymenopteran insects.

Reddy, A. L., Bryan, B., and Hildemann, W. H. Integumentary allograft versus autograft reactions in *Ciona intestinalis*, a protochordate species of solitary tunicate. *Immunogenetics* 1:584–590, 1975. Beyond the finding of discriminating immunorecognition, this pioneering study reveals the difficulties of working with these delicate marine animals.

Tanaka, K. Allogeneic distinction in *Botryllus primigenus* and in other colonial ascidians. In *Immunologic Phylogeny*, W. H. Hildemann and A. A. Benedict (eds.). New York:

Plenum Press, 1975, pp. 115–124. See also *Cellular Immunol.* 7:410–426, 1973, for genetic analyses of allogeneic "nonfusion" reactions.

Tartar, V. Transplantation in protozoa. *Transplant. Proc.* 2:183–190, 1970. Summarizes the classic work on unicellular animals.

Vertebrate Emphasis

Acton, R. T., Weinheimer, P. F., Hall, S. J., et al. Tetrameric immune macroglobulins in three orders of bony fishes. *Proc. Natl. Acad. Sci. U.S.A.* 68:107–111, 1971. IgM polymers of different sizes, probably reflecting J-chain polymorphism, distinguish different species and classes of vertebrates.

Benedict, A. A., and Yamaga, K. Immunoglobulins and antibody production in avian species. In *Comparative Immunology* J. J. Marchalonis (ed.). Oxford: Blackwell, 1976, pp. 335–375. An admirably comprehensive review of avian immune responsiveness in phylogenetic perspective. See especially addendum on pp. 375a and 375b for update on immunogenetic aspects. See also recent collection of articles in *Avian Immunology* A. A. Benedict (ed.). New York: Plenum Press, 1977.

Bloom, B. B., Diamond, B., Muschel, R., et al. Genetic approaches to the mechanism of macrophage functions. *Fed. Proc.* 37:2765–2771, 1978. A recent review of macrophage mutants, receptors, and functions in mammals.

Borysenko, M. Phylogeny of immunity: an overview. *Immunogenetics* 3:305–326, 1976. Especially useful on phylogeny of immunocytes and lymphoid organs.

Clark, E. A., and Harmon, R. C. The phylogeny of mammalian histocompatibility immunogenes. *Transplant. Proc.* 11:1113–1117, 1979. Examines the full array of immunogene loci and phenotypic traits of various mammals, including those linked and not linked to the *MIC.*

Cohen, N. Evolution of the MHC in vertebrates: a saga of convergent gene evolution. *Transplant. Proc.* 11:1118–1122, 1979. Evidence that *MHC* phylogenesis may reflect convergent gene evolution is analyzed in terms of functional *MIC* markers in vertebrates ranging from primitive fishes to mammals.

Gigli, I., and Austen, K. F. Phylogeny and function of the complement system. *Annu. Rev. Microb.* 25:309–331, 1971. See also Rosen, F. S., *Transplant. Proc.* 6:47–50, 1974, and Hildemann, W. H., *Dev. Compar. Immunol.* 2:45–50, 1978, for different perspectives on the phylogeny and significance of complement components.

Gitlin, D., Perricelli, A., and Gitlin, J. D. Multiple immunoglobulin classes among sharks and their evolution. *Comp. Biochem. Physiol.* 44B:225–239, 1973. Serum Igs of sixteen species of shark were examined and it is claimed, contrary to expectation, that sharks had not one but from two to four immunoglobulins.

Hildemann, W. H. Phylogeny of transplantation reactivity. In *Transplantation Antigens: Markers of Biological Individuality.* New York: Academic Press, 1972, pp. 3–73. A searching, comparative review of the immunogenetic bases of immunocompetence among invertebrates and vertebrates.

Hildemann, W. H. Specific immunorecognition by histocompatibility markers: the original polymorphic system of immunoreactivity characteristic of all multicellular animals. *Immunogenetics* 5:193–202, 1977. Existence of two immunorecognition systems, the H system of cell-mediated immunity and the Ig system of antibody-mediated immunity, is averred in phylogenetic perspective.

Litman, G. W., and Kehoe, J. M. The phylogenetic origins of immunoglobulin structure. In *Comprehensive Immunology,* Vol. 5, New York: Plenum Press, 1978, pp. 205–228. Focuses on the molecular evolution of humoral immune systems from invertebrates to mammals.

McMillan, M., Cecka, J. M., Murphy, D. B., et al. Partial amino acid sequences of murine Ia antigens of the *I-EC*$^{\beta}$ subregion. *Immunogenetics* 6:137–148, 1978. Highly variable amino acid sequences in polypeptides of Ia molecules in mice, guinea pigs, and

humans suggest that the multigenic system of the *I* region could serve as a source of *MIC* recognition receptors.

Raison, R. L., Hull, C. J., and Hildemann, W. H. Characterization of immunoglobulin from the Pacific hagfish, a primitive vertebrate. *Proc. Natl. Acad. Sci. U.S.A.* 75:5679–5682, 1978. Both hagfish and lamprey immunoglobulins are IgM-like with heavy chains of molecular weight similar to mammalian μ chains, but possess light chains of greater molecular weight than the mammalian counterpart.

Snell, G. D. T cells, T cell recognition structures, and the major histocompatibility complex. *Immunol. Rev.* 38:3–69, 1978. A detailed review of evidence bearing on the nature of T-cell recognition structures in relation to the functions and evolution of the *MIC*. See also Cunningham, B. A., *Sci. Amer.* 237:96–107, 1977, for other interpretations of the receptor/recognition functions of *MIC* cell-surface antigens.

Vitetta, E. S., Uhr, J. W., Klein, J., et al. Homology of (murine) H-2 and (human) HLA with a chicken histocompatibility antigen. *Nature* 270:535–536, 1977. There is increasing evidence of structural homology among *MHC* antigens of mammalian and avian species. See also *Proc. Natl. Acad. Sci. U.S.A.* 74:4002–4006, 1977, for evidence of structural similarities between heavy chains of *MIC* antigens and immunoglobulins.

GLOSSARY

ABO system: human blood group system discovered by Landsteiner in 1900. Humans belong to one of four main phenotypic groups: A, B, AB, or O.

absorption: the removal of a subpopulation of antibodies from a serum by mixing the serum with cells expressing an appropriate antigen. After antibodies bind to corresponding antigens, the cells are separated from the antiserum, which is now more specific.

adoptive immunity: immunity in an individual caused by the presence of immune lymphocytes donated from another individual of the same or similar genotype. In an *adoptive transfer* immune lymphocytes are inoculated into normal or unresponsive recipients.

adsorption: the attachment of antibodies to the surfaces of cells or inert particles.

agglutination: clumping together of cells usually by cross-linking of particulate antigens with antibody.

allele: one of two or more alternative forms of a gene occupying the same locus.

allelic exclusion: 1. phenotypic expression of a single allele in cells containing two different alleles for that genetic locus; 2. the synthesis of immunoglobulin of only one parental allotype by a heterozygous antibody-producing cell.

alloantigen: a genetic marker that elicits an immune response when inoculated into a host of the same species with a different genotype. Antibodies produced in response to alloantigenic stimulation are called *alloantibodies.*

allogeneic: members of the same species with different genotypes.

allogeneic disease: see graft-versus-host reaction.

allogeneic effect: 1. circumvention of the requirement for linked recognition in an immune response to a hapten-carrier complex by an allogeneic response; 2. nonspecific enhancement or suppression of an immune response by allogeneic T-lymphocytes.

allograft (homograft): a tissue graft from a donor of one genotype to a host of another genotype when donor and host are of the same species.

allophenic (tetraparental) animals: individuals who display an orderly arrangement of two or more alternative cellular phenotypes. Allophenic mice are formed by aggregating in vitro cleavage-stage blastomeres of different genotypes and then implanting this "mosaic" into a pseudopregnant female for further development.

allotype suppression: suppression of the production of allotype *a* bearing immunoglobulin in *a/b* heterozygous animals by inoculation of anti-*a* specific antibodies.

allotypes: antigens on immunoglobulin molecules or other serum proteins that distinguish inbred lines or different individuals of the same species; synonymous with alloantigens.

altered self: recognition by T-cells of self-*H-2* or *HLA* coded molecules which have been slightly altered by interaction with antigen.

amphotropic: a virus that can replicate in cells from its host species or cells from other species.

antibody (Ab): an immunoglobulin protein produced by B-lymphocytes or plasma cells in response to a foreign molecule (antigen) to which it can specifically bind.

antigen (Ag): a substance or molecule that can induce an immune response when introduced into the tissues of an animal.

antigenic conversion: 1. acquisition of specific cellular antigens by normal cells as a consequence of virus infection; 2. antibody-induced shift by certain protozoans or parasites to express a new cell-surface antigen and to cease expressing another. Synonymous in this sense with *serotype transformation*.

antigenic determinants: the sites or molecular constituents of an antigen to which antibody binds.

antigenic modulation: phenotypic suppression of cell-surface antigens in the presence of specific antibodies (e.g., Tla antibodies can cause corresponding Tla antigens to be suppressed).

antiserum: serum from an animal containing antibodies to antigen or antigens. A *conventional* antiserum is produced by immunizations with antigen and contains a heterogeneous group of antibodies. A *monoclonal* antiserum contains homogeneous antibody and is produced by inoculating an animal with antibody-secreting hybridoma cells.

associative recognition: requirement during an immune response for recognition of antigen in association with another structure, e.g., hapten and carrier, virus and self-H-2D, insulin and self-Ia.

autogamy: self-fertilization within a single cell by pairwise fusion of haploid nuclei; in ciliate protozoans, a process of self-fertilization resulting in homozygosis.

autogenous (autogeneic): derived from self.

autoimmunity: a specific immune response to the constituents of the body's own tissues. When this immune response is deleterious, *autoimmune disease* is present.

autosomal: associated with a chromosome(s) other than the X or Y sex chromosomes.

B-cell or B-lymphocyte: bone marrow or bursa-derived Ig-bearing lymphocyte which synthesizes and secretes antibodies.

backcross: a crossing of a heterozygote with one of its parents (e.g., A/B $\times$ A/A). The first, second, . . . backcross generations are symbolized by N1, N2, etc.

background: the residual genotype excluding specific loci being studied in relation to a particular phenotype (e.g., *H-2* congenic B10.BR strain mice share the B10 background with B10.D2 but have a different background from BALB.K).

β_2-microglobulin: a small polypeptide noncovalently associated with cell-surface histocompatibility molecules such as H-2 and HLA; it exhibits substantial homology to the C_H units of Ig molecules.

blast cell: a large immature cell that can synthesize DNA in response to mitogens such as concanavalin A (Con A blasts) or lipopolysaccharide (LPS blasts).

capping: process of redistribution of cell-surface structures to one region of the cell surface usually mediated by antibody.

carrier: immunogenic molecule to which a hapten is coupled that renders the hapten capable of inducing an immune response.

cell-mediated immunity: immune responses such as delayed-type hypersensitivity or allograft rejection principally mediated by T-cells rather than by humoral antibodies.

chimera: a host containing cells of genetically different origins, found in nature in cattle twins, or produced artificially, e.g., by reconstituting irradiated recipients with bone marrow of a different genotype.

chromosome: an extended DNA-protein structure characteristic of the nucleus of cells.

cis: mutations of two closely linked genes in the same chromosome ($a^1a^2/++$); genetic material from only one of two homologous chromosomes (as opposed to *trans*).

class (or subclass) of antibody: the major molecular types of immunoglobulin, i.e., IgA, IgD, IgE, IgG, and IgM.

clonal selection theory: theory describing how B-lymphocyte clones express characteristic membrane antibody receptors and are triggered (or selected) by their specific antigen to proliferate and differentiate into antibody-producing *plasma cells.*

clone (stock): a group of cells or organisms of identical genotype descended from a single common ancestor by mitotic division.

codominance: expression of both alleles of a gene pair in the heterozygous state.

coisogenic: genetically identical except for a difference at a single locus.

combining site: the area of the antibody molecule in the Fab region which is complementary to and thus can bind specifically to a particular chemical group or antigenic determinant.

complement: a group of serum proteins that combine with antibody–antigen complexes and thereby facilitate chemotaxis, phagocytosis, and cell lysis.

complementation: interaction of products of two (or more) genes to yield a trait or characteristic not obtainable with one gene alone.

complementation test: a genetic test used to determine if two gene mutations occur in the same functional locus, i.e., are allelic or nonallelic genes. Heterozygotes with the two mutations either on the same chromosome (*cis*) or on separate chromosomes (*trans*) are compared. If the mutations are nonallelic (a+/+b), the normal phenotype should be expressed, since each chromosome compensates or makes up for the mutant in the other.

congenic lines; a congenic line differs from its parental inbred strain only at a selected locus plus a small chromosomal region flanking this locus. Congenic strains can be produced by, e.g., a cross-intercross mating system or a cross-backcross-intercross method.

constant (C) region: the C terminal portion of Ig molecules which does not vary greatly from polypeptide to polypeptide in amino acid sequence, unlike the V (variable) region. The C region and V regions are encoded by distinct genes.

cross: mating of unlike homozygotes (AA $\times$ aa).

cross-match test: a procedure used for screening donor for blood transfusions or organ transplants. The recipient's serum and potential donor's cells are mixed, and if agglutination or cytolysis in the presence of complement occurs, the pair is not compatible.

crossing-over: the reciprocal exchange of segments at corresponding positions of paired homologous chromosomes by symmetric breakage and subsequent rejoining.

crossover suppression: a gene-coded effect or chromosomal structural change which reduces the frequency of crossing-over during meiosis. Genes in the *T/t* complex may suppress crossing over in the chromosomal region between *T/t* and *H-2*.

cross-reaction: combination of antigen with an antibody produced in response to a different antigen. The two antigens may be stereochemically similar or share antigenic determinants.

cytotoxicity: the process leading to lysis of cells. This may be mediated by antibody and complement, antibody and K-cells, or directly by T-cells, NK-cells, or macrophages.

deletion: loss of a section of genetic material from a chromosome. Deletion of the genetic material between a given *V* gene and a *J* gene is a probable mechanism for joining them.

differentiation antigen: a serologically detectable cell-surface antigen expressed at a particular stage of differentiation.

diploid: the chromosomal state in which each chromosome except the sex chromosomes is represented twice (2N).

domains (homology units): regions of the immunoglobulin light and heavy chains with similar amino acid sequences; each of these regions or units (i.e., V_L, V_H, C_L, C_H1, C_H2, or C_H3) consists of about 110 amino acids and has a centrally placed disulfide bridge.

dominant: an allele which manifests its phenotype in the homozygous or heterozygous state.

dual recognition: model suggesting T-cells have two receptors for recognizing antigen, one for self-*MIC* molecules and one for antigen; contrasts with the single recognition model, in which one receptor recognizes an interaction product of self and antigen.

ecotropic virus: a virus that replicates in cells of its host species but not in cells from other species.

endogenous virus: a virus which exists in an inactive form in a host cell and can be genetically transmitted from one generation to the next as an integrated part of the gametic genome (vertical transmission).

enhancement (immunologic enhancement): a prolongation or facilitation of allograft or tumor survival mediated by specific antibodies against the foreign tissue. See also *immunoblocking.*

episome: a genetic element, nonessential to a cell, that can exist in a free state or in an integrated state as part of a normal cell chromosome.

epistasis: a form of gene interaction in which one gene interferes with or modifies the phenotypic expression of another nonallelic gene(s).

epitope: an antigenic determinant of known structure.

excision and insertion: a model for joining *V* and *C* immunoglobulin genes, in which the *V* region gene is excised from the chromosome and later reintegrated near its *C* gene.

exconjugant: a protozoan that has just undergone conjugation.

exogenous virus: a virus that replicates in lytic cycle and is not vertically transmitted in a gametic genome.

F_1 generation: first filial generation of a cross.

F_1 test: Snell's test for distinguishing whether two congenic strains with the same background have different *H* loci or different alleles at the same *H* gene.

F_2 generation: second hybrid generation from an $F_1 \times F_1$ intercross.

F_{ab} fragment: the fragment of an immunoglobulin molecule obtained after papain hydrolysis consisting of one light chain and the N-terminal region of the heavy chain.

$F_{(ab')2}$ fragment: the fragment of an Ig molecule obtained after pepsin digestion containing two F_{ab} fragments connected by disulfide bonds in a hinge region.

F_c fragment: crystallizable fragment of an Ig molecule obtained after papain digestion consisting of the C terminal half of two heavy chains linked together by disulfide bonds.

founder principle (founder effect): the principle that a new population established in isolation will have a different gene pool from the parent population because of sampling error. These differences are enhanced by evolutionary pressures which lead to increased divergence of the two populations.

framework region: the highly conserved, relatively invariant portion of the V region as distinguished from the hypervariable segments of the V region.

gamete: the haploid cells (ova and sperm) which unite at fertilization to form a zygote.

gene: a distinctive hereditary unit located on a chromosome at a specific site or locus which codes for a functional product, e.g., tRNA, rRNA, or a polypeptide chain.

gene frequencies: the proportions of different alleles of a gene in a population. Changes in the gene frequency depend on random processes (e.g., nonrecurrent mutations) and systematic processes (e.g., mutation, selection).

gene dosage effect: quantitative action of the alleles of a gene on the phenotypic expression of a character.

generation of diversity: the mystery of how the immune system generates a vast diversity of antibodies to recognize some 10^6 to 10^9 different antigens.

genetic drift: any change either directed or undirected in given gene frequencies in a population, usually due to sampling error; most evident in small populations.

genome: a haploid set of chromosomes with their associated genes.

genotype: the complement of genetic material an organism inherits from its parents.

genotype frequency: the proportion of a particular genotype among the individuals of a population.

germ-line: the lineage of cells ancestral to the gametes which, during the development of an organism, differentiate into gamete-forming tissues.

globulin: serum protein fraction whose anodic mobility upon electrophoresis is less than albumin, consisting of α-, β-, and γ-globulins. Antibodies are found in the gamma-globulin fraction.

graft rejection: a cell-mediated response evoked by histocompatibility antigens on transplanted tissue which leads to the destruction of the graft.

graft-versus-host reaction (GVHR), allogeneic disease, runting disease: a cell-mediated immune response of immunocompetent lymphoid cells after inoculation into an immunologically incompetent, histoincompatible host; characterized by spleen and lymph node enlargement, eczema, diarrhea, weight loss, and often lethality.

H antigens: 1. the flagellar protein antigens of motile Gram-negative enterobacteria; 2. the histocompatibility alloantigens of multicellular animals.

H (heavy) chain: the heavy chain polypeptide of immunoglobulin which varies in molecular weight according to Ig class; linked to the light (L) chain by a disulfide bond.

H-2 complex: the major immunogene complex of the mouse.

haploid: the chromosomal state in which each chromosome is present in only one copy (N).

haplotype (phenogroup): a set of alloantigens coded by a single allele or by closely linked genes on a single chromosome. This term refers to the products of one gene complex that usually segregates as a single allele in matings.

hapten: a chemically defined determinant that can bind to antibody but cannot induce an immune response unless it is coupled to an immunogenic carrier.

Hardy-Weinberg principle: the rule stating that a large random-mating population, in the absence of migration, mutation, and selection, is stable in respect to both gene and genotype frequencies. The genotype frequencies here are determined solely by the gene frequencies of the parents and reach an equilibrium after one generation.

helper cells: a subpopulation of presumably specific T-cells that cooperate with or "help" B-cells produce antibody to thymus-dependent antigens.

hematopoiesis: the process of blood cell formation.

hemizygous: of genes present in a single dose, such as a sex-linked gene in the heterogametic sex.

heterogametic: sex that produces equal numbers of different gametes, e.g., X- and Y-bearing sperm.

heterogenetic: involving the same or similar antigens shared by several or many species (e.g., Forssman antigen).

heterozygous (heterozygote): the state in which a gene pair in a diploid organism consists of two different alleles, e.g., *A* and *a*.

hinge region: the portion of an Ig molecule in which the F_c region and two F_{ab} regions are joined together; it is flexible so that the antibody can open like a Y.

histocompatible: cells or tissues accepted and functional upon grafting from one individual into another.

histocompatibility (H) antigen: a genetically determined antigen on nucleated cells which, upon grafting into an individual with a different genotype, evokes an immune response leading to rejection.

histocompatibility (H) genes: genes coding for cell-surface H antigens, e.g., *H-1* and *H-3* in the mouse.

HLA complex: the system of genes in man constituting the major immunogene complex.

homology: structural similarity due to descent from a common form.

homology units (of Ig domains): units of similar amino acid sequence of about 110 residues in length in light and heavy Ig chains. There are two groups of homology units, the V region group and the C region group.

homozygous (homozygote): the state in which a diploid individual has identical alleles at a given locus or loci.

humoral: pertaining to serum factors or circulating serum antibodies of the immune response.

hybrid: an offspring of a cross of two genetically unlike individuals.

hybridoma: an antibody-producing hybrid cell line formed by the fusion of a nonproducing myeloma cell line and a normal antibody-producing cell; hybridomas can be grown as tumors in mice to produce *monoclonal* antisera.

hypervariable regions: portions of V_L and V_H immunoglobulin regions which differ considerably from one immunoglobulin chain to another as contrasted with the relatively invariant framework regions. The hypervariable regions comprise the antigen-binding sites of the antibody molecule.

hypogammaglobulinemia: a state of abnormally low levels of serum immunoglobulins present in certain immunodeficiency diseases.

I region: region in the *H-2* complex of the mouse coding for Ia antigens and containing immune response genes; homolog of *HLA D(DR)* in man.

Ia antigens: alloantigens coded by the murine *H-2I* region (or *HLA-DR* in humans) found in B-cells and some T-cells and macrophages.

idiotype: 1. an antigenic determinant on a specific antibody characteristic of that antibody and different from others even of the same *isotype* and *allotype;* 2. the specific antigen-combining site of an Ig molecule which itself can be identified as an inherited antigenic determinant.

Ig: abbreviation for an immunoglobulin (antibody) molecule.

immune response (Ir) gene: genes that regulate the "on-off" status of lymphocyte biosynthetic pathways and the cellular interactions of an immune response.

immune surveillance: theory that the cell-mediated immune system arose in evolution to continually monitor the body for spontaneously arising aberrant cells or foreign pathogens and to destroy them.

immunization: administration of an antigen to evoke an immune response to it.

immunoblocking: the promotion of allograft or tumor survival or of failure to respond actively to an antigen mediated by specific antibodies or antibody–antigen complexes. Preferred designation to older term *enhancement.*

immunodeficiency: condition in which the immune system is impaired due to an underlying disease either congenital or acquired.

immunodominant: refers to the monosaccharide or other small molecule which possesses the highest affinity for the antibodies in a specific serum.

immunogene: a gene or locus affecting any immunological characteristic.

immunogenetics: studies in which the principles and techniques of both genetics and immunology are employed together.

immunogenic: capable of stimulating an immune response. The immunogenicity of an antigen depends on its size, shape, and other structures with which it is associated.

immunoselection: antibody-induced genetic change in antigen expression, e.g., antibody-induced variations in influenza virus strains or mutations in cell lines leading to expression of a favorable cell-surface specificity.

inbreeding (inbred line): the crossing of closely related plants or animals. An *inbed line* is theoretically homozygous at all loci.

inbreeding, coefficient of: the probability that two genes at any locus in an individual are

derived from the same ancestor; the proportional reduction in heterozygosity in a random sample of individuals in a population as a result of inbreeding.

incompatibility: nonidentity between a pair of individuals at a locus (loci) coding for antigens which evoke a rejection or antibody response.

incross: the mating of like homozygotes, e.g., a/a × a/a.

interallelic effect: principle that the "strength" of a histoincompatibility response is a function of the interallelic combination rather than the genetic locus involved, e.g., $H\text{-}4^b \rightarrow H\text{-}4^a$ is a much stronger incompatibility than $H\text{-}4^a \rightarrow H\text{-}4^b$.

intercross: the mating of heterozygotes, e.g., $a/+ \times a/+$.

intervening sequences or *introns:* 1. DNA nucleotide sequences not coding for a polypeptide chain; 2. the DNA separating the C and V segments coding immunoglobulin polypeptides.

isotype: antigenic determinants on Ig molecules which distinguish classes of immunoglobulins.

J chain: a small polypeptide that binds the monomeric units in IgM and IgA polymers.

J region: a splicing DNA segment adjacent to *V* region genes that joins to particular *V* gene segments and activates them.

K-cell: effector or killer cell which mediates antibody-dependent cell-mediated cytotoxicity.

karyotype: the characteristic phenotype of the chromosomes of an organism, i.e., number, size, shape, banding, etc.

Kaufman-White scheme: a system used to classify the multiple serotypes of the bacterial genus *Salmonella* based on their H and O antigens.

L chain: the smaller or light chain of Ig consisting of one variable and one constant region.

linkage disequilibrium: the tendency of certain alleles of different linked loci to occur more frequently together on the same chromosome than would be expected based on the map distance between the two loci.

linked genes: genes located on the same chromosome which therefore tend to segregate together.

lipopolysaccharide (LPS): the active component of endotoxin derived from bacterial cell walls; a B-cell mitogen in some species.

locus: position occupied by a gene on a chromosome.

Ly antigen: polymorphic antigens found on subpopulations of lymphocytes; Lyt antigens are expressed on T-cells; Lyb antigens are found on B-cells.

lymphokines: biologically active, nonantibody proteins produced by T-lymphocytes.

lysis: the bursting of a cell by destruction of part of its membrane.

lysogenic conversion: predictable change in the O antigen serotype of *Salmonella* after infection with certain lysogenic bacteriophages.

macronucleus: the larger of the two types of nuclei in ciliates.

macrophage: a nonlymphoid, adherent mononuclear cell with important accessory functions in immune responses.

major immunogene complex: a genetic region containing loci coding for lymphocyte surface antigens, H antigens, *Ir* gene products, and complement components which has a major role in the regulation of the immune response, particularly T-cell-dependent responses.

memory (second-set response): the ability of the immune system to mount a specific strong and rapid response to an antigen it has previously encountered.

merozoite: 1. a cell developed from a schizont in the asexual reproduction of certain sporozoans; 2. the extracellular malarial parasite released after red cell rupture.

MHC or *MIC:* major histocompatibility complex or major immunogene complex. The latter terminology is preferred to emphasize the diverse immune functions of the corresponding gene products.

micronucleus: the smaller reproductive nucleus in ciliates as distinguished from the larger vegetative nucleus.

mitogen: a substance that induces cells to divide.

MLC or MLR: mixed leukocyte culture or response.

moderate (minor) H genes: loci coding for H antigens which evoke relatively moderate or weak histoincompatibility reactions. Most *MoH* loci map outside the *MIC.*

monoclonal: derived from a single clone. Hybridomas and myelomas secrete monoclonal immunoglobulin.

monophyletic: derived during evolution from a single ancestral stock or type.

monospecific: operational term used in reference to antiserums that appear to be reactive only with single antigenic determinants.

multigene family: a group of genes that exhibit close linkage, sequence homology, and related overlapping functions, e.g., V_λ or V_κ genes.

mutation: rare genetic change caused by an alteration in a DNA base pair (*point mutation*) or a loss of genetic material.

mutual exclusion: phenomenon among nonallelic ciliary antigens in which only one genetic locus for one serotype is active at a time. Changes in environment without a change in genotype can lead to a transformation of one serotype to another but only one is expressed.

myeloma: a tumor of bone marrow-derived cells, particularly Ig secreting cells; neoplastic plasma cells are called *plasmacytomas.*

network (immunologic): theory that receptor-antireceptor interactions regulate the immune response. Idiotype specific autoantibodies have their own new idiotypic determinants that evoke a new immune response; this process may repeat itself and lead to a network of idiotype-antiidiotype interactions.

NK (natural killer) cells: lymphocytes or monocytes from a previously unimmunized host which kill foreign target cells or virally-infected cells; NK-cell activity rapidly increases in response to foreign pathogens and may play a role in immune surveillance.

O antigens: somatic or cell wall antigens of enterobacteria such as *Salmonella* or *E. coli.*

oncogenic: able to induce events leading to the malignant transformation of a cell; giving rise to tumors or causing tumor formation.

ontogeny: the development of an individual.

operon: a unit of adjacent genes that function in a coordinated or sequential manner under the control of an operator and a repressor.

parabiosis: the experimental joining together of individuals; used to test for incompatibilities at the whole animal level.

paroral cone: in ciliates, a protected area to which micronuclei migrate during sexual conjugation. The nuclei in the paroral cones divide mitotically and then migrate to the cytoplasm where fusion of nuclei from opposite mating types occurs.

phagocytosis: the ingestion of large particles and cells by leukocytes. Macrophages and neutrophilic granulocytes are *phagocytic* cells.

phenotype: the observable characteristics of an organism, produced by interaction between the individual's genotype and the environment.

phylogeny: the evolutionary history of an organism; the evolution of a trait, species, class, or phylum.

plaque-forming cells (PFC): lymphocytes producing antibody to cell-surface antigens or against an antigen coated to red cells. Plaque assays are used to enumerate individual PFC.

polyclonal: arising from more than one clone; even simple antigens activate several B-cell clones, producing a polyclonal response and heterogeneous antibodies.

polymorphism: the presence of multiple alleles of a gene or of genetically different classes in the same interbreeding population.

polyphyletic: derived during evolution from more than one ancestral line; branching or independent lines of descent.

precipitation: the formation of a visible complex of lattice after mixing soluble antibody with soluble antigen. Precipitation reactions can be detected in agar gels and are used, for example, to identify immunoglobulin classes.

preferential association: theory that specific viral antigens or other surface antigens interact more readily or strongly with some *MIC* allelic products than with others. This preferential association may render the virus more immunogenic: hosts with the necessary allele would resist viral infections; those without it would be susceptible.

primed: an animal or cell population previously exposed to an immunogen and capable of mounting a memory response.

private determinant: a serologically defined alloantigen unique to one among numerous alleles.

processing (of antigens): the uptake and breakdown of antigen by host accessory cells leading to the presentation of antigen in an immunogenic form. Macrophages may *process* antigens and *present* them to lymphocytes in association with self-Ia molecules.

public determinant: a serologically defined alloantigen associated with two or more different alleles of a polymorphic locus or gene complex (e.g., *H-2* in mice).

recessive: an allele that exerts its phenotypic effect only when present in the homozygous state.

recombinant inbred (RI) lines: inbred lines independently derived from an F_2 generation of a cross of two unrelated, inbred progenitor lines. Each RI line has a characteristic combination of genes with a different pattern of alternative alleles at multiple loci.

recombination: any process giving rise to cells or individuals (*recombinants*) in which two or more genes are associated in ways different from their parents.

regulatory gene: a gene whose primary function is to modulate the "on-off" status of structural genes.

restriction enzymes: class of endonuclear enzymes which cuts unmodified DNA at specific sequences exhibiting twofold symmetry around a point, e.g., at $\begin{matrix}\text{GAATTC}\\ \text{CTTAAG.}\end{matrix}$

Rh system: a system of human red cell antigens under complex genetic control. Rh-negative mothers who bear Rh-positive offspring may develop Rh antibodies, which can produce hemolytic disease in newborn babies.

schizogony: asexual reproduction in which multiple division occurs resulting in numerous progeny (schizonts), as in malarial parasites.

segregation, principle of: Mendel's law, which states that alternative parental genes or alleles separate or segregate in the gametes.

segregation distortion: a distortion of expected Mendelian ratios in a cross due to dysfunction or lethality in one or more classes of gametes, caused by, e.g., the *T/t* locus in mice or *SD* locus in *Drosophila.*

serotype: antigenic type of bacteria or red cells identified by serologic methods.

serotype transformation: reversible induction of a new antigen type (e.g., ciliary antigen in *Paramecium*) and complete disappearance of the previous type resulting from switches in gene activity.

serovar: serofermentative types in *Escherichia coli* distinguishable by their surface antigens.

somatic: referring to the body or vegetative cells of an organism as opposed to the germ-line cells.

somatic cell hybridization: fusion of somatic cells of the same or different species in vitro to form hybrid cells which contain chromosomes from both parental types.

specificity: a selective reactivity between substances, e.g., between an antigen and its corresponding antibody or a hormone and its cell-surface receptor.

strain distribution pattern (SDP): the distinctive pattern of alleles at polymorphic loci in a recombinant inbred line.

structural gene: a gene coding for a messenger RNA which dictates the amino acid sequence of a polypeptide chain.

suppressor cell: cells that can suppress the immune response to an antigen, e.g., specific and nonspecific suppressor T (T_s) cells.

syngen (variety): a reproductively isolated, specific mating type in ciliate protozoans.

syngeneic (isogeneic): involving genetically identical individuals.

T-cell: class of lymphocytes derived from the thymus which mediate cellular immune responses and regulate immune responses.

T/t complex: system of genes on chromosome 17 in the mouse regulating embryonic development. T/t mutations have a range of phenotypic effects including embryonic lethality, short tails or taillessness, segregation distortion, and crossover suppression.

tissue typing: the identification of the histocompatibility antigens of transplant donors and potential recipients usually by serologic tests. Donor and recipient pairs with identical or very similar H antigens are *matched* in order to reduce the likelihood of allograft rejection.

tolerance: the absence or inactivation of an immune response to a specific antigen, usually as a result of previous contact with that antigen.

trans: the configuration in which two alleles or mutations of two linked genes are each in one of the homologous chromosomes ($a^1+/+a^2$) in contrast to the *cis* configuration.

transduction: the transfer of a genetic fragment from one cell to another, specifically the transfer of bacterial genes from one bacterium to another by a bacteriophage.

transplant: a piece of tissue taken from the body and grafted into another individual.

trypanosomes: any flagellate protozoan of the *Trypanosoma* genus, including blood parasites of man and other vertebrates.

V (variable) region: N-terminal region of immunoglobulin chain containing the antibody-combining site, in contrast to the constant C terminal region.

virion: a virus particle consisting of a nucleic acid core embedded in a protein coat or *capsid.*

wildtype: the allelic form of a gene most frequently found in nature, arbitrarily designated as "normal."

xenogeneic: involving members of different species. A *xenograft* is a transplant in which donor and recipient are members of different species.

xenotropic virus: a virus that replicates in cells from species other than its host but not in cells from its host species.

INDEX

(continued from front flap)

— continually emphasizes basic interrelationships among diverse topics

— provides annotated key references of recent research as well as citations of earlier work for more in-depth study

— presents self-contained chapters which can be taught separately or out of sequence—to best suit students' needs.

——**William H. Hildemann, Ph.D.,** is Professor of Microbiology and Immunology, and Director of Dental Research Institute, Center for the Health Sciences, University of California in Los Angeles. **Edward A. Clark, Ph.D.,** is at the Regional Primate Research Center, Department of Genetics, University of Washington in Seattle. **Robert L. Raison, Ph.D.,** is at the Department of Bacteriology, Immunology Unit, University of Sydney in Sydney, Australia.

Blackwell Scientific Publications Ltd
Osney Mead, Oxford OX2 OEL
8 John Street, London WC1N 2ES
9 Forrest Road, Edinburgh EH1 2QH
52 Beacon Street, Boston, Massachusetts 02108, USA
214 Berkeley Street, Carlton, Victoria 3053, Australia

Jacket Illustration is taken from figure 9.2
Jacket Design by Paul Agule Design