Autoallergy

Uptodate Immunology 11

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<Opening titles>

<Lachmann to camera>

Autoallergy describes the situation where an animal mounts an allergic response to components of is own tissues. From the time of Ehrlich, autoallergy has been held to be an abnormal and unallowable state. It was considered that any mechanism for the induction of allergic responses must contain provisions preventing reaction to autoantigens. Thus, Ehrlich talked about horror autotoxicus, and much later Burnet described as forbidden clones those antigen-reactive cells potentially capable of reacting with self antigens.

The thesis I would like to put to you today is that there are probably no immunological mechanisms specific to autoantigens, and that the various factors that influence whether a response leads to immunisation, on the one hand, or to immunological tolerance or unresponsiveness, on the other, apply to all antigens alike; and that autoallergic responses are, in fact, quite common and do not normally lead to disease. I'd like to consider first those factors that are known to favour the

development of immunological unresponsiveness as opposed to immunisation for antigens in general.

<Lachmann narrates over slide>

The first of these is the maturity of the lymphon. The lymphon is here taken to comprise the whole of the lymphoid organ, including the primary lymphoid tissues, the thymus and the bursal equivalent of mammals, as well as the peripheral lymphoid tissues in lymph nodes, spleen and the gut associated lymphoid tissues. It's well known that neonatal animals whose lymphon is not yet well developed are more likely to respond to antigen by non-responsiveness, and the same is true if animals have their lymphon depleted either by x-radiation or by cytostatic drugs. All these circumstances – it is far more likely that a given antigenic stimulus will give rise to immunological unresponsiveness. There are also a number of properties of the antigen itself which favour the development of unresponsiveness. The first of these is dose. It was shown by Mitchinson that doses of antigen either less than that optimal for providing immunisation and also those much greater, both tend to produce immunological unresponsiveness. It is now realised that, in fact, low zone unresponsiveness is restricted to unresponsiveness in the T-cell population in most cases, whereas high zone unresponsiveness affects both T- and B-cells.

The chemical nature of the antigen is a further parameter affecting response; in particular certain non-physiological polymers of amino acids and certain polysaccharides that are difficult to catabolise have a high tendency to give rise to immunological unresponsiveness. And though it's doubtful whether materials of this class are likely to be relevant to autoantigens, they are, nevertheless, of interest in this category.

The physical state of the antigen is also of considerable importance and here it may well be that resistance to phagocytosis is the parameter which determines whether an antigen is likely to give rise to unresponsiveness. With certain types of antigen, particularly serum proteins, entirely monomeric preparations that have been fully cleared of aggregated material by centrifugation will not raise an antibody response

at all at any dose but will only produce immunological unresponsiveness. This is related not only to their failure to be phagocytosed but to the related property of lack of adjuvanticity. This property of antigens, which is not clearly defined at a molecular level, is certainly something to do with their capacity to activate macrophages or to be taken up on the macrophage surface. And certainly, the lack of adjuvants in an antigenic challenge is also a feature of strongly predisposing towards unresponsiveness and conversely giving potentially tolerogenic antigens, together with powerful adjuvants such as complete Freund's adjuvant, or bacterial endotoxin, or silica particles, of all tend to make potential tolerogens act as immunogens and these adjuvants may indeed play some part in the creation of autoallergic responses.

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The route of administration of antigen is also important. Here again, phagocytosis may be one of the relevant mechanisms involved.

<Lachmann to camera>

It is known that giving antigens into the portal circulation has an increased tendency to induce immunological unresponsiveness and it is believed that this is due to immunological screening by the liver, the liver being an organ that has an ample supply of macrophages but no antibody-forming tissues. And this screening of potential antigenic material from the bowel may be an important physiological role of the liver in limiting antibody formation to such products.

Besides these properties of the antigen, it's also in recent years become clear that there are genetic properties of the host which determine whether an animal will respond to an antigen. This is not really a question of whether it produces unresponsiveness or not, but whether it will give any response at all. There are a series of characteristics transmitted as single Mendelian dominant or co-dominant characteristics that control the T-cell response of animals to particular, usually rather specific, antigens like polymers of polypeptides.

<Lachmann refers to chart and narrates over it>

And on this chart, you will see the response of various strains of mice to the polymer TGAL, tyrosine glutamic acid alanine lysine, measured on this side in percentage binding of the antigen. And you can see that the CBA mice, on the whole, on immunisation fail to make any antibody to this material, whereas C57 mice raise a very good antibody titre. The F1 hybrids between them are intermediate and the back crosses behave as you would expect: if back crossed with CBA, you get low responses; if back crossed with C57, you get high responses. A factor of great interest about these so-called immune response or Ir genes is that they are linked to the principal transplantation antigen of the host, the H2 system in mice.

<Lachmann to camera>

In man, although the evidence for definite immune response genes is not yet really very hard, the linkage of particular immunological phenomena to the HLA system is often regarded as evidence that immune response genes may, indeed, be involved, and attempts to link particular human autoallergic conditions with particular HLA phenotypes have been undertaken now in a number of diseases.

I'd now like to go on to discuss the mechanisms of immunological unresponsiveness.

<Lachmann narrates over slide>

Of these, the one originally postulated by Medawar and his colleagues, and the one that almost certainly correctly accounts for classical transplantation tolerance is that here designated as clonal elimination. When an antigen-responsive cell meets antigen under appropriate circumstances, it gets a signal which either kills the cell or causes its permanent inactivation and this is known as clonal elimination. This clonal elimination can affect both T-cells and B-cells, which is, of course, of some importance since T-cell elimination alone can give rise to what appears to be a state of complete tolerance in situations where T- and B-cell cooperation is required to give antibody responses.

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A second mechanism of unresponsiveness which has received much attention in recent years is the possibility of blocking factors.

<Lachmann to camera and then narrates over a display board, adding illustrations>

Here it is postulated that a product of allergic reaction, the product of an antigen-sensitive cell, can cause the blocking or the depression of activity of other antigen-sensitive cells. And a system which has been studied in vitro by Dinar and Feldman has shown that if you take cells, particularly B-cells, in vitro and you treat them with antigen, and you treat them then with quite modest amounts, in fact, critically small amounts of antibody, you can render these cells unresponsive or apparently tolerant, and it is postulated that this works by an agglutination of the receptors causing their elimination or causing these cells to become permanently inactive. And although it is not clear that such a mechanism exactly like this works in vivo, there is a considerable corpus of evidence, largely derived from the work of the Hellströms, that in any rate immunity to tumour and certainly other systems, this antigen-antibody complex type of blocking can render harmless the effects of an allergic response, in this case quite possibly of a T-cell response.

There are other mechanisms of blocking known which are not really due to reactions of an antigen-sensitive cell but which, nevertheless, show that it is possible to irreversibly block a potential antibody-forming cell. It's been shown, for example, that if an antigen is coupled to an affinity label, which combined covalently to a cell membrane, the end result of this is that these cells become irreversibly blocked and incapable of taking part in antibody formation. Perhaps an analogous system occurs, as shown in the experiments of Mitchell and Humphrey, if an antigen, in this case pneumococcal polysaccharide type 3, that cannot be recognised by T-cells, is coupled to a hapten. Under these circumstances too, the B-cell may become blocked

and incapable of responding to this hapten on other more normal carriers. The relevance of this type of blocking in in vivo situations remains to be evaluated.

The third mechanism on the slide refers to suppressor T-cells […]

<Lachmann narrates over previously shown slide and then further diagrams, interspersed with talk to camera>

[…] and this concept is new but has been gaining ground recently, the idea being that T cells, instead of as is their normal custom, cooperating with B-cells and antibody formation, may in certain circumstances modulate in a negative sense the activities of these cells and, in fact, suppress the production of antibodies. There are a variety of lines of evidence in favour of this view; perhaps, the most impressive of which concerns the phenomenon of allotype suppression in some strains of mice. <*Next diagram*> And some experiments from Dr Ethel Jacobson and her colleagues are summarised here. If one mates two strains of mice, having two different immunoglobulin allotypes, **b** and **a**, one produces **a/b** F1 hybrids of phenotype **a/b**. If, on the other hand, the **a** mother is immunised with **b** immunoglobulin before she becomes pregnant, and therefore has circulating anti-b, the hybrid which is still genotypically **a/b** will, in fact, be phenotypically **a**. In other words, the expression of the **b** allotype in its immunoglobulin has been suppressed.

Now, evidence from a variety of experiments was adduced to show that, quite surprisingly, this suppression is an active phenomenon. And cells taken from the suppressed animals can, in fact, suppress the formation of **b** allotype immunoglobulin in normal mice. And this work has recently been extended to in vitro studies and it's been shown that supernatants taken from the spleens of suppressed F1 hybrids, when added to cultures of normal primed F1 normal cultures, suppresses the normal formation of anti-sheep erythrocyte antibody containing the **b** allotype. On the other hand, if the same supernatants are taken from cultures which have been treated with anti-theta serum and complement, prior to being set up in culture to remove all the T-cells, in that case these supernatants have no suppressive activity.

<*To camera*> And this really shows that the T-cells in the system are producing a soluble factor that is capable of influencing, in a negative sense, secretion of immunoglobulin by B-cells. This type of experiment is, I think, worth labouring in a discussion of autoallergy because, as you will see later, there is growing evidence that autoallergic phenomena and autoallergic disease may be associated with a failure of T-cell function. And it is presumably the failure of suppressor T-cell function that is involved.

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Having now briefly reviewed the factors which in the whole animal tend to give rise to immunological unresponsiveness, and the mechanisms of the unresponsiveness that can be produced, I think we might go on to consider the state of immunological reactivity that, in fact, normally obtains in response to an animal's own antigens.

<Lachmann narrates over slide>

And just for a start, I would like to make the point that autoantibody formation is, in fact, very common and that there must be many situations where, in fact, there is ready antibody formation to autoantigens, and I have listed here a number of situations where autoantibody formation is more or less ubiquitous. Almost all animals have circulating antibodies to spermatozoa that are potentially cytocidal and the reason they do not, in fact, destroy the spermatozoa in vivo is that in vivo spermatozoa enjoy the basement membrane protection afforded by the testes. Rabbits normally have in their serum the so-called Kidd-Friedwald antibody, which is an antibody to a sedimentable intracellular component of normal rabbit tissue. There are also antibodies normally found reacting with cell membrane antigens. There are the anti-I cold antibodies reacting with the I antigens of the human erythrocyte that occur at low titres in almost all humans and, although high titres of these antibodies is pathological and associated with a haemolytic anaemia, low titres are present quite normally and give rise to no adverse effects. And it has even been postulated that these low titre, normal anti-I cold agglutinins play a physiological role in the process of blood coagulation.

There is a further antibody that reacts with an autoantigen that can be demonstrated on human erythrocytes by neuraminidase treatment. This is the so-called T-agglutinin, another normal antibody. Finally, there are these two groups of antibodies, the rheumatoid factors and the immune agglutinins, that react with determinance, that can be found in normal serum proteins after they have taken part in immunological reactions: rheumatoid factors reacting with bound immunoglobulins, and the immune agglutinins with bound complement components.

<Lachmann to camera>

There is therefore nothing at all uncommon about autoantibody formation, but in considering the state of unresponsiveness to autoantigens, one can conveniently divide them into three groups. The first of these is a group of autoantigens to which there is no demonstrable self tolerance at all. And in terms of mechanism, one can say that immunisation with such antigens will give rise to a normal antibody response, and furthermore will probably give rise to delayed hypersensitivity as well, in other words, they can raise both the T-cell and a B-cell response.

<Lachmann narrates over slide >

In this situation, presumably, antibody formation is not normally seen because the antigens are sequestered, and one can picture three different groups of sites where such sequestration occurs. Firstly these antigens may be located in what are known as privileged sites. Privileged sites being those where one can administer antigens, both autoantigens and external antigens, and they will not give rise to an allergic response. And it is now known, largely from the work of Bellingham and his colleagues that the anatomical feature of privileged sites, that confers privilege on them, is predominantly the absence of an afferent lymphatic drainage. The anterior chamber of the eye is perhaps the best example of such a privileged site and the eye lens of an autoantigen to which no tolerance is enjoyed. Injection with lens protein or accidental spillage of lens protein at cataract operation gives rise to autoantibody formation and can produce eye lens damage in vivo, the so-called phacoanaphylaxis.

Another site of sequestration where antigens are protected from the reticular endothelial system is in the inside of the cell. And there are probably a wide variety of intracellular antigens to which there is no proper tolerance because the reticular endothelial system has no proper experience of them. And situations of tissue damage will then give spillage of these antigens and may give rise to autoantibody formation. And it's quite likely that the anti-heart antibodies found after cardiac surgery are an example of this.

And finally, as already mentioned, one can get antibody determinance sequestered inside molecules, and it requires a change in the molecule, brought about by involvement in immunological reaction or proteolytic cleavage, to expose this antigenic determinant to which then the animal is not tolerant. It's of interest here that rabbits, for example, have another ubiquitous autoantibody, the so-called homo-reactant, which reacts with an antigenic determinant exposed by pepsin digestion of its IgG.

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<Lachmann to camera>

So here there is a group where there is no demonstrable tolerance, perhaps a larger group, and perhaps, apropos of autoallergic disease, a more important group is the situation where you may get T-cell tolerance to an antigen in the absence of B-cell tolerance. Now, this is a situation where the autoantigen can react as a hapten rather than as a complete antigen. In other words, it can react with B-cells, but it cannot react with T-cells.

<Lachmann narrates over further slides and diagrams, interspersed with talk to camera>

That one can get situations where T-cells are tolerant and B-cells are not, has been shown in a number of experimental systems, both by Mitchinson and by Weigel and

his colleagues. This is some data of Weigel's showing that if low doses of tolerogen are given, and this is a serum protein antigen, one induces tolerance in the thymus cell population but not in the bone marrow cell population. You can see that in this system in the mouse, 100 micrograms of tolerogen give you complete unresponsiveness in the thymus cell population, whereas it requires about 25 times this amount of antigen to give you a large amount of unresponsiveness in the bone marrow population.

Furthermore, it is not only a question of dose, the time relations are of T-cell tolerance and B-cell tolerance after giving tolerogen are also quite different. <*Next diagram*> This is another experiment of Weigel and his colleagues investigating the tolerance in mice to deaggregated human gamma globulin. And they give their mouse a dose of this gamma globulin and you can see that throughout the period of this experiment, this animal is completely unresponsive to HGG. But if by cell transfer experiments, they investigate the time course of tolerance in the T-cell population and the B-cell population, you can see that they're quite different. T-cells rapidly and completely become tolerant and remain so throughout the duration of this experiment. The B-cell, on the other hand, and this is the B-cell of the bone marrow, and perhaps in the spleen they become tolerant a bit quicker, takes about a week to become tolerant, remains tolerant for a shorter time and loses its tolerance again in a shorter period of time as well.

So, in these experimental systems, there is no doubt that one can produce situations where one has this situation of T-cell tolerance without B-cell tolerance, which would provide such a nice model for our various autoallergic situations. And Weigel and his colleagues went on to demonstrate <*next slide*> that this does, in fact, happen to at least one autoantigen and that is thyroglobulin. They were able to show that whereas native thyroglobulin does not give rise to an antibody response, if thyroglobulin is altered so as it is to get a carrier function of a non-self kind recognised by the animal's T-cells, either by chemically-modifying thyroglobulin or by using cross-reacting thyroglobulin from related species, or even in some cases by using antibody to thyroglobulin as a carrier, they were able to make an animal produce autoantibodies to thyroglobulin.

It's further been shown by Allison and his colleagues that in situations where antibodies to thyroglobulin are formed in vivo, there are no T-cells reactive to thyroglobulin, showing that, indeed, this is a situation where there seems to be T-cell tolerance but no B-cell tolerance. In this sort of situation, one can envisage the stimulation of antibody production being due to cross-reacting antigens. Now, although in the case of thyroglobulin the nature of this cross-reacting antigen in vivo may not be altogether clear, in the next example here, that of heart muscle antigens, we know that there are cross-reacting antigens to this in the streptococcus and it seems highly plausible that the anti heart muscle antibodies seen in rheumatic fever are, in fact, due to stimulation by streptococci which have a foreign carrier but a cross-reacting hapten. And this so-called streptococcal mimicry of mammalian antigens extends to other antigens as well and may be responsible for the formation of a number of autoantibodies, perhaps, even in some circumstances to other basement membranes such as kidney.

<*To camera*> It's quite possible that this mechanism of defective T-cells, T-cell tolerance, but without defective B-cells, the presence of reactive B-cells, is quite important in that kind of human autoallergic disease characterised by the formation of organ specific autoantibodies.

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Now, the third group of examples of antigens, autoantigens, is where full tolerance both in the T-cell line and the B-cell line seems to exist and this can be demonstrated by the fact that immunisation is not capable of raising antibodies even using cross-reacting antigens or antigens from a related species. <*Next slide*> The examples of this are, for example, of the native serum proteins of one's own species. But even here, it's necessary to put in a caveat: in man, saliva contains antibodies to bound complement, immunoconglutinins, that react with his own native C3. So that in this extra circulatory site where antibodies are formed in the serum-free medium, it is possible to form antibodies that react with one's own native serum proteins, showing

curiously enough that in some circumstances tolerance is a local phenomenon rather than a whole body phenomenon.

Similarly, one would expect the membrane antigen to one's own circulating cells to confer full tolerance and, indeed, they normally do so. But, as I've already mentioned, one can quite normally form low titres of low affinity antibodies to ones own red cell membrane antigens. And this presumably shows that the mechanisms that cause tolerance induction require a contact between antigen and antibody that is meaningful in the sense that it must give a certain size of energetic stimulus and that these very low affinity antibodies allow the coexistence of antigen and antibody without any mutual interference. A third example of such antigens is a double-stranded DNA to which many workers have tried to induce antibodies by experimental immunisation with uniform lack of success, but this is an antigen to which antibodies are regularly formed in humans with systemic lupus erythematosus. And in the mice with the related disease, which we're about to talk about.

<*To camera*> The mechanism causing stimulation of antibody formation in this situation, where there's full tolerance, is by no means clear, but it's probably related to some disturbance of lymphoid function which has certainly a number of factors involved in it. Some of these factors are apparently genetic and some are environmental. And in this connection, it becomes meaningful to talk about an autoallergic diathesis. A diathesis being a condition where there is a genetic soil which allows certain environmental stimuli, the environmental seed, to produce certain disease states or autoallergic phenomenon. A good example of a diathesis is glucose-6-phosphate dehydrogenase deficiency which renders people capable of getting autoallergic haemolytic anaemias in response to certain environmental stimuli, such as eating pepper beans. And one can probably talk both in mice and men of autoallergic diathesis. And I would really like to discuss this first in relation to the diseases of the New Zealand mice, which have been so intensively studied, and which have given so much information about the systemic lupus erythematosus group of diseases in man.

<*Next slide*> The New Zealand black mouse is a strain of inbred mouse which suffers spontaneously from an antiglobulin positive haemolytic anaemia which gets increasingly severe with age. The F1 hybrid between the New Zealand black and New Zealand white mice suffer in addition from a disease closely resembling human SLE with antinuclear antibodies, including antibodies to double-stranded DNA and with an immune complex type of glomerulonephritis. Investigation of the pathogenesis of the disease in these mice has shown two at least major factors. The first is that the onset of this disease appears to be associated with a deficiency of T-cell function. Thus they show a premature loss of the so-called serum thymosin-like activity that can be demonstrated in the serum. Then the disease can be accelerated by injecting syngeneic spleen cells from old hybrid mice into young hybrids. And conversely, the disease may be prevented or delayed by the repeated injections of young syngeneic thymocytes into New Zealand mice, all suggesting that as there is a premature and rapid decrease in T-cell function that these autoallergic phenomena develop.

The second major association with this disease is that these animals carry a leukaemia virus as do many strains of mice, but these mice carry what appears to be a perhaps specific type of leukaemia virus named by Dr Dixon and Lerner as the Scripps leukaemia virus, which appears to be a necessary if not a sufficient cause of the production of disease in these animals.

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<*Next diagram*> And Dr Lerner and Dixon have pointed out from studies of these animals that if you look at the replication cycle of C-type leukaemia virus, in a susceptible cell, at the various antigens that can be formed: the cell surface antigens, the single-stranded RNA, the RNA dependent DNA polymerase, the RNA-DNA hybrid, the single-stranded DNA, the double-stranded DNA, the virion polypeptides, the virion RNA, and the intact virus; in all these which have this white frame around them, antibodies to all these components have been found in the New Zealand mice, so that it really does seem that perhaps antibodies to this virion dependent material

is responsible for quite a lot of the autoallergic phenomena found, including perhaps some of the antibodies to the double-stranded DNA which are so characteristic.

The nature of the genetic element in the New Zealand mice is not yet wholly clear, but it seems likely to be an immune response gene, and if it is an immune response gene it is not by any means clear that this is an immune response gene related particularly to response to autoantigens. It is perhaps more likely that this is an immune response gene which causes or permits or facilitates the persistence of this C-type virus to an unusual extent, and, in fact, the immune response gene may be producing an immunity deficiency in these animals allowing the proliferation of the virus, allowing the persistent latent virus infection, which then plays the major role in the development of the autoallergic reaction.

In man there is a related diathesis system as seen in the New Zealand mice <*next slide*>, the so-called lupus diathesis, comprising clinically the tendency to familial incidence of systemic LE, of rheumatoid arthritis and other 'connective tissue' diseases, and of the antibodies normally associated with them: <*Next slide*> antinuclear antibodies, rheumatoid factors and of hypergammaglobulinaemia in general. <*To camera*> It is not so far clear that there is a persistent virus infection associated with human SLE. Structures resembling C-type virus particles have been seen in the electron microscope in studies of the tissues of these patients, but they have not so far convinced all electron microscopists. Furthermore, it has not been possible to show so far that there is a convincing relationship between systemic lupus and a particular HLA phenotype. Nevertheless, it is still a strong possibility that underlying systemic LE, the diathesis associated with it, is an immunity defect to some form of persistent viral infection, and perhaps this would account for the known association between the diseases of this diathesis and other immunity deficiency states, particularly hypergammaglobulinaemia where it's been known for a long time that there is an increased incidence of diseases of this group, although it is, perhaps, not quite as large as was originally claimed. And also the much more recently recognised association between genetic deficiencies of the complement system and diseases of the lupus erythematosus type, which is really a quite striking association.

<*Next slide*> In man, there is a further autoallergic diathesis which is quite distinct from the lupus diathesis and is worth pointing out for this reason. This is a tendency to a familial incidence of thyrotoxicosis, autoallergic thyroiditis, pernicious anaemia, idiopathic Addison's disease, and autoallergic gonadal failure. And to the particular autoantibodies that are associated with these diseases, which are, of course, the organ-specific antibodies, the production of which may well be related much more to the mechanism where there is T-cell type tolerance but no B-cell tolerance. And exactly what the genetic predisposition to this group of diseases is, is so far entirely obscure.

<*To camera*> If I can sum up what I've said, it's that in most cases at least the origin of autoallergic reactions is explicable in terms of the same mechanisms that apply to all other antigens. Secondly that autoantibodies are quite common, that in some cases, for example, the immunoconglutinins, they may actually be useful, that in any case, in many cases they are quite harmless. And that abnormal autoallergic reactions associated with the autoallergic diseases occur in certain apparently genetically determined circumstances where there is an interplay of genetic factors, possibly related to immune response genes, and possibly related to immunity deficiencies, and that these two groups of factors may have a final common pathway in facilitating a persistent virus infection.

<End credits>