Lymphokines

The Scientific Basis of Medicine

Presented by Dr DC Dumonde, Head, Immunology Division, Kennedy Institute.

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<Opening titles>

<Dumonde to camera>

Ladies and gentleman, welcome to the field of lymphokines. Today we're going to discuss a field of immunology that has its roots in delayed hypersensitivity, but actually which is only about 10 years old. What I'm going to do is develop the concept that there are special molecular mediators of delayed hypersensitivity and the cellular immune response and to put across, I hope, some of the current excitement that this new field of work has generated amongst experimental and clinical immunologists.

Now, I shall start by discussing some of the general principles of the cellular immune response. And in the first place, we have as the basis of the cellular immune response interaction of sensitised lymphocytes with antigen. The effects you get clearly depend upon the biological environment in which this interaction is taking place: whether it's in the test tube or the whole animal; for example, in the skin or a specific organ. Now, the magnitude of the observable reaction can be taken as an index of sensitivity of the lymphocyte population which is entering into the reaction of

cell-mediated immunity. When we look at the broad spectrum of phenomena that comprise cell-mediated immunity, we can say they fall into five categories. Firstly, resistance to intracellular infection; secondly, mechanisms of delayed hypersensitivity and allograft rejection; thirdly, restriction of tumour growth; fourth, association with autoimmune disease; and finally, number five, the facilitation of antibody production.

My starting point is that the biological and the clinical importance of the cellular immune response arise from two principle mechanisms. Firstly, a direct action of sensitised lymphocytes, or shall we say T-cells, upon an antigen which is sequestered or stuck in the local biological environment. And secondly, an indirect action in which this lymphocyte, T-cell, function is expressed or amplified by means of soluble non-antibody mediators. And this general concept brings in with it the idea that cellular cooperation is fundamental to the expression of cellular immunity.

Now, historically speaking, the idea of an indirect action that sensitised lymphocytes arose from two observations. The first was made in the mid 1950s by Dr Sherwood Lawrence working in New York, who showed that small molecular weight extracts of sensitised lymphocytes could apparently transfer or augment the state of tuberculin and delayed hypersensitivity in man when injected into healthy but tuberculin negative subjects. Secondly, however, in the mid 1960s, David and Bloom, working also but independently in New York, and ourselves, here in London, showed that larger molecular weight products of specific lymphocyte activation could inhibit the migration of cultured macrophages in the guinea pig, thus producing an apparent in vitro correlate of delayed hypersensitivity. The active principle was first called MIS.

<Dumonde refers to slide, showing migrating macrophages, and then narrates over photograph of Drs Lawrence, Al-Askari and David>

And here is what is now a very familiar picture of macrophages migrating out of the ends of capillary tubes. If you add the lymphocyte activation product to the culture medium then effectively there is no macrophage migration. Now, for sentimental reasons, it's interesting to look at the scientists that initiated these discoveries. This

is a snapshot that I took in 1962 of three scientists on the Boardwalk at Atlantic City, where, in fact, the battle for mediators of cellular immunity was eventually fought out. And you can see, on the left of this slide, the two people working at Bellevue Hospital in New York. In the centre is Dr Sherwood Lawrence, the discoverer of transfer factor. On the left is Dr Al-Askari, a transplantation surgeon, who helped him. And on the right is Dr John David, one of the co-discoverers of migration inhibition factor. Now, I thought you'd like to see that slide at least for its sentimental value.

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<Dumonde to camera>

Now, following the discovery that soluble lymphocyte activation products could inhibit macrophage migration, people started to see whether they had other effects that might be relevant to the expression of cellular immunity. And a whole host of discoveries were made round about 1967 to 1969. It was found that these lymphocyte activation products could inhibit macrophage and polymorph migration, they could enhance lymphocyte DNA synthesis, they would produce an inflammatory response if they were injected into the skin, they were cytotoxic for fibroblasts, they agglutinated macrophage suspensions, and if they were added to macrophages in culture, they would enhance their metabolic, phagocytic and microbicidal properties, they would be chemotactic for various classes of leucocytes, and they would even interfere with virus pathogenicity in cell culture – a sort of interferon-like effect.

Well, you can imagine that a whole host of acronyms, such as MIF, IF, LMF and all sorts of unpronounceable names, were applied to these various factors. And so we thought that we would simplify the field. And round about 1968, we invented the term lymphokines as a generic term to describe what was obviously a growing field of a study of non-antibody mediators of cellular immunity. We proposed that this term lymphokines would indicate the general source of these materials being produced by lymphocyte activation and the kinds of biological activities that they would be expected to be producing in vivo. And it was a proposition that lymphokines would be found to be auto-pharmacological mediators of lymphocyte or T-cell responses to

antigen, which would function both in expression and regulation of the cellular immune response. Because, obviously, every time the lymphocyte met its antigen, if it was the right kind of lymphocyte, you might expect lymphokines to be produced, and because of their many activities upon all sorts of cells found in connective tissue, including lymphoid cells, you might expect them to modulate or modify the immunological response. And so, we set up a classification of mediators of cellular immunity into two broad classes. Firstly, lymphokines produced by specific lymphocyte activation and later found to be produced by lymphocyte activation with phytomitogens. Once produced, these act like local hormones or auto-pharmacological mediators and they don't need the inducer or lymphocyte activator for their expression. On the other hand, there are the smaller molecular weight lymphocyte extracts, known collectively as transfer factor, which are a completely different kettle of fish and which we won't talk about in this discussion.

In general, then, there are two broad classes of mediators: lymphokines and transfer factors. And the simple proposition is that they function in mediation and regulation of the cellular immune response, the transfer factors viewed as triggering the early stages of cellular immunity, and the lymphokines viewed as amplifying or modulating the extent of the cellular immune response in the local biological environment.

<Dumonde narrates over slide listing areas for investigation in the field of lymphokines>

Now, having said that, the field of lymphokines, which has grown up, is obviously a really complicated one. And this really ends my introduction which now leads me to select five principal areas of investigation in the field of lymphokines. And what we shall talk about are the following five topics: firstly, the distinction of lymphokines from immunoglobulins; secondly, how to design experiments to test their role in the expression of cell-mediated immunity in vivo; thirdly, how to design experiments to test their role in immunological regulation if, indeed, this is the case; fourthly, what is their clinical significance; and fifth, how do you measure or bioassay them? Because quite clearly if you can't measure them then you're experiments can only be at the level of descriptive biology.

<Dumonde to camera>

Now, to take the first of these points, the biochemistry of lymphokines; well, the last 10 years of work has indicated that they are real rather than imaginary molecules, which was first suggested. They turn out to be anionic glycoproteins, or proteins which are anionic at alkaline pH. And they have a molecular weight range between 20 000 and 100 000 according to the species and according to the circumstances of production. They are certainly separable from immunoglobulins via a variety of biological and biochemical fractionation procedures and when you've got them, you know you've got them because they're active in quite small amounts, for example, at the level of 1 microgram per ml or 0.1 microgram per ml.

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<Dumonde refers to photomicrograph demonstrating delayed hypersensitivity and narrates over it>

Now, the next important feature is to design experiments to test their role in the cellular immune response and here is a typical reaction of delayed hypersensitivity in the guinea pig. And here you see the skin of the guinea pig with a variety of inflammatory cells infiltrating it. And the question arises as to how we can design experiments to test the role of lymphokines in this very complicated cellular and vascular response of delayed hypersensitivity and what relation this might bear to other phenomena of cell-mediated immunity.

<Dumonde to camera>

Now, in the first place, it's clear that lymphokines have many effects on macrophages – oh, about 20 different effects, I suppose, have been described in the last few years, from the inhibition of spreading to the increase of bactericidal activity and even to the activation of macrophages to make them cytotoxic in vitro for cultured tumour cells. But there are other cell types affected by lymphokines, for example, lymphocytes,

polymorphs, fibroblasts, vascular endothelium and tumour cell lines directly. Then a number of experiments have shown that lymphokines certainly have a part to play in vivo, for example, intradermal or intra-articular injection of lymphokines produces an acute inflammatory response, either in the skin or the joint, with exudation of protein and cells. If you inject these things into the skin together with mononuclear inflammatory cells, you mimic the time course and, to some extent, the histology of delayed hypersensitivity. If you inject them into the peritoneal cavity, you aggregate peritoneal exudate macrophages, and if you inject them repeatedly in the joint, you get a chronic synovitis which looks a bit like rheumatoid synovitis.

You'll find them in the afferent lymph draining a primary immune response, suggesting they have a role in immunological sensitisation. You find them in the efferent lymph, coming out of the lymph node, draining a tuberculin or delayed hypersensitivity response, suggesting they certainly are playing a part in the lymphoid system's expression of delayed hypersensitivity. You'll find them in the serum during desensitisation of cell-mediated immunity. You find them in synovial fluid in rheumatoid arthritis and in explant culture systems.

When you inject them into the lymph nodes themselves, as I shall show you, you get an acute tension of lymphocytes followed by lymphocyte activation in the node, and this is accompanied by adjuvant effects of lymphokines if you also give an antigen at about the same time. So, it's clear that there are a variety of activities of lymphokines which you can pick up in the intact animal. So the first point, which arises from a consideration of the effect of lymphokines on macrophages, is that they would appear to maintain the microbicidal, cytotoxic and adjuvant activities of macrophages. They might restrict the metastatic spread of parasitized macrophages from one part of the body to another. They might be recruiting or mobilising macrophages to sites of infection or inflammation and they might be interfering with virus replication in macrophages.

<Dumonde narrates over slide showing diagram of experiment set up to study delayed hypersensitivity>

Well, that's quite a lot to talk about and the second question is whether we can design experiments to study their role in delayed hypersensitivity in the intact animal. Here is a guinea pig – a diagram of a guinea pig in a box – and you can see that a skin fold has been raised on the back of the guinea pig and placed in front of a counting device. The guinea pig is filled up with radioactive serum protein and then a delayed hypersensitivity reaction is elicited in this skin fold for any inflammatory reaction, for example, for the intradermal injection of lymphokines.

<Dumonde to camera and then narrates over photograph of guinea pig in delayed hypersensitivity study. Then back to camera>

And now you can see the actual guinea pig in the box. This guinea pig is effectively asleep but remains in this happy state for about 24 to 48 hours, whilst we're looking at the skin reactions. Now, on this basis, having discussed the experiments to study the role of lymphokines in the expression of cell-mediated immunity, we're now going to go on to talk about their role in immunological regulation and this brings up two kinds of situations. The first is to study the traffic of lymphocytes, and the second is to study cellular cooperation between lymphoid cells, which, everybody now agrees, is fundamental to the immunological response itself.

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<Dumonde refers to and narrates over slides showing various images of lymphatic system, using indicator stick. Interspersed with talk to camera>

Now, the lymphatic system, which you can see on this slide in a figurative fashion, shows you rather nicely the two main classes of lymphocytes and figuratively the way the lymphocytes circulate around the system and enter tissues. Now, clearly this entry of lymphocytes from the vascular compartment into the extravascular compartment must involve interaction between recirculatory lymphocytes and vascular endothelial cells. And so we can now speak of a physiological lymphocyte endothelial interaction in which a normal lymphocyte would be expected to pass between or through the microvasculature and to re-emerge on the other side in a

relatively quiescent state <*next slide*>. And this interaction between lymphocytes and microvascular endothelium will take place normally and presumably involves some sort of control mechanism. What we would like to suggest is that lymphokines may be influencing or even perhaps regulating lymphocyte endothelial interaction and the traffic of recirculatory lymphocytes through microvascular endothelium, and one or two of the following pictures will show you this in more detail.

Now, when lymphocytes pass through the microvasculature of the lymph node <*next slide*>, they seem to do so in special segments of the microcirculation which are known as postcapillary venules. And this is a figurative diagram of Anderson's showing you the vascular endothelial cells as cushions and the lymphocytes in various stages of emigrating through the microvasculature and then moving off through extravascular connective tissue. This probably takes place to a limited extent in microvasculature outside the lymphoid system, <*next slide*> but one of the very interesting features of Anderson's work is that by scanning electron micrography he demonstrates small projections between the lymphocytes and the microvasculature endothelial cells, which resembled the kind of situation you would get if lymphocytes were being reversibly activated. The interaction between these two cell types is obviously of fundamental importance for lymphocyte recirculation, and if it involves lymphocyte activation, there's clearly a chance it may well involve the local production of lymphokines with a very short-term action on microvascular endothelium, which it will be the object of these experiments to study.

Now, perhaps the most figurative representation of what we could investigate is provided by an anatomical drawing of the lymph node <*next slide*>, which, as you can see, is comprised essentially of sets of lympho-endothelial tissue, and in the lymphoid system it would appear that lymphocytes and vascular endothelial cells, at sites of lymphocyte recirculation, may be actually forming biological tissues. Now, if we want to study this kind of situation in the laboratory, clearly we have to investigate the action, or the interaction, between lymphocytes and vascular endothelial cells. And what we want to show you now is some experiments which we've recently been doing in the Kennedy Institute to study this very phenomenon. We do this by taking pig aortic vascular endothelial cells and studying the interaction between pig

peripheral blood lymphocytes and these cultured vascular endothelial cells in monolayer culture.

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<Film clip featuring Dumonde in a laboratory, speaking to camera and narrating over procedures showing scientists culturing vascular endothelial cells, separating out lymphocytes from pig blood, and addition of lymphocytes to vascular endothelial cells. Sound quality poor with background hum of laboratory apparatus>

Firstly, the aortas are taken out of the pig at the slaughterhouse. Then the aortas are taken into the laboratory under sterile conditions and they are cut out effectively with scissors. They are then treated with enzymes, which enable the vascular endothelial cells to come off the inner surface of the aorta. The cells are collected and washed and then plated out as a cell suspension in the little chambers, and the cells grow out and they divide rapidly, and in 2 to 3 days they have covered the floor of the chamber. At this time you have what is effectively a blood vessel in culture, in a figurative sense, because you have a carpet of vascular endothelial cells covering the floor of a small chamber.

The second stage of the experiment is to take pig blood and to make lymphocytes from it. This is done by a technique called differential sensitisation[?] and sedimentation. The blood is taken and a special sedimenting agent called dextran[?] is added to it. Gradually, the red cells sediment away from the white cells. To the top layer is added another sedimenting agent called [unclear word]. And together this procedure separates out the lymphocytes in a purified form. And, in fact, the suspension we have here, which we've just counted, is comprised of 99% of small round cells of the core blood lymphocytes.

The first stage of the experiment is, therefore, to add the lymphocytes to the vascular endothelial cells in culture. And now, we're going to draw up these lymphocytes in this tiny capillary tube. And here they come, up into the capillary tube, okay, and

we're going to pull the capillary tube out, like that. Then we're going to plug the end of it with wax and wipe it and put it in another little tube to spin down.

<Scientist, off-screen>

Beautiful.

<Dumonde>

They're all right?

<Scientist, off-screen>

[Unclear words] at least, my memory's not as good as it was.

<Dumonde>

No, they're beautiful.

<Scientist, off-screen>

They look alright to me.

<Dumonde continuing narration over film>

Well, what we are now going to do is to cut these little capillary tubes at the cell interface and we're going to mount them, rather like a macrophage migration experiment, but this time onto the floor of a tissue culture chamber which contains the carpet of vascular endothelial cells. The lymphocytes are going to migrate out of the ends of these capillary tubes and they are going to simulate, in a figurative sense, the passage of lymphocytes across the vascular side of microvasculature endothelium. What actually happens in this situation is that as the lymphocytes move across, they also burrow in between the vascular endothelial cells, and after

overnight culture of mixed lymphocytes and vascular endothelial cells in this fashion, […]

<End of film clip. Cut to Dumonde in studio to camera>
[…] what you now see is effectively tissue formation between lymphocytes and vascular endothelial cells.

00:24:28:11

<Dumonde refers to photomicrograph and narrates over it>

This is the result of that three-stage experiment. Under the phase-contrast microscopy, you can see that the nuclei of the vascular endothelial cells are lying in the same plane as the nuclei of the lymphocytes which are migrating into them. And what we have effectively done is formed a lympho-endothelial tissue in culture. This means that having the system for studying lympho-endothelial tissue formation, we can now alter the lymphocytes, we can alter the vascular endothelial cells, and we can alter the medium in which lymphocyte endothelial interaction is taking place.

In some more recent experiments, we have found that lymphokines can actually damage vascular endothelial cells.

<Dumonde to camera>

So, we would have to say that if lymphokines are released in the normal process of transmural migration, then they have a very short-term and possibly transient effect on vascular endothelium, perhaps assisting the passage of recirculatory lymphocytes across microvascular endothelium. Now, clearly if this process takes place, this represents a most important potential physiological role for the lymphokine system in traffic regulation. And so we have coined yet another expression to convey this physiological idea of lymphocyte endothelial interaction. We have coined the term lympho-endothelial system to describe a body system concerned with regulating the traffic and activation of recirculatory lymphocytes through microvasculature

endothelium, and that this body system, or LE system, must involve close interaction between lymphocytes and endothelial cells. And we would like to suggest, at short-term and short-distance, lymphokine production may be involved as physiological event, and there is some mechanism for switching off potential lymphocyte activation which might occur during this process. If you can recall those electron micrographs of Anderson's, you will remember that the projections on the lymphocytes look as if the lymphocytes were being transiently activated.

<Dumonde narrates over animated diagrams of lymphocyte migration>

Now, how may this process go wrong in pathological situations? Clearly, the process of control of lymphocyte migration could go wrong, either because of something wrong with the lymphocyte, for example, an irritable or activated lymphoid cell population in the vascular space; or, secondly, something wrong with the blood vessel endothelium, perhaps infections or toxins, viruses localised inside vascular endothelium; or, thirdly, something wrong with the plasma milieu in which lymphocyte-endothelial interaction is taking place, for example, the presence of vasoactive mediators, circulating immune complexes or abnormal hormone levels. Now, in this situation, one might expect to get abnormal lymphocyte-endothelial interaction, and many pathological consequences of this could be the emergence and persistence of activated lymphocytes immediately outside the microvasculature – with local activation, with proliferation and with persistent lymphokine and immunoglobulin production; the sort of situation, for example, that one sees in the tissue lesions of rheumatoid disease.

<Dumonde to camera>

And so here is a pathological role, if you like, in which lymphokines may be involved in the pathology of the lympho-endothelial system and we have to find some way of investigating how lymphocytes are normally kept in a happy and depressed state during lymphocyte migration.

<Dumonde refers to diagrammatic slide of prostaglandin E action on lymphocytes>

Now, one of the possible mechanisms is viewed on this slide. We know that mononuclear phagocytes, as well as vascular endothelial cells, can produce E-type prostaglandins, and it is known from culture experiments that prostaglandins of the E series are capable of suppressing lymphocyte activation mechanisms, for example, lymphocyte transformation and even lymphokine production. So there may be a feedback system here in which prostaglandin production by vascular endothelial cells and or associated macrophages may normally suppress the activation of recirculatory lymphocytes at the time of transmural migration.

<Dumonde to camera>

Investigations of this feedback system will obviously require experiments in which lymphokines are studied for their effects on the vascular endothelium, and the auto-pharmacology of this system explored.

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Now, having discussed the potential role, an important role, of lymphokines in lymphocyte recirculation, let us now turn to what might happen in the lymphoid system itself when lymphokines are actually injected. I want to show you very briefly some experiments which we did a few years ago on the intra-lymphatic injection of lymphokines. When guinea pig lymphokines are injected into the afferent lymphatic of the guinea pig ear, the auricular lymph node of the guinea pig reacts very quickly by enlarging to 3 or 4 times its size within 24 hours, together with an acute retention of lymphocytes in the paracortex and plugging of corticomedullary sinuses with small lymphocytes. This is followed by a mitotic activation of lymphocytes in germinal centres together with follicular hyperplasia and, at the same time, there is an adjuvant effect on local antibody production if antigen is injected into the same regional drainage area.

<Dumonde refers to photomicrographs of lymph nodes and diagrammatic slides, interspersed with talk to camera>

Now, this slide shows you the blown-up lymph node at 24 hours, and this is a situation in which the marginal sinus of the lymph node has been outlined with carbon. The lymphokine has been injected 24 hours previously, and you can see that the thing looks like an ovary with a lot of Graafian follicles; a greatly distended lymph node with follicles pushed to one side.

Now, the next slide, which is a histological preparation of this, shows you how the paracortex of the lymph node is distended in a wedge-shaped fashion at the site of the drainage area of the lymphatic which is coming in, into which the lymphokine has been injected. And you can see how the follicles have been pushed aside, there and here. This great retention of lymphocytes takes place within 24 hours and is equivalent histologically to the kind of cell-mediated immune responses that one sees in lymph nodes after days or even sometimes weeks of response to an infection or a graft.

A day later the lymph node starts to develop follicular hyperplasia and you can see now, and this slide is taken at 48 hours, that there is persistent paracortical distension in the centre of the lymph node, but round the outside now the follicles have started to become hyperplastic, and, in fact, autoradiographs of these follicles show mitotic activation of cells in the germinal centres.

This is a very dramatic effect on the lymph node itself and the phenomenon of paracortical distension can be explained simply by a sort of migration-inhibition-factor-like activity of the lymphokine on the paracortico-medullary junction. If this were so, then lymphocytes which were passing through the motorways of the lymph node would be effectively held up. And at the bottom of the slide, you can see how this might result in ballooning of the paracortex with small lymphocytes. The phenomenon of paracortical distension during the induction of cell-mediated immunity is then viewed in terms of an intra-lymphatic effect of lymphokines on the lymph node.

<Dumonde to camera>

Now, quite clearly this situation where lymphokines are using the architecture of the lymphoid system raises the question as to how they might operate physiologically. There are three principle possibilities. Lymphokines might affect lymph node architecture by acting on macrophages, for example, those lining the paracortical sinuses and blocking up lymphocyte traffic through the node, or they might be acting on lymphocytes to have a mitogenic effect which may be relevant instead of the cooperation. Or thirdly, they might be acting on the microvasculature of the node, and we've discussed how they act on vascular endothelial cells, and in the lymphatic system, in the node itself, this might well result in the selective accumulation of lymphocytes across the microvasculature. In these situation, then, the lymphokines could be viewed as making good use of the architecture of the lymph node and we can say, figuratively speaking, that in view of the number of cells which are retained in the node, it would appear that the lymphokines are having an amplification effect about a million-fold when introduced into the lymphoid system. You might say this was a very efficient use by the body of its own lymphokines.

<Dumonde refers to diagrammatic slide and narrates over it>

Now in terms of cellular cooperation, there are various ways in which, well, you could really draw a diagram as to how lymphokines might be operating. You could view them, for example, as to coming out of T-cells and acting directly upon B-cells to stimulate antibody production. Or secondly, you could view the possibility that macrophages might be involved in this, and you could say that the lymphokines were acting on macrophages to help the macrophages to process antigen which was stimulating B-cells better than it would do otherwise. Or thirdly, you could view lymphokines as promoters of cytodifferentiation of antibody-producing cells in this way: you could say that lymphokines were acting on antibody-forming precursor cells of any specificity which in the presence of the specific antigen to which these B-cells were, if you like, sensitised or programmed, then there would be accelerated

cytodifferentiation of these antibody-forming precursor cells in the presence of their own antigen towards a valuable and useful immunoglobulin.

00:35:30:18

<Dumonde to camera>

Now, this thesis, which is very prevalent in experimental immunology, views lymphokines in a very unidirectional sense and research is now developing on the possible role of some lymphokines as suppressor agents in limiting cellular cooperation because, obviously, when presented with any given antigen, one doesn't want to turn into an uncontrolled immunological response. And very recently lymphokines have been discovered which would appear to switch off lymphocytes, and so we have a homeostatic system in terms of cellular cooperation.

What I've been discussing, then, is the role of lymphokines in immune regulation, and it therefore appears that these can act potentially as traffic regulators of cells crossing the microvasculature and within the lymphoid tissue, and that they might be acting also as cell co-operators. And so lymphokines in immune regulation could be viewed as controlling cell traffic through the regional lymphoid system in the microvasculature; facilitating cooperation between lymphoid cells during immune induction and expression; possibly the overproduction of lymphokines might underlie some aspects of high dose tolerance, or desensitisation of delayed hypersensitivity, or the production of non-specific immunoglobulins; and maybe as a long shot, we might suggest that lymphokine production and action maintains, if you like, the lymphoid system up to scratch. It might maintain lymphocyte hypermutability, to use the semantics, despite the tendency for phenotypic restriction during the immunological response.

Now, having said all that, what about the clinical significance of lymphokines? Clearly there are many experiments that one can do in the lab, but we have to come, at some stage, to assessing their potential role in man and in human disease. In the first place, one could say that there are three main expressions of the cellular immune response, allergic inflammation, immune surveillance, and adjuvant or

regulatory expressions. You could sit down in an armchair and you could arrange the thirty-two different lymphokine factors, as they might be called. in any combinations you like, and suggest, for example, that the cytotoxic and chemotactic and macrophage adherent factors might be important in inflammation, that macrophage activating and interferon-like factors might be important in immune surveillance, and that mitogenic and lymph node activating activities may be important in immune regulation and adjuvants, but there's really more to it than that. And that is the problem of designing experiments to really assess the clinical significance of these materials.

In the first place, we wish to get together with the clinicians to measure mediator activity against defined clinical criteria. And this is one of the big problems. Secondly, we need to develop quantitative and parallel bioassays for these different lymphokine activities and to develop in the process biological standards of animal and eventually human lymphokines in order to assist the development of this work. And then we have to go on to study the heterogeneity of production of lymphokines and to see if there, in fact, is a molecular pathology of the lymphocyte mediator system that can be useful in diagnostic criteria of immune dysfunction. This process will have to involve the isolation, purification and characterisation of lymphokines, and eventually the development of agonists and antagonists of mediator production. And eventually we would hope to recognise and classify abnormalities in lymphokine production or responsiveness to lymphokines by man to develop diagnostic reagents to assist the quantitation of lymphocyte function in clinical immunology, and eventually to develop therapeutic agents to control defined aspects of the immunological response in man, which, after all, is what immunology, I suppose, is all about even in 1977.

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Well, that's, I suppose, a rather tall order and in terms of the progress of clinical immunology and its application to situations such as autoimmune disease, cancer and microbial infection, these being some of the three principal areas in which progress, it is hoped, will be made in the last quarter of this century, we might view lymphokines as being the property of recirculatory lymphocytes, which enables them

to express their potential on the kind of cells and the kind of environment in which they spend most of their time. Now, recirculatory lymphocytes presumably spend about 75% of their time, or even 80% of their time, coursing through connective tissue. And in view of the fact that they have a molecular system available for exerting short-term effects on all the four major cell types found in connective tissue, that is to say macrophages, lymphocytes, fibroblasts and vascular endothelial cells, we could at least suggest that diseases such as inflammatory connective tissue diseases may not be actually diseases of connective tissue themselves but diseases of the lymphocyte or abnormalities of lymphocyte regulation – of that kind of lymphocyte subpopulation which actually moves through connective tissue, the recirculatory lymphocyte. Now, this is clearly a field for the future to interpret disease states according to the pathology of lymphocytes and the pathology of lymphokine production.

<Dumonde refers to diagrammatic slide and narrates over it>

And finally, I would like to turn to the problem of the measurement of lymphokines. Quite clearly, in order to make these interpretations, we must be able to measure their biological activity. This slide shows four little tubes with microphages migrating out of them. And figuratively speaking, the more lymphokine you add, the less the macrophage migration, so you could turn this into a sort of bioassay and develop biological standards of these lymphokines against which measurements of mediator activity could be made.

<Dumonde refers to camera>

Now, quite clearly bioassays can be developed according to classical pharmacological and endocrinological criteria which can be made to turn themselves into situations which measure precision of assay as well as a reproducibility of assays. Now, we've been doing this during the last five years, and we have developed situations in which these assays are reasonably precise and that we can measure four of the major lymphokine activities with a certain amount of precision and reproducibility. Now, we've been delighted to involve some other laboratories in

inter-laboratory study of the usefulness of lymphokine standards between different laboratories.

<Dumonde refers to slide showing standardised freeze-dried lymphokine sample and narrates over it>

The people at Hampstead, at the National Institute, were very kind and freeze-dried large batches of lymphokines for us, and here you see a picture of one of the early standards which is a standard guinea pig lymphokine, which we have found to be quite useful in the assessment of cellular hypersensitivity between different laboratories and within given laboratories.

<Dumonde to camera and then refers to diagrammatic slide and narrates over it>

So this is the way the field is developing and one can undertake complicated comparisons of lymphokine activities; for example, here is a 3-dimensional plot of inflammatory activity on the vertical axis, lymphocyte mitogenic activity on the horizontal axis, and macrophage migration inhibition activity on the oblique axis. Of six different lymphokine preparations against a standard, which you can just about see starred with the coordinates 1,1,1 in the middle of the 3-dimensional diagram, each block represents the three separate lymphokine activities of that particular lymphokine, and the fact that these blocks are quite distinct from one another means that parallel bioassay has shown us that the inflammatory macrophage migration and mitogenic activities can't be due to the same molecular substance.

<Dumonde to camera>

Now, this is very clearly important in the development of the lymphokine field that it is possible to manufacture large batches of lymphokine standards and to develop parallel bioassays, which can be improved and whose precision can actually be stated.

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So, I suppose, in conclusion, this field of lymphokines will move very rapidly in the next two or three years. One would say that the lymphocyte activation products, as a whole, could be viewed as mediators of cellular immunity in the sense that there are a large number of biological activities which mimic various aspects of the cellular immune response. We've seen that it is a reasonable working hypothesis that lymphokines are involved in both expression and regulation of lymphoid cell activity, in mediating cellular cooperation and in regulating the traffic of lymphoid cells through, possibly, the microvasculature and through connective tissue within the lymphoid system itself. They presumably represent this indirect pathway for T-cell action which we talked about at the beginning of our discussion, and indeed their activities would be ascribable to one or more molecular substances, the exact number of which will depend upon the application of brighter biochemistry with brighter bioassay. This, in fact, is an interdisciplinary or multidisciplinary problem and requires the getting together of scientists with backgrounds in biochemistry, immunology, pharmacology and, if you like, microcirculatory physiology to sort it all out – a tough problem, perhaps one which we can expect will be resolved to our satisfaction in the latter quarter of the 20th century.

Now, of course, it's no news that people have been studying auto-pharmacological mediators for 50 or 60 years. And one of the most famous investigators of all time was, of course, Sir Henry Dale, and he and his colleagues established a set of criteria which came to be called the Dale criteria for assessing the physiological status of proposed chemical mediators in the organisation of the autonomic nervous system and eventually, if you like, in the organisation of the endocrinological system. And we could develop Dale criteria for considering lymphokines as physiological mediators of the immune response or the cellular immune response. We would have to show that there were mechanisms for production, destruction and inactivation in situations where mediator production in vivo appear to correspond with the definition of lymphokines in vitro, that the lymphokines themselves would have to mimic the physiological process which they are supposed to mediate, and they would have to be experiments which showed that the specific depletion and specific antagonism

would abolish not only the lymphokine production and action but also the physiological event or that component of the cellular immune response which the lymphokines was supposed to mediate. The application of parallel bioassay, together with biological standards of lymphokines, may be expected to resolve some of these difficulties just as it has done in other areas of biological chemistry.

Now, in conclusion, I think it is very interesting to view the field of lymphokines as pointing to a more general field of the molecular pharmacology of cell-mediated immunity. There must clearly be physiological systems to generate lymphokines and other mediator molecules. There will be physiological systems to respond to them. We can potentially investigate not only the mediators themselves but also the cellular receptors for mediators. We can apply methods of biological measurement to the field and potentially we can assess its clinical significance in terms of the molecular pathology of the lymphokine system.

Now, it's nearly 1981, and in 1881 the late Thomas Henry Huxley used the following words in describing what he thought was the developing field of pharmacology: sooner or later, he hoped that the pharmacologist would supply the physician with the means of effecting any physiological element of the body; he hoped that it would become possible eventually to introduce into the economy a molecular mechanism which like a molecular torpedo would find its way to some living elements and cause an explosion among them leaving the rest untouched.

I think, when we consider the development of research on the lymphokine system and when we consider the complexity of the lymphatic system as a whole, with all the problems of lymphocyte traffic and cell cooperation that this engenders, we have a hard task ahead of us, and I don't think that even by 1981 we're going to find the answer to this molecular torpedo. However, its clinical significance is emerging and I would hope that, in this discussion, I have conveyed a little of the excitement that this whole field is engendering in the minds of experimental and clinical immunologists.

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