Wellcome Film Project

Cellular Mechanisms in Delayed Hypersensitivity, Part 1

The Scientific Basis of Medicine

Presented by Prof John Turk.

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Introduced by Dr Ian Gilliland.
Produced by David Sharp.

Black-and-white
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<**Opening titles**>

<**Gilliland to camera** >

Professor Turk is now Sir William Collins Professor of Pathology at the Institute of Basic Medical Sciences. He was previously Reader in Immunology at the Institute of Dermatology. He is the author of many well-known papers on immunological subjects and has made particular studies in the cellular mechanisms in delayed hypersensitivity. Professor Turk.

<**Turk to camera**>

A hypersensitivity reaction is the reaction which occurs in the tissues as a result of the release of pharmacological agents during the process of rejection of a foreign antigen.

Now there are 2 types of hypersensitivity reaction – those which are mediated by humoral antibody and those which we call cell-mediated humoral reactions. Humoral antibody reactions are of 2 further types – that which we call anaphylactic and that which we call the Arthus reaction. And we refer to humoral antibody reactions as immediate-type hypersensitivity and cell-mediated hypersensitivity reactions are delayed as a function of the time that they take to develop.

Now, the prototype of all delayed hypersensitivity reactions is the tuberculin reaction and this can be compared with the Arthus reaction which […]

<**Turk narrates over table comparing response times of different hypersensitivity reactions**>

[…] takes 4-8 hours to develop because the tuberculin reaction, being delayed, takes 24-48 hours to reach maximum response.

<**Turk to camera**>

These reactions can also be distinguished by their macroscopic appearance.

<**Turk narrates over series of tables comparing appearances of different hypersensitivity reactions**>

The tuberculin reaction is associated with erythema and induration, whereas the Arthus reaction is an oedematous reaction with a considerable amount of haemorrhage due to the local vasculitis.

Microscopically these reactions differ – the tuberculin reaction has a mononuclear cell infiltrate whereas the Arthus reaction is associated mainly with a polymorphonuclear leucocyte infiltrate.

<**Turk to camera**>

Furthermore, the basic difference between these reactions can be found when one attempts passive sensitisation.

<**Turk narrates over table comparing passive sensitisation of different hypersensitivity reactions**>

Because the Arthus reaction can be transferred passively by serum-containing antibodies, the immunoglobulins. But the tuberculin reaction cannot be passively transferred with serum but needs suspensions of sensitised lymphocytes derived from lymphoid tissues.

<**Turk narrates over series of tables listing causes of delayed hypersensitivity**>

Now, delayed hypersensitivity to microbial protein antigens can be produced by a number of different substances. Tuberculin is the prototype that we've been discussing but there are also other soluble antigens such as the reaction that occurs to diphtheria toxoid which results in the "pseudo" Schick reaction which can make the interpretation of a Schick test difficult. Then we have the reaction of immunity to vaccinia virus and we also have reactions to fungal soluble antigens such as histoplasmin and coccidioidin; and to protozoal antigens as in the Montenegro test for Leishmaniasis.

One may get delayed hypersensitivity to heterologous serum proteins following small doses of foreign antisera and these reactions are called Jones-Mote reactions and we shall be talking about this in my next talk. Then we come across some of the more common forms in which delayed hypersensitivity can take – we're all familiar with the delayed hypersensitivity reaction that occurs as the result of an insect bite, and we are familiar with contact sensitivity to simple chemicals which is the cause of a major proportion of industrial morbidity.

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< **Turk to camera** >

These all are skin reactions of the delayed hypersensitivity type. But delayed hypersensitivity can undergo, can take part in a number of other biological phenomena.

< **Turk narrates over table listing information on other types of delayed hypersensitivity reactions**>

For instance the homograft reaction, the rejection of skin allografts, is mediated by cell-mediated immune processes, the same processes that produce delayed hypersensitivity in the skin. And the same processes also underlie the mechanism that we have to reject certain micro-organisms which have a facultative intracellular behaviour. Examples of these among the bacteria are mycobacteria and brucella, and it also underlies the rejection processes of fungi, certain protozoa such as leishmania and a wide range of viruses.

Finally, the same cell-mediated immune processes that play a role in delayed hypersensitivity can collaborate with humoral antibody in the development of organ-specific autoimmune lesions such as those which occur in thyroiditis, in cephalitis and adrenalitis.

< **Turk narrates to camera, stands, walks to large chart displaying illustration with movable parts which shows phases of delayed hypersensitivity. Turk uses indicator and manipulates order of parts on chart**>

Now, as all immune reactions, cell-mediated immune reactions can be considered to be like a reflex arc with an afferent limb and an efferent limb. We have the afferent limb on one side of the regional lymph node and the efferent limb on the other. And the first phase of recognition occurs out in the periphery and then we have a phase of proliferation in the central lymphoid tissues, and a phase of rejection of that which is foreign which results in the manifestation of the inflammatory reaction.

Now, all immune reactions need lymphocytes and the lymphocyte is the cell which recognises that which is foreign, and one may take a lymphocyte and it recognises the antigen as foreign in the periphery and then it can pass down to the regional lymph node where it finds the right milieu for proliferation and for differentiation.

And from that point onwards we have the specifically sensitised lymphocyte which can pass out into the periphery and react with antigen, or can pass down to other areas of lymphoid tissue where it can undergo further proliferation. And from these 2 points one may then get lymphocytes reacting with antigen in the periphery again and producing the hypersensitivity reaction.

<**Turk narrates over table listing stages of hypersensitivity reaction**>

There is reaction of the lymphocyte with antigen and as a result of this there's the release of soluble substances, the pharmacological mediators, which have a direct effect on macrophages and can cause the initiation of the inflammatory reaction.

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<**Turk narrates while standing by series of charts, diagrams and illustrations, uses indicator**>

Now, lymphocytes are derived from stem cells which may be found in the bone marrow. These stem cells may be influenced by the thymus in which case we call them T lymphocytes; or they may be influenced by the bursa of Fabricius in the chicken or its equivalent in mammals to become what we call B lymphocytes.

T lymphocytes are the cells which, when they are stimulated with antigen, proliferate and differentiate into sensitised lymphocytes which take part in cell-mediated immunity and produce delayed hypersensitivity reactions.

The B lymphocytes on the other hand respond by proliferation and differentiation to antigen to become plasma cells which make immunoglobulins and form the humoral

antibodies that take part in immediate hypersensitivity reactions. We know there is some interaction between T lymphocytes and B lymphocytes and that the T lymphocyte response can augment and amplify a B lymphocyte response. Similarly, a B lymphocyte response occurring at the same time as a T lymphocyte response may modulate the T lymphocyte response.

Now, these 2 cell populations may be found in defined areas of the lymphoid tissue, and this is illustrated in this next picture which is a section of a lymph node. The B lymphocyte areas are the germinal centres, their marginal cuff of small lymphocytes, a narrow rim of small lymphocytes round the edge of the cortex and the lymphocytes of the corticomedullary junction, and the medullary cord where one may find plasma cells being formed. The other rather diffuse area of the cortex we call the paracortical area of the lymph node, and this is the T lymphocyte area and is depleted of T lymphocytes in neonatally thymectomised animals and animals treated with antilymphocyte serum. It is also depleted in children born with an absence of the thymus in Di George syndrome or the Nezelof syndrome.

<**Turk, standing, to camera**>

Now, we can illustrate this departmentalisation of lymphocytes by looking at actual histological sections and we can see how the different cell compartments respond in a cell-mediated immune reaction as opposed to a humoral antibody reaction.

<**Turk narrates over series of slides of lymph nodes, uses indicator, camera returns briefly to Turk's face between slides**>

Now the first slide, which is of a normal lymph node which has not been stimulated at all. We may see in this slide a narrow rim of cortex which are the B lymphocytes, the lymph follicles which are B lymphocyte areas, the paracortical area which is the T lymphocyte area and here we have the medulla and the corticomedullary junction. This is the appearance of a lymph node of an animal that has received very little stimulation.

Now, one can produce, as I said before, depletion of T lymphocyte areas and by a number of ways and this is shown in the next picture which is of a lymph node where the T lymphocyte area here, the paracortical area, has been depleted by treatment with antilymphocyte serum. On the other hand, the germinal centres and the marginal cuff of small lymphocytes are normal, and the narrow rim of cortex is undisturbed. So also are the cells at the corticomedullary junction and in the medullary cord. We can look at the depleted area, here, in the next picture at higher power.

Now this is the higher powered picture showing the paracortical area depleted of T lymphocytes and all one sees are the background reticulum cells. And if one moves further down towards the medulla, shown in the next picture here, one can see the depleted paracortical area and the beginning of the B lymphocytes at the beginning of the corticomedullary junction and where they are transforming, differentiating into plasma cells. And if one looks in this area down here at a little bit higher magnification one can even see the typical appearance of the plasma cells. Here's one with a cartwheel nucleus right up there, and there's a typical plasma cell down there. These cells, the B lymphocytes and the plasma cells, are unaffected by processes where one gets depletion of the T lymphocytes.

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Now, in a B lymphocyte response, a pure B lymphocyte response, one gets massive germinal centre formation and the proliferation of plasma cells in the medulla. This is a lymph node responding to the injection of a T-independent antigen pneumococcal polysaccharide. Up here one can see a paracortical area but it is not particularly well developed and there's no evidence of lymphocyte proliferation within this paracortical area. One can see marked activity, however, at the corticomedullary junction and this is shown in the next picture where we've got the corticomedullary junction up at the top and then the medullary cords running down here where one has plasma cell proliferation.

The next picture I'd like to show you is one of an electron micrograph of a plasma cell because I'd like to show you the difference between a plasma cell and the cells that are proliferating in the paracortical area in delayed hypersensitivity. Now this is the plasma cell and the point that I want to make is that in the electron micrograph there is a very marked network which we call endoplasmic reticulum on which the ribosomes can be found and where the protein antibody is being manufactured for export.

<**Turk to camera**>

Now, that is the picture that we find in a pure T-independent humoral antibody production. And now I'd like to show you a series of events which may be found when one produces a relatively pure cell-mediated immune reaction as following a contact sensitisation.

<**Turk narrates over series of slides of lymph nodes, uses indicator, camera returns briefly to Turk's face between slides**>

This next picture is of a lymph node 4 days after sensitisation with a contact agent and here one sees no proliferation of lymphocytes in the lymph follicles, the B lymphocyte areas, and the other B lymphocyte areas down here, which are at the corticomedullary junction show no proliferation. But this pale area here is the site of massive proliferation of lymphocytes and this is the paracortical area responding by proliferation of T lymphocytes.

Now we can look at this area at higher power in the next slide and we can see here these large blast cells, the cells that the lymphocyte transforms into prior to division. And down here one can actually see a mitosis. Now, evidence that these cells are proliferating into other small lymphocytes can be found from studies using the radioactive trace labelled thymidine, we use tritiated thymidine, and we inject the animal with the tritiated thymidine. Usually 1 hour or 24 hours before examining the tissue. Now, if one looks at the lymph node 1 hour after the injection of tritiated thymidine, at the peak of cell proliferation, one can see that these blast cells have all

taken up tritiated thymidine, but the small lymphocytes are free of the DNA precursor. And then 24 hours later one sees that the thymidine is mainly in small lymphocytes and the grain count in the small lymphocytes is half of the grain count found in the blast cells 24 hours previously. And this would indicate that these are the cells that the blast cells have divided into.

These small lymphocytes in ordinary preparations don't look any different from other small lymphocytes in other tissues. One can look at these cells also by using electron microscopy. And this picture is of one of these blast cells under the electron microscope. And the point I want to make is that these are strongly basophilic cells, like the plasma cells, but they differ from the plasma cells in that in the cytoplasm there's none of the endoplasmic reticulum but it is full of polyribosome granules where the protein is being made, not for export, but as a part of the process of cell division. And this is shown at higher power in this picture where we see the polyribosome rosettes and one can also indicate that these are the cells that are undergoing division because one can find cells of this type in the prophase or in metaphase.

And this is shown in the next picture where we have one of these cells in division and you can see the chromatin breakdown as part of the early stage of cell division, and the many polyribosome granules in the cytoplasm.

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Now the type of cell that these blast cells divide into can also be examined under the electron microscope and we can see 2 types of cell and this is shown in this next picture where we have a small lymphocyte which is typical of all lymphocytes in their resting phase and also an intermediate cell; a cell which is intermediate between the small lymphocyte and the blast cell has many of the features of the blast cell such as the polyribosome granules and may also have a Golgi apparatus. Now, one sees both of these cell types in the lymph node following the proliferative phase and both these cell types may be involved in the sensitisation process.

<**Turk to camera, then walks to a wall charts, uses indicator; graph shown in close up while Turk narrates**>

Now, the process of proliferation takes 4 days to develop and it is on the following 4 days of proliferation that the animal shows the first signs of sensitivity. One can see in this chart the process of proliferation as is shown by the development in size of a lymph node draining an area of application of a contact sensitising agent. The lymph node increases in size until the 4th day after sensitisation and then after that rapidly decreases in size. And there is a parallel increase in the size of the paracortical area. Now, sensitivity develops between the 4th and 5th day and it is as though a plug had been put in the lymph node and an influx of cells, and also as a result of cell proliferation, there is an accumulation of lymphocytes in the regional lymph node. But between the 4th and 5th day it is as though the plug got pulled out and the sensitised cells passed out into the periphery to be able to take part in an inflammatory reaction when they react with antigen and also to maintain the sensitisation process in other areas of lymphoid tissue.

<**Turk narrates over further graph, showing cell concentrations in lymph nodes**>

Now the proliferation can also be illustrated by looking at the proportion of blast cells in the draining lymph node. This also increases up to the 4th day after sensitisation which is again the day before the animal shows sensitivity, the day before the animal has lymphocytes in the periphery capable of reacting with antigen.

Now, the 4th day is critical because if one removes the regional lymph node before the 4th day, one can block the development of generalised sensitivity and the lymph nodes taken after the 4th day contain lymphocytes which will transfer reactivity to recipient animals.

<**Turk narrates over diagram**>

We can recapitulate these lymph node changes in this diagram here. Here we have a lymph node where there has been no immune response and this may be stimulated or geared over to develop a cell-mediated immune response by the application of a contact sensitising agent, and then one has the development and increase in size of the paracortical area without any changes in the B lymphocyte areas. One can gear over to a pure B lymphocyte response by the injection of a T-independent antigen such as pneumococcal polysaccharide. In this case one gets no development of paracortical area, no proliferation of T lymphocytes but one gets changes in the general centre formation in the B lymphocyte areas and proliferation of plasma cells in the corticomedullary junction and in the medulla.

But with most antigens, both protein antigens, bacteria, one gets a mixed response, one gets not only T lymphocyte proliferation but also B lymphocyte proliferation. And under these conditions one may get proliferating T lymphocytes helping to augment a humoral antibody response and proliferation of B lymphocytes in some situations helping to modulate or damp down a T lymphocyte response.

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< **Turk returns to large chart, from earlier, displaying illustration with movable parts which shows phases of delayed hypersensitivity. Turk uses indicator and manipulates order of parts on chart**>

Now, so, if we go back now to the board here, we see what we've dealt with – the recognition of antigen by lymphocyte. The lymphocyte then comes down to the lymph node and we've seen the different type of immune responses that can develop and how the response in delayed hypersensitivity differs, particularly in the response from humoral antibody production; that these lymphocytes, once they've proliferated and differentiated into specifically sensitised lymphocytes pass out into the periphery where they're capable of reacting with antigen, or passing down into other areas of lymphoid tissue where they're capable of maintaining the sensitisation process.

In my next talk then we will talk about what happens after the specifically sensitised lymphocyte has reacted with antigen and what agents it produces to produce the, or to show, the inflammatory reaction in the periphery.

<**End credits**>