Immunity

Uptodate: Immunology, 6

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University of London Audio-Visual Centre, 1974.

Produced by Peter Bowen and David Sharp.

Made for British Postgraduate Medical Federation.

Black-and-white

Duration: 00:32:18:07

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<Opening titles>

<Dr AC Allison to camera, then over chart showing different forms of immunity against infectious disease>

This talk will be concerned with immunity against infectious disease. There are different aspects of this and these are shown in the first chart.

Here we compare the difference between the innate immunity which certain individuals have against infection, as contrasted with acquired immune responses. In relation to the innate immunity, we know that viruses will only grow in certain species, for example, if we take polio virus, which is a well-known pathogen for humans, this will not grow in non-primate cells – it grows readily in the cells of monkeys but not in those of bovines, for examples, or chickens and so forth. And we know that there are many other such examples so this is an important aspect of immunity: the limitation of growth of viruses in other organisms. One other example which we've been interested in are the genetic differences in haemoglobin types which are present in

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the red blood cells. And we know that, for example, one is called sickle cell haemoglobin and this produces abnormal red blood cells which are common in Africa and other parts of the world. And these cells, these red cells, resist the growth of malignant ocean malaria parasites, so this is another example of genetically controlled innate immunity against certain types of infections.

Now, in addition to this there are a whole series of acquired responses which are involved in resistance against infection. Now some of these are relatively non-specific and the best known example in the case of viruses is interferon. Now interferon is produced when cells are infected with viruses; this is released from the cells and protects other cells against infection by viruses. Now, the challenge virus, the second virus, can be either the same virus or another one and this is different from the antibodies which are produced as a result of infection because these are entirely specific; they confer protection only against the organism which raised the antibodies in the first place, or elicited their formation, and not against others. But in relation to antibody formation we have then a host of responses which are due to the immunocompetent cells, or lymphocytes, to their proliferation. This is in part, of course, antibody formation, but in addition to this we know that T-lymphocytes are involved in a variety of responses which play a role in resistance against infectious disease.

<**Allison over table showing interactions**>

Now we have then, in the course of considering immunity against infection, to consider four main components. We have to consider antibodies and the role which they play in the limitation of virus infections and certain bacterial infections and, in particular, the pyogenic organisms which seem to be particularly susceptible to inactivation by the interaction of antibodies and complement and phagocytic cells. Then we have to consider complement components themselves. The phagocytic cells which cooperate in this particular system, not only in immunity against bacteria, as everybody knows, but also in immunity against viruses.

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Now the phagocytic cells which are especially important in immunity against viruses are mononuclear phagocytes. We know that in virus infections you commonly have a considerable infiltration of mononuclear cells, that is to say, lymphocytes and macrophages. You have perivascular infiltrates in the brain where you have encephalitis, and in the liver where you have hepatitis, and in other organs. And the interactions of antibodies and complement in phagocytic cells are just as important in immunity against viruses as they are in immunity against pyrogenic bacteria. Then in addition we have cell-mediated immunity which is important in immunity against intracellular growing bacteria, that is to say, tubercle bacilli and others, which will be discussed by Professor Turk. Cell-mediated immunity is also important in resistance against certain viruses as we shall see a little later.

Now in order to analyse the action or relative roles of antibodies, complement components and different cell types, one can either look at experimental animals or at human patients with specific deficiency syndromes. So what we try to do is to eliminate one or other of these components of immunity and then to replace them, selectively, as far as we can.

<**Allison over table listing the role of antibodies and complement**>

Now, it's possible to produce immunodeficiency experimentally in animals, either by x-irradiation or by treatment with an immunosuppressive drug, of which cyclophosphamide is a commonly used example. Now, cyclophosphamide is very useful because it markedly diminishes antibody formation against viruses and against a wide range of other organisms; in fact, it reduces antibody formation of the IgG class down to zero, you have a limited early IgM response under many conditions but this does not go over to the standard IgG response. And you can compare the severity of the infection in the normal animal and contrast it with those in the immunosuppressed animals. And then in addition you can restore certain functions by giving antibodies to the immunosuppressed animals or by transferring syngeneic cells, that is to say, cells of the same genetic type, and one usually uses immune spleen cells or immune lymph node cells and you can then see what difference this makes to the infection. And we shall see examples of this later.

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Now, the natural immune deficiencies have been considered in some detail by Professor Soothill – I am chiefly concerned with these insofar as they illustrate the mechanisms of resistance against different types of infection. Now we have the three main types: those which involve gamma globulin, and these are either classically the x-linked type of Bruton agammaglobulinaemia where the levels of gamma globulin of all classes are very low indeed; sometimes you have selective deficiencies of IgG and similar results are obtained in these two conditions as far as viral and bacterial infections are concerned. In addition, you have a series of infections of complement components of which the important ones seem to be those affecting the third component of complement, what is known as C3. And we know that if individuals are deficient in C3 for a variety of reasons, either because there's a genetic lack in the production of C3, or because C3 levels are decreased because the inactivators of the complement component are themselves deficient – under these circumstances there's a greatly increased susceptibility to recurrent bacterial infections. Now, you have also defective cell-mediated immunity which, in exceptional cases, is specific. For example, you have the so-called Di George syndrome where children are born without normal thymus functions, you have the Swiss form of agammaglobulinaemia, as it's called, where there is a stem cell defect and where both cell-mediated immunity and antibody formation are markedly impaired.

<**Allison over table comparing infections arising from defective immunoglobulin levels with those arising from defective cell-mediated immunity**>

And so that one can compare the different types of infections which exist in these natural experiments of nature, so to speak, in order to find out whether the results which are obtained in experimental animals are applicable to man. Now, we know that, broadly speaking, where you have selective deficiencies of immunoglobulin formation but you have normal cell-mediated immunity, or nearly normal cell-mediated immunity, that there are three types of infections which are greatly

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increased in severity. First of all, these patients are susceptible to recurrent infections with pyogenic bacteria, and this is the reason why, until fairly recent years, they died; now we know that if we give them gamma globulin and protect them with antibiotics and other drugs, many of them will survive, so that the antibody plays a very important role, then, in protection against pyogenic bacteria. Now, secondly, we know that such children are highly prone to paralytic poliomyelitis, that the incidence of paralytic polio in these children is much higher than in comparable children having normal immunoglobulin levels. And not only this, that such children are also sensitive to the ordinary vaccine strains of polio; we know that they can actually have a paralytic disease resulting from infection with the vaccine strain, so that such individuals should be protected passively by the administration of antibodies and if active immunisation is attempted, this should be done with a killed virus rather than with a live virus. We know also that some children are also very much prone to echovirus meningoencephalitis – we have one such case under observation of my colleagues at Northwick Park Hospital and there are other cases described in Australia and in the United States. So these are very difficult to treat, the infection goes on for long periods of time so that this enterovirus appears to get from gut to the central nervous system and appears to persist in the central nervous system when antibody levels are abnormal.

Now, in contrast, where you have cell-mediated immunity you have increased susceptibility to tuberculosis, for example, children with the Swiss type of agammaglobulinaemia and other defects of cell-mediated immunity have been found to have generalised infections when they are exposed to BCG. We know that in the case of leprosy, the lepromatous variety of leprosy, you have very deficient cell-mediated immune responses against organism in question and this allows large numbers of organisms to proliferate. We know that individuals with defective cell-mediated immunity are highly susceptible to mucocutaneous candidiasis. Now what we are especially interested in, in this talk, is their susceptibility to certain viruses. We know they are susceptible to herpesvirus infections, that is to say herpes simplex which is unusually severe, and varicella zoster, which gives, again, extremely severe infections in some of these children and sometimes fatal infections. Also cytomegalovirus infections are more severe in these children than they are in normal

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children. Now the same is true of pox virus infections, that if you take children that have selective deficiency of gamma globulin production, the ordinary vaccination takes place perfectly normally and early childhood infections with measles and with herpes simplex, for example, or varicella zoster, are quite normal. Now, in contrast when they have defected cell-mediated immunity, you have greatly increased sensitivity to vaccination and a number of cases of severe progressive vaccinia growth have been observed in such children. These are not readily treated by giving them antibody and so this is one piece of evidence which fits with the experimental evidence we'll be talking about a little later which suggests that in virus infections, such as those produced by herpes viruses, pox viruses and measles, that the cell-mediated immunity plays an important limiting role.

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<**Allison seated to camera, then over chart on board next to him, uses indication stick** >

And now we're going to go on to see in more detail what happens when you have this interaction between antibody, complement component and cells in immunity against virus infections.

And we can begin by just showing diagrammatically the relationship between a virus and a phagocytic cell. Now, if this were a non-phagocytic cell, an ordinary cell which were the target in which the virus grows, we know that the virus consists of two essential parts. The first is the protein coat, these are so-called capsomeres which form the coat outside the virus and inside this you have nucleic acid and the nucleic acid has to get inside the cell in order to initiate infection and replication of the virus. Now because these are proteins on the outer part of the virus, in the capsomere, or in the case of some other viruses you have an even more complicated system where you have an envelope which contains lipid as well but also contains antigens; these are antigenic and these are the components which elicit the formation of specific antibodies. Now, these antibodies can then become attached to the surface of the virus and they do so in the usual fashion by the so-called Fab components of the

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antibody molecules – this is the antibody combining site. Now in this diagram, of course, we have just shown a very small virus in relation to a very large antibody molecule; if the whole thing were done to scale the virus would be very much larger. And so the antibody then becomes attached to the surface of the virus and this can prevent infection in a number of ways. One of the ways is that it can actually agglutinate the virus, or in other ways prevent the attachment to the target cell in which the virus would normally grow. But the other thing which antibody can do is actually to deviate the virus from cells in which it grows, the so-called target cells, into phagocytic cells where the virus is inactivated. We know that viruses do not grow in polymorphonucleocytes at all, and for the most part they do not grow in mononuclear cells as well; there are certain exceptions to this and it is interesting that those viruses which can readily grow in mononuclear phagocytes are, in fact, either virulent if they destroy other cells of the body, or else they are persistent; these are the viruses which tend to persist for long periods of time in cells other than neurones. And many of these are known in experimental animals: there's lymphocytic choriomeningitis and Aleutian Mink disease and others that appear to persist because they are able to grow in macrophages.

Now, here we have then the surface of the phagocytic cell and these, either polymorphonuclear or mononuclear phagocytes, are known to have receptors for the Fc components of immunoglobulin and also for the third component of complement, the so-called C3 receptor. And this is true for certain sub-classes of immunoglobulin – we know this is true for IgG_1 and IgG_3 in the case of the human phagocytic cells, but not for IgN and not for IgA, so that you have to have antibody of the appropriate type in order to carry the virus to the mononuclear phagocytes or polymorphonuclears in which the virus can then be inactivated.

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Now, the same is true of bacteria. We know that if bacteria are opsonized for phagocytosis, either by having antibodies of the appropriate classes which then become attached to the surfaces of the receptors on the surface of the phagocytic cell; or again you have C3 complement receptors which facilitate phagocytosis, or facilitate attachment followed by phagocytosis, in a number of cases. We know then that the bacteria are taken into the so-called lysosomal system, they are taken into large membrane-lined vacuoles which are formed by invagination of the plasma membrane; the lysosomes which contain hydrolytic enzymes then fuse with this phagocytic vacuole, they are then responsible for killing and digesting the bacterium and that is the way in which the pyogenic bacteria are normally prevented from multiplying. You have then the interaction between antibody and complement and the phagocytic cell. And as we said before, if you have defects in any one of these three parts of the whole system, you then have an increased susceptibility to infection.

Now the same is true in the case of viruses. We know that in the case of viruses, and complexes with antibody, in many cases are taken up by mononuclear phagocytic cells. We know that in the case of some viruses the process of uptake and neutralisation is, in fact, increased by having complement present. This is true, for example, of herpes viruses where the early antibody of the IgM class, and also some of the later antibody, is made more effective by the presence of complement. And we have then the same type of interaction of these three major components.

Now exactly how the virus is inactivated in these cells we still don't know. The thought is that if the virus gets in by attachment directly to the plasma membrane it can then go into a cytoplasmic compartment and can multiply, whereas if it gets in attached to an antibody, or to an antibody plus complement, it is then deviated again into the lysosomal system where it is exposed to the damaging enzymes which are present in the lysosomal system and is there degraded.

Well now we can go and consider a few examples of the way in which antibodies and cells collaborate in resistance against infection. And I want to discuss, first of all, an infection which is called coxsackie virus which is studied in mice. I mention this simply because we've done some model studies to illustrate the importance of

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antibody and mononuclear phagocytes in resistance against infection with this type of virus. And this is used as a model of an enterovirus which infects the gastrointestinal tract and, provided the infection is limited to the gastrointestinal tract, there is very little damage or pathology produced in the host, very little disease. But if the virus can actually escape from the gastrointestinal tract, through lymph node where it is exposed to a macrophage barrier, and then enter the blood stream, it can pass to the myocardium, to the heart muscle, and it multiplies there and produces a lethal myocarditis. So we have then two factors to consider. First of all, we have these macrophages which form a barrier to the spread of the infection and, secondly, we have antibody because the virus of this particular case seems to pass directly in the serum of the blood rather than within blood cells, so that if you have antibody which is present, this can then limit the spread of the virus from the initial site of infection, which is in the gastrointestinal tract, or if one is experimentally inoculating the virus into the perennial cavity, you again have limitation of spread from the initial site of multiplication in the abdomen to the heart, which is the sensitive target organ.

Now this will illustrate the kind of experimental result which is obtained. If you take an adult mouse and you expose it to coxsackie virus, you see that there is no mortality at all, that the virus infection is limited, it does not pass in large amounts, in fact, to target organs which are highly susceptible, such as the heart, and the animal recovers perfectly well. Now, in contrast, if you cyclophosphamide treat the animal in order to prevent antibody formation, and then infect it with the virus, you see that all of the mice die. And it's possible to show that antibody is the critical factor which limits infection in this particular case because if you take the immune-suppressed animals and you transfer antibody to them, you inoculate antibody in amounts which are comparable to those which are seen in the normal animals, you find that you prevent the mortality entirely. So in this case, the antibody is playing an important role in limiting the spread of the virus.

And it's possible to show this experimentally because this is the usual situation, that if you look at virus in the blood stream, and if you look at antibody formation, you find that antibody is demonstrable already two days after infection; there's quite a lot, four days, and by five days you are actually as high as you ever obtain, which is a

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relatively high antibody titre. Now, if you look at the virus you find that initially there is a viraemia, the virus gets into the blood stream, but very rapidly this viraemia disappears. And so you have a transient viraemia and the antibody plays the critical role in this phenomenon because in the immunosuppressed animals you find that there is no change from the early IgM antibody formation to IgG and, in fact, you have no late antibody at all, but you have a persistent viraemia which persists until the animals die. And they die because the virus spreads to the heart and grows there in large amounts.

And this is shown in this chart where: if you look at the heart in the normal animal, you find that no virus is recoverable. In contrast, if you look at the heart in immunosuppressed animals, after the fourth day you have rather high titres of virus which persist until the animal dies. So this illustrates, then, the critical role of an antibody in limiting the spread of a virus infection from the primary site of multiplication in the gut to a susceptible target organ which in this case is the heart.

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<**Allison seated to camera, then over charts on board next to him, uses indication stick** >

And one has comparable things exactly in polio. We know that in the case of poliomyelitis you have infection both of the oropharyngeal region and you have then the possibility of virus spreading into the blood stream. Or you usually have infection of the gastrointestinal tract. And again you have the possibility that the virus will spread from there into the blood stream, and from there the virus can spread into the nervous system and produce paralytic disease. So provided that the infection is confined to the oropharyngeal and gastrointestinal tract there is no danger – that you have a transient infection which normally clears up, and then the virus is no longer excreted and is a danger to the rest of the community.

Now it looks as though secretory immunoglobulin plays some role in the limitation of the excretion of the virus so this is one factor in immunity of the community in

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general, but that IgG antibody which is present in the bloodstream provides the important barrier to the spread of polio from the gut to the central nervous system. And, as I mentioned earlier on, the fact that children that are agammaglobulinaemic and have very low levels of IgG are much more prone to paralytic disease supports this view. This is the human counterpart to the experimental infection with coxsackie virus which we have just discussed.

Now there's another point which I would like to raise in this connection, and this is the effect of age on susceptibility to these enterovirus infections. You find that very young animals are highly susceptible to parenteral inoculation, that is to say intraperitoneal or subcutaneous inoculation of the virus, whereas fairly rapidly in the course of the first few weeks the animals become resisted and by three weeks they're entirely resisted to this intraperitoneal inoculation of virus. And the question we can ask ourselves is what is the difference between these young animals which makes them highly susceptible and the older animals which are highly resisted?

And it looks as though there are two factors which are mainly involved. And one of these is antibody – in the young animals there is some delay in antibody formation, and the second is the mononuclear phagocytes themselves; as I mentioned there is a collaboration between antibody and mononuclear phagocytes in limiting infection and this can be shown by transfers of cells from older to younger animals.

Now here we see an experiment in which the virus is given to the young animals and you find this illustrates survival occurring under different circumstances. This is the time after virus infection, and you find that in these newborn animals, very rapidly they die, and so that 8 days after infection, you have, in fact, all of them already dead as a result of the coxsackie virus infection. If you take macrophages, mononuclear phagocytes, from the peritoneum of the adult animals of the same genetic strain and you introduce them to these young animals, you find that you increase survival time – you don't actually increase the number of survivors, they all die, but they only die after 20 days, in contrast to 8 days. Now if you give them at the same time very small amounts of antibody you find that, in fact, all of the animals will survive, so the deficiency in the young animal is partly because antibody formation is itself delayed

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and partly because the cells that collaborate with antibody are immature in these newborn animals. And this immaturity can be made up by transfers of cells from the older animals.

Well now we can contrast this type of situation which is typical of the enteroviruses, in which there is a major role of antibody in limiting infection, with that which is seen in herpes simplex virus. In the chart you will see plotted here the survivors of mice, a group of mice, that have been given cyclophosphamide to suppress their immune responses, and infected with herpes simplex virus. You see that in a control group there are very few survivors. And if you give antibody to these mice, you do not improve the survival rate to any significant degree. In contrast, if you transfer immune spleen cells from donor animals of the same genetic strain which are immune against herpes simplex virus, you find that nearly all of the animals survive. And if you treat these with complement, you see that this makes no difference at all. If, on the other hand, you treat these cells with anti-theta serum and complement which eliminates T-lymphocytes you find that you markedly reduce the survival, in other words the protective effect. Now this type of experiment shows formally that in the case of herpes simplex virus, antibody does not play a major role in limiting the infection, but that T-lymphocytes, which have been sensitised against the virus antigens, do make a very considerable contribution to the survival of the experimental animal. And this, as we've said, is analogous to what is found in the human situation where you have severe infections by herpes simplex viruses in, by herpes simplex viruses type I and type II and also by other herpes viruses, in individuals that have deficient cell-mediated immune responses.

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<**Allison seated to camera, then over charts on board next to him, uses indication stick** >

Now the way in which all this works is not yet fully understood but at least some factors are known and it's interesting to speculate about the remainder. We know that in the case of herpes simplex virus, for example, that the virus can actually

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spread from infected cells to neighbouring cells even in the presence of antibody. This can easily be shown if you have a monolayer of cells on the bottom of the Petri dish and you have antibody in the medium up above the cells, and you can then find that whole foci of infection can actually be generated underneath the antibody. So that although the antibody neutralises virus which appears on this surface of the cell, the virus can evidently spread by intercellular contacts and in some cases actually syncytial formation between these different cells, and this is presumably why the antibody is so inefficient at controlling this type of infection. The same sort of thing happens exactly in the case of measles virus and certain pox virus infections, that you have this spread of infection from cell to cell in the presence of antibody, and this is, as we have just said, perhaps explains why antibody is also insufficient to control those infections too.

Now what happens is that evidently the T-lymphocytes come into the lesions, and they may in fact be recruited partly because of damage done to cells which occurs in the neighbourhood, releasing inflammatory mediators, but once they are in there they can then meet antigen viro-specific antigen, and then they can transform and differentiate into blast cells and they liberate a whole series of so-called products of activated lymphocytes as discussed by Professor Turk. Now these are responsible for the recruitment and immobilisation of macrophages in the lesion. We know that there are certain products of activated T-lymphocytes which are chemotactic for macrophages and there is the well-known macrophage immobilisation factor which would immobilise macrophages once they have got into the lesions. So in this way you have the recruitment of mononuclear cells into the lesions which is produced by a cell-mediated immune response.

<**Allison over series of photomicrographs**>

Now this is shown in a chart in a series of photomicrographs. On the left-hand side you see the liver of a mouse which is infected with herpes simplex virus, and to the top left you see a small focus of infection which is characterised by the infiltration of mononuclear cells. Now on the right-hand side you see the infection in an immunosuppressed animal; this is a little bit more heavily stained to show that you

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have spaces – these are regions where the virus has grown and has damaged the hepatic parenchymal cells so that you have areas of necrosis of cells but there is no cellular infiltration at all. And, if these animals are then restored, if their immune function is restored by transfers of sensitised lymphocytes, you again recover the mononuclear infiltrate which is characteristic of those lesions. So this then suggests that […]

<**Allison seated to camera, then over chart on board next to him, uses indication stick** >

[…] an important aspect of T-lymphocyte function is actually recruitment of mononuclear cells into the sites of virus infection.

Now we know also that these cells, the T-lymphocytes which are exposed to antigen, produce interferon. There is, in fact, quite a large amount of interferon generated by these cells and this must play some role in limiting the spread of virus infections. And there may well be other factors which are not yet fully understood – products of macrophages which interfere with the proliferation of viruses in other cells.

Now of course if you have macrophages of antibody in the lesions themselves, you have the possibility that much of the virus will actually be deviated into the macrophages themselves and cannot grow in macrophages, as we have said earlier on, and so that this would be one way in which the virus would be limited.

<**Allison to camera**>

So these, then, are ways in which the different types of immune response can collaborate with complement and with cells to limit bacterial and virus infections. Other people, particularly Professor Turk, will be discussing the collaboration of T-lymphocytes in macrophages in the limitation of intracellular bacteria and protozoal infections.

<**End credits**>