Pathogenic Mechanisms in Rheumatoid Arthritis

The Scientific Basis of Medicine

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Photomicrographs courtesy of Dr Leonard Glynn.

University of London Audio-Visual Centre, 1973.
Made for British Postgraduate Medical Federation.

Produced by Peter Bowen.

Black-and-white
Duration: 00:35:37:20

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<Opening titles>

<Dr Gilliland to camera>

Professor Ivan Roitt is Professor of Immunology in the Middlesex Hospital Medical School, London. He achieved international distinction with his work on thyroid autoantibodies with Dr Deborah Doniach. Since then he has extended his interest in autoantibodies to many other disease processes. Today he is going to talk upon the pathogenetic mechanisms of rheumatoid arthritis. <*Tape jumps*>

<Professor Roitt, seated, to camera>

In addition to affecting the joints, rheumatoid arthritis also has other manifestations in the body, such as the more systemic effects caused by vasculitis; there are also the subcutaneous nodules found at certain pressure points, but the main burden of the disease really clearly falls on the joints and I want to really talk about the events which seem to be responsible for causing the lesions which we find in the joints themselves.

<Roitt stands and walks towards a projected slide. Narrates over a series of photomicrographs of joints with rheumatoid arthritis>

Perhaps, we could start off by giving ourselves an idea or refreshing our minds about some of the features seen in the inflamed joint in rheumatoid arthritis. Here we have, for example, a joint from a patient with rheumatoid arthritis and you see here the hyperplasia of the lining cells of the synovial membrane and there's also some plasma cell infiltration, not quite so visible at this magnification. We'll have another look at that soon. <*Next slide*> Now, here's another view of a synovial membrane, again showing hyperplasia, here, of the lining of the cells. And in this region there is a dense infiltration with mononuclear cells, lymphocytes – and histiocytics are the typical chronic mononuclear inflammatory cell in infiltration.

<*Next slide*> And this is an example of villus hyperplasia in the synovial membrane. You see all the very active villi, which have been formed as the cells have gone into division, become active. And, in addition, here very prominently you see these lymphoid follicles with germinal centres, these just very much the same as the ones you would expect to see in the cortex of an active lymph node responding to an antigenic stimulus.

<*Next slide*> And we mentioned before the plasma cell, and here we get a closer look at them. Here you see very large numbers of typical plasma cells, mature plasma cells, in the deeper part of the synovial membrane. And these, of course, are cells which are actively producing immunoglobulins. So we keep on being reminded that we have here many of the features we associate with immunological reactivity.

<*Next slide*> And another feature frequently seen, here on the surface of this synovial membrane, one finds fibrin deposition.

<Roitt narrates over slide showing section through patella in a case of rheumatoid arthritis>

And this is a gross picture of the patella and the lower end of the femur of a PM case. And while not very easy to see here, there is hyperaemia of the synovial membrane and the beginning of the granulation tissue, which we call the pannus. And this granulation tissue is responsible for eroding the cartilage and eventually leading to erosion of the bone, as we can see in this picture here.

<Roitt narrates over photomicrograph slide and then X-ray, both showing signs of rheumatoid arthritis>

Here we can see the cartilage and here, in this region, the margin: this is a proximal interphalangeal joint. We can see here in the margin, there is this erosion of the cartilage and eventually of the bone itself by osteoclasts in the granulation tissue. Now, if we look at the X-ray picture that mirrors exactly this feature here of the marginal erosion of the bone. That's a quite typical feature.

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<Roitt, seated, to camera>

Now, we've seen plasma cells. We've seen lymphoid follicles, the germinal centres, this infiltration by mononuclear cells; all these things, all these factors, as I say, do link us up and make us think about immunological processes. And I think the important question, sorry, the important feature of rheumatoid arthritis is the operation of immunological factors, and I want to really discuss the way in which we think the role of immunological factors plays a part in causing these lesions that we've just been looking at.

Now, the one really central feature about rheumatoid arthritis that comes to you straight away, when you think about it, is the fact that a large number of these patients with rheumatoid arthritis are making an autoantibody response against their own immunoglobulin. This is the typical rheumatoid factor seen in such classical tests as the latex test and the sheep cell agglutination test, and let me just remind you of how those work.

<Roitt draws a diagram and narrates over it>

The principle is that a particle – and that particle could be latex – is coated with human immunoglobulin gene. And the rheumatoid factor, which we've agreed is, or which I've told you, is an autoantibody to IgG, cross-links the particles of latex, which bear the immunoglobulin on the surface so that latex agglutination test will show this rheumatoid factor, the anti-immunoglobulin gene. The other test, you'll recollect, involves the sheep cell coated with rabbit antibody. And that rabbit antibody is, of course, an IgG. And, again, the rheumatoid factor will cause agglutination of several sheep cells coated with the immunoglobulin on the surface. The central feature here, let us look again, is the rheumatoid factor: it's an antibody against IgG.

<Roitt to camera>

Now, we may well ask the question: how is it that one can develop antibodies to one's own IgG? There's no doubt that some of the antibodies are clearly against native IgG, that's the IgG as it's circulating normally as a 7S molecule in the blood, but it also seems to react very much better with aggregated immunoglobulin G. You can either heat aggregate it or particularly when it's aggregated in the form of an antigen-antibody complex, when your IgG forms a complex with antigen, there are many IgG molecules in the complex and that would be counted as an aggregated IgG entity. And rheumatoid factor reacts very well with that. And there is some considerable controversy at the moment about whether there is something special, some new determinant on aggregated IgG, which cause rheumatoid factor, or

whether rheumatoid factor reacts against a determinant present on native IgG but which can become effective because the native IgG is aggregated.

Now, I'd just like to spend a moment discussing – this is little bit of a divertissement for the cognoscenti, but it would be quite interesting to look at possible ways in which these two different types of view can be examined. And let me just say that if it's a little bit beyond you, don't lose too much sleep about it because it won't deflect the major thrust of our discussion today.

<Roitt walks towards display board and places attachable images on board to illustrate narration>

Now, let us suppose that we have a B-cell, a B lymphocyte, which is a precursor of the cells which are going to form antibody. That's the wrong one, of course, needless to say. Here we are, that's the one we want, that is a B small lymphocyte, which is gong to become an antibody forming cell. It needs to be stimulated. Now, if we take native IgG with just, say, one determinant, that can't stimulate the B-cell because it's rather like a hapten trying to stimulate a B-cell. But we know that we could bring in T-cell cooperation, if we can provide another determinant on the IgG. And we could argue that if aggregated IgG has a new determinant, which I've represented by this little square here, then the T-cell, not being tolerant of this new determinant, would recognise, and we have the classical system which would enable us to stimulate the B-cell to make antibodies against what is a determinant on natural native IgG.

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But the proponents of the native IgG stimulation, of the, in other words, people who don't consider there is a new determinant on aggregated IgG would have us accept a different viewpoint. And they say the following: that aggregated native IgG could stimulate our B-cell – here's the same B-cell but I've put the receptors together – by having a multivalent attachment. You see here are some native IgG molecules; by themselves they can't do anything, as we've just said before, they're rather like haptens, but if we aggregate them, you see, in some way by putting them in a

complex so this will be on their Fc part, we would have a multivalent bond which is very much stronger than a univalent bond.

Now, I just want to illustrate the reason why we think this is so. That multivalent bonds, you know, for example, something with three bonds in it is very much better than three times one bond. It's probably more like something like a thousand times better than one bond. The reason for it is this: each antigen-antibody interaction is a reversible interaction. That means that supposing my hand is an antibody-combining site for antigen. Now, here's the antigen, this roll. Now, the antigen-antibody reaction is reversible, that means it's making and breaking. Now, if I'm only holding this with one arm, one arm here, as I make and break, as I let go, we drop the antigen. Now, let's suppose we're using two arms in the process and we're making and breaking with them at different times. That means that when I'm letting go with this hand, it's more likely that I'm still keeping hold of the antigen with the other arm, the other antibody arm, and therefore I'm not likely, I'm not going to drop this. And we're in a good position spatially for the antibody to bind again with its antigen. So, and therefore, it's very unlikely now if I'm holding it with two arms, two hands even though they're making and breaking at different times, it's unlikely that I'm going to drop it, so I've got a much better grasp on the antigen by multivalency. And if we add up to three then it's even better. And so this is the strength of the multivalent bond versus the single bond. And this view would hold that by having a multivalent structure, there is a direct stimulation of a B-cell.

Now, we see the same sort of argument coming in to play when we try to consider why rheumatoid factor does react very much more strongly with polymeric, that is, aggregated IgG than with monomeric. And if we, let's produce ourselves a rheumatoid factor. Here's a rheumatoid factor that is reacting <*drops diagram*>, (guess we always have our bloody problems), rheumatoid factor, (too much gravity), rheumatoid factor here reacting against native. And native by itself, a single determinant, that's a fairly weak bond, but let's build it up again into an aggregate and we have a multivalent attachment. And we've just agreed, I hope you've agreed, that a multivalent attachment, many, many times stronger than a single attachment. So that would be OK, that would be a possible reason for the better combination with

aggregated. We don't have to invoke the presence of a new determinant. However, with a new determinant, we would have no trouble either. There we would have our rheumatoid factor, that's an IgM rheumatoid factor – we've made it pentavalent – reacting with a new determinant on aggregated IgG.

Now, it's not been resolved though, I say, this particular question, it is perhaps easy to see the cross-reaction with the, important cross-reactions, with other immunoglobulins of different species, such as rabbit immunoglobulin if there is a new determinant produced in the aggregated gamma. But I thought it worthwhile to just point that out as one of the major points of controversy and this will be important in trying to analyse how rheumatoid factor arises.

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<Roitt returns to seat and narrates to camera>

Let's get back to the main thrust of our discussion, the question of rheumatoid factor responding to, well, to aggregated gamma. Now, there is, in fact, good evidence that there are aggregates of IgG, aggregates which contain IgG, in the synovial fluid. Now, we have a number of different ways of detecting these aggregates. One of them would be just simply by looking with ultracentrifugation; we see peaks which are intermediate between 7S and 19S and which we think are due to these aggregated IgGs. But that's fairly indirect. We much better evidence from the precipitation by rheumatoid factor […]

<Roitt narrates over slide and then back to camera>

[…] or by the first component of complement. These are reagents which will react very well with multivalent aggregated IgG, and when we add either rheumatoid factor or this first component of complement to synovial fluid from a patient with rheumatoid arthritis, we get a precipitation reaction when it reacts with these aggregates. So there's one piece of evidence in favour of aggregates.

Now, another one is the very frequent occurrence of cryoprecipitates, those are precipitates that form when you take the synovial fluid and place it in a refrigerator around 4 degrees and you get this cryoprecipitate forming. You warm it up – goes back into solution, and the cryoprecipitate is very frequently an indication of antigen-antibody complexes or indeed of aggregates of IgG combined with, say, an antiglobulin. Another telling feature for the occurrence of these aggregates in synovial fluid is the fact that the complement levels are very low indeed, which you would expect if you have aggregates of IgG there fixing complement. And not only are the complement levels low, but we see a very significant conversion of the third component of complement from the so-called beta-1C to the beta-1A form. And this is a very significant observation because that occurs during the activation of complement.

Now, if we look in the polymorphs in the synovial fluid, we can actually see evidence visually, by the fluorescent antibody technique, for these aggregates being taken up by the polys. And you can stain those aggregates in the polymorphs for IgG, for IgM, […]

<Roitt narrates over slide and then back to camera>

[…] for rheumatoid factor itself and this third component of complement, which we are calling here beta-1C. Now, all the features which we might expect to see in an immune aggregate which has antiglobulin, that is the rheumatoid factor, and complement and IgG can all be seen in the actual polymorphs within the synovial fluid of an inflamed joint of a patient with rheumatoid arthritis.

<Roitt returns to display board and places further labels to illustrate narration>

Now, the presence of aggregates does lead to an inflammatory reaction. And let's just look at the way in which this can happen. And we start here: our antigen-antibody complex, or our aggregated IgG. Now, this will activate complement by more or less the classical pathway, moving in through C1 and so on, and bring us eventually to the activation of the third component, the complement. One of the

things that happen when we get to the third component complement is we chop off a little fragment, a little peptide called C3a. And C3a already has important biological activities which are related to the inflammatory process. Now, one of these activities is something we call anaphylatoxin. Now, anaphylatoxin releases histamine from the mast cell so, lo and behold, one of the things that's happening, we get histamine release and that changes vascular permeability and brings in more serum proteins, more antibody, more complement components and feeds the complex situation here, giving us more complement to feed into this pathway. And then we get hyperaemia and so on.

The other thing that C3a has as a biological activity is the fact that it is chemotactic for polymorphonuclear leucocytes. They attract polymorphs and they come out of the blood vessels into the site here of the lesion, into the site where the antigen-antibody complex activated the complement. C3a is formed, chemotactic for polymorphs – they come into the lesion. And these polymorphs start chewing up, trying to phagocytose the antigen-antibody complex; that is their job, they come in and they are attracted to the complexes, or the complex is attracted to the polymorphs, whichever way you want to look at it. And – we've got slightly short of arrows, but let's not worry too much about that – there is phagocytosis and a simultaneous release of proteolytic enzymes from those granules. Now, let's just look at how the way in which we think this might happen, or at least the way some people think it happens.

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<Roitt returns to seat and draws diagram, narrating over it>

You see what happens is that we have here our phagocyte, and here we have the antigen-antibody complex. And here lurking in the background, we have a lysosome with its hydrolytic enzymes. Now this, the surface of the phagocytic cell, in response to its contact with the antigen-antibody complex, starts to put out arms of cytoplasm, like that, to engulf the antigen-antibody complex in a phagocytic vacuole, and at the same time the lysosome seems to come up to the edge of this potentially forming

vacuole and it releases some of its enzymes. Now, this isn't a non-specific thing: other enzymes in the cytoplasm, such as lactic dehydrogenase, for example, don't come out of the cytoplasm when a phagocytic cell is doing its job of phagocytosing something. But the enzymes of the lysosomes, the acid phosphatase and other enzymes we'll talk about in a moment, are released and then, of course, this goes through the usual process of closing off the vacuole and fusion with a lysosome. We don't want to worry about that just at the moment.

<Roitt narrates to camera>

It's these enzymes that I want to draw attention to because those enzymes, or these components of the lysosome, include, now we recognise, some neutral proteinases and also, most importantly, collagenases. And here, of course, we have materials which can start to break down the surrounding tissues; particularly the collagen could be broken down by the collagenase and other components of the connective tissue would be eaten into by these neutral proteinases. So what is happening is that when we are… when these phagocytic cells, not only the polymorphs but also some of the phagocytic cells which make up about half the lining of the cells in the synovium, if these are phagocytosing complexes and releasing these nasty enzymes at the same time, you can see how you can start to build up a process in which we are damaging the surrounding tissue during this inflammatory response.

Now, many of you will say, well, seronegative rheumatoid arthritis is a very common thing, I mean, there are no rheumatoid factors there; why are you making all this emphasis on the antiglobulin aspect and its relationship to IgG aggregate? Well, what we have to recognise is that the reason why we call some patients seronegative is that they are negative in the classical test that I described to you earlier in the programme. Now, if we use a different type of test, one that incidentally Torigian and I myself developed using an immunoadsorbent, you can show the presence in virtually all seronegative patients of antiglobulins. They aren't always of the kind you see in seropositive, they are very often IgG instead of IgM, but, nonetheless, they do show reactivity toward aggregated IgG.

<Roitt narrates over graphs showing antiglobulin levels>

And if you look at the situation there, you find that all our patients, or at least very many of our patients, with seronegative rheumatoid arthritis have these antiglobulins to a level higher than we see in the normals. Each of these little dots represents the serum of one patient and we've plotted here <*indicates y-axis*> the antiglobulin as IgG in µg/ml. And here you see the group of normals; it's not abnormal to have antiglobulins, it presumably reflects largely a physiological response to the antigen-antibody complexes you normally form in the process of defending yourself against invading infectious organisms. Well, that's not a surprising thing to happen, but what you do see is that this is grossly extended or exaggerated in patients with seronegative rheumatoid arthritis just as much as in those with seropositive. Not only are these adults with seronegative rheumatoid arthritis but also Still's disease of children. These are some studies we did with Barbara Ansell at Taplow, looking at some of her patients with Still's disease. And these children, when they were active, they had antiglobulins quite clearly higher than the controls. Whenever these were inactive, these were rather lower.

<Roitt to camera>

So, I think, one can see that the place of aggregated IgG really is quite central in this whole process of developing rheumatoid arthritis. And we have to think really now: how is one going to aggregate this IgG to provide the stimulus to set all this process off? Well here, of course, we start getting rather speculative again. There might be some non-immunological process which causes aggregation of IgG, something funny happening by release of an enzyme or other within the joint – very speculative, not much evidence for this at the moment, or there might be some acid type pH change which causes alterations in the IgG. It's possible, again, not much evidence.

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There is a natural feeling, I think, perhaps because of so many immunologists are involved in the study of rheumatoid arthritis to put their money on the idea that the

aggregation comes from the interaction of immunoglobulin G with some antigen. And then, of course, we have the big question: what antigen? Well, it might be an autoantigen present within the joint. It could be a component of cartilage or what have you. That's not been clearly established yet, but it must remain a very definite possibility. But, quite naturally, people have tended to think of the possibility of microorganisms of different kinds localising in the joint and setting up a localised immune response. And there have been many, many claims throughout the last few decades for people being convinced that they have found the infectious agent that is going to be the answer to the aetiology and pathogenesis of rheumatoid arthritis. And we've had different viruses, we've had mycoplasmas, we've had diphtheroids. Not much agreement yet, not much consistency in the way in which different labs vie with each other to make their isolations, but we were particularly interested, perhaps because Mervyn Williams was in our department at the time, in his observation on the isolation of a mycoplasma, *Mycoplasma fermentans*, from the joints of patients with early rheumatoid arthritis.

I have to say that there has been no confirmation from other laboratories since his initial observations. That is one point we have to make. On the other hand, you might say, well, if there is an immunological response in the tissues to an agent like a mycoplasma and you're producing lots of antibody, you wouldn't expect the agent to live very happily outside the cell in the extracellular fluid bathed in this antibody. The antibody should clobber the organism if it ever comes out of whatever cell it's been living in, so we tend to think that if there is an organism, we may not be able to find it easily, but it may be having an intracellular life, as many of them do, and occasionally pops out for a breath of air and divides and causes more hypersensitivity reaction, but is rapidly neutralised and killed by all this ferocious immunological reactivity around it in the joint. So maybe, you say, let's not worry too much about not finding the organism, let's see if we've got any evidence for an immunological reactivity against any joint antigens. And that is one thing that Brostoff and I did together with, Dr Brostoff and I did, together with Mervyn Williams. And we looked for evidence of immunological reactivity against this organism, this *Mycoplasma fermentans*.

Now, looking at the humoral antibody response, there really wasn't much difference between normals and rheumatoid arthritics. But then we looked at a test of cellular hypersensitivity called the migration inhibition test. Now, I'll just describe it, I expect most of you know it already, but let me just rapidly describe it and put those of you straight who are not too familiar with it.

<Roitt draws diagrams and narrates over them>

Now, the essential basis of this test, particularly in the human, is to fill a capillary tube with buffy coat cells. And this is an open end here and we place that in a large little chamber. And if incubate the capillary tube for overnight, we'll say, the cells inside migrate out and you get a fan of cells like that, spread out over the floor of the tissue culture chamber. Now, if instead you have cells which are sensitised to particular antigens, we have the antigen present within the fluid, then the cells instead of migrating out this far, as they would in the absence of antigen, migrate out to a lesser extent. And the area of migration, the presence of antigen, is smaller than the area in the absence of antigen. Absence of antigen. And you put that over that, multiply by 100, you get a percent migration/inhibition.

<Roitt walks to display board, refers to and narrates over chart illustrating migration of leucocytes>

And, let's have a look and see what happened in this test with patients, the buffy coat of patients with rheumatoid arthritics, and we look and see the percentage migration; 100% should mean that there has been no retardation of those leucocytes. And here are our controls, normals, you see that they spread around the 100. Obviously, the mycoplasma antigen that we're putting in here, that we're taking *Mycoplasma fermentans* membranes, which have been washed in urea and various other sorts of little treatments, and you see that we get this range of values.

And when we look at untreated rheumatoid arthritic patients, there's a very definite lowering of the migration. There's an inhibition of migration compared to the controls.

And if they're treated with gold or chloroquine and they're in remission, then you see that their migration inhibition comes back comparable to the normal level.

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<Roitt returns to seat, narrates to camera>

Now, it's not easy to work out the real significance of this yet. First of all, I should say that we're not entirely clear about whether this migration inhibition test measures a T-cell hypersensitivity, that is, delayed-type hypersensitivity, which we're used to as typified in the Mantoux test. Does it really reflect that sort of reactivity or is this due to some sort of special antibody bound onto the leucocyte and a sort of cytophilic antibody and we get a migration inhibition? We're not exactly sure, I mean there are lots of cases where you have great deal of circulating antibodies, for example, in Hashimoto's disease you can get tremendous amount of antithyroglobulin antibody, and yet you can put thyroglobulin in with the leucocytes from a patient with Hashimoto's disease – they migrate quite happily as if the thyroglobulin wasn't there. So if it is a cytophilic antibody, it's got to be a very special one.

Well, we better not go any further with the controversy of whether this represents a T-cell reaction or not, this migration inhibition, but it does show a differential effect when the *Mycoplasma fermentans* antigen is in the system. Now, we have another problem, that I should draw attention to here, that the organism, when we culture it, is cultured in the presence of horse immunoglobulin G. It's in horse serum; it does pick up some horse IgG on the surface of the membrane and we do try and get rid of this with urea, and we have tried other methods in which we think we've reduced the horse IgG to a very low level in these preparations, and still got very active migration inhibition, so we have fractions very low in IgG which still give very good results in these tests. But I have to say that aggregated IgG itself can cause inhibition of rheumatoid patients' migration. The leucocytes of patients with rheumatoid arthritis are inhibited in this test in the presence of aggregated gamma.

So, we've really got this problem now of deciding: is this migration inhibition which so characteristically seems to separate the rheumatoid arthritics from the normals? Is that due to an antigen which is characteristic of *Mycoplasma fermentans* or a similar organism, or is it due entirely to these little smidgeons left behind of IgG on the surface of these membranes, placed in perhaps a cunningly, very effective way, which is giving us this reaction? In other words, is this a sensitivity to IgG or is to mycoplasma?

Well, as you'd expect, we haven't got the answer completely to this at the moment. I can say that our results, at the moment, seem to indicate a far better correlation of the mycoplasma migration inhibition with the degree of activity of the disease as compared with the results obtained with aggregated gamma, which doesn't seem to discriminate very well between quiescent and active phases of disease, whereas there is quite a good correlation, which you see in some of it on the last chart, between quiescence under the influence of treatment and the active strongly affected joints. They show much greater migration inhibition with mycoplasma.

Furthermore, we can obtain very good inhibition with amounts of the mycoplasma antigen down to about 10 micrograms per ml, whereas we need something like 120 micrograms of aggregated gamma to produce a comparable inhibition. Well, you could say that what tiny vestige of immunoglobulin there is in our mycoplasma might have been very effective in causing inhibition. I think a great deal more work is needed. One really grasps at any new way into the problem of trying to find where this whole process starts, and I think that's just about as much I'll say for now.

<End credits>

Extra Author's note:

Aggregated IgG may also provide the correct type of antigen lattice needed to trigger B-cells without T-cell cooperation.