Induction of the Antibody Response

Uptodate: Immunology, Part 4

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<**Dr Melvyn Greaves to camera**>

When antigen is injected into an experimental animal, over a few days antibody is produced and appears in the serum and can be measured by a variety of serological tests. What I want to discuss in this programme is the cellular basis of this antibody response. To a large extent, our knowledge of what happens during antibody production is based on experiments in mice and also, to a large extent, on tissue culture or in vitro experiments. Also to a large extent, the story of antibody production is the story of cell collaboration in which three players appear to take part: the macrophage and two populations of lymphocytes. These lymphocytes are called T cells or thymus derived lymphocytes and B cells or bursa derived, or sometimes called, bone marrow derived lymphocytes. And much of what I have to say today concerns the way in which these populations interact with each other during antibody production.

Now much of what we now know about antibody responses has been dependent very much upon technological advances over the past few years, and I want to

comment on two of these very briefly. The first is the availability of marker systems which enables one to distinguish different populations of cells. For example, the T6 chromosome has been used extensively by Dr Davies and his colleagues to identify different populations of lymphocytes in transfer experiments. Another group of markers have been very extensively used in the types of experiments I'm going to be discussing, these are cell surface markers, cell surface binding sites receptors and so on, which are selectively expressed by different populations of cells so that one can use these markers to distinguish T cells from B cells, for example.

Now I want to comment in a little more detail about two of the technological points before we talk about experiments. The first concerns tissue culture methods. Now, over the past few years there's been a tremendous development and refinement of tissue culture methods. From the point of view of this talk, the main thing of importance is the development of methods that have enabled antibody responses to be both induced and expressed in vitro, in tissue culture. And these responses have paralleled in magnitude, at least, responses induced in whole animals, so one feels reasonably confident about using these in vitro systems as models for investigating cell interactions.

<**Greaves, shown seated, turns to charts to his left showing the Marbrook chamber, narrates over it**>

Now I've shown on the first diagram here one popular method of tissue culture that's been applied to antibody responses, particularly in the context of looking at cell interactions. It's called the Marbrook chamber and it was introduced by John Marbrook in Australia. What it consists of is an Erlenmeyer flask, here, that has about 100ml of tissue culture medium, serum supplement medium, inside it. Here we have a cylindrical glass hollow open-ended tube, on one end of which, the lower end, is placed the dialysis membrane. Now the cells can sit on here, on this membrane, and be bathed in a large volume of liquid and this is really the advantage of this type of tissue culture method; the cells have a ready supply of essential nutrients and toxic metabolic products can diffuse away from the area where the cells are most concentrated. I've actually shown on here a variety of this tissue culture method

that's been extremely important in investigating cellular interactions. It's a double chamber method where, inside of this chamber here, we have an additional smaller hollow tube in which there is also membrane on the end, it happens to be a different membrane with different sized pores. Now, with this kind of method one can co-culture different types of cells in different compartments and despite the fact the cells in these two compartments will be physically separate, they can communicate by means of soluble diffusible products which will pass through this Nuclepore membrane – the membrane will, nevertheless, prevent cell transfer. Using this sort of method one can add antigen to the cells and produce antibody responses.

<**Greaves to camera**>

Now I want to say something very briefly about the way in which one assays these responses, in which one measures quantitatively what is happening in terms of antibody production. I'm not going to say anything about serological methods that are commonly used for measuring antibody in the serum but I wanted to illustrate, just very briefly, the way in which antibody producing cells are enumerated in the context of these tissue cell experiments, and I've illustrated the method on the next picture.

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<**Greaves, shown seated, turns to chart to his left detailing process of haemolysis in gel, narrates over it**>

The method is commonly referred to as a yurner[?] method, in fact it's more correctly called local haemolysis in gel which describes exactly what it is. If we take lymphoid cells from lymphoid tissue, let's say the spleen of an immunised animal, let's take the example of the mouse immunised with sheep red blood cells, and we mix these lymphoid cells with a great excess of sheep erythrocytes (here's a lymphoid cell surrounded by red cells) we mix them with gel and pour them into a Petri dish, or alternatively put them into a shallow glass chamber formed by two glass slides. The real lymphoid cell will be making antibody, directed towards the sheep erythrocytes – this diagram illustrates such a cell. The antibody diffuses readily and will bind to red

cells in close proximity to the antibody-producing cell. Now, if one then adds fresh serum as a source of complement components, any red cell that has bound a sufficient amount of antibody will actually be lysed, it will be destroyed, and its haemoglobin will diffuse out. Now, macroscopically what one sees when that happens is shown on this side of the picture. We have a carpet of red cells, here, with apparently clear holes of lysis, punched into that carpet monolayer. If one looks at these plaques, so-called lytic plaques, under high power microscopy, one can usually find essential lymphoidal plasma cell that is responsible for producing the antibody which has lysed, so we talk of plaque-forming cells and plaque assays for antibody-producing cells. This method detects, with certain modifications, all types of immunoglobulins – IgM, IgG, etc.

<**Greaves to camera**>

That's all I want to say at the present moment about the technology that surrounds antibody responses, the investigation of antibody responses. I want to move on now to talk about collaborative phenomena and what we know about the nature of cell interactions. And before these culture methods were developed, we already had fairly sound reason for suspecting that complex interactions occurred during antibody production in vivo. We suspected that macrophages were important, although they didn't produce antibody themselves, for a number of reasons. Firstly, the size and digestibility and other properties of antigen, other general properties of antigens, were obviously very important in determining the nature of antibody responses, the magnitude of the responses and whether immunity or tolerance was induced. And this implied that initial interaction in macrophages and antigen was important, perhaps the macrophage degradation of antigen, and presentation of antigen to lymphocytes of ion[?] macrophages was crucial. And this was supported by various in vitro experiments which showed that macrophage processed and digested antigen was much more immunogenic, was much more potent, than non-processed antigen at inducing an immune response, either after following transfer back into an animal or totally in vitro.

Other experiments suggested that different populations of lymphocytes had to interact with each other and there was one crucial experiment, reported in 1966 by Henry Claman and his colleagues, from Denver, that I'd like to mention in some detail. Irradiated mice were repopulated with bone marrow cells from syngeneic donors, that is to say, mice of the same strain, bone marrow cells. Alternatively, mice were repopulated with thymus cells of the same strain. And a third group of mice received thymus plus bone marrow lymphocytes. All mice were then given sheep erythrocytes as an antigen and seven days later the spleens were excised and this local haemolysis in gel assay used to enumerate the antibody response.

Now what Claman observed was extremely important. He found that recipients of bone marrow lymphocytes made no antibody, recipients of thymus cells made no antibody, but recipients of bone marrow plus thymus cells made considerable amounts of antibody, comparable to that of a normal, intact animal charged with antigen. This experiment suggested two very important things. It suggested that although these cells were unable to make antibody by themselves, in the presence of T cells, which we now know the thymus was supplying in these experiments, B cells were able to make an antibody response – in other words, T cells and B cells synergised to give an antibody response. The experiment also suggested very strongly, and subsequent experiments proved conclusively, that lymphocytes actually gave rise to plasma cells which were the most active in producing the antibody.

Now, an obvious follow-up question from the experiment was: which cell actually makes the antibody – is it the bone marrow which I've just implied, or is it perhaps the thymocyte? And this is where markers, cell surface markers in particular, became extremely important. Tony Davies using T6, and Mitchell and Miller in Australia using histocompatibility antigens, cell surface antigens, showed conclusively that it was the bone marrow population which supplied the precursors of the antibody producing plasma cell, that the thymocytes did not go on to produce antibody. This was the sole function of B derived lymphocytes, and many subsequent experiments have confirmed these observations.

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So, what this experiment and subsequent experiments demonstrated very clearly was that the B cell population produces antibody, but somehow it requires the help and co-operation of T cells in order for this response to be fully expressed. Now this collaborative phenomenon seems to be true of all antibody classes and what I want to do now is to go on to discuss in some detail what we feel may be happening in this collaborative response in terms of interactions.

Subsequent tissue culture experiments demonstrated, as was anticipated, that not only T cells and B cells, but also macrophages, the third cell type, were essential for an antibody response, for example, to sheep cells in tissue culture.

Now, at present, the current state of affairs is that there are multiple or at least several different competing hypotheses which purport to explain the cooperation between T cells, B cells and macrophages and I can't present all of these in detail. What I'm going to do is present one current hypothesis which is fairly popular and, I think, very plausible and I will mention some of its competitors.

<**Greaves, shown seated, turns to charts to his left showing the Marbrook chamber, narrates over it. Then to camera**>

Now let's go back to the first picture of the Marbrook chamber. Now this method has been used by an Australian scientist by the name of Feldman, who now works at University College in London to investigate cell interactions and as I suggested earlier, this system has the advantage that one can grow one type of cell in one compartment, another type of cell in the other and study interactions. And what Feldman was able to show was that if T cells are in the upper compartment and B cells in the lower and antigen is present in both compartments, then a perfectly good antibody response can be induced and this suggests that the collaborative phenomena between T and B cells is probably carried out via the agency of a soluble factor which diffuses across from the T cell compartment through the membrane, to the B cell compartment.

Now, further experiments have substantiated that suggestion and we now know something about that factor. It's an antigen-specific substance which is its most important property, in other words, that the factor from the T cell induces an antibody response in the B cell population that is specific for the same antigen as activated the T cells, so it's a specific form of collaboration. The factor is probably a molecular weight of something like 200,000, is almost certainly an immunoglobulin since it binds to anti-immunoglobulin absorbents. One further property is of considerable interest: it binds very avidly to macrophages, it's cytophilic for macrophages, and this suggested a role of macrophages in this collaboration phenomena.

And if I turn over two pictures, we'll come to another experiment by Feldman which demonstrated the role of macrophages in T-B interaction. I should say first of all that what Feldman found in his double chamber system was that if macrophages were absent from the lower B cell compartment, and rather than inducing an antibody response, the T cell factor, migrating across the membrane, actually induced or facilitated B cell tolerance, whereas if macrophages were present, immunity developed and this suggested that macrophages were extremely important in governing the type of B cell response one measured.

<**Greaves, seated, refers to next chart on board to his left, showing a further experiment which suggests how macrophages might function in antibody response**>

And here we have, illustrated, an experiment which suggested a way in which macrophages might function. T cells were grown in the upper compartment and macrophages in the lower, there's no B cells in this system. Antigen is put in to trigger the T cells, the antigen-specific factor diffuses across the membrane and binds to, as shown by labelling experiments, macrophages. These are then harvested, the macrophages are taken out of here, they are washed thoroughly and transferred to a single chamber system containing now additional B cells. The question is: can macrophages that have been influenced specifically by T cells induce a B cell response. And the answer was yes, one could get a perfectly good B cell response in this system suggesting that the T cell factor had interacted with

macrophages first and had then influenced the B cells in a secondary manner which correlated with the fact that the factor was actually cytophilic.

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<**Greaves, seated, refers to further charts on board to his left, showing experiments which suggest models for cell interaction. To camera in between charts**>

Now, this suggested, as shown on the next diagram, a model for cell interaction that Feldman himself has suggested which is as follows. T cells interact with antigen via cell surface receptors of an unknown kind – this whole area of T cell receptors is extremely controversial, I shall say nothing more about that. Antigen receptors plus antigen or intracellular antibody released from these cells, in the form of a complex, binds to the surface of the macrophage. B cells, a small proportion of B cells of course because this is a clonal phenomena, rub up against or come in contact with the surface of the macrophage and are therefore triggered. Now, what this model suggests, and what it places special emphasis on, is the precise physical nature of the initial interaction between the antigen and the B cell surface. It suggests that some sort of multivalency or lattice type of presentation is optimal for triggering a B cell. And, as I said, if macrophages are absent from this system, these three complexes are very deficient at producing tolerance in the B cell population.

Now, as I'll say in a moment, there are other ways of looking at this type of phenomena and other suggested mechanisms. I want to digress for a moment to mention a different type of experiment which supports this general type of model. It's illustrated on the next picture, diagrammatically. It's an experiment by Mitchison and Rajewsky carried out in 1969, which concerns the antibody response to haptens. Now, as you all probably know, haptens are not immunogenic, it's not possible to get an antibody response to a hapten unless it's conjugated to a macromolecule. And here we're looking at an antibody response to DNP (dinitrophenol) on the carrier of ovalbumin. Mice immunised with this conjugate, other mice of the same strain immunised with another potential carrier, bovine serum albumen (BSA). Spleen cells

are harvested from these animals a few weeks after initial immunisation and are injected together into a syngeneic, lethally irradiated animal, whose own lymphoid system is defunct. These animals are then challenged the following day with a variety of conjugates, as we'll see in a moment, and a week or so later the anti-hapten, or anti-DNP response is measured serologically. Now, the design rationale of this experiment rests on the fact that B cells are going to recognise the hapten DNP and T cells are going to recognise the carrier molecule, either the ovalbumin or, in particular experiments, the BSA. Now if we could see the results of this experiment now <*results superimposed over chart in white lettering*>. Animals immunised with the hapten alone, DNP on a lysine molecule, do not give an antibody response. Animals immunised with the hapten on a completely irrelevant, in the context of this experiment, carrier – chicken gamma globulin, also give no antibody response; this is equivalent to giving a free hapten, there is no response during this time period, eventually there will be a primary response, of course there's no secondary response. Animals immunised with DNP-BSA give a very good antibody response, in other words what has happened here is B cells, primed to the DNP, have been helped by T cells primed to the BSA, despite the fact that these T cells and B cells were initially in separate animals. Collaboration has taken place.

Now the real point of this experiment is in the last line. DNP-chicken gamma globulin, the relevant hapten on an irrelevant carrier, plus separate BSA injected into the same animal is not immunogenic, despite the fact that there are T cells that can recognise the carrier and B cells that can recognise the hapten. And what this experiment suggests, what it demands of any model, is that the hapten and the protein carrier, which are going to be recognised, have to be physically joined. It's no good putting them in as separate molecules and a logical extension of that interpretation is that the recognition units for DNP on B cells and the recognition units for BSA on T cells themselves have to be physically joined.

So if we go back to, again, the Feldman model. If we imagine this little dot, rather than being a simple antigen is, in fact, hapten on one side and carrier on the other, it suggests that on one side we have to have T cell recognition units, on the other B cell recognition units for the phenomena to work. Now, the simplest variety or version

of that interaction would be to have T cells and B cells directly interacting – with T cells getting hold of one end of the carrier and B cells grabbing hold of the other end of the antigen, the hapten. Now, that's very unlikely to be true because these cells, the T cells and B cells, reactive to those particular determinants, are comparatively rare on a clonal basis; it's therefore statistically extremely unlikely that there would be an opportunity for appropriate contact of these two relevant cells. Now this suggestion itself that a soluble receptor molecule of some sort might be involved, that's the reason why I mentioned, I introduced these experiments of Mitchison because they fit in very nicely with Feldman's interpretation. The T cell recognition functions via the agency of the macrophage surface which permits direct contact, or physical bridge as we say, between the T cell recognition unit, now on the surface of a macrophage, and the B cell recognition unit here.

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<**Greaves, seated, to camera**>

Now that's quite a nice and plausible story. There are two qualifications that I have to go into in a small amount of detail. One of them concerns the presence of soluble factors derived from T cells which, unlike the Feldman factor, are not, in fact, antigen specific. These are factors that are produced in very large amounts, for example, in mixed lymphocyte reactions and in reactions in vitro where T cells are triggered by lectins, concanavalin and so on. Now, the important characteristic of these factors, as their name suggests, is that they will trigger a B cell response to an irrelevant antigen, that's to say, one can trigger the T cells with A, B, C or D, they will facilitate a B cell response to antigen T or V or whatever you like. They're antigen non-specific. Now, the way in which these factors work is not at all understood. Now they could act the initial triggering phase of the B cell, alternatively they might act to amplify the response of a B cell that's already been triggered by the type of interaction I've just described. Personally, I think that's the more likely explanation, that these factors are amplification or potentiation factors which increase the clone size of the B cells once they've been activated by cell surface antigen. Nevertheless, these factors are, I'm sure, very important.

The other qualification that I want to discuss, which is also quite important, is the existence of certain antigens which appear not to require these complex type of cell interactions. In other words, they can trigger cells to make antibody in the absence of macrophages and B cells in tissue culture of course. And we assume also, from in vivo experiments, they can do this in whole animals. Now, these antigens have certain properties in common and I might give you three examples of them. The first group are bacterial polysaccharides, for example pneumococcal polysaccharide, or levans. The second group is one unique example, in fact, Salmonella flagellin, which is the polymerised version which is a protein molecule. The third group are synthetic amino acids containing D-isomers and this is crucial point – synthetic amino acids, polymers, containing L-isomers are not thymus independent. What that statement means is that they cannot produce an antibody response independently of the presence of T cells and of macrophages, whereas if they contain D-isomers and they are indigestible, which is the crucial point, they can directly trigger B cells.

Now if we look at the common properties of these so-called thymus-independent antigens, hopefully one might discover why it is that they bypass the normal requirement for T cells and macrophages, and hopefully one might also learn something about what it is that T cells and macrophages give to the B cell in response to a normal thymus-dependent antibody response.

If one looks at the common properties, there are one or two things that are fairly obvious. The first is that these antigens are polymeric, or large, and what is more important, as I've already suggested, is that they are indigestible which means they stay large and this is a very important point, particularly in vivo. For example, the L-amino acid form – they start off in a syringe equally as large as the D form but after a few hours will be reduced to a small sized antigen. Such a small L polymer will be thymus dependent. Also the monomeric flagellin, the sub-unit of polymerised flagellin, is thymus dependent in contrast to its polymeric counterpart.

Now quite apart from the size, there's another factor associated with size that appears to be crucial; that is the fact that in contrast to conventional protein antigens,

these bacterial antigens in particular have repeating identical determinants, that's to say the same determinant appears many times in the same molecule in a linear array. And this suggested, as shown on my next picture here, […]

<**Greaves, seated, refers to chart on board to his left, showing an interpretation of thymus independency**>

[…] an interpretation of thymus independency which fits with the type of antigen presentation model that Feldman devised for thymus dependent antigens. Here we have the model of the thymus dependent antigen, being presented via T cell antibody on the surface of the macrophage. Now here we have one of these unusual thymus independent antigens, let's say a bacterial polysaccharide, with these repeating determinants. Now what this antigen can do is to bind multivalently to the surface of the B cell. The only way a normal antigen, a serum antigen, could do that is via the cross-linkage on the surface of a macrophage with T cell immunoglobulin – in other words, this thymus independent antigen has an intrinsic ability to form multivalent bonds with the surface of the B cell. And Feldman suggested that this is the reason why it is thymus independent, in fact, there is no real difference between thymus independent and thymus dependent antigens in the way they actually trigger cells, it's just the mechanics and the physical form of this multivalent lattice that makes the difference; the final presentation pattern of these determinants, in fact, is identical as far as the B cell is concerned. So that's a rather nice unifying hypothesis if you like. I've also illustrated on here, that I should comment on briefly, the comment I made before, that if these small non-pylovalent antigens interact directly with the B cell without the assistance of the macrophage and the T cell cross-linking antibody, rather than nothing happening which would be a trivial event, tolerance is actually induced; that's to say that small antigens without T cell help tend to turn off B cells such that they are unresponsive to subsequent charge.

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<**Greaves to camera, then refers back to previous chart**>

Now that's all very nice but I must comment on some alternative explanations of this phenomenon here. There's another category of explanations that have been put forward to explain why thymus-dependent, large polymeric substances can directly trigger these cells. And these really fall into the category of so-called second signals. It's been suggested that the reason why pneumococcal polysaccharide, for example, can trigger B cells directly is that it can supply two signals to the B cell, one of which is the obvious direct one with the antigen receptors, the second one is an indirect signal of some sort which depends upon some other general property of these substances, these molecules. And there are two candidates for second signals in thymus-independent antigens. The first one, if we can go back to this model here, relates to the polymeric structure again, although it's perhaps not absolutely dependent on polymeric nature. All of these substances, with very few exceptions, can activate complement. And since we know this B cell has on its surface a complement receptor, quite separate from its antigen receptor, it's been suggested by Peter Dukor in Basel, in Switzerland, that maybe for a B cell to be triggered you need two signals. First of all, the obvious signal between the determinant and the antigen receptor, and a second signal which this molecule is able to generate which involves complement activation by the bypass mechanism on the surface of the B-lymphocyte.

An alternative mechanism, but with the same principle, has been suggested by Antonio Coutinho who is a Portugese immunologist working in Stockholm. What he suggested relates to the observation he's made that all of these molecules with a polymeric nature have an intrinsic mitogenicity, which means that if they are added to B cell populations, purified B cell populations, a very large B cell proliferatory response will develop that's far in excess of what one would expect on normal clonal grounds. In other words, there's a non-specific type of interaction here, very much like one sees with lectins and lymphocytes, and he's suggested again that two signals are important for B cell activation – one is the initial interaction here between specific antigen receptors and determinants, the second one is more of the nature of a non-specific signal, but dependent on the initial specific interaction of the antigen receptor. This second mitogenic signal triggers the B cell.

So if we were to try and put together these thymus-dependent and thymus-independent models, one would say that in the case of a thymus-independent model, two factors or two signals are involved. One is the simple interaction of the determinant with the receptor and, secondly, are more complex secondary interactions or overall two-dimensional interactions which are dependent upon macrophages and T cells which could constitute, in a general sense, a second signal. For the thymus-dependent polymeric antigens one has the first type of interaction with the antigen determinants, and a second type of signal generated either by mitogenic sites here or other sites which are able to activate complement.

<**Greaves to camera**>

Now, obviously from what I've said this is a controversial area, these questions are not resolved and I think it will take a year or two before we understand fully the way in which these cells are triggered into antibody production.

I haven't said anything about what actually happens to a B cell after multivalent antigen presented in these different ways interacts with its surface receptors. Indeed, we know very little about it. I just want to make one comment and that is that it now appears from the work of the past year that there are a good many similarities between the triggering processes at the cell surface with antigen and lymphocytes and other ligand-induced changes, for example hormone target cell / drug target cell interactions and two important tasks for experiment that I think are worth just mentioning briefly. One is the observation that totally insolubilised antigen will trigger B cells to make antibody, just as insoluble insulin will trigger, and mammary epithelial cells, for example. Now what this experiment demonstrates is that the role of antigen is restricted entirely to the cell surface, that the initiating events in antibody production are cell surface phenomena, since the insoluble antigen obviously can't penetrate the cell. What happens after this initial interaction is very unclear, but there are very strong interesting clues that cyclic nucleotides are involved as second messengers as they are in many other cellular regulatory systems, and there's evidence that cyclic ANP plus cyclic GNP are extremely important in controlling B cell responses, whether they be immunologically productive antibody responses or

tolerance. And I think over the next two years we're going to see a great deal more work on cyclic nucleotides in antibody responses.

The final point I want to make concerns the overall complexity of these systems, that one feels one should almost apologise for. I don't think one can offer a particularly convincing rational reason why these systems should be so complicated. You might say why wouldn't it be simpler for antibody to be produced by a simple interaction of antigen directly with B lymphocytes – why is it so complicated, why is there so many cells involved, why so many interactions and factors involved. I think all one can say is we are looking at a server or regulatory system that is very finely controlled, and in that sense it doesn't differ from other physiological systems. There are positive and negative feedbacks involved, quite clearly. One might say that this is a little more complicated than most systems we're used to in physiology and the only point one can make is that one is dealing with an absolutely crucial defence mechanism and it's absolutely essential that this is regulated in a fine way, both quantitatively and qualitatively. Also, we're dealing with a mechanism, a response, that has an intrinsic capacity to turn against the host, to attack oneself, to give an auto-immune reaction. I think, there again, precise regulation is crucial.

I think the picture I'd like to leave you with of cell collaboration in antibody production is one in which the B cell is the producer cell in the centre of the picture, the T cell is primarily the regulator cell controlling the specificity and magnitude of the B cell response.

<**End credits**>