Cytotoxic Lymphocytes

The Scientific Basis of Medicine

Presented by Dr Ian MacLennan.

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<**Dr Ian Gilliland to camera**>

Dr MacLennan is Lecturer in Immunology at Oxford University in the Nuffield Department of Clinical Medicine at the Radcliffe Infirmary. He was previously a Research Fellow in the Medical Research Council's Rheumatology Unit at Canadian Red Cross Memorial Hospital, Taplow. He is the author of many papers on clinical and experimental immunology and has made a special study of antibody dependent cytotoxic lymphocytes, or K cells. Dr MacLennan.

<**Dr Ian MacLennan to camera**>

The analysis of the ways in which the immune system can bring about the destruction of foreign cells has wide spread application in relation to medical practice.

Now, the clinical situations in which this arises are numerous. There is graft rejection, the destruction of neoplastic cells which, at least potentially, often carry antigens which are recognisable as foreign by the host. There are virus infected cells and tissues which, for some reason or other, carry antigens which have lost tolerance, to which the body's immune system has lost tolerance. Examples of the latter are well provided by the experimental system of autoimmune allergic encephalomyelitis where the injection of brain tissue into animals, in the presence of complete Freund's adjuvant, brings about damage against normal brain tissue, and similarly, polymyositis will do the same.

Now, it's not been possible to reconcile all experimental data to fit a single mechanism of cell destruction and perhaps this is best exemplified by taking examples from total body experiments. Now Mitchison, in 1954, independently from Billingham, Brent and Medawar, who were studying just the same thing, described the way in which it was possible to bring about accelerated graft rejection by transfer of lymphocytes from animals which had been sensitised to that graft previously. Now, they found it impossible to reproduce this by transferring serum factors and they therefore concluded that it was likely that the damage was being mediated by lymphoid cells from the transferred animal.

Now, as opposed to this observation, Gore and Amos, in the following year, studied the way in which lymphoma cells were rejected from mice. Now this lymphoma was a transplantable lymphoma called EL4 leucosis and grew in an inbred strain of mice and it was possible to raise antibody against this in a genetically different inbred strain of mice. Now they took the antiserum from this third party strain of mice against the EL4 leucosis and then passaged it through the tumour-susceptible animals in order to absorb out all the normal histocompatibility and anti-histocompatibility antibodies and were left, hopefully, with tumour-specific antibody. And under these circumstances with very small amounts of antibody, they were able to significantly delay the time of onset of the appearance of a subcutaneous nodule of this lymphoma when it had been injected. Furthermore, they were able to do this by injection of antiserum some time after the original leucosis. It therefore seems clear that two different mechanisms are at play in rejection of the tumour on one

hand and grafts on the other. But this is by no means easily classifiable into tumour or graft and there are good examples of antibody rejection of grafts on the basis of histocompatibility antigens, and similarly, tumours by direct cell-mediated killing.

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Now to complicate the situation further, the work which was started really by Nathan Kaliss, again in the 1950s, showed that if one pre-sensitised animals to tumour antigens, subsequent exposure to that tumour sometimes resulted in enhanced growth of the tumour and this was termed immunological enhancement. Now, analysis of this system showed that the enhanced growth of the tumour was caused by humoral factors in the serum and were, in fact, antibodies directed against tumour-associated antigens. So, to summarise, there were three different types of killing, three different types of mechanism, which were operative in relation to graft rejection. One was a direct killing, apparently by lymphoid cells which didn't involve the presence of antibody but which could be blocked by antibody, second the destruction of tumour cells by antibody transfer, by some mechanism unknown, and thirdly, that antibody could, in fact, protect grafts or tumours from rejection.

Now, it was obvious at this stage that further analysis would be a benefit if it could be taken into tissue culture and the advent of the use of antibiotics and disposable culture made this feasible. But it wasn't until 1960 that the first positive report of this sort of cytotoxic effect was described by Govaerts who was able to show that kidney grafts in dogs resulted in the production of lymphoid cells in those dogs which would destroy graft donor cells in tissue culture. Following this there were multiple reports of similar cytotoxicity and until 1965, it was assumed that this sort of killing was independent of antibody.

<**MacLennon stands and moves over to narrate over board showing diagram**>

If we move on to this diagram, you can see here that the hypothesis at that time was the target cell which bore recognisably foreign antigens caused a reaction in the specific immunological systems, from which came out an effector cell which would, in

turn, go back and kill the target cell which carried the original antigen. Now, this type of killing has now been well established and certainly exists and has perhaps been analysed in the greatest detail recently by workers in Lausanne, namely Brunner and Ceroltini. They have shown that the effector cell, shown up here, is in fact thymus dependent. It appears to develop independently of non-thymus dependent lymphocytes. It kills target cells because it recognises the antigens on the target cells and there appears to be an intimate contact between these cells before killing can occur. Furthermore, there is no evidence that soluble factors are released to kill cells at a distance, although it's not excluded that some factor may pass between these cells and bring about the demise of the target cell.

<**MacLennon, seated, to camera**>

Now, it's quite clear, from the work of Ceroltini and Brunner, that this system works independently of antibody and, indeed, is remarkably prone to blocking, by antibody, for as you can imagine, antibody coming and sitting on the target cell's antigens will prevent the cytotoxic T cell from gaining access to those antigens and so inhibit killing.

Now, in 1965, Werner Müller in Stockholm designed an experiment in which she took an antibody which was directed both against lymphocytes, which were potentially cytotoxic, and against the target cell, hoping that a bridge would bring the target cell and lymphocyte together and bring about killing. And, in fact, this is what happened. And, a couple of years later, Peter Perlmann and Göran Holm in Stockholm and Joe Loewi and myself, working in this country, showed that, in fact, there was no necessity for antibody to be directed against any effector cell in this system, but that it was possible that the target cell-specific antibody could induce killing against the target.

<**MacLennon stands and refers to diagram**>

Now this is exemplified here, instead of this simple system in which the effector cell recognise the target cell antigens, we have a messenger substance working in

between. Here we see the target cell become sensitised by antibody directed against the target cell antigens. Now, the free antibody has no capacity to react with any cytotoxic cell but once it has reacted with target cell antigens in an appropriate fashion, some change, which is not fully understood, occurs in the end portion of the molecule, which is known as the Fc portion, which enables this effector cell to home on to the target cell and, again, in exactly the same sort of direct contact system, bring about the demise of the target cell.

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<**MacLennon sits, talks to camera**>

Now, this system has now been established in relation to a very wide number of situations. It was first described in relation to xenoantigens, that is, antibody and lymphocytes from one species killing target cells from another. But more recently it's been shown in syngeneic systems, against human histocompatibility antigens in particular, which is a contradistinction to the situation in mice, and also against virus-associated antigens on tumour cells.

Now, by no means all cytotoxic systems are mediated through antibody and we just do not know what it is that dictates that either this T-mediated killing or the antibody-mediated killing is going to be effective in any one situation. And this is one of the points which I would like to bring up later on. But in the first instance, I think it would be profitable to try and define the nature of this effector cell.

Now, to start with, it's necessary to be able to quantitate the cell. Now, cytotoxic lymphocytes of lymphoid cells of this type are usually in a minority. That is, that for each cytotoxic cell in a given bag of lymphocytes, you're going to have a high proportion of cells that are not cytotoxic. Consequently, nobody has been able to observe this cell with confidence and say this is a cytotoxic cell that I have under the microscope. It is therefore necessary to resort to physiological methods of quantitation, and the way that we've found convenient of doing this in Oxford has been to measure the number of lymphocytes from a given lymphocyte population,

which may or may not contain effector cells, which are required to produce a given level of killing. Now we use a standard target cell which is sensitised with antibody, in our case it's Chang cells which are a source of human liver cells, and the antibodies produced in rabbits against the Chang cells. We then add varying numbers of lymphoid cells and measure the amount of killing which is produced by these various numbers of lymphocytes. And the picture we get is somewhat like this.

<**MacLennon over graph measuring levels of cytotoxic cells in blood of normal human individuals**>

Now we measure the killing by the release of chromium from the target cell. We label the target cell with sodium chromate, 51 chromium, and this is covalently bound to structures within the cell and is relatively stable – although there is a low spontaneous release of this. If the cell is damaged, however, as the result of an autolytic process the chromium is released and goes into the soluble tissue culture medium and is no longer centrifugical. Now, log increase in the number of lymphocytes produces an increase in killing shown on a sigmoid curve like this. Now, this curve is based on Andrew Campbell's data in our laboratory and is the result of the study of a very large number of normal human individuals. And this is, in fact, the normal mean number of lymphocytes, 10^5, which are required to produce 50% killing of 10^4 target cells.

Now, this represents the standard deviation of the position in humans and this is a standard deviation of the slope. From a practical point of view we can ignore the variation in the slope and it is possible by measuring the position of this curve to find out just what cytotoxic activity is found in a unit amount of blood. If there is a lower proportion of cytotoxic cells, then this curve will be shifted to the right because we will have to add more lymphocytes to produce the same amount of killing. If, on the other hand, there is a greater proportion, the curve will be shifted to the left, and by dropping a perpendicular from the 50% kill point, we can measure the log number of lymphocytes required to produce a given amount of killing, and this we term the SC50. Again, if we then add this to the logarithm of the peripheral blood lymphocyte count, we can get a measure of the cytotoxic capacity which is found in the blood.

<**MacLennon over graph showing levels of cytotoxic cells in the blood of people on long-term Azathioprine**>

Now, to give you an idea of how this works, I've taken an example, again from human studies, in which we measured the cytotoxic capacity in the blood of individuals who had been on long-term treatment with the cytostatic drug Azothioprine. Now, when on this drug, patients almost universally show a depression in the level of their cytotoxic activity; 100% here representing normal. And this vertical line represented stopping the drug and shortly after stopping the drug there was a dramatic rise in the level of cytotoxic cells until, at the end of 3 months, all patients fell within one standard deviation of normal.

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Well, without going into the details of that experiment, it enables us now to understand just how we are able to quantitate the number of cytotoxic cells. The next step, having achieved a satisfactory way of quantitating these cells, was to find out what relative proportion of cytotoxic cells were found in various different lymphoid populations in the rat. And if we look at the slide now […]

<**MacLennon over table showing levels of cytotoxic cells in different lymphoid populations, then to camera**>

[…] you will see this tabulated in a diagrammatic form. The various lymphoid populations are shown on the left of your picture and on the right is the relative cytotoxic capacity. The thymus has no cytotoxic activity, as far as we can see, towards antibody-sensitised target cells and neither does the thoracic duct lymphocytes, in the latter I may say this observation also applies to man. Lymph nodes have relatively little cytotoxic activity as does bone marrow, but the blood is a good source of cytotoxic cells, especially in man. And peritoneal exudate is also a good source as is the spleen. And I should mention that peritoneal exudate cytotoxic

activity is not diminished by prior absorption on glass beads or removal of phagocytic cells with carbonyl ion.

Now, if you now look at the central column in that slide you will see the relative mitotic response to phytohaemagglutinin which is seen in the same population as in the rat, and you will immediately notice a marked difference. Now if we assume that the PHA response, soluble PHA response of lymphocytes, is predominantly due to T cells in the rat, for which there is a considerable amount of evidence, it makes it extremely unlikely that all thymus-process lymphocytes have the capacity to kill antibody-sensitised target cells. As you will see, the thymus is an extremely rich source of PHA responsive cells but has no cytotoxic cells. On the other hand, taking the simple populations it's not possible to say that cytotoxic cells can be present in the absence of PHA responsive cells. In order to test this a little further, Brian Harding and I did an additional experiment in which we subjected rats to chronic thoracic duct drainage. By doing this we hoped to remove the PHA responsive cells from the spleen while not depleting the rat of cytotoxic cells, and you will see that, in fact, the thoracic duct has no cytotoxic cells but an extremely good level of PHA responsive cells. And the results of this experiment are shown in this graph.

<**MacLennon over graph showing results of experiment on rats' spleens** >

The normal spleen shows a good response to phytohaemagglutinin, this is without phytohaemagglutinin on the left. After 3 days drainage, these are mean results of a large number of experiments, the PHA response had fallen to negligible levels. What then happened to the cytotoxic capacity of the spleen? Well, this is shown on the right-hand side. This line here shows the cytotoxic effect of normal spleen cells from the rat towards antibody-sensitised target cells, this is spleen cells without antibody-sensitised target cells, and this discontinuous line here shows the cytotoxicity which was mediated by spleen cells from rats drained of thoracic duct lymphocytes for 3 days, there was, in fact, a considerable rise in the capacity of a given number of lymphocytes to kill sensitised target cells. But at the same time there was a fall in the number of spleen cells as a result of the drainage and when one calculated the total capacity of the spleen to kill it was, in fact, the same before and after drainage. This

side here simply shows the control of a normal spleen cell population, both with and without antibody, as compared with the cytotoxic capacity of thoracic duct lymphocytes, which is negative.

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<**MacLennon, seated, to camera then over graph**>

Now, this information then told us that the cytotoxic cells didn't have the capacity to respond to soluble PHA, at least by the technique which we used. But we wanted more direct evidence against these cells being thymus processed lymphocytes – for at the time we were doing these experiments, the speculation in general was that most cytotoxic cells would prove to be with thymus-processed lymphocytes. And the information that we got from this experiment confirmed our earlier findings. We depleted rats of thymus processed cells in the following way. This was a technique which was suggested to us by Tony Davis from the Chester Beatty Hospital. Now, he suggested that we thymectomised the rats at 6 weeks of age and then gave them serial low dose irradiation, in fact, 200 rads at fortnightly intervals until the rats had had 1000 rads. The idea of this was so that we could take down bone marrow-derived lymphocytes by small stages but in the 2 weeks between irradiation, we'd hope that the bone marrow-derived lymphocytes would, in fact, reconstitute themselves. But the thymus sites, we hoped, would be serially depleted because, in the absence of a thymus, it was assumed that they would not be able to repopulate.

Now, this appears to have been the case. And when we measure the cytotoxic capacity in various groups of animals after this treatment we found there was no change. The N here represents the normal cytotoxic capacity, here is the cytotoxic capacity in thymectomised only animals, here irradiated only, and here thymectomised and irradiated; these results expressed as a deviation from the normal. Now, if we looked at the mitogenic capacity to PHA in these animals, there was a dramatic change. As compared with the normals there was a slight drop in the thymectomised animals, remembering that they'd been thymectomised some 3 months before being tested. The irradiated animals showed a remarkably heavy loss

of PHA responsiveness, a fact which has now been well documented in relation to man, that irradiation selectively destroys T cells, or it certainly selectively prevents the recovery of T cells. And the thymectomised and irradiated group showed a drop in the PHA responsiveness to less than 0.3% of normal. And from these data we felt fairly confident in suggesting that, in fact, cytotoxic cell which killed antibody sensitised target cells, a cell which has now been termed a K cell, was probably capable of developing in the absence of the thymus.

<**MacLennon to camera, then over earlier table showing levels of cytotoxic cells in different lymphoid populations**>

Now, if this cell is not a thymus-dependent lymphocyte, what is it? Now, I've already suggested, in passing, that it's not a macrophage and the evidence here is fairly strong, but not absolutely convincing, certainly in relation to the rat and man against sensitised Chang cells we have failed to provide really strong evidence to suggest that macrophages can do the killing. For instance, the cytotoxic cells are not removed by pre-absorption on glass, or by treatment with carbonyl ion. In addition, the antibody which induces cytotoxicity against target cells by K cells isn't acting in quite the same as the antibody which induces phagocytosis by macrophages. So, really there is very little evidence, in rats and man at any rate, in relation to this target cell, that we are dealing with a macrophage or a macrophage precursor.

This then leaves us the possibility of it either being a B lymphocyte or a cell which is independent of any known lymphoid population and merits its own independent term, and hence the term, now, of K cell. The evidence against it being a B lymphocyte is, I think, equally strong.

If we return to this slide, I would like to point out 2 points – one is that thoracic duct lymphocytes contain no cytotoxic activity. The experiments of Gowers and his collaborators have really substantiated beyond all doubt that the precursors of antibody producing cells do circulate in the thoracic duct. Their experiments have been based largely upon lethal irradiation in rats and reconstitution with thoracic duct lymphocytes which has enabled the rats to subsequently produce normal antibody

responses. And, more recently, Jonathan Howard, again working in Professor Gowers' laboratory, has been able to show that the actual antibody-producing cells derived in this situation come from the donor thoracic duct cells. Secondly, if one considers the primary site of antibody production after injection of Chang cell into rats, in the skin, the lymph nodes produce by far the most antibody. And yet the lymph nodes are a relatively poor source of these cells and this, again, mitigates against these cytotoxic K cells being antibody producing cells.

<**MacLennon to camera, then stands and refers to diagram**>

Equally convincing evidence has now been derived from human studies where it has been shown that many patients with hypergammaglobulinaemia have relatively normal levels of K cells in their peripheral blood. To conclude, therefore, at this point it seems likely that the cytotoxic cell, which we have been looking at for some time now, is neither a T cell nor a B cell, it probably isn't a conventional macrophage and I think we must consider it to be a cell apart, a cell in its own right, which is capable of killing antibody-sensitised target cells, and the name K cell seems as reasonable as any.

Now, I just want to take you over to a diagram which I've made up to show that, in fact, K cell lysis is not as simple as it may appear. If we consider the total biological system by which this one mechanism is working – a large number of cells are involved in bringing about an effective kill. First of al the target cell will release antigen. We know that the antibody response in rats is a thymus-dependent antibody response, so we require T helper cells which will help by presenting antigen, in some way which is not fully understood at the moment, to the antibody-producing cell's precursors. These then mature into antibody-producing cells, secrete antibody which will then sensitise the target cell and the sensitised target cell then becomes vulnerable to lysis from the K cell and death ensues.

Now, if one considers this complicated series of steps to bring about this lysis and then considers that more than one cytotoxic mechanism may work in graft rejection, the clinical problem of interfering with this or augmenting this is very considerable

and I think we will have to bear in mind all these things if we want to rationalise therapeutic manipulation of the rejection or protection of grafted cells.

<**MacLennon to camera, then stands and moves pieces from earlier diagram to previous diagram, then narrates over it**>

Now, in the second half of this talk I want to go on to discuss the way in which the antibody, which induces lysis by K cells, works and particularly study this in relation to other systems which are triggered by antibody.

Now, if we go back to this picture which we had at the beginning of the T effector cell, the relatively simple system, and take these two elements out and place them over here, we put them into the immunoglobulin-mediated effector systems, or the immunoglobulin-induced effector systems, and here the specific reacting system is responding to antigen injected in and immunoglobulins of various types are produced. Now, the antibody can be directly put into two functional types: namely, that which sticks directly onto effector cells, and here we think particularly of macrophages, basophils and mast cells, and that antibody which circulates free and then first sensitises target cell antigens before calling in various other effector mechanisms, here, in which we have the complement system, phagocytosis and effector lymphocytes of the K type.

Now, cytophilic antibody, that is, antibody which sticks directly on effector cells, does not induce any cytotoxic effect, again, until it is triggered by the combination with antigen. So there are two distinct steps: one is reaction with the effector cell and then triggering. Looking on the free immunoglobulin system, the target cell antigen comes into contact with the antibody and following this there is some change which may be associated with aggregation of a number of antibody molecules together, or possibly a conformational change in the actual structure of a single antibody molecule which enables it to trigger these other effector mechanisms.

Now, the best known of these mechanisms is perhaps the complement system, and this has been described particularly in relation to the lysis of sheep red cells. And

here, fixation of a cascade of protein substrates results in the subsequent lysis of the target cell through the C8 and 9 punching holes in the cell membrane. The complement system also acts in relation to phagocytosis, for, after fixation as the first component of complement C4 and 2 are fixed and then a very large number of C3 molecules are deposited on the cell membrane. Now in the case of phagocytosis this is more often going to be, in fact, bacteria than total nucleated cells, but the neutrophil and macrophage have receptors for the 3rd component of complement and this is an extremely efficient system for inducing phagocytosis.

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Now, IgG has the capacity to react directly with neutrophils and macrophages and so can also induce phagocytosis. And finally, we have antibody which sensitises the target cell and brings into play the K cell to bring about target cell lysis and this cell lysis, here, should be taken very generally as to mean, in fact, the effector function of these agents.

Now, one of the questions I want to ask now is: are these 4 antibodies, represented A,B,C and D here, in fact, different or are they the same antibody showing a heterogeneity of function, indeed, is this antibody the same? And the answer, I'm afraid, is a bit of both. To show the biological system which is often found – if we sensitise a target cell with antibody, we may not necessarily see it being vulnerable to all effector mechanisms.

<**MacLennon over previous diagram of experiment on rats**>

Now, if we look in this old system which we've been dealing with – the Chang cell in rats, and take rat and Chang cell antibody in the presence of a source of complement, fresh guinea pig serum, we see that complement-mediated lysis is lost by a dilution of 1 in 1000 antibody. But the lysis, mediated by the spleen cells from rats against the antibody-sensitised target cells, is only titred out at dilutions in excess of 1 in 1,000,000. Now, in many situations it is not possible to demonstrate any complement-mediated lysis but still get K cell lysis, and yet other situations one

can get complement-mediated lysis without K lysis, and the last situation is most obviously seen with IgM which is capable of inducing complement-mediated lysis but not lysis by K cells.

<**MacLennon, standing, to camera, then over diagram of IgG model**>

Now, I'd like to confine the rest of my argument to IgG because this is the antibody which induces lysis by K cells and we can consider a number of different effector mechanisms which are known to be triggered by IgG, namely, the complement system through the first component of complement, the macrophage phagocytosis (and this is a cytophilic antibody of course), phagocytosis of bacteria by neutrophils and lysis of target cells by K cells.

First of all, it would seem reasonable to tackle this on the basis of the structure of the IgG molecule. Now this is extremely diagrammatic but it's helpful for our purposes. You can see here we have the antibody combining end of the molecule, the variable part of the light and the heavy chain here – denoted by VL and VH respectively. And then we have constant portions of the heavy chain - CH1, CH2 and CH3 and the constant portion of the light chain. Now, it's possible to cleave these molecules by various proteolytic processes and by doing this we can find out which portion of the molecule is responsible for which function.

Now, as I have already suggested, the antibody combining activity is confined to the variable region, but the effector activating determinants are more or less totally confined to this portion of the molecule known as the Fc portion. The only biological activity, effector mechanism, which is known to be triggered by this portion of the molecule, which is left after pepsin digestion to yield 2 light chains and 2 heavy chains to this region, retaining this thiol bond, namely this is F(ab')2, the only effector function which is left in rabbit IgG is the alternative pathway of direct C3 fixation, and this was described by Ken Reid.

Now, if you cut the molecule here, the Fc portion which remains from papain digestion has the capacity to react with K cells, with neutrophils, with macrophages,

with the first component of complement, so all those effector mechanisms are found along this region of the molecule. Now, George Connell, from Toronto, was over in Professor Porter's laboratory in Oxford some time ago and he conducted some experiments and found that digestion of IgG with plasmin, after pre-treatment of its low pH, resulted in a cleavage here, leaving a large portion which contained the variable regions CH1 and CH2 and cut off CH3 – this representing the mere 110 terminal amino acid residues. And by doing this he produced an antibody which had a normal antibody-combining activity but which failed to react with K cells, with neutrophils or with macrophages, but the capacity to combine with complement through the first component C1Q was absolutely normal and this, I think, clearly indicates that the C1Q must be on these 80 amino acid residues between the papain cut and the plasmin cut. We then are left with the reactivity of macrophages, neutrophils and K cells in this end portion. Well, we know that IgG can react in its native form with macrophages but it acts in a rather different way with K cells and neutrophils; there is very little evidence that the antibody will react directly with either K cells or neutrophils.

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<**MacLennon to camera**>

And we have done some experiments to find out what physical changes you have to produce in normal antibodies, in the absence antigen, in order to mimic the changes which occur in antigen binding and this is remarkably different for different effector mechanisms. For instance, the capacity to react with K cells is acquired in the purification step of putting IgG on an ion exchange cellulose, DEAE cellulose. After this a proportion of the antibody reacts well with K cells, however, it has very little capacity to react with neutrophils and it is still pretty inactive in the complement system. If, however, we heat the IgG to 63° for 20 minutes, it then acquires the capacity to react with neutrophils, also the capacity to react with complement is considerably increased.

Now, different sizes of aggregate produced by this heating mechanism react with these different systems in different ways, and I haven't time to go into that at the moment, but this sort of experiment shows that physical changes which mimic antigenic combination are, in fact, different for different effector mechanisms. Now, I'd now like to swing over to the final slide just to show what heterogeneity there is in relation to IgG and their function.

<**MacLennon over table showing different sub-classes of IgG, then to camera**>

Now, it's possible to recognise 4 different sub-classes of IgG which are conveniently named IgG $_{1, 2, 3}$ and $_4$. Now, these have been detected as a result of analysis of myeloma proteins; these are proteins produced by malignant development of a single clone of antibody-producing cells and consequently pretty well identical immunoglobulin molecules are synthesised in large quantities. It's therefore possible to study a single type of immunoglobulin in some detail and when we analyse the capacity of these different IgG molecules to react with different IgG sub-classes, to react with the 4 effector mechanisms you see shown there, IgG_1 and IgG_3 were able to react with all 4 effector mechanisms, but IgG_2 only really reacted with neutrophils and K cells, while IgG_4 appears to only react with K cells.

From this information it is clear that IgG_1 and $_3$ molecules appear to have a capacity to react with at least 4 different effector mechanisms and there are others which haven't been tested in this series. Therefore there must be heterogeneity of function within a single molecule but the different sub-classes clearly show that there are different types of immunoglobulin which have a greater capacity to react with one effector mechanism than another.

Now, these studies have really got us rather a long way from the initial starting point in which we were discussing the way in which immunological processes could bring about cell rejection or cell death, but it is helpful, because, as I said at the beginning, there is no one single mechanism which can be taken to be the only important cytotoxic mechanism in graft rejection, tumour rejection or autoimmunity. We have to

consider several of these and we don't really know why any one of these mechanisms is switched on at any time. And probably the key to this is the antigenic pattern on the target cell itself and we have to know why certain antigenic patterns switch on certain immunological mechanisms and others switch on others.

Now, this is an extremely difficult problem to analyse, although it's relatively easy to state, and I would suggest that the study of antigen antibody combination in fluid phase with myeloma proteins may very well make an easier starting point than trying to unravel the different antigenic patterns which are capable of triggering various different immunologically competent cells. Thank you.

<**End credits**>