Essentials in Immunology

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Lecture No. # 02

Cells and Organs of the immune system – Part 1

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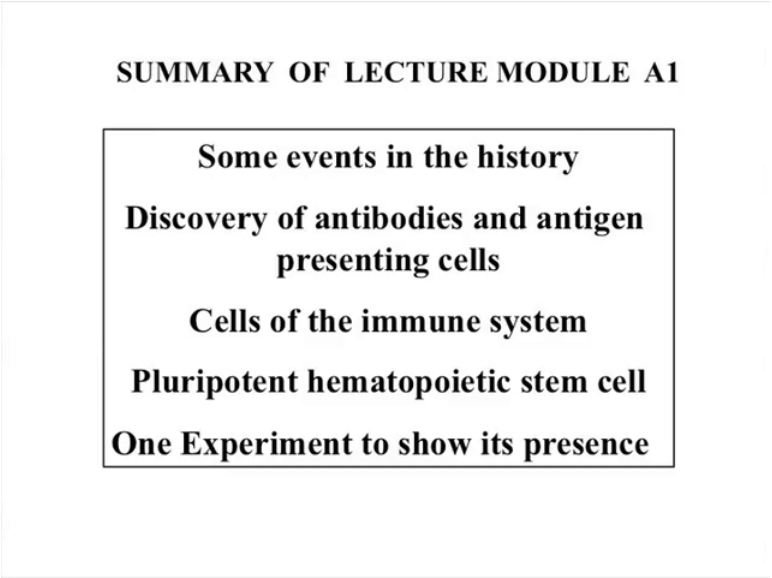

Hello and welcome. Let us summarize some of the points that were dealt with in the previous lecture. In the previous lecture, we dealt with some of the events in the history of immunology, which led to the discovery of antibodies and macrophages, and then we looked at the different kinds of the cells in the immune system and the concept, that all these cells arose from pluripotent hematopoietic stem cell. Due to lack of time in the last lecture, we were not able to go into the experiment that was done to show the presence of this hematopoietic stem cell.

Before showing the presence of the hematopoietic stem cell as events took place, there were experiments that were done in order to find out if in fact, the cells, that arose and populated the humeral and the cellular immune system, were actually derived from immigrating cells. In other words, once the thymus was differentiated, the question was

asked, whether these cells actually arose from cell, that were immigrating from elsewhere or was it possible, that all these cells, that make up the T-cells coming from the thymus or for that matter, from the Bursa, did they arise within the tissue itself or within the organ itself, like thymus and the Bursa?

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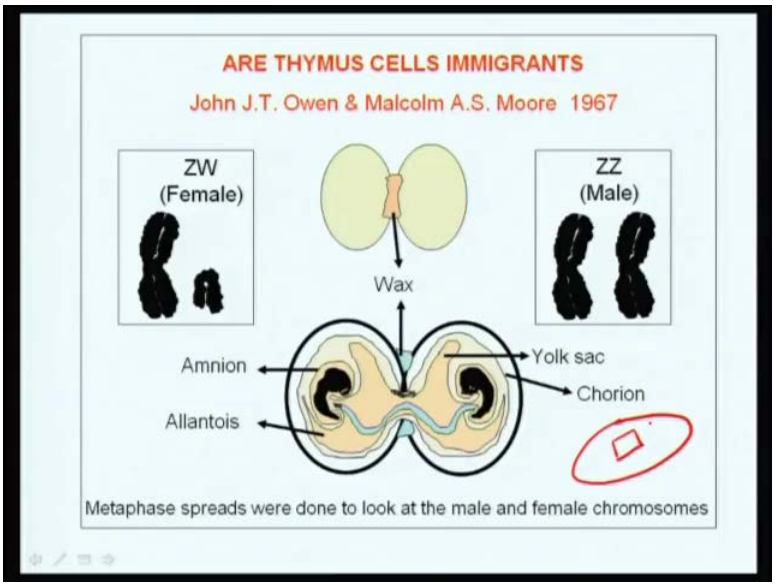

So, experiments were done to find out, if the cells, that were coming into the Thymus were coming in from elsewhere? Another point in history, several experiments were done using embryos of chicken and chicken eggs. So, if in embryology, the several experiments, that are done to look at, how embryo genesis occurs? How the embryo develops? And one of the favorite models is to look at the chicken egg; so, one takes a chicken egg and looks at it under the light and cuts a small window, cuts a small window to find out, excuse me let me go on to the pen, so if you had a chicken egg, one would look at it under the light and see, which part of the egg has got embryo and then cut a small window over there, and then put a ((cover slip)) on top, so that you could observe, how the embryo developed inside.

Similar, such experiments were done using 2 different chicken eggs, one of them being derived from the female and one of them being derived from the male, or so to say, that these embryos were male and female. The objective of trying to use the male and female was that, a lot of experiments during those days were done using metaphase spreads looking at the appearance of chromosomes. And if one looks at the female and the male

set of chromosomes in chicken, you find that the female one has got a smaller one associated with the bigger chromosome, and that is shown clearly over here and it is referred to as Z and W. In contrast, the male cells, you have 2 chromosomes, which are similar and they are called as Z and Z. And therefore, if you had cells that were derived from the female, one could distinguish by doing a metaphase spread and ((searching)) for this smaller chromosome.

So, if, when you took, took these eggs, then cut open a small area and make a small window and then juxtaposed these 2 eggs and then seal it with wax, the underlying membrane would fuse together and blood vessels would fuse allowing the circulation to be mixed and then have continuity between the two embryos. And with time, there would be a joint circulation, that is established between the 2 embryos and then, once the, once the birds developed or chickens hatch, you could look at, took out the Bursa or took out the Thymus, because earlier experiments had found, that thymectomy, as I told you in the last class, the thymectomy and bursectomy led to a decrease in the number of small lymphocytes, that are present by morphological examination by staining these blood preparations.

So, they took these developed or hatched chicks and then looked at these organs, the Thymus and the Bursa, and took out these cells and then did a metaphase spread, and found that actually, there was a mixture of cells. That means there were cells that were derived from other one, would have the other chromosome that was there. And therefore, this (( )) experimentally is that in fact, cells were actually immigrating from the embryo to the one, that was fused to it.

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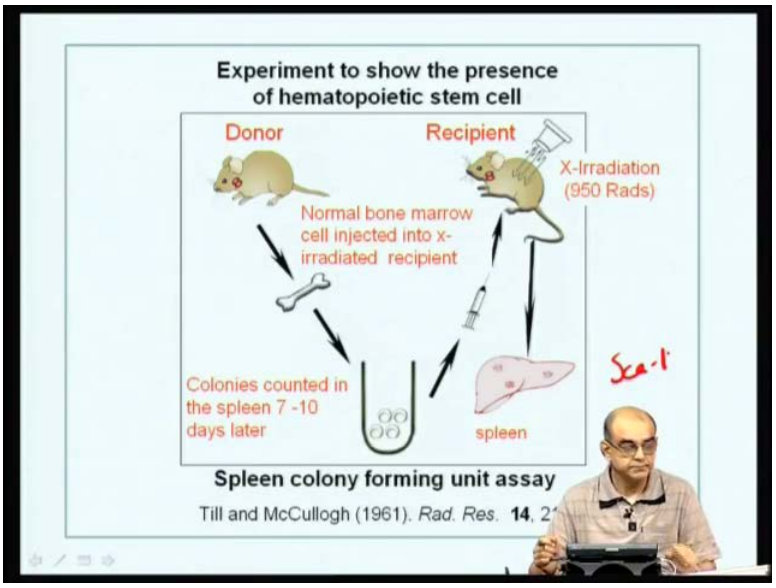

So, once this immigration aspect of cells going into the Thymus was, was, was shown, then the question was, how can it show, that a single hematopoietic stem cell actually gives rise to all the rest of the cells, all the rest of the hematopoietic cells in the, in the mouse, or for that matter, for the immune system? Now, this was an experiment that is called as, spleen cell, spleen colony forming unit assay, which was shown by Till and McCullogh in 1961.

Now, as I told you earlier, that in order to find out the function of various organs, experiments aimed at removing that particular organ, for example thymectomy, bursectomy, hypophysectomy, so on and so forth, and then see, what could happen once that organ is removed? What sort of cell would be absent, what sort of hormone would be absent, and so on and so forth?

In a similar experiment, Till and McCullogh found, that you could remove all the immune cells and body of the mouse by doing or subjecting them to X-irradiation at 950 Rads. The dose of exposure is very important because this is the dose that could allow the mouse to live; as you all know, higher dose of irradiation would actually kill the mouse, or for that matter, even human beings. And this exposure of irradiation was done at 950 Rads, and this would affect all cells that would, that have the capacity to divide or proliferate.

Whereas, those cells that did not have the capacity to proliferate, would be more resistive to this dose of, this dose of irradiation. So, what they did was to take 2 mice, one was called donor, the other was called as a recipient because it was receiving the cells from the donor. Now, this recipient was exposed to 950 rads and then, after certain amount of time, they received normal bone marrow cells, that were derived from the donor.

So, in other words, you could take the femur of this donor mouse and break it and inside the pulp, if you remove the pulp, you would have cells in it that would come out into a petri-plate, take out those cells and adaptably transfer or inject it into this mouse that is a recipient.

Now, after about 7 to 10 days, one would remove the spleen, which is located in this area; take out the spleen and then look for colonies, that have, that are there in the spleen, for example over here, which I have designated in this organ and there would be several other colonies, that would form. So, all these would form from these cells, that had the ability to immigrate into the spleen, and once the cells immigrated into the spleen, it would start to proliferate and therefore, the colonies. So, this was the assay that was done to show, that when you took out bone marrow, these cells were derived from the bone marrow, these bone marrow cells have the ability to repopulate the spleen, which is actually a secondary lymphoid organ and given time, these cells would actually form different kinds of lymphocytes.

But this, this is an assay or model, that you must remember, that exposure to irradiation would result in mutations and therefore, one could not have a wide model at the end of the sometime, except to show in a short duration, that these cells were actually forming colony in the spleen, that they had ability to show you certain characteristics, that were evident for lymphocytes. So, this (( )) is called as the spleen colony forming unit assay, and it was discovered in 1961.

Now, in modern days, you have, as I told you in the last class, there is an antigen called, called as stem cell antigen-1, so one could look at the presence of this antigen. And there are methods to isolate these stem cells by using advanced machine, you could isolate these themselves and then give it to various kinds of mice, for example mice that are mutated, called as skid mice - severe combined immuno deficient mice - which do not have lymphocytes. So, you could actually, one could actually, isolate humus stem cells

and put them into skid mice, and humurize that mouse in order to see, how human lymphocytes behave in a, essentially in a mouse bag? So, so much for a spleen cell colony forming unit assay.

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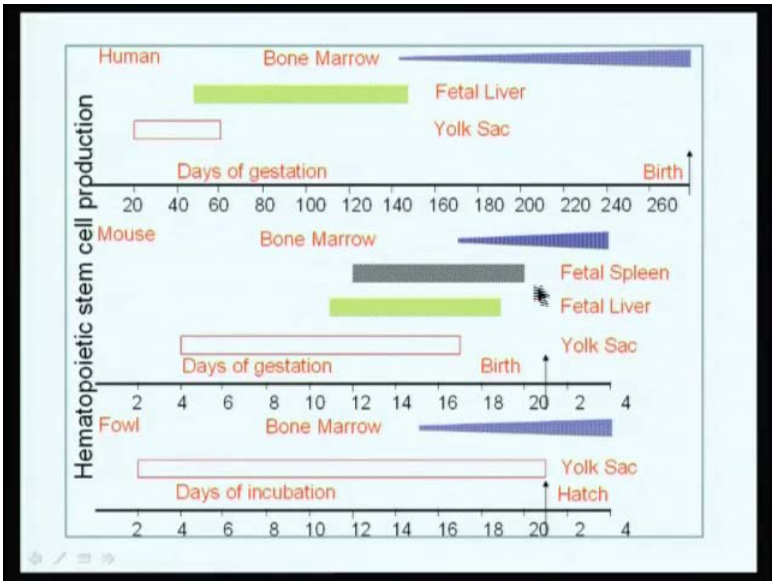

Now, as we go on, we find, that this capacity to form lymphocytes from a hematopoietic stem cell or the generation of the hematopoietic stem cell, as it was a (( )) from the bone marrow in the previous slide, one sees, that in different animals it is not only the bone marrow, that, but different organs also take part in hematopoiesis earlier on, during gestation. For example, in the chicken, you find that in addition to the bone marrow, you had the yolk sac that participated in hematopoietic stem cell, before hatching. After hatching of course, you had bone marrow participating and providing those hematopoietic stem cell precursors.

In the mouse, you find, in an addition to the yolk sac, you had the fetal, fetal liver as well as the fetal spleen playing a roll, an optimal roll, approximately during these times before birth, as I have indicated to you in the colors. Finally, of course, the bone marrow takes over the function of hematopoietic stem cell production in the adult stage.

In the humans, you find that the fetal liver plays some, some roll in addition to yolk sac. Finally, the bone marrow takes over the function of hematopoietic stem cell production after birth. So, these are of course, the approximate time-table that are involved in the hematopoietic, just hematopoietic stem cell production. This slide, just to, has been put

in here, just to tell you, that in addition to bone marrow, other organs do play a part in a hematopoietic stem cell production, earlier on during gestation.

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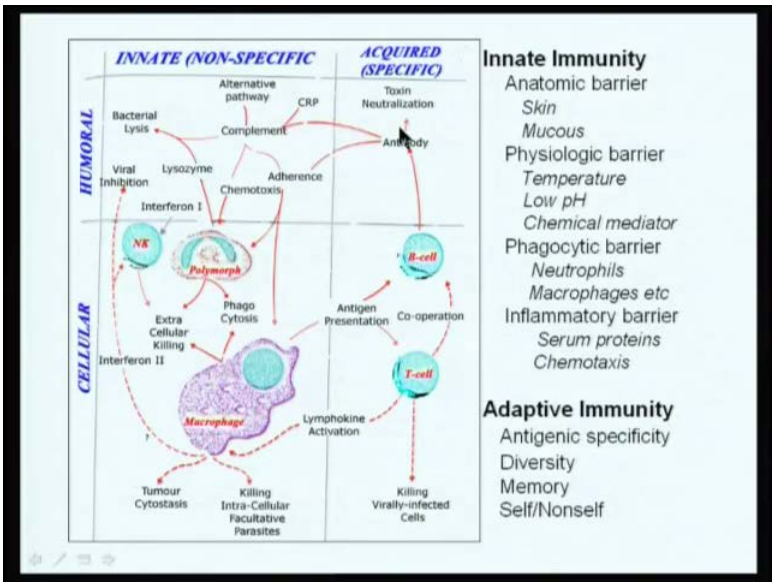

Now, to put all these things together, now that we know, there are types of cells, lymphocytes, and how do these lymphocytes participate in the immunological reaction or the immune system. So, the immune system has been known to have 2 arms, so to say, and that is called as the innate immunity and the acquired unity. The innate immunity is more known, specific in nature while the acquired immunity is more specific in interactions. As I told you earlier, these 2 categories can be visualized as humeral, as well as, cellular.

Now, what happens in innate immunity? What is innate immunity? Innate immunity is the immunity, that you have got during birth or you have received as you were born. Now, these are, as I told you earlier, that these are mostly nonspecific in nature. For example, it consists of anatomic barriers, for example the skin and the mucous membrane. Now, the skin accesses the barrier for a variety of infections because of its nature and also, perhaps because of the pH that is there in the skin. So, certain viruses and bacteria do not tolerate the pH that is present in the skin, certain areas of the skin and therefore, they were not able to infect.

In the mucous that is, that is present, that is present in many of these cavities, this is also associated with what is called as malt or mucosal associated immune system, mucosal associated lymphoid tissue (malt), that you will see in many of the text books.

So, you, you see, the mucous has got several immunological molecules, like for example IgA-secretions. Now, IgA-secretions are a barrier to infections because of their very nature, they have lot of polysaccharides and they agglutinate various kind of organisms and do not allow them to infect. And therefore, you have in your nose mucosal secretions, which act as a barrier. Then, you have of course, the physiological barrier, for example temperature. Many of the viruses, bacteria cannot divide or cannot metabolically function in the temperature of the 37 degrees and therefore, you have, for example, whenever you get an infection, you have fever and this is essentially, to block certain infections. And of course, you have the low pH as I needed to, earlier on, that might be present in certain areas of skin; then, you have variety of chemical mediator.

What are these chemical mediators? These chemical mediators are, for example, certain of these proteins like interferons, and so on and so forth, which actually, like example lysozyme. Lysozyme, actually it cleaves the cell wall bacteria and causes inhibition of bacteria, bacterial infections because it leads to bacterial lysis.

And therefore, you see, these are all nonspecific in nature. So, when you look at all these, all these in total, you find, that they have a kind of a barrier towards infections. In addition to that, you have what is called as a phagocytic barrier, which all of you know, like neutrophils and macrophages, which we saw in the last class, that they had the ability to phagocyte those bacteria; they were chemotactic in nature and this chemotactic is part of innate immunity.

In addition to that, you had inflammatory barriers, like for example, what you call as an acute phase reaction or acute phase proteins that are synthesizing response to infections in mammals. These contains several serum proteins, in addition also has certain proteins like complement, which I told you in the last class and we will going to that little bit in this class also, and also, a protein called the (( )) active protein. So, all these help in blocking infections because complement leads to bacterial lysis or inactivation of viruses that have the ability to infect.

In addition to that, I have already told you about chemotaxis in the previous lecture, about how the cell walls have the ability or have chemical that, that can cause chemotaxis of macrophages from inside; they can cross from inside, the capillaries can come out into the area of infection.

As opposed to adaptive immunity, which is most specific in nature, which we spoke about in the last, last class, that they produce antibodies and these antibodies was specific to the antigens that they would react to. And therefore, this toxin neutralization that we referred to in the last class, are all part of acquired immunity.

Now, this acquired immunity actually involves, not only antibody production by B cells, it also involves interactions between T cells and B cells, because T cells also have the ability to recognize antigen and the T cells would cooperate with the B cells, help them to proliferate, help them to make these antibodies.

All these interactions are specific in nature and in addition to that, you have the phenomena called as antigen presentation, which will be going into in, in, in a small way in the coming few slides. So, you had antigen, you have antigen specificity in adaptive immunity and of course, the diversity of these specificities are involved in the antibody generation, which you will come to learn in the classes on antibodies, as well as, an aspect of immunological memory, which I will tell you in the next line, and of course, self and non-self-discrimination.

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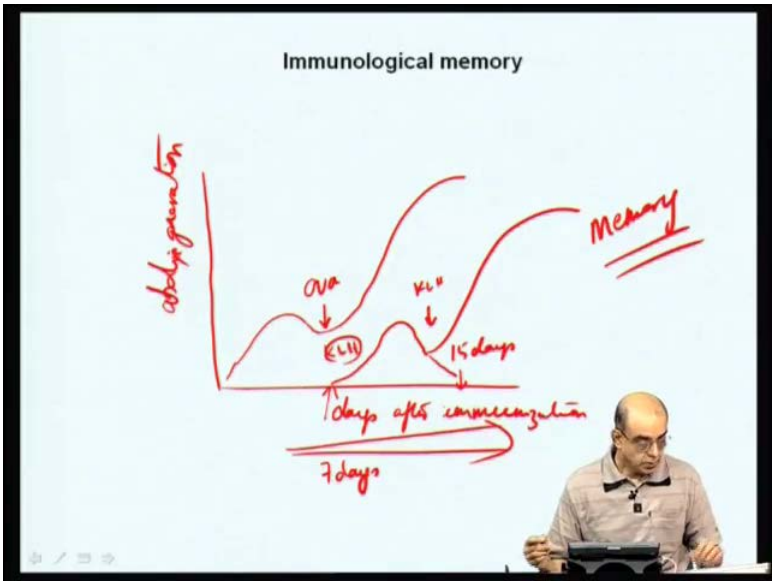

So, what is immunological memory? Immunological memory pertains to the fact that if one gets over or overcomes a particular disease, for example during those days, small pox, the individual would never again come down with small pox; so, what is this? This said was because of immunological memory.

So, if you were to look at as, as were confirmed during those days, by injecting various kinds of antigens, like sheep red blood cells or KLH or ovalbumin, they would find, that if you were to look at the antibody responses to, let us say for example, ovalbumin and you were, you were to look at antibodies to ovalbumin over a period of days in, let us say, any of the higher animals, for example chicken or for that matter rabbits, and so you had days after injection or immunization; so days after immunization and it would go roughly like that.

Then you looked at the ability to form antibodies. Antibody generation let me put it as, of course, this antibody generation could be measured by the antigen-antibody complex, that you learned how it could be precipitated in the previous class and that was in fact, some of the first few experiments that were done. But if nowadays, one can follow these antibody titers and looking at lysis by taking the blood or bleeding these animals and then looking at the lysis, titers of the antibody against the particular antigen, that you had used to immunize.

So, if you are to immunize and take out this blood at various times, bleed these animals and look at the antibodies in the blood, you found, that if you had the antibody, look at the antibody titers, you would find, that there would be an increase in the beginning and there would be a drop. Then, if you administer antigen again at this point, you would find, that these antibodies had a property of showing a higher response. So, this was, this was after immunization 2nd time with the same antigen.

For example, if you were using ovalbumin, so you would find that the antibodies would pick up rapidly. So, if these were referred, let us say, if you were to have 7 days over here, so in the same, approximately same, around 15 to 16 days, you would find, that the titers of anti-ovalbumin antibody rose much higher than this, than this 1st phase. On the other hand, if one immunized, at this point, if you immunize with another antigen, such as, let us say KLH, you would find, that the same small peak would come and after 7 days, at the end of, at the end of 7 days after this initial injection with KLH, if one must

be injected at 2nd time with KLH, then you would have the same secondary response coming up. So, this is called as memory because these cells retain the memory, that they had seen the antigen earlier and they were able to kick in a response, much rapid and much higher response to the same antigen.

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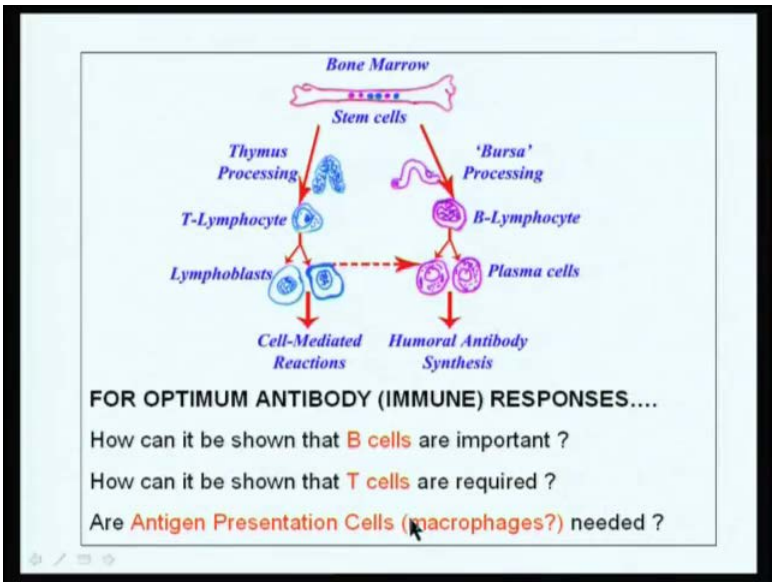

So, this is a phenomena of immunological memory, which is characteristic of acquired immunity. So, going on further, to look at other aspects of the immune system, we need to know, how can it be shown that B cells are in fact, important? And how can it be shown that T cells are in fact, important? And more importantly, we allure it to the discovery by Elie Metchnikoff, and how important these macrophages were, that he found in the star fish larva, and in higher animals, these, these, these are now come to know as antigen presenting cells.

Now, what is, what are the experimental proofs to show, that these 3 major players are very important for an immune response? So, just to summarize in this slide, we have the bone marrow giving rise to hematopoietic stem cells and these stem cells, if they migrated to the Bursa or in higher manuals, if it was the bone marrow itself, it would give rise in that differentiating environment, in that milieu of the availability of kinds of growth factors or cytokines, they would give rise to a B lymphocyte, which would go on to produce antibodies upon stimulation with an antigen, that we will come to learn later on.

On the other hand, if these stem cells go to migrate into the Thymus, which is a primary lymphoid organ, the Thymus and the Bursa are primarily lymphoid organs, whereas the spleen and other lymph nodes are called as secondary lymphoid organs. So, migration into the Thymus resulted in the differentiation of these precursors or stem cells into T lymphocyte, which had the property of differentiating into other kinds of T subset, which would in turn help B cells or B lymphocyte, to make more of antibodies, by virtue of the type of cytokines they secreted in response to antigen mediated activation of T lymphocytes.

What this antigen mediated T lymphocytes activation means, we will see in the next few slides. So, therefore, in order to see, that the B cells, T cells and antigen presenting cells are important for the immune system, let us see, what are the experiment that were done to show that importance?

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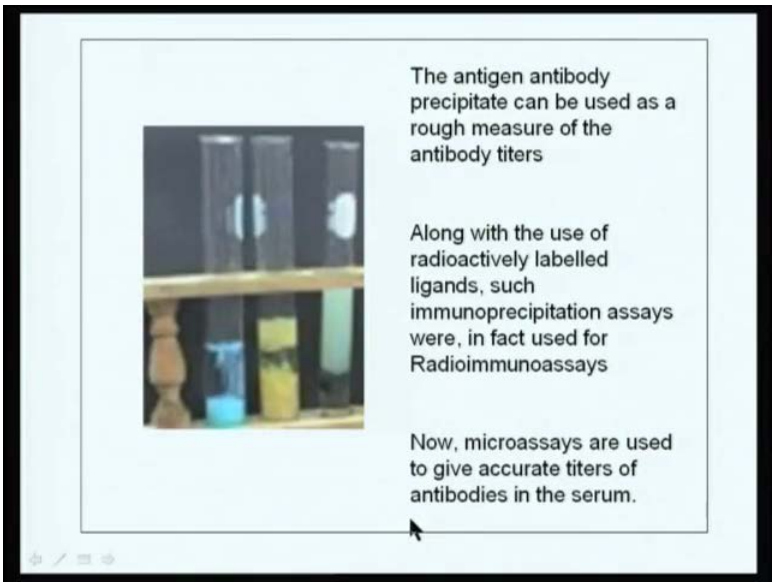

As I told you earlier, all these experiments developed in the earlier stages by looking at the antibody titers. So, as the history of immunology developed, as I told you in the last class, all these developed in the initial stages by the ability of the antigen and antibody to form clouding or precipitate, and these would of course, as I told you, precipitate at a certain concentration, and this was evident, even in Ouchterlony double diffusion test, which I showed you in the last class.

Now, of course all these assays later on, developed into much more really accurate assays, called as radioactive, radioimmuno assays. So, if one follows all these different ways of measuring antibody titers, one could follow, what are the different, what are the ways by which an animal responds to different kinds of antigens. And therefore, of course, one could know, whether it was a primary response or it was a secondary response?

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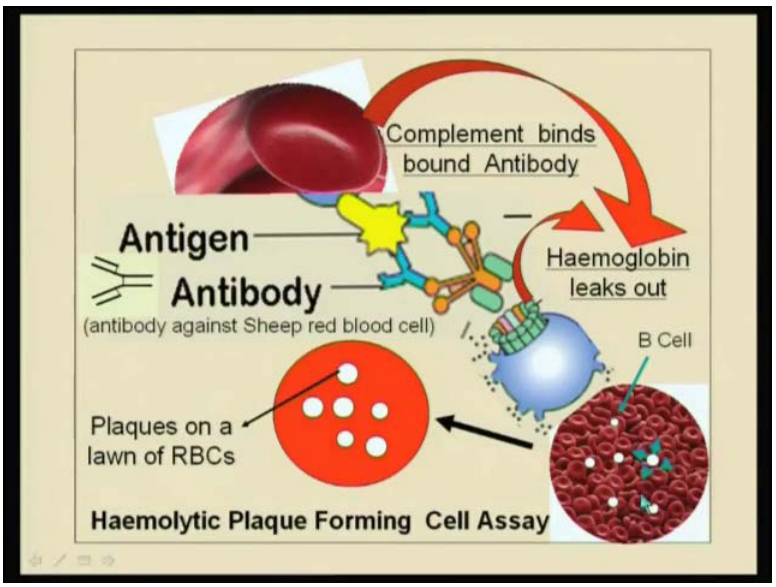

Now, in order to know, whether actually cells would secrete antibodies, there were other assays that were done in order to find out, whether there were more cells and more B cells that would secrete antibody? And this, one such, as I say, is called heamolytic plaque forming cell assay, so or a PFC assay. What this shows you is that the cells that are actually producing these antibodies and in order to understand this assay, let us go into, what type of antigens, that were used during those days as immunogen? I (( )) to you earlier, that the sheep red blood cell was easily available and therefore, was used as a powerful immunogen because it is quite good in activating various kinds of lymphoid cells.

So, if you look at the response to sheep red blood cell, one can make antibodies to sheep red blood cell in the different animal, like the rabbit and so, when you, main antibodies and also (( )) to you in earlier classes, that serum factor, additional serum factor, called as a alexine or now named as complement, also responsible in activating bacteria. So, how

do these antibodies, antigen and complement function, in order to make this assay a viable one.

So, what one does is to take the sheep red blood cell, it has got haemoglobin, so it is red in color, and therefore, if you had antibodies generated to the sheep red blood cell, rabbit and you put the serum along with the sheep red blood cell. As I told you earlier, the antibody can be represented in a, as a Y shaped structure like this, and it had 2 heavy chains and 2 light chains, which are connected to each other by disulphide balls in this manner; so, these are your antibodies.

And let us say that this is the antigen against which the antibody has been raised. And in sheep red blood cell, one of the powerful antigens is actually carbohydrate in nature, in addition to protein antigens. So, these antibodies would bind to this antigen, and complement has the property of binding to an antigen that has bound to an antibody; it has the property of binding to the antibody that is bound to an antigen.

So, antigen-antibody complexes have the property of activating complement, the (( )) is a complement cascade. Now, what does this cascade do? The first component of complement binds to the constant portion of these antibody molecules of the stem portion of this y, and then starts to activate the cascade, which finally leads to punching of the hole into the cell, that is binding the antibody.

So, in this case, this is an RBC, so therefore, you have haemoglobin and the punching of the hole into these RBCs, like what I have shown you over here, causes a leeching or leaking of the haemoglobin and therefore, this area clears out. So, where is this antibody that is going to be there, that will bind to this sheep red blood cell? These antibodies are going to be produced by a B cell, that is, that is found in this place. Therefore, what one does is to take the sheep red blood cell, mix it, sheep RBC's mix it along with different dilutions of the lymphocyte, that have been taken, taken from the animal's blood.

So, you can isolate cells from the blood, or for that matter, if it was a small animal, like the mouse, you could take the spleen, which is an excellent source for B cells, as well as T cells. So, if you had different dilutions of these B cells, you would have, these are, I have represented this as, each white one, as a single B cell over here. So, this B cell would secrete the antibodies, the antibodies would then bind to your RBC, and then they would form a complex of RBC with the antibodies.

So, all this is done in a petri-plate, which has got a semi-solid medium like agarose. All you have to do is to take molten agarose, mix your sheep red blood cell and the serum or the preparation, that is containing the cells, that is derived from the animal that was (( )), lymphocytes, that have been derived from the immunized animal and then allowing this agarose to solidify, after which, allow for some time for the antibodies to be secreted and binding to the red blood cell, after which you overlay it with complement, that is derived from the rabbit serum. So, when you freshly bleed rabbit, the serum is an active source of complement. So, the complement in turn binds to this complex, and the haemoglobin leaches out leading to formation of clear areas, white areas in a red lawn.

So, these are the plaques; therefore, these are called as haemolytic plaque forming cell assay. So, therefore, one could actually evaluate the number of B cells in an immunized animal against sheep blood cell. Now, as I told you, this is an assay based upon the use of sheep red blood cell, but sheep red blood cell can in turn, surface of the red blood cell can in turn, the coupled to various kinds of antigen by using certain chemical or chemical coupling agents, which people used to use at that time.

So, one could couple KLH, one could couple ovalbumen or other kinds of antigens to the surface of the sheep red blood cell and therefore, you had an assay again, or for an antibody, that is being made against the antigen, that was coupled to the sheep red blood cell. For example, ovalbumen is coupled to the sheep red blood cell, you had to immunize mice with ovalbumen, you could take those, these cells and look at antibody, that were binding to the sheep red blood cell, that would couple to ovalbumen. So, you had an antigen specific assay, also in addition to just having the sheep red blood cell as a, as an immunogen.

So, in addition to this, one could also differentiate between 2 kinds of antibodies, in what are called as a direct assay or an indirect assay, suffice it now to say, direct assay gives raised or gives an estimate of IgM producing B cells and the indirect assay gives raised to an estimate, that will show you the IgG forming ability of the immunized cell.

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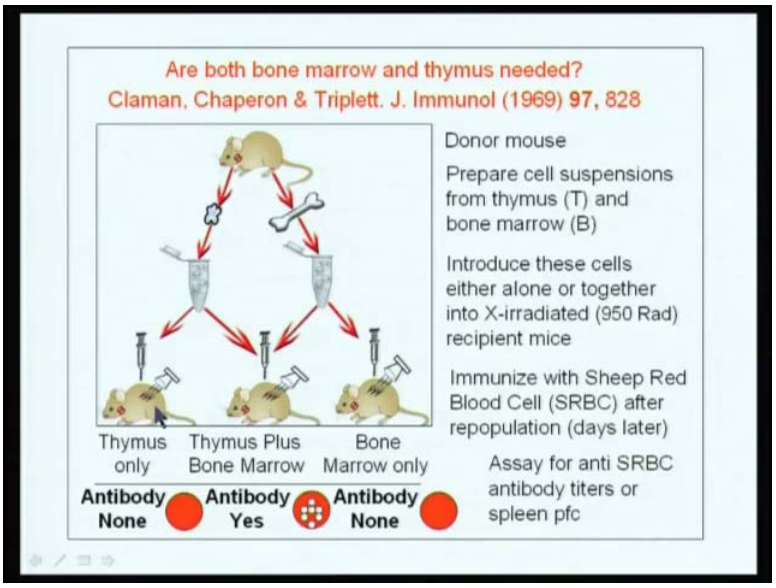

So, using such assays, one could do an experiment, that was actually done by Claman, Chaperon and Triplett in the year 1969. So, they asked the question, whether both the bone marrow as well as the Thymus, what they needed for active antibody responses by using this plaque forming cell assay, that I described to you.

So, they took a mouse, they took out the thymocyte and they took the bone marrow cells. So, you had thymocytes isolated and you had the bone marrow cells isolated. The bone marrow cell would be the source of B cells from the mouse and they took that into and injected into animal, that were irradiated at 950 rad, which I showed you earlier, was the dosage, that was required to coat un-coat, to (( )) or incapacitate immune cells on the body.

So, therefore, one could try and see, if the thymocyte would repopulate this animal, the bone marrow would repopulate this animal and challenge them with an antigen such as sheep red blood cell and do a plaque forming cell assay.

So, what did they do? They took a donor mouse and they prepared cell suspensions from Thymus and the bone marrow, then they introduced these cells into mice, recipient mice that were irradiated at 950 rad. So, either, they did this alone or in combinations. For example, here in this mouse, this mouse is only Thymus derived cells or the irradiated mouse, this receives Thymus cells only, this mouse had a preparation of both Thymus as

well as bone marrow cells being injected into it and this mouse had only bone marrow cells injected into it.

So, therefore, in a short term assay for example, just days later for example, may be 7 days later, just remember, that these bone marrow cells, the ability of the hematopoietic stem cell to form for all rest of the cells in the body, would be much more, it would take much more time and the experimental time that was used for this experiment. So, here, when they did, when they took these mice, took out the spleens and met at the ability of these spleens sense to make antibodies, so they found, that when they took the spleen from the animal that had received the Thymus only, they found, that there was no antibody, may, although, there was all these mice were immunized with sheep red blood cell. So, there was no cell that had the ability to make antibodies.

As opposed to, when they had the Thymus, as well as the bone marrow being injected, they found, that there was an optimal produsion of plaque, as indicated by these white circles over here on a red lawn. And when they injected the bone marrow alone, they found, that the ability to make antibodies was very, very minimum, comparable to what was there in the Thymus only. As I told you earlier, in the short term duration of these reconstitution experiments, this bone marrow alone, would take a longer time to differentiate into both, B cells and T cells in this animal and therefore, you would evaluate only the ability of the B cells in the bone marrow preparation to make antibodies.

So what (( ))? This is the kind of an overall or normalized summary, so to speak. One could ask the questions, whether the B cells and this animal would make antibodies or not in this experiment? What was being done, that is the optimal ability of this animal to produce antibodies? And this optimal ability to produce antibodies is achieved only when there are T cells as well as B cells. As I told you earlier, T cells cooperate with B cells and therefore, the optimal production of antibodies was found in this recipient mice, which had deceased both, Thymus as well as bone marrow cells, and therefore, the conclusion by Claman et al, that bone marrow and Thymus were needed for an optimal antibody response in an immunized animal.

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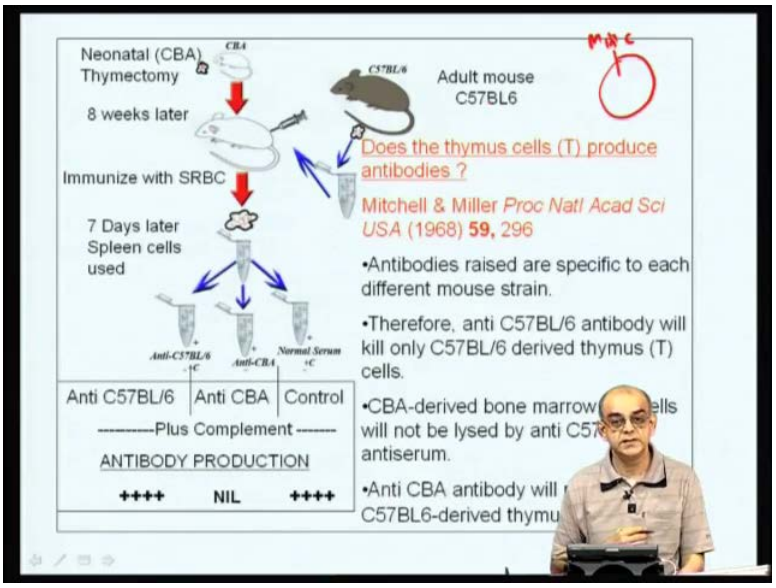

And then came the question, what, how to show whether actually Thymus cells produced antibodies or not? What if B cells, there is a lot of information telling that B cells are producing antibodies, how was it soon, that T cell was not producing antibodies? So, this was actually answered in the year 1968 by an experiment that was done by Mitchell and Miller.

So, what they did was to take 2 different kinds of mice over here, indicated a CBA strain, which is normally it is brown in color and C57 black 6, which is black in color. So, these inbred strains of mice, we will talk about it later on, when we come to the class on major histocompatibility complex, suffice it now to tell you, there are different kinds of mice and some of them are black in color, some of them are brown in color and the common laboratory mouse perhaps, you may be aware of, is white in color.

So, what they did was to take out the Thymus at a worthy early stage, neonatal thymectomy; that means, within 3 days after being born. So, this mouse pup was operated upon to remove the Thymus and therefore, it had no ability to produce T cells, but it had the ability to produce T cell and the adult B 6 mouse or C 57 black 6, which is otherwise called as B 6 was the donor, they gave rise to the adult thymocytes in this animal.

Now, some of the principle that was involved in this experiment should be enumerated for you to understand the outcome of this experiment. The, just like it was found, that

you could make antibodies to sheep red blood cell, if you take these cells that are derived from different kinds of mice, like for example, a black mouse and a white mouse and immunize each other with each other cell, you could make antiserum, that are specific to the other strain's mouse. This is because of the presence of these markers on the cell, called as MHC antigens.

So, the MHC antigen is actually a self-signature molecule; so, what is a self-signature molecule? A self-signature molecule is something, that indicates cell or something like a fingerprint that is unique to each one of us. So, you could make antibodies to these self-signature molecules and therefore, one could raise antisera against the B6 mouse cells and one could raise antisera that would react only to the CBA mouse cells. In other words, the antisera that are raised against these cells or so to say, anti B6 antisera would not recognize anti-CBA or would not recognize CBA cell, and on the other hand, anti-CBA antisera would not recognize B6 cells. So, one could use this antibodies in a complement mediated depletion assay, which you came to learn in the previous slide.

So, what they did was, in this experiment? So, 8 weeks after thymectomy, they repopulated this mouse, which had now grown and become an adult. After that 8 weeks, they injected these thymocytes that were taken from a different mouse, a black mouse, B6 mouse, from that they took of the Thymus and they removed this thymocytes, made a suspension of it and injected into the CBA mouse. So, because there were, there were no T cells here, these theymocyts would actually differentiate here and come to tolerate the CBA mouse cells. In other words, at the way this experiment was done, that these T lymphocytes would not react against the CBA mouse cells and the CBA would not react to the T cells because this was already, the Thymus was already removed.

So, once these T cells repopulated this mouse, so you had T cell, Thymus being the organ where T cells differentiate. Therefore, if these T cells differentiated in this mouse, you had all the T cells derived from B6 mice and therefore, if you had antisera, that were specific to B6 cell, you could actually recognize the presence of B6 T cells that are circulating in the CBA mouse. So, in this adult, CBA mouse was then immunized with sheep red blood cell, so one could then try to remove the B6 derived T cells or the CBA derived bone marrow cell or the B cell because only the Thymus was removed here, the bone marrow was intact, giving rise to CBA derived B cells. So, one could raise

antibodies to those CBA mouse cells and remove, that by using complement in a way, that I told you in the earlier slide.

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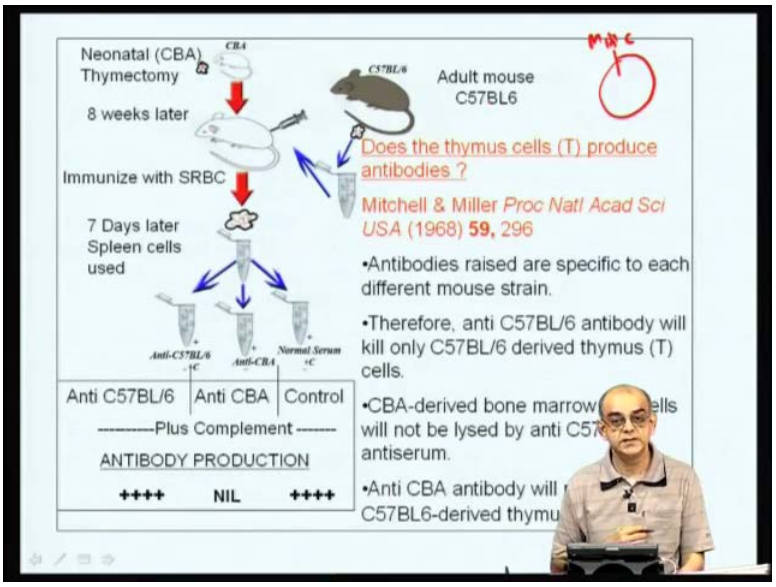

So, after 7 days, after immunizing with sheep red blood cell, they took off the spleen in this animal, made 3 aliquots or 3 batches of these suspensions. So, one was treated with anti C57 serum plus the complements and therefore, in this reaction, one would assume that all the B6 derived T cell would be, would be removed when you added complements because complement would lyse those T cell. On the other hand, if you took out batch of cells from here and we added an anti-CBA anti-serum plus the complement, it would remove all the bone marrow derived B cells, that was there in the CBA mouse. And of course, you had the control, which is very essential for all experiments and all experimental endeavours.

So, we had this normal mouse serum, which would not have any antibodies to B6 or any antibodies to CBA mouse, then added complement to see, what would happen just with that, as a control, normal serum was added? So, you have now these 3 tubes; so, you added anti-B6 serum, so therefore, there would be no T cells over here; and then, you had removed the B cells over here because this was the recipient, recipient bone marrow, you had removed the B cells and therefore, you lysed all the B cell over here; and then, the control, which would have both, the T cells as well as the B cells, then they added the

complement, removed the lysed cell, then looked at the ability of the remaining cells to produce antibodies, as I told you in the previous slide.

Now, we found, that when you added anti B6 serum, still the ability to producing antibody was retained, whereas when you removed the B cell, there was no production of antibodies. And when there was, just the control antiserum was added, which did not have all these specific antibodies still that served as a positive control.

Therefore, showing that because you had removed the anti-T cell or antisera that were raised against the B6 derived T cell, despite which, antibody, ability to produce antibodies in a plaque forming cell assay was still intact.

Whereas, when you removed the B cells, the ability to produce antibody was gone and therefore, the conclusion, that Thymus was not the cell that produced antibodies, it was a B cell that actually produced antibodies. Of course, this conclusion, that the B cell production of antibody was also fortified or supported by earlier observation, that if you remove the Bursa, you would have antibody production, that was being inhibited.

So, all these experiments, in fact, went to show, went to show, that the B cell actually make antibodies and the T cells were also required, but how the T cell was required to produce optimum antibody responses, was kind of, still not yet very, very clear. And the production of the antigen presenting cells in the roll of antigen present cell of the macrophages was still unclear at that time.

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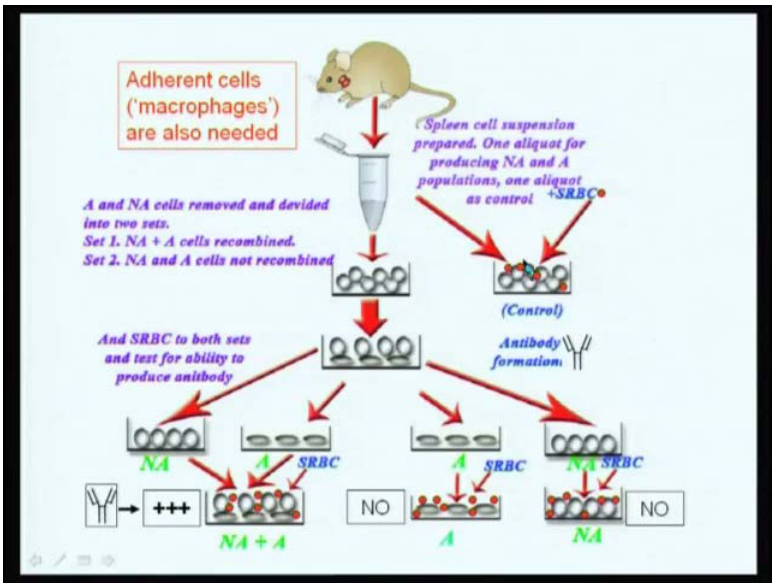

So, this experiment show, that in fact, that if you remove these adherent cells, now adherent cells are nothing but macrophages, you have all these adherent cells being, have the ability to stick to a substrate. So, if you took blood cells and then put them in a petri-plate, you would have all these macrophages sitting tight on the substrate after 37 degree incubation for, let us say, an hour also. And if you shook all this, then you had the floating cells and then decant all those floating cells, you had in the petri-plate, stuck macrophages.

So, what was, what was the need for macrophages? Did they play roll in immune response at all? So, this was the experimental, but actually show, that the optimal production of antibodies, you required these macrophages or adherent cells. Also, the experiment that was done was similar to, in fact, to the previous experiment. So, you had donor mouse giving you the spleen cell, so they took the spleen cell population and put them in glass petri-plate, so when you put them in glass petri-plate, you had the separation after some time at 37 incubation, at 37, you would call them adherent population and the non-adherent population.

So, after adherence for about an hour, you could shake off and whatever shook off, which was not adherent, not adherent called as the NA, or non-adherent population and the A stands for the adherent population. Now, there was a separate control, as I told you

earlier, control are very much required, so control cell, control preparation not subjected to the separation by incubation on the solid substrate or a petri-plate.

So, after separation of this adherent and non-adherent population, they were mixed back in several combinations. Now, as opposed to the earlier experiments, this was kind of an in vitro experiment, they looked at the ability of these cells, either combined or isolated for their ability to make antibodies in vitro against the sheep red blood cell. So, they took these preparations and they added sheep red blood cell and looked at their ability to make antibodies to the sheep red blood cell. So, what they found was that if you had the non-adherent population alone for example over here, when you added the sheep red blood cell, incubated it for some time, there was no in vitro production of antibodies.

Similarly, when you took the macrophages alone, an incubated sheep red blood cell, they also would not produce antibodies. On the other hand, when you mixed the non-adherent population and the adherent population and mixed sheep red blood cell along with it, you would have the optimal production of antibodies. So, for these optimal antibodies, in the optimal working of this particular experiment, one could also use immunized animals over here and get the non-adherent population from a non-immunized animal.

In other words, if you took the non-adherent population, which contains the T and B cell, which was isolated from an immunized animal, let us say for example, an animal, that immunized against sheep red blood cell, so the B cells were already activated and the T cells were activated and they could cooperate to make production of antibodies. On the other hand, if you removed the macrophages, these T cells and B cells would not produce optimally these antibodies, but you could combine non-adherent population from a naive mouse, which was not immunized and mix them with this non-adherent population to produce optimal production of antibody. So, in other words, these macrophages did play an important role in the production of antibody.

So, how actually these macrophages play a role in the production of antibodies, we will actually explore in the next class. For this, you need to understand a few aspects of how these macrophages or how these macrophages actually, take in these antigens and how the T cell functions? Because the assay, that was used in order to show the function of macrophages, involve the property of T cell activation.

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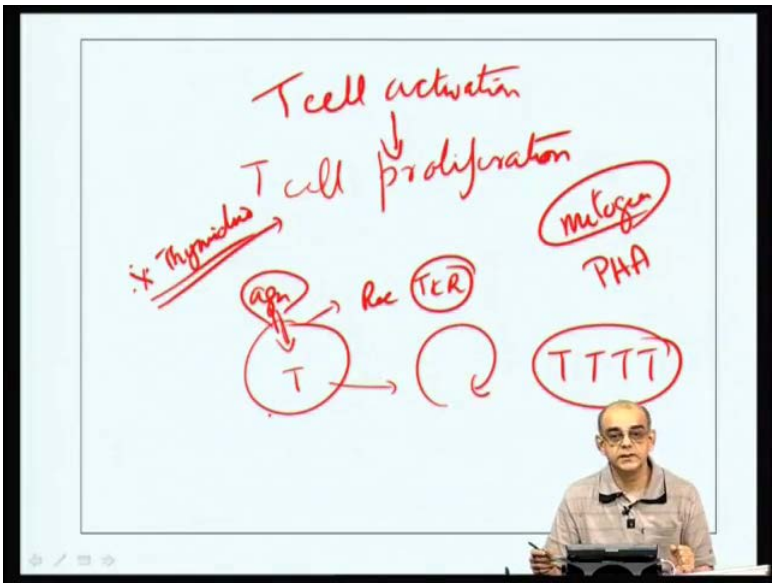

So, what does T cell activation mean? And what does T cell activation lead to? Now, whenever a T cell is activated, one of the primary results of T cell activation is what you call as T cell proliferation.

In other words, once a T cells sees its antigen, the nature of the antigen that it sees, we will discuss in the coming classes; once a T cell sees an antigen, it is recognized by a particular receptor, for now let us call it as a T cell receptor, so the combination of specific antigen with a specific T cell receptor gives a signal to this particular T cell and the signal results in the activation of proliferation. And therefore, there is a cycling of these T cells and more T cells are produced.

So, the T cell proliferation is in fact, a hallmark of T cell activation. So, T cell activation, whether this activation is produced by virtue of an antigen binding to a receptor or by virtue of a mitogen; a mitogen is something, like for example phytohemogloblin, these have the property of activating T cells by themselves and leading to T cell proliferation. Therefore, some of the points, that I would like to leave you in this class, is that activation of T cells leads to T cell proliferation.

And how do you follow T cell proliferation? One could follow the division of cells by adding radioactive thymidine, why? Because thymidine goes in specifically into the DNA and if it is labeled by a radioactive, by a radioactive precursor, so you can use radioactive thymidine, like for example, tritiated thymidine, and that tritiated thymidine

would be taken up by the T cell and incorporated into the DNA as the T cell proliferation.

So, the extent of T cell proliferation can be followed by the extent of T cell or extent of thymidine in tritiated thymidine incorporation into these T cells. And CPM or accounts per minute read out of from the preparation of these T cells, would give you an indication of T cell proliferation. So, we will leave it at this for this class and we will go into this assay in the next class.

So, to summarize, we looked at some of the experiment, that were done to look at, how T cells and B cells were in fact, very important for immune responses and how it was shown, that a hematopoietic stem cell precursor actually gives rise to other kinds of colonies in this plain.

Thank you very much.