Essentials in Immunology
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Lecture No. # 07
Development and differentiation of B cells

Today's lecture is on the Development and differentiation of B cells. We are surrounded all the time with millions of bacteria, viruses, pathogens, and if it was not for our very developed, well developed immune system, we would be succumbing to various infections from time to time.

You have already been, by now, introduced to the several cells of the immune system as well as the several interconnections they make with each other, to provide now, this very well balanced immune system which can keep us safe from the myriads of antigens. I would like to recapitulate your memory and go back to the basics of immunology, immune system, which can be studied under two separate headings: one is the innate and the other is the acquired. Now, though as I have already said that there is a network between the innate and the acquired immune system, in fact, all the cells of the immune system innate is different from the acquired in that the cells, for example, neutrophils, eosinophils, basophils, macrophages - all of them act to protect us, but they do so by recognizing patterns which are common amongst viruses, bacteria, etcetera. So, there are set of patterns that these cells recognize.

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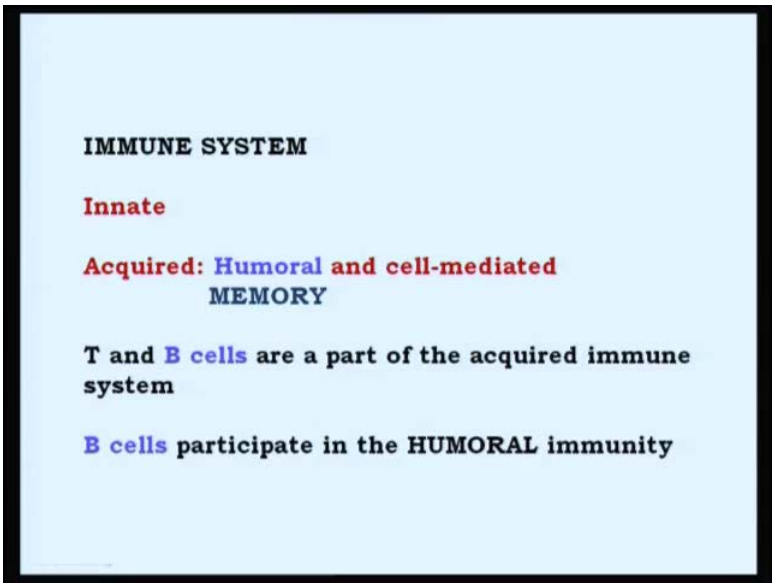

On the other hand, the acquired immune system which comprises mainly of T and B cells, and of course, several accessory cells, which we will come to, later. The T and B cells are specialized cells that recognize not only immune assay sequences of a particular protein, but also the confirmations associated with this, these sequences. These cells can also recognize carbohydrate structures. So, like I said T and B CELLS are a part of the acquired immune system, but they do need help from cells such as monocytes or macrophages. These cells are known to participate in the humoral immunity.

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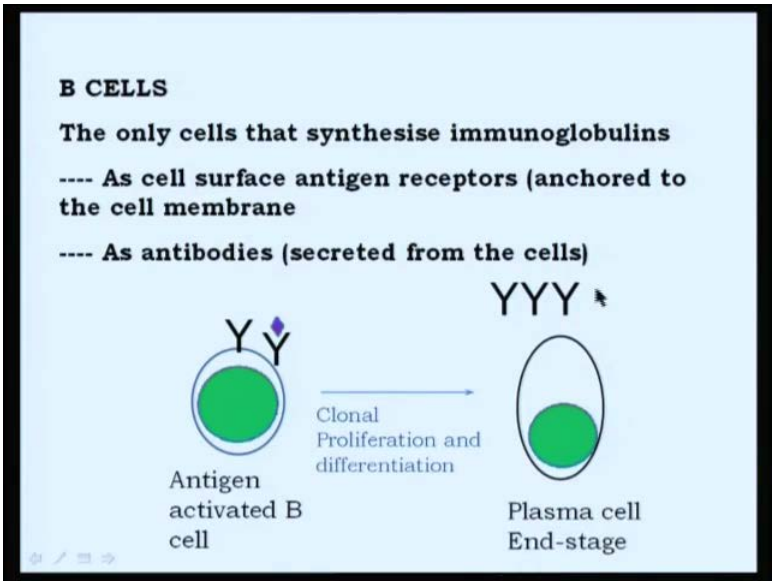

And let us look at what are B cells. right You probably have been already introduced very briefly, I would like to talk a little bit more with respect to the markers on the cells as well as what the cells actually do with respect to their function.

B cells are the only cells of the immune system that synthesize immunoglobulins. What are immunoglobulins? You perhaps know these molecules as antibodies. Immunoglobulins are either present as cells of its antigen receptors, which would mean that they anchor to the cell membrane of B cells. Alternatively, immunoglobulins are antibodies which are secreted from the cells.

I have here a picture that depicts an antigen activated B cell which has these cells of its receptors - the immunoglobulins, recognizing this antigen in response to this recognition and binding, the B cell gets activated, and through a large number of processes which in fact happen in between, which is clonal proliferation and differentiation, the ultimate cells are plasma cells which secrete immunoglobulins. Now, when they are secreted, these would be called antibodies.

I would like to go into several of these processes when I deal with the development of B cells. So, when I talk about development, it also describes the weight, as cells are synthesized in the bone marrow and their cells of receptors to the stage where they have started to now become plasma cells to secrete antibodies.

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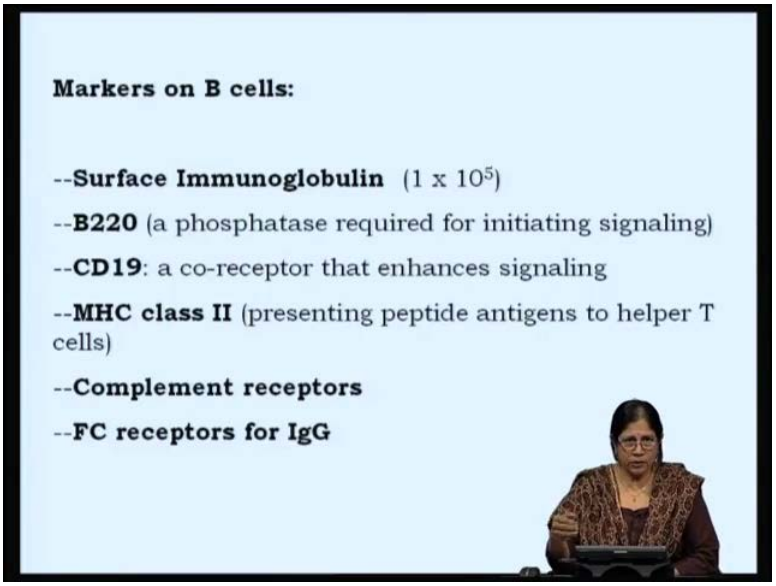

I already told you that B cells have surface immunoglobulin and there are a very large number of these surface immunoglobulins on every B cell, and the number appears quite large, close to 0.1 million.

I just like to mention here, though we will go into this in detail. Every B cell has these 0.1surface immunoglobulin as antigen receptors which are absolutely identical. I will be coming to this over and several times, but everyone B cell recognizes only one type of antigen, and therefore, all the immunoglobulins or the antigen receptors are identical.

Apart from surface immunoglobulin, there are several other markers of B cells. I will just list them out. B220 which is a phosphatase; it is an enzyme which is required for initiating signaling. I will be dealing with signaling in my next class.

So, I would like you to remember, apart from B220 - the phosphatase, there is another molecule which is very specific, only to be precise, which is CD 19, and it is suffice you have to remember that CD 19 is a co receptor.

MHC class II molecules - MHC (Major histocompatibility complex), you have been introduced to this molecule as well and you probably recall that MHC class II, I mean MHC class II molecule are present on antigen presenting cells. Just to remind you that MHC class I, on the other hand, are molecules that are present on all nucleated cells; MHC class II molecules are very necessary for the activation of B cells. Apart from monocytes as well as dendritic cells, MHC class II molecules are present also on B cells. So, B cells are also antigen presenting cells.

Apart from these, there are also molecules called complement receptors. As the name suggests, these receptors are able to fix complement. Now, again, all this will be discussed in the subsequent lectures. I would like you also to remember FC receptors. So, from all these molecules, B220, CD19, MHC class II molecules, FC receptors for IgG - all these participate in the signaling that is initiated by the surface immunoglobulin, after the surface immunoglobulin or the antigen receptor here (Refer Slide Time: 07:20) binds to its cognate antigen.

Why do we call these markers on B cells? That is because one can have antibodies or reagents which recognize separately each one of them, and one can simultaneously bind

all the reagents to these molecule on a particular B cell. And if you have ways by which you can detect each one separately, on any B cell you will be able to detect each one.

Where are these B cells synthesized? First of all, let me talk a little bit with respect to evolution. Now, during the course of evolution from a huge cellular organism to a multi cellular organism, there have to be a parallel development of our system which would be able to keep the self from the non self; in other words, keeping the self, protected from invasion by a non self. So, the immune system, in fact, is developed likewise; it is obvious then, that as the complexities in evolution grew, even the immune system developed very slowly to meet with all the challenges that multi cellular organisms had, trying to keep the unicellular organisms from invading time.

When do you see the appearance of B cells, and therefore, the immunoglobulins in the evolutionary scale, if you remember, you have non-cordata and cordata or the phyla, you might remember that in vertebrata, you have the jawless versus jawed fishes. So, it is in the jawed fishes that B cells first make the appearance, and you see a few isotypes of the immunoglobulin, and of course, isotypes is the new word now, but I will be discussing that later.

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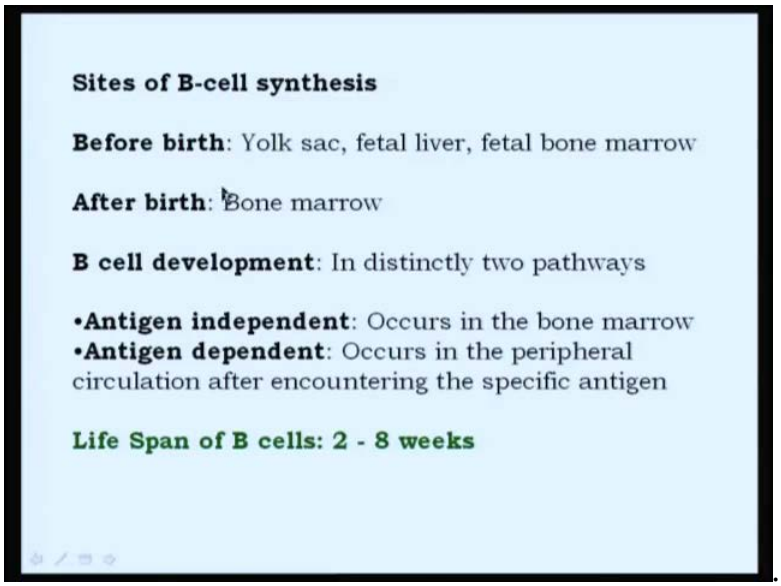

Site of B cells synthesis, therefore, can change; however, in mammals, in mice and human, both of which have been studied extensively. So, what is true of mouse is essentially true in humans; before birth, B cells make the appearance in the yolk sac; that

is the first site of synthesis, this is taken over subsequently by the fetal liver, and then on to fetal bone marrow.

After birth, it is only the bone marrow that is the site of B cell synthesis. The B cell development itself is the complex process. These cells start of as stem cell; well, stem cells are these starting cells for almost all cells of our system, but the good part of our B cell development as for all lymphocyte cells that there is a common progenitor. So, the common progenitor is the stem cells of the bone marrow and B cell developments can be looked in distinctly two pathways: one that is called antigen independent, which would mean that inspite of no antigen being present, B cells develop to their fully formed surface immunoglobulin expressed cells, which are ready to recognize the following antigen.

Antigen independent phase occurs in the bone marrow. The second phase is the antigen dependent phase which occurs in the peripheral circulation after encountering the specific antigen. Are B cells immortal? No. The life span of B cells just 2 to 8 weeks. Of course, it depends on what you call the life span, as I will be coming to in the next slide.

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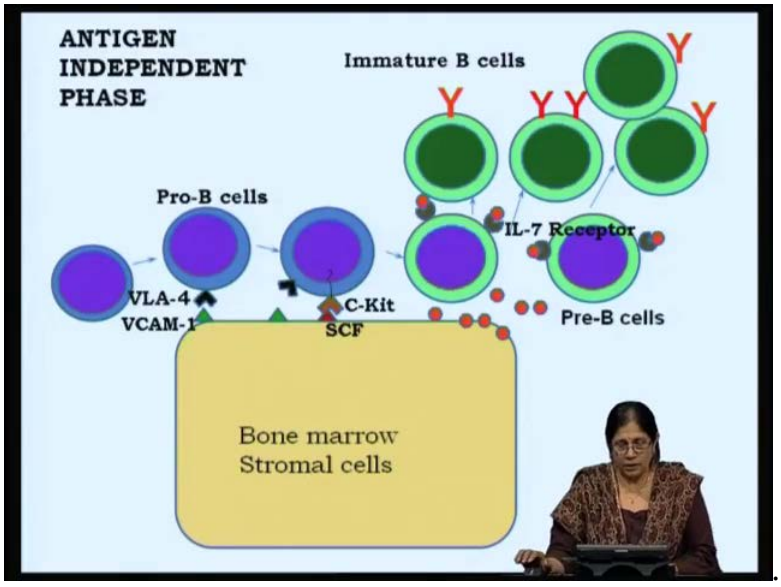

Every stem cell or the progenitor cell, when it is committed to becoming a B cell goes through these several stages and I will be describing each and every one, but there is always a large number of cells that are generated from a single stem cell or single B stem cell.

Now, this is in the scenario for bone marrow where you have large number of stromal cells and it is absolutely essential that stromal cells are in the vicinity of the dividing developing B cell.

People have tried to look at the development of B cells outside of the bone marrow. And if only the stem cells are taken out and even if they are provided several of the cyto kinds that are required for development and growth factors, the cells in fact do not develop into mature B cells. Therefore, people started looking at what goes on between the developing B cell as well as these stromal cells, and have come to the following information.

A stem cell starts to express certain cell addition molecules on the cell surface. What are these cell addition molecules? On the B cell it is VLA 4 and on the bone marrow stromal cell its VCAM; that is actually cell addition molecule. These two molecules interact with each other, rather specifically, and brings the pro B cell in close proximity to the bone marrow stromal cells. So, one can look at this molecule is something like tethering. So, the B pro B cell is tethered to the bone marrow stromal cells, such that they can be the second set of interaction.

The second set is between the receptor which is called C kit on the product B cell and the stromal cell, stem cell factor. The specific interaction between these two cells of this protein now signals the pro B cells to start dividing. Because of lack of space here, I have not been able to show more than two cells, but in fact, this can go to several thousands. What happens? Also in response to this C kit binding to the stem cell factor, there is expression of the receptors on the B cells which were not there earlier. These receptors are called interleukin 7 receptors.

Stromal cells constitutively express the ligand for these receptors and these are called interleukin 7. Now, you have binding of the interleukin 7 which is being synthesized by the stromal cells that bind specifically on the interleukin 7 receptor, which now the B cells have acquired. Once the B cells, pro B cells have acquired the interleukin 7 receptors, these are now called pre B cell.

After the IL 7 receptor binding to its cognate like and that is for the development of the pre B cells to immature B cells. During this process, not only are the cells proliferating to make more and more of their kind, but they also undergo a recombination process which

happens in the genes of the immunoglobulin. The recombination, event that happens in these cells to, now, tell the cell that you make a surface immunoglobulin and of the type that can recognize a specific antigen. This is a very complex process and I think I will be dealing with that, and discussing with you 3, 4 lectures from now.

Just like you to remember, that at this stage, the reorganization of the immunoglobulin gene because of which, now, this transcription of the immunoglobulin gene and cells surface expression - first of the isotype IgM. All the cells here that you see on the screen are immature B cells which express only IgM. This is only about one molecule or two molecules per cell which are depicted here, but remember, each cell would have about 10 to the power 5 or 0.1million immunoglobulin on the cell surface.

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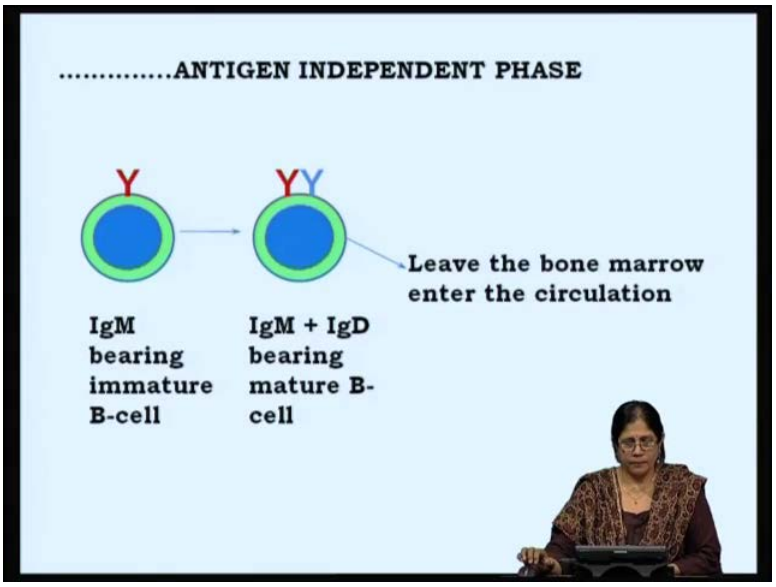

We are still with the antigen independent phase which is still in the bone marrow up to here (Refer Slide Time: 16:20). So, we go back now to a cell which is immature B cell which has only one isotype, which is capable of recognizing or interacting with this cognate antigen. However such a immature cell is not ready for the immune response that is seen, generated upon activation. It requires another immunoglobulin molecule of the isotype IgD, and now from the immature B cell, when two molecules are two different molecules expressed on the cell surface IgM and IgD, then such cells are mature cells.

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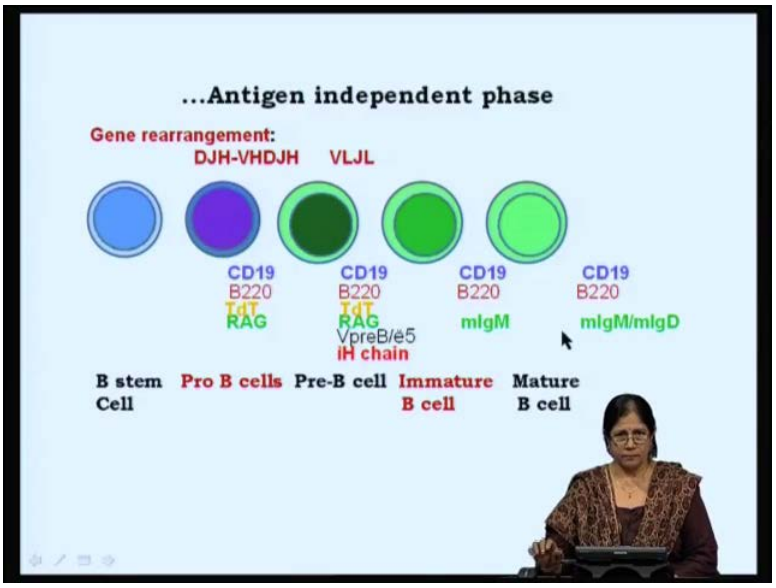

I would like to emphasize here, however though the isotypes are different, the capacity for each of the molecules here to bind to only one epitope or one antigen still remains. Therefore, now every B cell has the capacity to recognize only one type of antigen. Once the cell has acquired even the second isotype, IgM plus IgD cell leave the bone marrow and enter the circulation. I have talked about several of these molecules so far. So, let us just go over the antigen independent phase once more, to know that you have the stem cell which becomes a pro B cell which then becomes the pre B cell, immature B cell, and mature B cell.

The characters have moved a little bit away from the cell, but this is the final cell which is a mature cell bearing cell surface immunoglobulin IgM, Immunoglobulin IgD, it also had that CD19, just to make you remember that it is a co receptor for this, and also that phosphatase. Now, you have the same on the cell which is just before, which is the immature B cell, which has everything this cell has, except does not have IgD. When you go back to the previous, you have cell surface expression of CD19, B220, but these cells have also the expression in the cytoplasm of two enzymes RAG 1 and RAG 2. RAG - recombination activating gene, one and two.

Before that, the cells have again RAG 1 and RAG 2, and again the same cell surface markers which keep which are there, you can see throughout the cell development. There is also expression in these two stages of an enzyme called terminal deoxyribose

transferase or TDT. Both RAG 1, 2 as well as TDT participate in the immunoglobulin gene rearrangement. You have the immunoglobulin rearrangement taking place in the pro B cell as well as the pre B cell.

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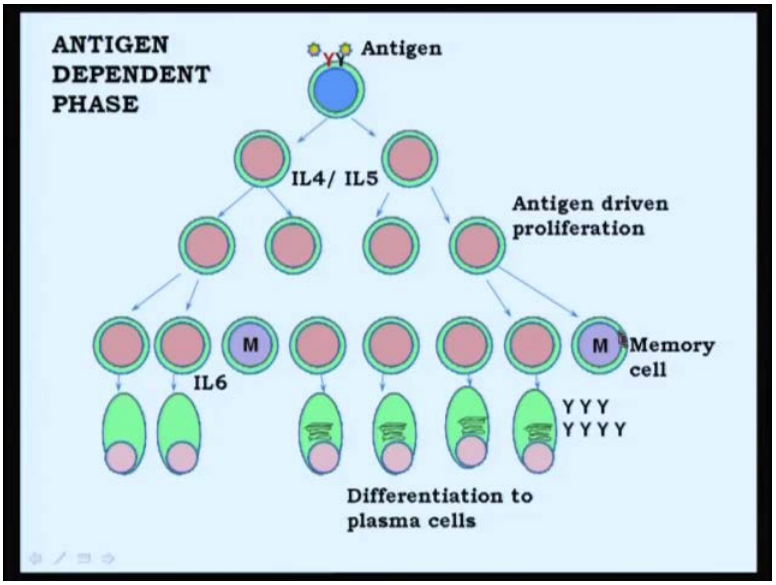

Like I told you earlier, this complex process of reorganization of the immunoglobulin genes will be dealt with later. Now, we come to the antigen dependent phase. Though I have said this a couple of times earlier, I would like to reemphasize that everyone B cell has receptors; does not matter if it is both IgM and IgD, both of them have the capacity or the antigen binding pocket which are identical.

Therefore, they both would be able to recognize only one type of antigen or one set of amino acid sequences. This is the mature B cell now. It is in the circulation in the periphery out of the born marrow; it comes across its cognate antigen. This is a very specific binding and the strength of the signal that is sent to the cell as response to this antigen receptor binding, determines how much of an immune response in terms of antibody secreted happens. Let us look at that little bit closely. You have the antigen binding, now, to the cognate receptor on the B cell, in which it the cells now acquires an activated phenotype.

I have not shown here, but I like you to remember that the activated phenotype would mean that there would be larger number of MHC class II molecules which are expressed. There are also other molecules which are cells of its receptors for two key cyto kinds

called IL4 and IL5, interleukin 4 interleukin 5 which cell synthesizes. These are synthesized by activated T cells. Therefore, one can imagine that the same antigen, now it is activating a B, a T cell. The T cell, now, in its activated state starts to make interleukin 4 and 5 which is recognized by the cell and the cell starts proliferating.

So, the interleukin 4 and interleukin 5 are also known as B cell growth factors. They allow the cell to start proliferating. All the cells of the acquired immune system, T as well as B cells have this one property that before they becomes effective cells, in case of B cells the effector cells are plasma cells, they need to undergo proliferation at first and subsequently differentiation.

Now, the proliferating cells are differentiating cells are very different from each other in the way they go on with their lives. Plasma cells are the antibody producing cells or the n state cell. So, once a B cell becomes a plasma cell is destined to die; these cells do not live for more than 3 to 4 days, and even with in experiments where there are support systems that are given, these cells would be able to live maximum for 15 days.

What I have shown here is one cell which is becoming 2, then becoming 4, after binding to interleukin 4 and 5, but in fact they are one cell could become as many as 100 1000 cells. So, there would be a clone of cells which is quite identical to the first cell. There is one more aspect to B cell differentiation that not all cells differentiate to antibody producing plasma cells.

Now, what strikes you here I think would be, what I have shown here as rounded cells during proliferation, now become oval; then I call them plasma cells. In fact, that is precisely what happens. You have each of these cells which is proliferating till here (Refer Slide Time: 20:03) we start binding to or they develop receptors to interleukin 6 first. So, then they are ready for the differentiation and the proliferating cells develop receptors for interleukin 6, and interleukin 6 binds to these cognate receptors. They differentiate now to antibody producing cells. They cannot proliferate any longer.

Now, most of the cells have become antibody producing cells, and as you can see that, the nucleus has become polar as compared to the nucleus which is occupying the center of the proliferating cell, and it is also covering almost covering the entire cytoplasm. You can see that the cytoplasm is increased and there is a very well developed network of

endoplasmic reticulum which is absolutely required for the protein synthesis here. These cells need to synthesize and secrete immunoglobulins.

Another important aspect here is that not all cells become antibody producing cells, plasma cells. A few of them which could be because of lack of adequate I l 6 become what are known as memory cells. I will talk about the memory cells a little later, but memory cells are very important part of the immune system, the acquired immune system. You do not have memory cells in the innate immune system.

These memory cells, they are long lived; they stay on quietly until the body is exposed to the same antigen again; the good part of the memory cells then is that they already have receptors which can allow them to get activated and differentiate very fast. Therefore, memory cells are the ones that actually generate a very fast and a robust immune response.

Just like to mention here that in during the development of vaccine, it is important to have adequate amount or numbers of memory cells generated; otherwise, the vaccine, well, is of not much importance.

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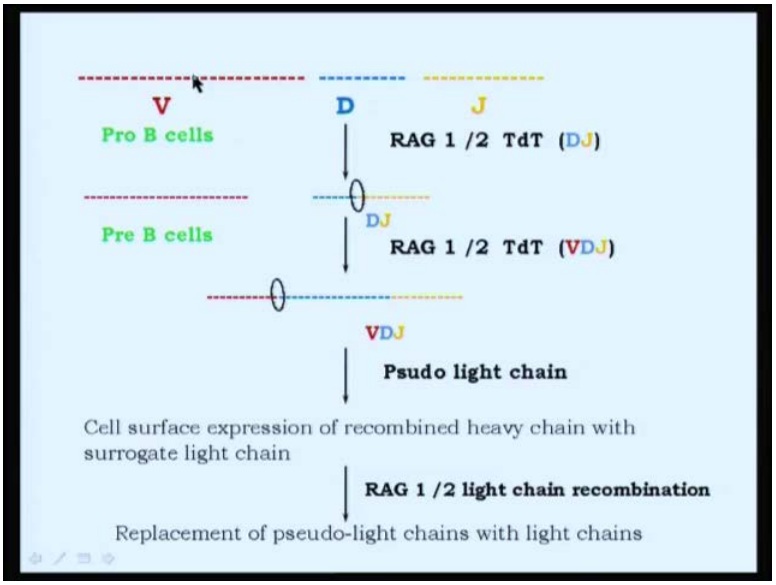

Recombination - as I have already told you, recombination of the immunoglobulin genes is going to be discussed in a later lecture, but just to give you a flavor of what happens in the pre B cells before they become immature and mature B cells, here, I am depicting

only the heavy chain, as you all probably already know that the immunoglobulin is made up of two heavy and two light chains. Both the heavy chains are identical in the amino acid sequence and both the light chains are also identical. It is the combination of the light chain with the heavy chain that forms the antigen binding pocket. Each of the heavy chain is made up of three different gene segments at the antigen binding site. You have the V, D and J.

There are three 100 to 1000 different V gene segment; there are fewer of D. In human, they could be as many as 6 to 11, and J which are even few may be 5 to 6. Nature has it that during the recombination process, any one of the J now binds to any one of the D. So, you have a DJ segment which is initiated by the two enzymes are talked about a little while ago; the recombination activating gene one and recombination activating in gene two. After DJ combination, there is recruitment any one of the V. Therefore, now you have VDJ recombination. The entire event from pro B cell to pre B cell and the immature B cell is under the directive of this recombination which is through these specific enzymes.

Now, VDJ that is heavy chain, after it is ready, this transcription and you have generation of only the heavy chain. At this stage, the cell recruits a Psudo light chain before the assembly of the light chain recombination event that takes place. Now, the Psudo light chain along with the heavy chain is expressed on the cell surface. Until now the D cell cannot recognize antigens. It is only after recombination of the light chain light chain which is again needed through RAG 1 and 2 that you have the entire heavy and light chain coming together as a cell surface molecule.

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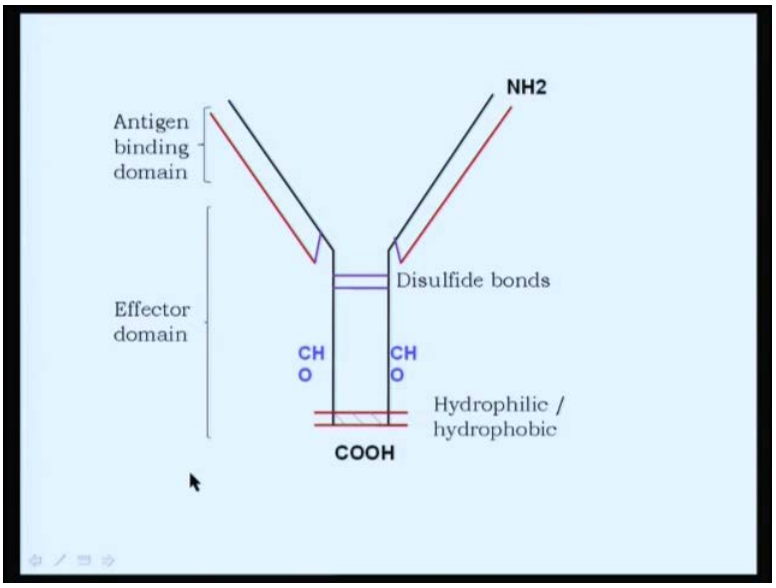

Let us look at the basic structure of the immunoglobulin. You have the two heavy and the two light chains. The heavy chains are as in black and the light chains are shown here in red. The light chains are bound to the heavy chain through disulfide bounds. also the two heavy chains are held together tightly by disulfide bounds. The light the heavy chain also has two distinct carbohydrates, there is a slight move. Here, it is CHO denoting carbohydrate.

This part of the immunoglobulin, it is forms the antigen pocket, antigen binding pocket, and therefore, it is called the binding domain and two-thirds of the molecule is the effector domain. We will be dealing with the functions of immunoglobulins in subsequent lectures.

Important here is the facts, that the C terminals of the immunoglobulin can either have hydrophilic or hydrophobic regions. And if the replacements of a short stretch of hydrophobic to hydrophilic determines whether this molecule should stay as cells of its receptor or should be secreted from the cell as antibody.

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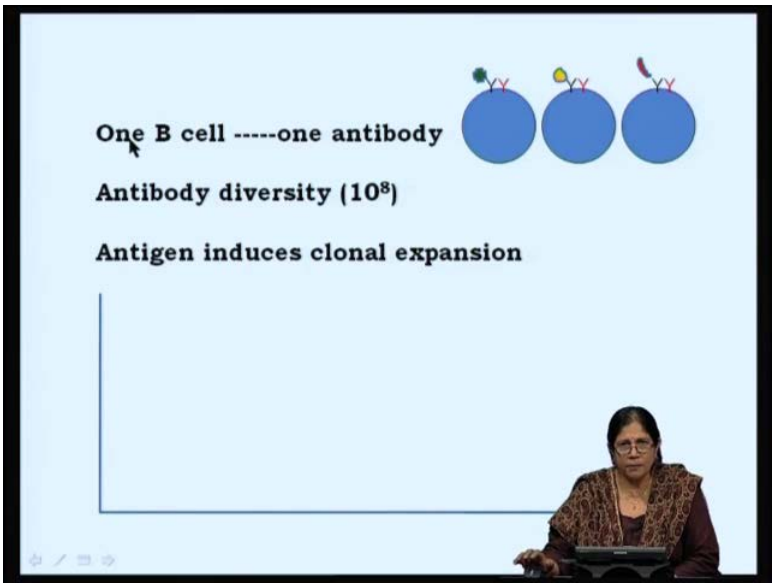

Again, to keep happing on the same thing, one B cell can make only one type of antibody, which would mean that once the recombination process of the immunoglobulin gene has happened, then the variable domain remains the same, and therefore, now every B cell and its progeny will be able to recognize only one type of antigen. In fact, let us be a little bit more specific, will be able to recognize only one set of sequences.

Like you to see this, the antibody diversity that we have, mammals have, we have the capacity to recognize a 100 million different epitopes or antigen determinants. Antigen, the third point I have on the slide says antigen induces clonal expansion. What does this mean? The B cell, when it is already when it is produced in the bone marrow and comes into circulation, in fact, already is predetermined with respect to the kind of antigen it recognizes because the variable domain has already been established. There could be some amount of receptor editing, but that is minimal.

What I like, what I try to show here, there are thee B cells here; each one of them seems to have the same type of IgM and IgD on their cell surfaces because these are mature cells, but the small region are in the amino terminus which actually binds, makes the antigen binding pocket is very different, so that now cell number 1 can recognize this type of antigens, cell number 2 recognizes these yellow antigen, and cell number 3 red. And it is when, such as number 1 manages to recognize its antigen; it comes across it is cognate antigen; then there is clonal proliferation.

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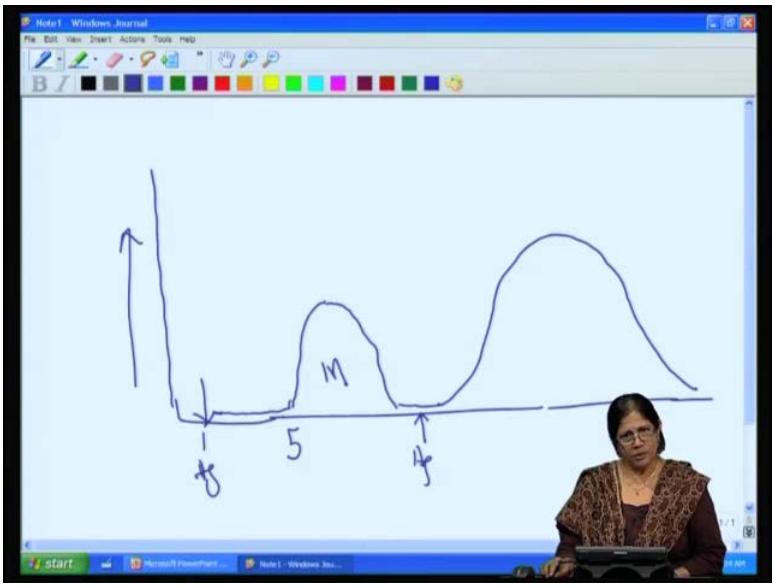

I would like to just show you this. So, in the form of, let us say, an experiment where a rabbit has been injected with an antigen. Let us say it is (( )). If one injects the rabbit on day one and one starts to take blood samples from the rabbit every other day, and then does an immunoassay to find out what is the level of antibodies specific to this antigen which has been injected on day one. Now, this is the pattern one would see most of the time. You see almost no antibody in the circulation for something like 5 days, this would, of course, depend on the sensitivity of the immunoassay.

Let us say one is using a Lisa to detect. So, there is nothing, let us say, till about day 5, and then slowly the amplitude increases, which would be maximum at around day 9, 10 and then rapidly falls down. If the antigen is now withdrawn subsequently, if the anomaly is given with same antigen, then one can see there is almost no lack phase from day 1 to day 5, and if one measures the immunoglobulin here again, sorry antibodies here to the specific antigen, this could not only stay in circulation for a longer time, but also the amplitude of the response also increases.

Now, why is there no lack phase here? There is no lack phase here because of the generation of memory cells during the course of the primary stimulus. This is the primary stimulus and this is the secondary stimulus (Refer Slide time: 34:38), and you have B cells which are already now mature B cells which recognize the antigen. Only those cells which have cognate receptors for this antigen would recognize, start to

proliferate, differentiate to plasma cells and one clone of cell were to make now antibody to that particular antigen.

There are memory cells generated here, which sit quietly in the secondary lymphatic organs which are, as you have already learned, the spleen and the lymph nodes. Once the animal sees the antigen, the same antigen for the second time, then the memory cells get into action. They have receptors already for the interleukins. So, they proliferate extensively very fast, and of course, you have not only number of cells increasing, but you also have the memory cells which allow the memory to remain for a longer period, and therefore, you have this antibody which is large amount of antibody over a larger period of time.

What would happen if you were to inject the animal for the third time? The amplitude of the response would again perhaps increase. This is determined by the antigen, but this cannot go on. There will be a decrease in the amplitude with subsequent immunizations and we will discuss that when we come to this signaling events that B cells have.

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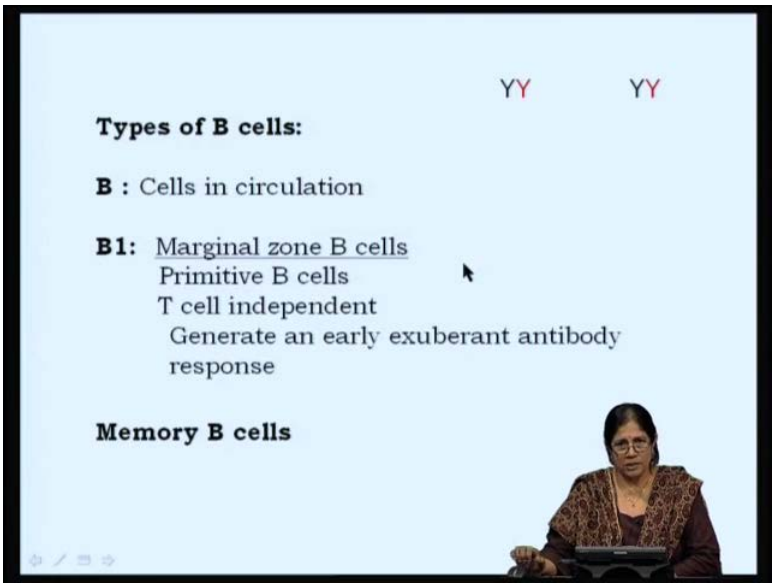

Now, let us go back to the antigen induces clonal expansion. The clonal expansion, in fact, it is what now gets translated to amount of antibodies secretion. So, if you have more antibody circulation, you can imagine that there are more clones that have been expanded and you have a very large number of plasma cells. Always the primary

immune response and the secondary immune response would have a difference in the isotopes.

Again, we will be dealing with that detail later, but suffice you have to say that the primary immune response usually has IgM type of antibody that is synthesized, and in the secondary response you can have the other isotype; you can have IgG you can have IgA and you can also have IgG. Are there any different types of B cells? I have been talking about only one type, but in fact, there are another type of B cells - marginal zone B cells. The type of B cells apart from B cells and memory cells you also have marginal zone B cells. Now, these I should have probably talked about earlier, but these have been discovered about something like seven to eight years ago.

Marginal zone B cells are also called primitive B cells. They are totally T cell dependent. Now, I have not talked about dependence of T cells so far, but will be talking about that in my next slide. Now, marginal zone B cells are usually found in the outside of the germinal center of the spleen. Germinal center - you have been introduced to germinal centers; you know these primitive B cells or marginal zone B cells are present at the out skirts of this germinal center and they generate and very early very fast exuberant antibody response.

These cells are called primitive because they do not take the time for clonal proliferation the way I have explained in my last slide, but they already have large number of interleukin 6 receptors which allow them to immediately become plasma cells and start making antibodies.

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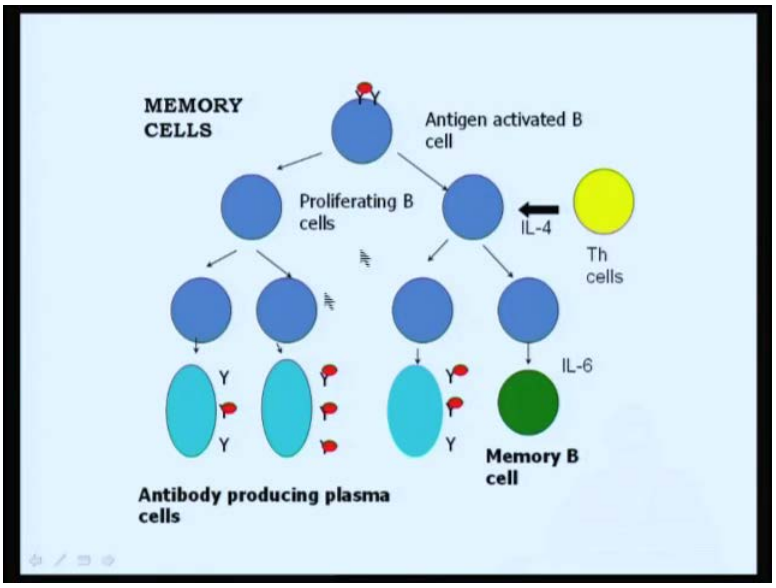

A little bit about memory cells. Are they different from the B cells? because I said earlier, I did say that. You know what I have been talking about so far are these B cells and memory cells, but I did not say much about the memory cells, memory cells which are generated. You can see that these are very long lived because I told you that they participate again in a very fast immune response. When in the experimental animal the antigen is injected for the second time, memory cells have a very good number of these interleukin 6 receptors as well as interleukin 4 receptors, both of which are absolutely essential; interleukin 4, first for proliferation. That is how they are quite different from the naive B cell; B cell which is not encountered is antigen. People have tried to look for specific markers on memory cells, and as of now, there are not too many which designate or differentiate the memory cell from the naive B cell.

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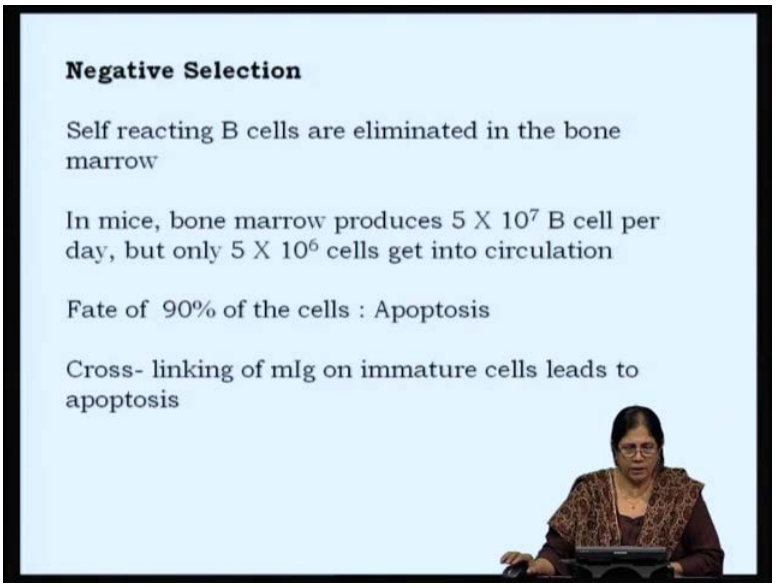

Another important aspect of the B lymphocyte development is negative selection. We always talk of the immune system with respect to self versus non- self that the immune system is able to recognize non-self from self, and is able to mount an immune response only to non self. Now, how should this happen? It is very it is very difficult to envisage that B cells will be able to recognize each and every pattern on each and every molecule, and say and go through some kind of a computer driven program, that says, yes, this these are foreign and not self.

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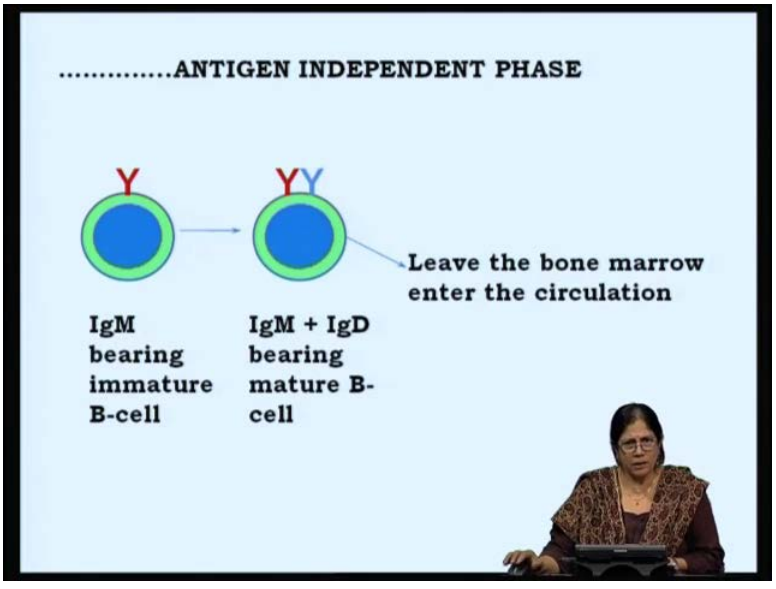

Therefore, we come down to looking at how B cells can do this, and this process is known as negative selection, in fact, quite a simple process. In the bone marrow, self-reacting B cells are eliminated. How does this happen? Now, in the previous slides where I talked about the differentiation sorry the development of B cells, there are these I g yeah there are these IgM bearing cells which are called immature B cell and there are these mature B cells which have both IgM as well as IgD. Now, if such a cell which has only IgM on its cell surface, if such a cell encounters its cognate antigen, this cell is destined to undergo apoptosis; apoptosis or program cell.

Perhaps you will be dealing, we will be dealing with apoptosis at a later stage, epitopes itself is, probably it is a lot to learn in this process, but the program cell that would mean that the cell which undergoes apoptosis, it kills itself signals to kill itself, such that the cell is very nicely the death cell is taken away very efficiently by microphages, and death cells do not stay back which can cause inflammatory responses.

So, if such an immature cell which has only IgM innate cell surface is now exposed to its cognate antigen, there is a signal for such a cell not to undergo proliferation, but to undergo apoptosis. Now, soon after that, this stage, when it is double positive which would mean as IgM and IgD on the cell surface, and such a cell now recognizes the antigen; that is the positive signal.

Now, what does IgD do? How is it that IgD can rescue IgM bearing cells from apoptosis? People were trying to look at several molecules inside the cytoplasm, tried to see whether the IgD receptor is anyway involved in anti-apoptotic signal. The only thing that one was able to decipher that the study is that during the stage of single immunoglobulin bearing cells to double immunoglobulin bearing cell, there are large regulation of large number of molecules which are inside the cell and which are nothing to do with the receptor, but they give the anti-apoptotic signal or one can say pro survival signal.

So, when such a cell meets with this cognate antigen, the cell can undergo proliferation. So, it is only when the cells leave the bone marrow or rather when they have these two receptors, can they leave the bone marrow.

In the bone marrow, one should have to think in terms of total compartmentalization bone marrow versus the circulation and the secondary lymphatic organ. Though bone

marrow is the primary lymphatic organ, it is well connected with the entire circulatory system and you do have antigens that travel from rest of the body to the bone marrow. There would always be cells which have only IgM on the cell surface, and If this particular cell for example, is destined to recognize self-antigen, then the self-antigen would have entered the bone marrow and now induce apoptosis in the immature cell.

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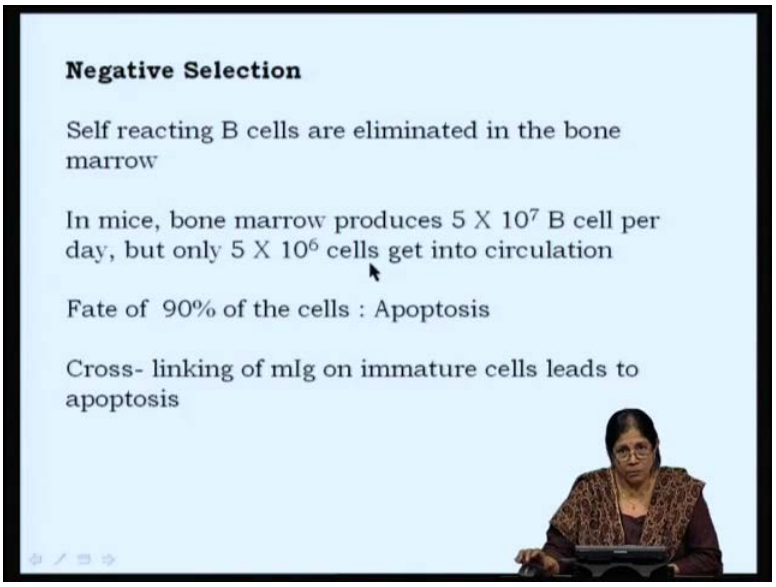

So, this is the basis for negative selection, all well I cannot say all, because we do have atomin disorders. Very many, most of the self-reacting B cells are eliminated in the bone marrow at the stage when the cells have only IgM on the cell surface.

In mice, this has been studied greatly in mice. In mice, the bone marrow produces 50 million B cells per day; that is very large number for a small number mouse that is about 15 grams, but of these, only 10 percent get into circulation. So, only 5 million get into circulation and it has been shown 90 percent of the cells undergo apoptosis.

Now, the apoptosis here is not because of only negative selection. There are other reasons and we will be dealing with that when we come to recombination of the immunoglobulin genes. People have shown this innumerable number of times that if you take immature cells and you cross link the receptors of the of these cells by anti IgM, the cells undergo apoptosis.

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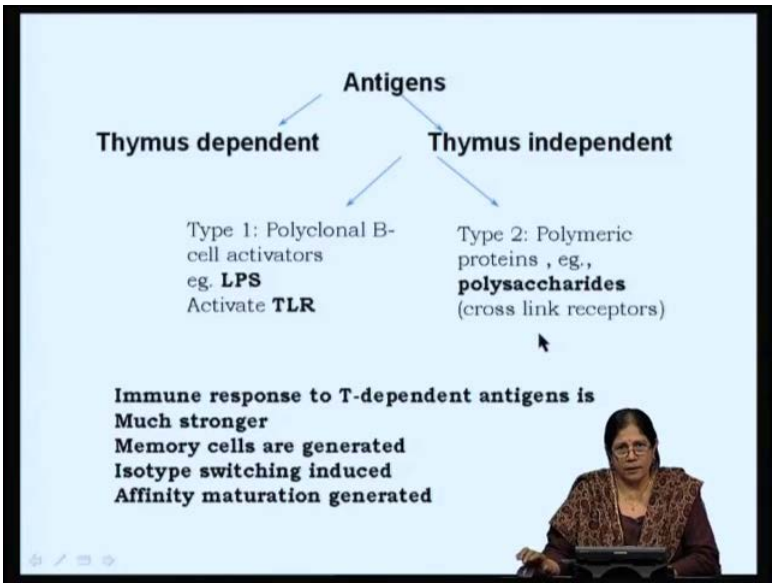

Now, my next class is going to be signaling, but before that I would just like to introduce to you antigens which would be thymus independent and thymus dependent. What does this mean - Thymus dependents versus independents? B cells also bearing those type of receptors for either the let us say polysaccharides or a globular protein would determine whether that that B cell would require T cell help or not.

Thymus dependent, actually one can even think it with their also commonly known as T cell dependent T cell independent antigens; of the two antigens, let us just look at the independent one. 95 percent of the B cells require T cell help. You remember where the T cell help comes? It is in the generation or of the interleukin 4 and 5 receptors on the B cell upon activation, which require interleukin 4 and 5 synthesize and provided by the T cell. In fact, not only the T and B cells interact with each other, and that is what we will see in the next class.

Thymus dependent antigens, like I said, are T cell dependent. Antigens constitute over 95 percent of the total antigens that we can we have. People have studied thymus independent antigens are very few. They also can be looked at two different types: one is type one which polyclonaly activate; they are called polyclonal B cell activators are just like lipopolysaccharide. These are recognized by toll like receptors. Toll like receptors, you may have already studied, are receptors which are present on the innate the cells of the innate term of the immune system. Type two, on the other hand, is polymeric

proteins or polysaccharides. These are able to cross link receptors and activate them. Therefore, they do not need all this. This will become much more clearer when I start with my signaling class.

I would like to, however, point out that of the two, the thymus dependent versus thymus independent antigens of B cells, the immune response to T dependent antigens is much stronger, which would mean that the amplitude of the antibody response, that I talked about little while ago, increases much is much higher for T cell dependent antigens.

Importantly, it is only the T cell dependent antigens which induce B cells to generate memory cells. Another important aspect is that of isotype switching. I have not talked too much about that except I just mention that a B cell makes IgM type of antibodies first to the same antigen, after it becomes the memory cell and generates a second response, the IgM isotype is switched to IgG or any one of the three other isotypes. It is only the thymus dependent antigens that induce the B cells to switch the isotype and other important part is it is only the T cell dependent antigens that induce the cells B cells to undergo affinity maturation.

All these I am going to able to discuss in detail after I talk about signaling, and as we deal with also the recombination events that take place for the different regions of the immunoglobulin gene to come together, and make a complete heavy chain or a complete light chain. Another thing what I would like to mention here before we go to the next class is, and perhaps you might like to look at or at least get to know about the different signaling molecules that are present in the B cell.

Now, though B cells and T cells are part of the acquired immune system, the way they recognize antigens are totally different. Both of them are able to recognize antigens of different types, different sequences of amino acid, but whereas, T cells require presentation of the peptides or amino acid sequences in the context of major histocompatibility complex one or two, B cells recognize antigens in the native confirmation. So, let us look at the difference between B and T cells now.

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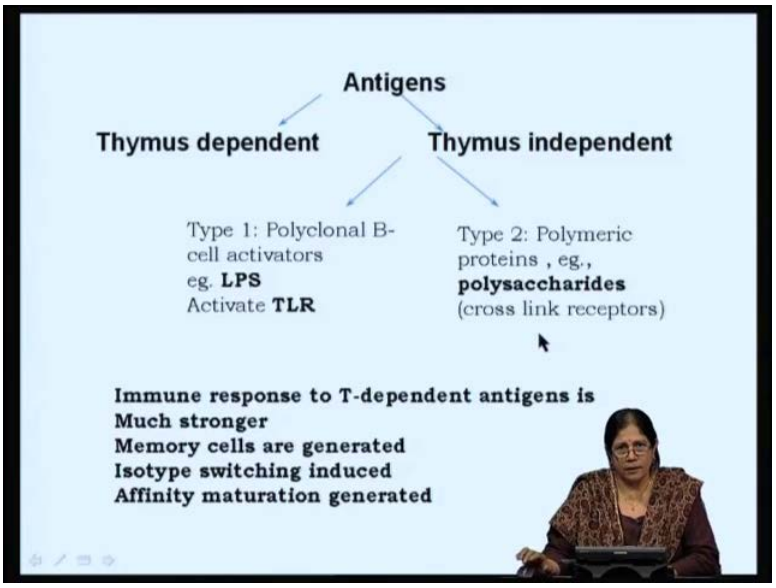

When there is a pathogen that enters the body, B cells which are in circulation, and if they have the cognate receptors for this antigen bind to them get activated, start proliferating and differentiating. The same B cells can internalize this antigen again after binding to the surface immunoglobulin or the antigen receptors, internalize the antigen, process it with respect to making small fragments or peptides of the antigens. This is loaded on the class II molecules and presented to (( )). So, you can see that B cell receptors are so different from T cell receptors in this one or the first event that happens, that is recognition of the antigen.

In my next class, I will be dealing mostly with B cell signaling, but one can imagine that B cells and T cells are so similar in the way they divide and they differentiate to their final differentiated or the n cell stage. The signaling molecule should be very similar. However, in my next class, I will be able to bring all this out and discuss this in detail. alright.

Thank you.