Essentials in Immunology
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Lecture No. # 08
Signaling in B cells

Today's lecture is on the signaling in B cells.

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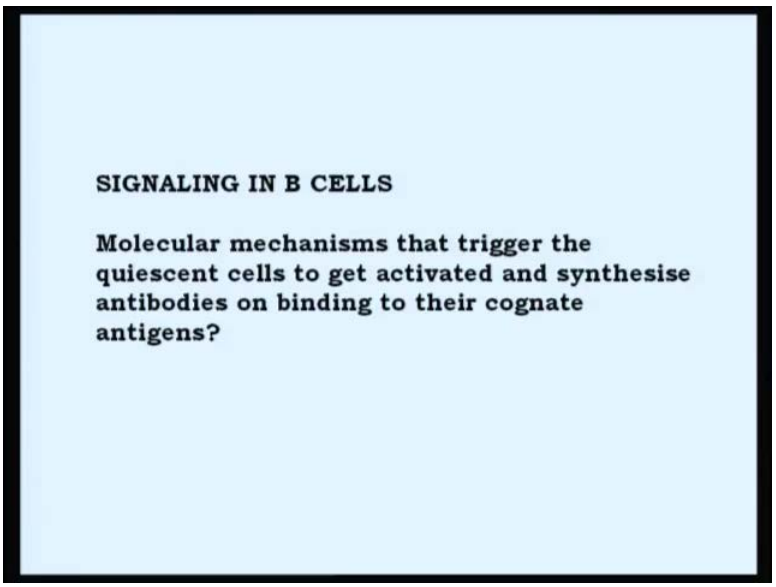

There are several molecular mechanisms that need to occur for the quiescent cells to get activated and start proliferating and synthesise antibodies on binding to their cognate antigens.

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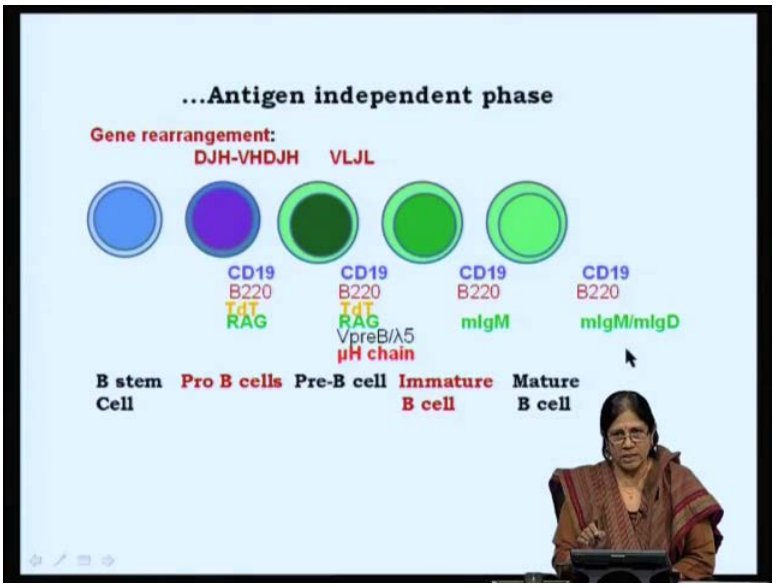

Just to recapitulate your memory of the last lecture of mine, I would like to again talk about the two distinct phases that these cells undergo in their development; one is the antigen independent phase where a B stem cell goes to pro B cells, pre-b cells, immature B cell where there is suffice expression of one isotype of immunoglobulin. Later on, the same cell undergoes development to express two different isotypes of the same immunoglobulin, the same antigen specificity.

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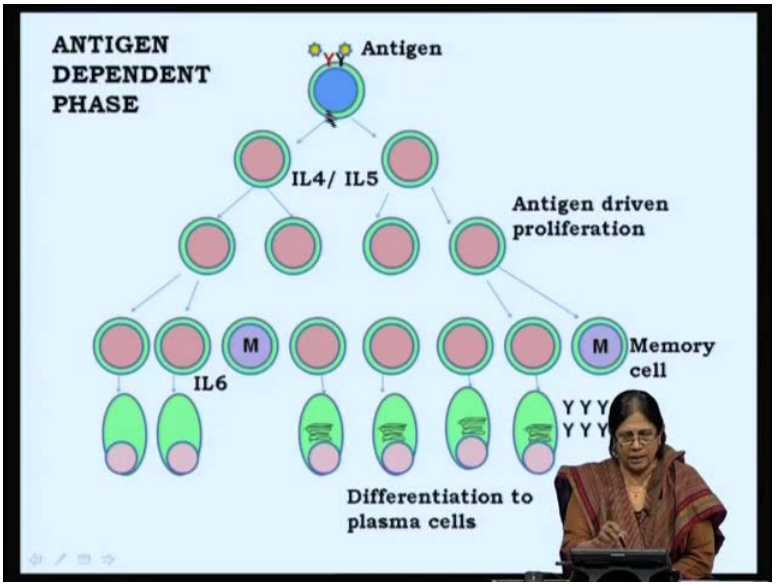

Such a mature B cell then comes into the circulation and the secondary lymphoid organs where each B cell is able to recognize its cognate antigen. The cells undergo an activation upon binding to the cognate antigen; they undergo proliferation; subsequently, they go what is known under the state of differentiation to antibody producing cells.

I like to emphasize again that the same specificity of the antigen binding to the antigen receptor on that B cell is kept to the antibodies produced or secreted from these differentiated plasma cells. So, the previous one, where the development occurred in the bone marrow up to the stage of surface expression of the B cell receptors which is antigen independent phase. We come to now the antigen dependent phase where those very same mature B cells recognize antigen and become plasma cells. Now, needless to say, the immunoglobulins or the antibodies here are the effective molecules of the humoral element response.

I like to also reiterate what I said in the last lecture that the generation of a few memory cells when some cells start to differentiate also is important and inherent to the immune system.

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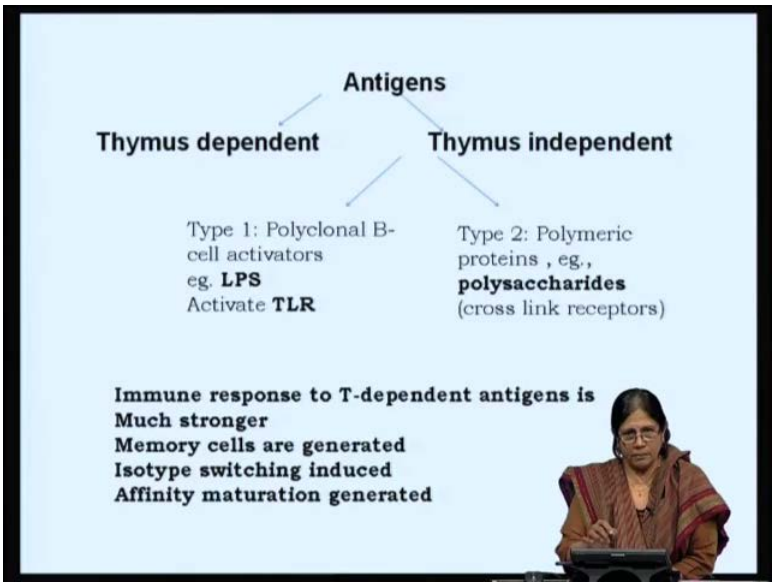

This slide is what I also showed you last time, but I would like to tell you again that antigens can be classified as those that are thymus dependent or T cell dependent and those that are thymus independent. 95 percent of the antigens that we encounter are by C cells that are T cell dependent. And therefore, the signaling mechanism that we are going

to discuss today is of the B cells which are thymus dependent. Again, to reiterate, the immune response to T dependent antigen is much stronger; memory cells are generated in this response; isotype switching is induced and affinity maturation is generated, but the last two that I just discussed, isotype switching and affinity maturation - these two we will be covering in the next class.

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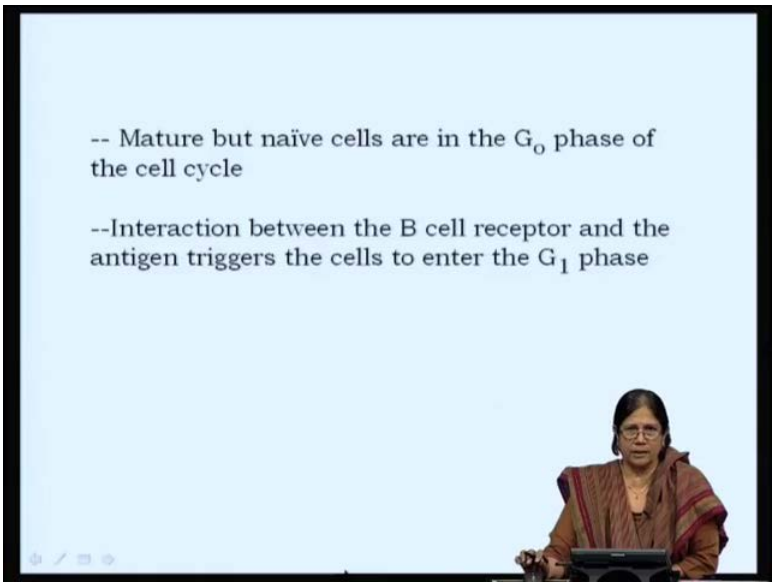

Now, let us go back now to the mature B cells, but which are still naïve which are in circulation. And since they already have the cognate receptors, pre-determined receptors on their cell surface, they are waiting now in the G0 phase of the cell cycle to recognize their or meet with their cognate antigen. The interaction between the B cell receptor which is again, I will say, immunoglobulin with the cell surface and the antigen triggers the cells to enter the G 1. So, all mature naïve cells are in the G 0 phase of the cell cycle; also the memory cells are in the G 0 phase of the cell cycle.

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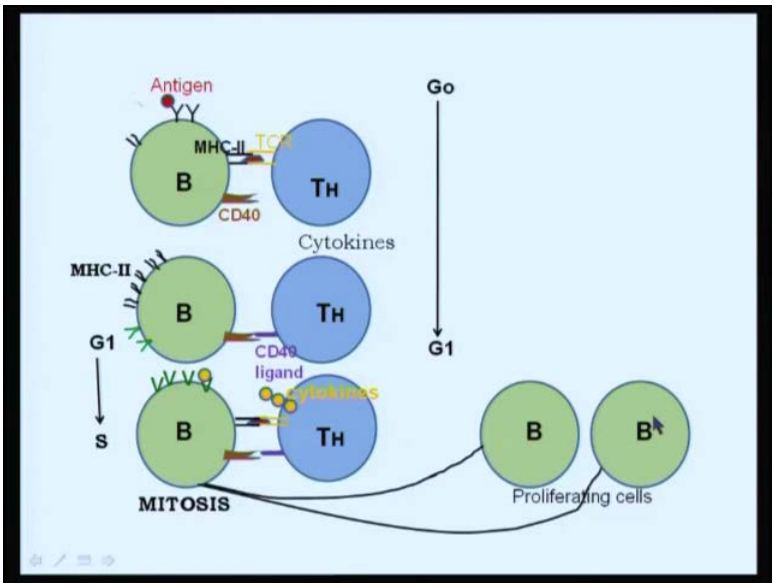

Let us see what happens now, when a B cell a mature B cell binds to its cognate antigen. Now, this is the quiescent B cell; recognizes the antigen; this, as a result of this interaction, the antigen is phagocytosed, taken into the B cell; I should sorry I should not say phagocytosed; instead, I should say receptive mediated endocytosis. As soon as this happen, these cells are known to be in the activation phase. When such a cell is activated, there is surface expression of MHC class II molecules.

Now, I have shown only one here, but there are several of them that are expressed. MHC II molecules, as you might remember from your other classes on the cells and organs of the immune system, are molecules that are present on all antigen presenting cells such as monocytes, dendritic cells as well as B cells.

When the antigen and the immunoglobulin or the antigen antibody complex is now endocytosed, the antigen is broken down into small peptides by large number of enzymes that are resident in the B cell cytoplasm and then the peptides are presented in the concept of MHC II molecule to the T helper cells. The T helper cell recognize specific sequences on the peptide, again in the concept of class II molecules, and this allows the cell T cell also get activated. The activation of T cells thus induces the expression of a molecule called CD40 ligand. The CD40 ligand binds to its partner - the CD40 receptor present on the B cells and this interaction gives the competence signal.

What does this competence signal do? It makes the B cells, now, competent to receive to interleukins or cytokines synthesized by the T helper cells. These cytokines are interleukin 4 and 5 which are required for B cell proliferation.

How do the B cells become competent? Upon CD40, CD40 ligand interaction, there is expression of receptors which are seen here in green, receptors for these cytokines. I have told you that the T helper cell is already activated. And by another mechanism, which I am not going to deal with here, that T helper cells starts to synthesize these cytokines which are in yellow.

The cytokines bind to the B cell, now giving such a B cell a proliferation signal. So, the proliferation signals now it results in mitosis and generation of daughter cells. And though there are only two daughter cells which are shown here, there are in fact, a large number of clonal proliferation that happens and you can have from one such B cell, you can have even a 100, 1000 daughter cells identical with respect to the mother.

The G 0 phase that I talked about, the quiescent cell being in the G 0 phase first gets to the G 1 phase upon the expression of MHC class II molecules as well as receptors for the cytokines, and subsequently from after receiving the cytokines goes from G 1 to S phase which now allows the cell cycle to be completed with respect to mitosis. And now, this phase keep on interacting with more and more cytokines and these cells keep proliferating.

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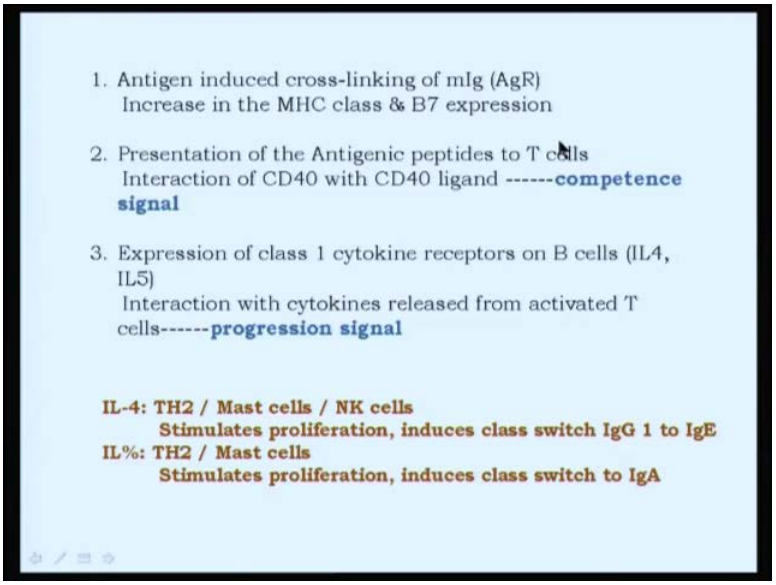

Just to sum up what I said, antigen induced cross linking of the membrane immunoglobulin or the antigen receptor gives rise to increase in MHC class II, sorry the II is missing here, and B7 expression which is this B7 expression on the B cell is required for the T cells, but MHC class II is what is required for the B cells to present antigen to the T cells.

Presentation of the antigenic peptides to T cells now is concomitant with expression of CD40 ligand on T cells and interaction of CD40; receptor on the B cell and the CD40 ligand now gives a competent signal. Expression of class 1 cytokine receptors on B cell happens in response to the CD40 receptors CD40 ligand interaction, now allowing the cells to interact with interleukin 4 interleukin 5 which are synthesized by the activated T cell and this gives a progression signal for the B cells to start proliferating.

What are interleukin 4 and this should be interleukin 5. What are these two? Interleukin 4 and 5 are also known as B cell growth factors and these interleukin 4 is synthesized by the TH2 cells, mast cells, and NK cells. You have already been introduced to these cells; so, I will not describe them further. And interleukin 4 stimulates the proliferation of the cells induces class switching from IgG and from IgN to IgG 1 and IgE.

So, the only difference between these two is that in case of interleukin 5, this should be 5 which is also produced by TH2 and mast cells. This also stimulates the proliferation, but the difference is that this cytokine induces class switch to IgA instead of I G G1 and IgE.

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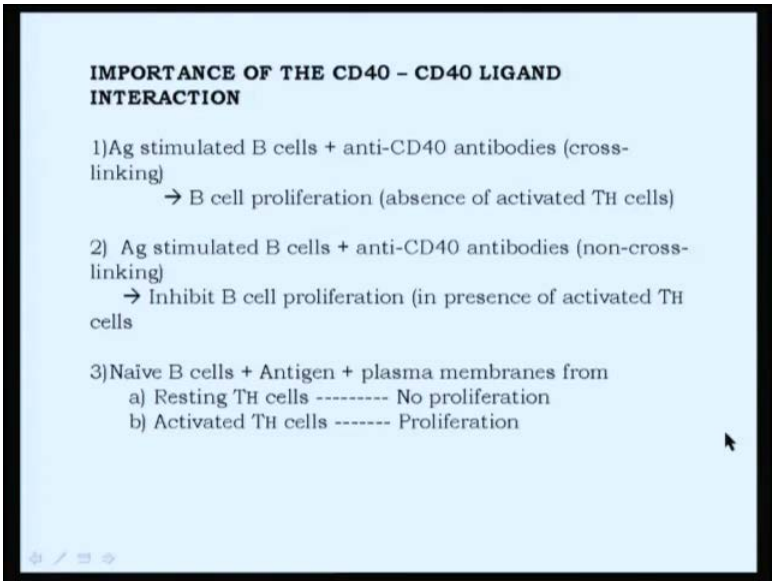

CD40 receptor and CD40 ligand interaction is very important. As I have already mentioned, this gives a competent signal. I would like to also tell you how people have determined that. In the absence of CD40 ligand interaction with the CD40 receptor, there is no B cell proliferation. This has been shown by more than one way. If antigen stimulated B cells are taken in vitro and antibodies to CD40 are added, this results in cross linking of the CD40 receptor.

So, this is in the absence of the ligand in the absence of activated T helper cells, but just a cross linking of the receptor is able to induce the signalling such that B cell proliferation takes place. Similarly, now if you have antigen stimulated B cell, but blocking antibodies to the CD40 receptor are added so that even though there is presence of T helper cell which are activated and have CD40 ligand. In such a situation, B cell proliferation is inhibited.

The third experiment is even more interesting because if naïve B cells are taken and induced, activated with their cognate antigen and now plasma membranes are taken from the activated T helper cell, there is proliferation. that When membranes are taken from resting helper cell which are which are which would have all the receptors except CD40 ligand, there is no proliferation.

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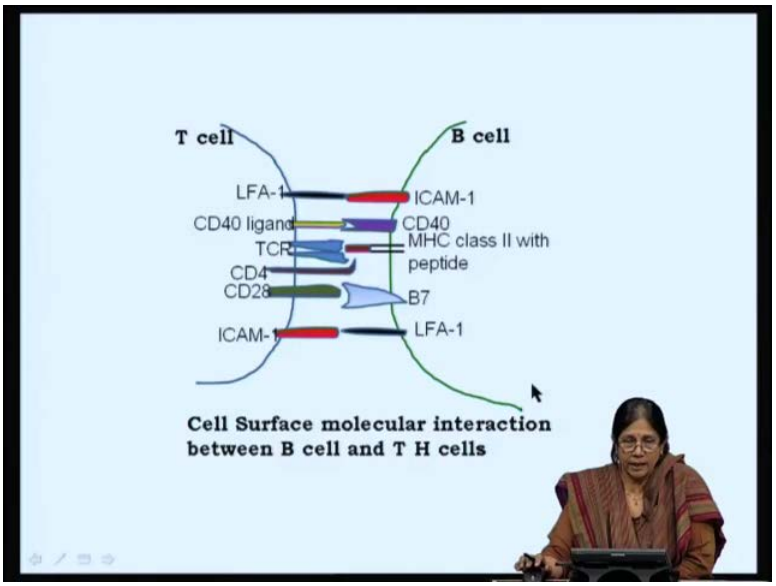

So, CD40 receptor CD40 ligand interaction is absolutely essential for the B cells to gain the competent signal to make cytokine receptors to accept the cytokine so that they can

get proliferation signals. This also tells us that there is a very good T B cell interaction that takes place and this interaction is essential for an optimum response. In the previous slide what I showed, in all these experiments, which tell you that CD40 receptor CD40 ligand interactions are essential, but none of these gave a very good immune response with respect to proliferation. However, if you have T cell interacting with the B cell, this interaction gives a robust proliferation response to the B cells.

Let us look at some of the molecules that allow the original interaction between the antigen receptors on the T cell and the MHC class II of the B cells with the peptide corresponding to the TCR or the T cell receptor. Now, when these two cells come together, in fact, when the T cell receptor recognizes a specific peptide in the context of MHC class II molecule, this interaction, the cell-cell interaction is stabilized by addition molecules which are present on both T cells and B cells, but it is important to remember that these addition molecules have partners. For example, LFA 1 which is the cell addition molecule shown on T cell interacts with another cell addition molecule B cell ICAM 1. Now, ICAM 1 can also be present on T cell and this would interact with the LFA 1.

So, these partners are these partners are absolutely essential for keeping the cells together. This allowing the cell addition molecules to come together, you allow therefore, the CD40 receptor present on these cells to interact with the CD40 ligand which the activated T cell has. Another interaction which I did not deal with in my earlier slide is the CD28 which is present on T cells and the expression of B7 molecules in the B cells. This interaction allows the T cells to get a competent signal and start producing cytokines.

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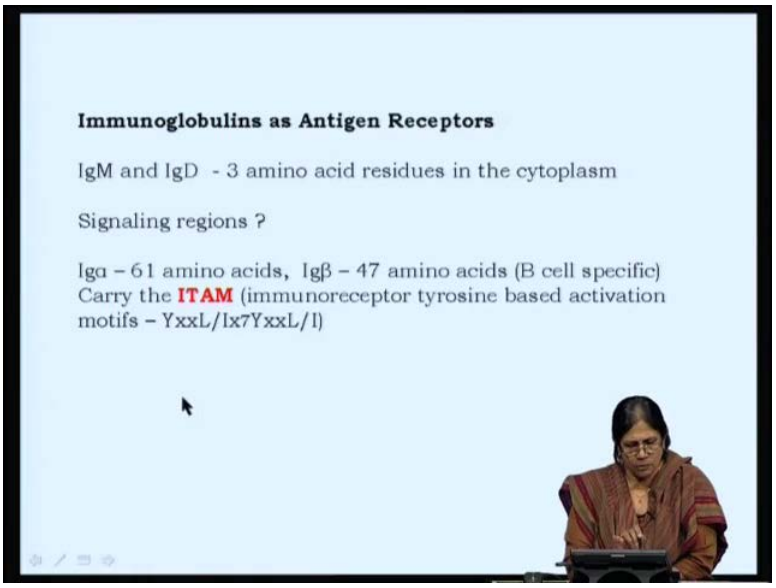

Let us look at the immunoglobulins as antigen receptors. What are receptors? Receptors are those molecules which are which participate in signalling. Receptors bind to their cognate ligands or molecules with a very specific interaction. When immunologists started looking at IgM and IgD which are the cell surface antigen receptors B cells, they found that in the inter-cytoplasmic domain, both IgM and IgD had only three amino acid residues which would mean that there are no signaling molecules inter-cytoplasmic. Now, they were quite intrigued because by then it was already known that B antigen B cell antigen receptor belongs to the phosphotyrosine receptor family.

So, how do these molecules now trigger a signal when from the outside to the inside when there are only three amino acids inter-cytoplasmic? So, immunologists started looking for these molecules, trying to look for what is present in the cytoplasm if there are any co-receptors which might be associated with the antigen receptor.

It is only when detergent such as chaps which is a zwitterionic detergent became available, immunologists were able to solubilize B cell membranes and were able to pick out the immunoglobulin antigen receptor and found two molecules associated with this; immunoglobulin alpha and immunoglobulin beta. Immunoglobulin alpha and beta are the larger and smaller peptides which are linked to each other by disulphide bond and these are very B cell specific. These have inter-cytoplasmic domains which are much longer

than present on the as antigen receptors and these are ones that carries what are known as ITAM. ITAM is the acronym for immune receptor tyrosine based activation motifs.

Now, these motifs can be tyrosine xx leucine isoleucine x7times tyrosine xx leucine for isoleucine where xx can be any amino acids. So, what would be important here the spacing between the two tyrosines and the isoleucine, and the leucine.

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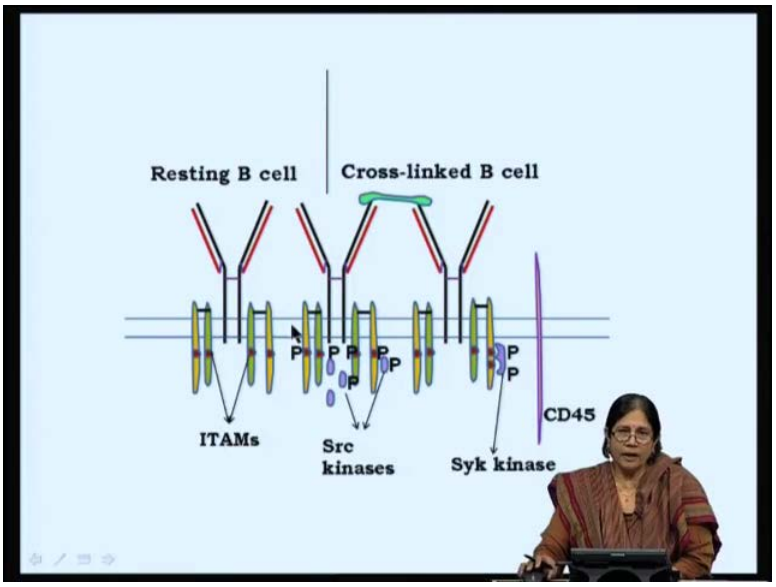

Having identified immunoglobulin alpha and beta, of course, they were then the antigens were corresponding to these molecules were described and it was found that both immunoglobulin alpha as well as immunoglobulin beta carry these ITAMs in the cytoplasm.

Let us look at the resting cell and let us look at an activated cell. In a resting cell, you have the antigen receptor as well as these two in a state where there is absolutely no availability of the ITAMs. Once the antigen crosslinks two receptors, two immunoglobulin receptors, by specific interaction between the epitopes and the antigen binding pocket induces a conformational change in this molecule. This conformational change now induces a conformational change in the immunoglobulin alpha beta, the two co-receptors, such that the hidden ITAMs which are shown here in red become available for further activation. I already said that the immunoglobulin as antigen receptor belongs to the tyrosine phosphate kinase sorry kinase receptor. Therefore, phosphorylation has to be induced.

Now, let us look at another receptor which I mentioned in my last class as CD45 and this receptor is also known as B220 on the B cells. This is a phosphatase. So, what is it doing here? Because I said that the antigen receptor is a tyrosine kinase receptor that means activation would allow addition of phosphate molecules rather than removal. So, what is the CD45? In fact, this is a very important molecule in the signalling because it removes a phosphate and it actually kick-starts the phosphorylation of all the molecules which I am going to describe right now.

Inter-cytoplasmically, there are mainly two types of kinases involved in this particular activation process; a family of protein kinases called Src kinase and another called Syk kinase. The Src kinases are simpler of the two; Syk kinase is more robust in its phosphorylating activity. As the name suggests, these two are enzymes and kinase would mean that they are able to add a phosphate group; in this case to tyrosines. And let us go back.

The antigen receptor - it undergoes crosslinking by the presence of… let us say this is a bacterium which has several of these - the same antigens spread. So, two of these antigen receptors are in close proximity to other; they are binded to the same antigen which brings about crosslinking, inducing conformational change in these molecules, the receptor molecules which now is transmitted to the immunoglobulin alpha beta, so that the ITAMs are exposed.

At the same time, the CD45 molecule which is in the vicinity, it is a phosphatase. This gets activated and it activates in turn the Src kinase by removing an inhibiting phosphate. Now, when I come to the structure of these enzymes, it will be clearer, but it removes a phosphate, an inhibiting phosphate, opening up the Src kinase in a manner, so that auto phosphorylation can happen and these Src kinases get phosphorylated. Now, phosphorylation is what causes the Src kinase to get activated and now these can phosphorylate Syk kinases and also the ITAMs. So, you can see that there is addition of phosphate molecules to the ITAMs; in turn, Src kinases and Syk kinases can both after getting activated, they can start activating ITAMs and also each other following which there are a few molecules which are present in this membrane to get activated.

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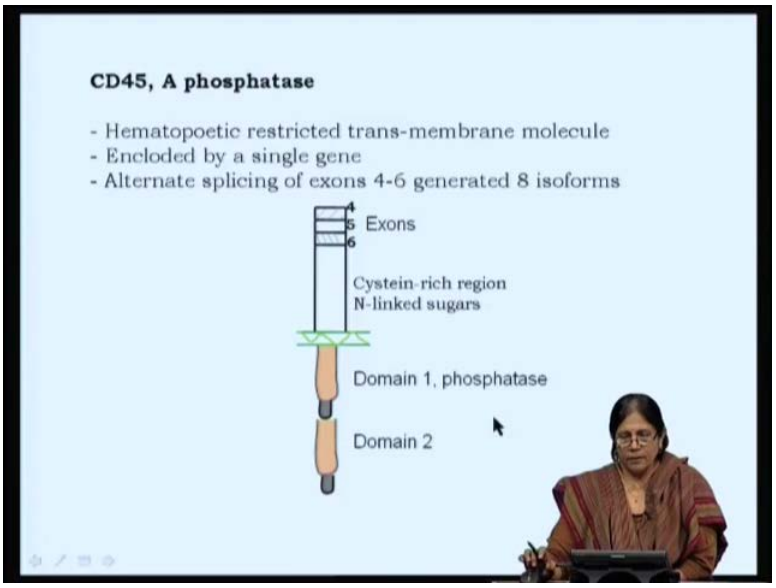

We will come to that, but before that let me just describe the enzyme CD45 which is a phosphatase. What kind of a molecule is that? Because this seems to be a very important molecule because this is what starts an inactive kinase to become an active one, by the removal of phosphate. Interestingly, CD45, it is a hematopoietic restricted trans-membrane molecule and it is encoded by a single gene. This should be encoded sorry for the mis-spelling, encoded by a single gene. There are 3 exons and alternate splicing of these exons generate at least 8 different isoforms of this molecule. The extra-cellular domain changes from one cell to another; the intra-cellular domain remains almost the same. Therefore, CD45 molecules present on T cells differ from that present on B cells or from monocytes only in the exo-cellular domain. The intra-cellular domain and the function remain intact.

Now, there is a cysteine-rich region which is highly glycosylated. Now, it is the extra-cellular domain which is formed by the exon 4 5 6, which in fact give the distinct specificity with respect to recognition let us say of antibodies to the CD45; suffice you have to remember that CD45 is a phosphatase.

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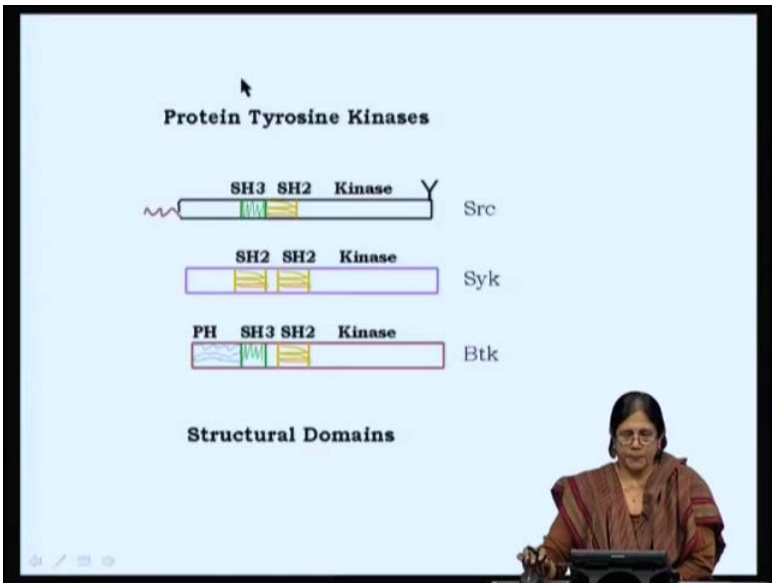

Let us look at the structural domains of the kinases that I have already talked about, Src and Syk, and there is yet one more Bruton's tyrosine kinase and I will I will talk about it. If you look at the three structures, they look very similar to each other, where there is in this C terminus, there is the kinase domain. All three have kinase domain; each one of them has different types of interacting domains SH2 or SH3. SH stands for Src homology and these allow the protein to interact with another protein.

Now, Src and Btk - both of them have only one domain each for binding to another protein whereas, Syk which I told you is more efficient with respect to its function that is phosphorylation. This has two protein binding sites, and therefore, when Syk kinase bind to ITAMs, this interaction is quite robust and therefore Syk is more efficient in phosphorylation.

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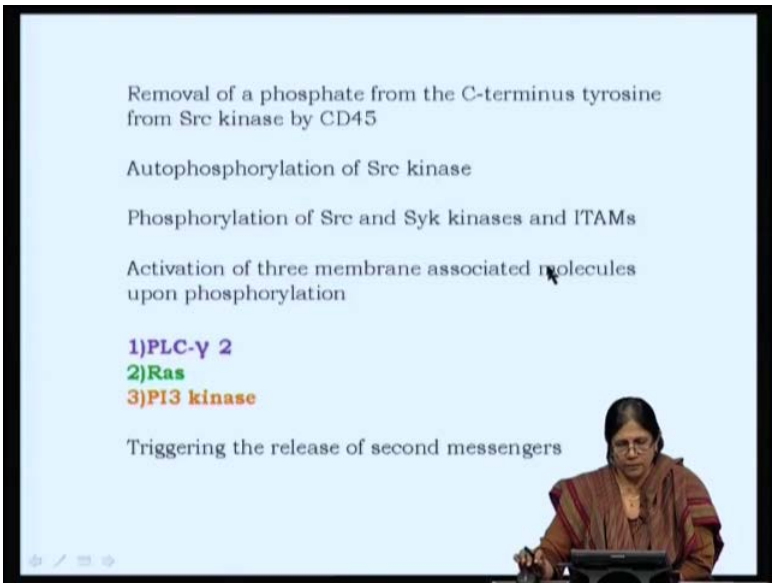

To go over again, what happens after signalling, you know after the conformation alteration takes place in the immunoglobulin alpha beta, removal of a phosphate from the c-terminus tyrosine from Src kinase happens by CD45, the phosphatase allowing the Src kinase to get phosphorylated. This now allows phosphorylation, intense phosphorylation of Src and Syk kinases as well as ITAMs, and they can phosphorylate each other when they interact with each other, and in turn Syk and Src kinases activate three different membrane associated molecules upon phosphorylation.

So, now, these kinases can transfer phosphates to three molecules PLC phospholipase C gamma 2 which is specific for B cells, Ras as well as PI3 kinase. Interaction of activation of all these three molecules now triggers the release of second messengers. Just one small experiment I would like to suggest to show that phosphorylation, intense phosphorylation, is something that happens soon after this activation process.

Now, I have been telling you that this crosslinking of two antigen receptors by the cognate antigen, but what I have shown is a specific example when there is a particulate antigen which would have more than one, the epitope occurring more than one, so that crosslinking is possible. Now, how have people, how have immunologists studied all this interlink process? They have done that by bringing about crosslinking of these surface immunoglobulin M or surface immunoglobulin D. It just one reagent, an antibody that has been made.

Let us say one is looking at a mau system. When one would be using antibodies made to mau's immunoglobulin M or mau's immunoglobulin D, such that when two B cells from let us say spleen sites of mice this reagent anti IgM or anti IgD, so that crosslinking of receptors and then the further signaling. So, each step of the signaling has been studied like this and it is believed that this is exactly what happens even when crosslinking or binding of antigen to its cognate receptor happens.

Now, let us go back to the experiment that I was talking about. If such a reagent is added to the cells made from spleen sites, after let us say ten seconds, a minute, two minutes, the cells are lysed and extract is made electrophoresed. And now, western blotting is carried out to find out how many molecules are phosphorylated before and after the activation experiment. One can see there are a whole lot of modules, tens and twenty different molecules are phosphorylated and one can in fact identify specific phosphorylated proteins also by using the specific reagents.

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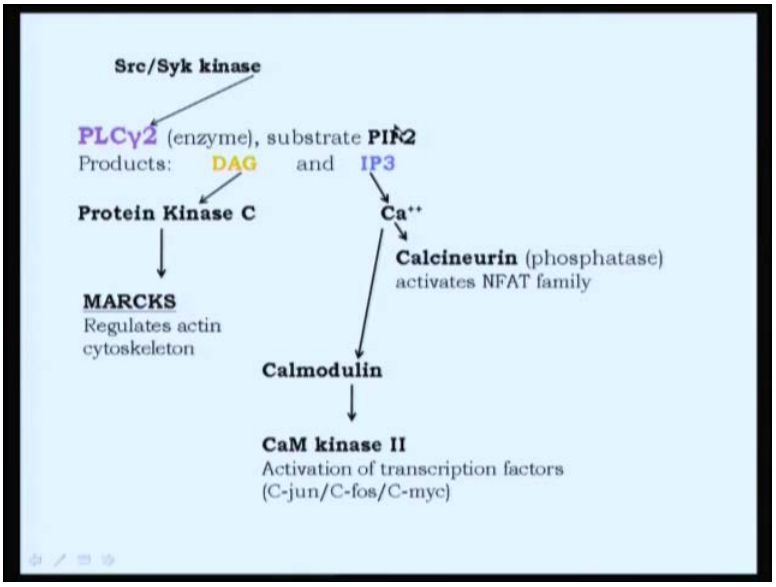

Let us come now to the signalling process that takes place of the second molecules. In all of them, the common factors are these kinases. So, you have the kinases which are activated in response to antigen and antibody interaction where you have phosphorylation of these kinases, activation of these, and now these kinases can activate the first molecule we deal with this - phospholipase C gamma 2. This is an enzyme and once this enzyme gets activated upon phosphorylation, it can bring about the hydrolysis

of phosphor phosphatidylinositol bisphosphate PIP2 to the products diacylgycerol and IP3. Diacylgycerol and IP3 are the second messengers; diacylgycerol binds to protein kinase C, activating this enzyme, now, which in turn activates a molecule called Marcks. Marcks is required for regulation of the actin cytoskeleton. Now, why could this be important?

Remember that activation of B cells now lead to proliferation and during the proliferation phase, the cytoskeleton has to undergo tremendous reorganization because every time there is also increase in the cell size during the synthetic phase. Therefore, regulation of actin cytoskeleton is very important, and therefore it is important to have activation of this regulation which is done through protein kinase C.

Now, the second messenger IP3 inositol triphosphate - this it binds to its receptor on the endoplasmic reticulum of cells allowing a signaling there, bringing about release of stored Calcium in the cytoplasm and is believed that from something like a 100 nano molar of Calcium in the cytoplasm, this can increase to as high as one micro molar concentration of calcium. What does calcium do? It binds to calcineurin which is a phosphatase; activates thus which in turn activates NFAT family of transcription factors. Calcium also activates another protein calmodulin which in turn activates a kinase, yet another kinase, called calmodulin; specific kinase two. What are these kinase two? It activates another set of transcription factors C Jun, C Fos, C Myc and all these transcription factor upon getting activated enter the nucleus and start regulating a whole lot of genes which are required for proliferation as well as for differentiation.

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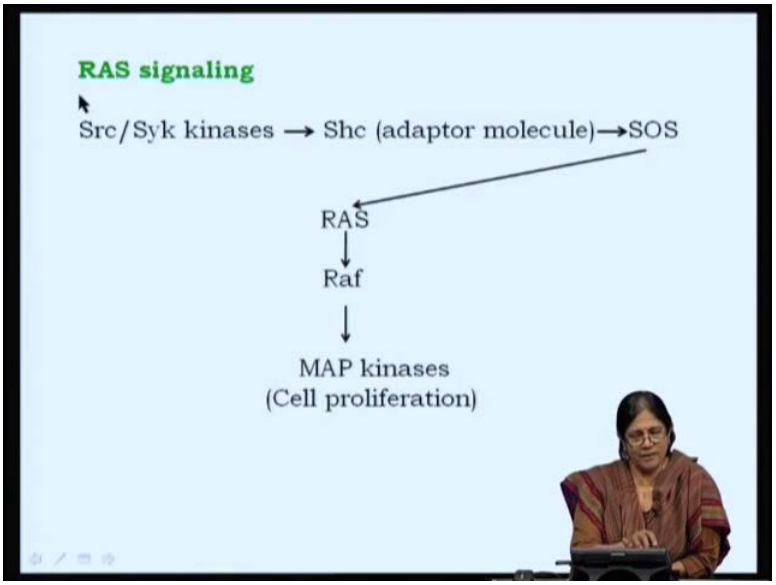
RAS signaling
Src/Syk kinases → Shc (adaptor molecule)→SOS
RAS
Raf
MAP kinases
(Cell proliferation)

The third molecule that I talked about… the second molecule sorry because we just finished p l C gamma 2, the second molecule which gets activated upon phosphorylation is Ras signaling. I would like you to just… where have you heard this Ras? Ras is a molecule that is cited along with several cancers. Ras signaling leads to cell proliferation through another set of kinases which are map kinases; mitogen activated protein kinase.

How does this happen? Once again the common factor is Src and Syk kinases which get activated after they are phosphorylated. These in turn activate an adaptive molecule called SHC. Now, once this gets phosphorylated, it undergoes a conformational change, so that it can bind to another protein called SOS - son of seven less. After SOS binds to SHC, SOS gets phosphorylated and after this gets phosphorylated, SOS again has sites which can bind to Ras. Ras gets activated upon receiving the phosphate. It activates Raf which is Ras-associated factor, which in turn activates the kinases. These map kinases can induce cell proliferation. You can see there are so many molecules that are generated in response to antigen-antibody interaction which gives the cell a proliferation signal and therefore, you have this intense proliferation of B cells.

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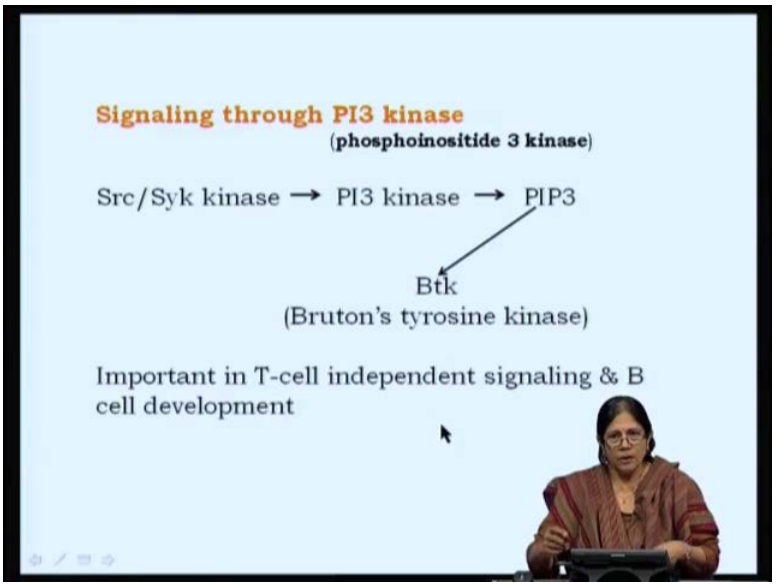

The third molecule that I said also gets phosphorylated by the kinases the Src and Syk kinases, and this one is phosphoinositide 3 kinase; PI3 kinase. This it phosphorylates PIP3 which in turn phosphorylates Bruton's tyrosine kinase. Now, Bruton's tyrosine kinase is not required as Src and Syk kinases, but these are very important. This is an

important molecule in T cell independent signaling and B cell development. It has been seen mice that lack an Bruton's tyrosine kinase or mutant Btk have very low numbers of B cells. Therefore, Btk is a kinase which is required during the development stage as well.

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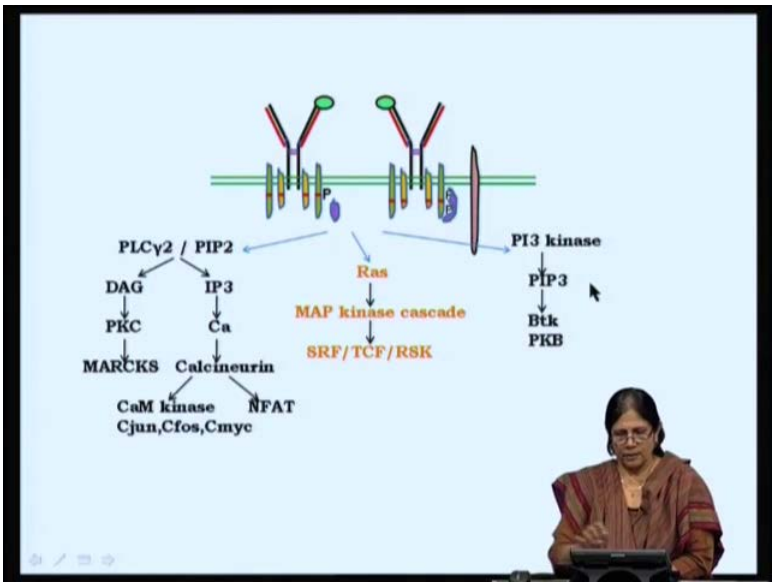

Now, let us look at all the three signaling molecules in one slide. Antigen, here, I have shown that it is not crosslinking. Therefore, like I said that whatever has been studied so far with respect to crosslinking of the receptors induced by antibodies to this would be similar to what happens when you have even soluble antigens binding to their cognate receptors. You have activation of Src and Syk kinase. This leads to activation of three molecules PLC gamma 2, Ras, PI3 kinase which allow now a cascade of events that takes place, such that you have proliferation of cells as well as you have differentiation and change in morphology of these cells.

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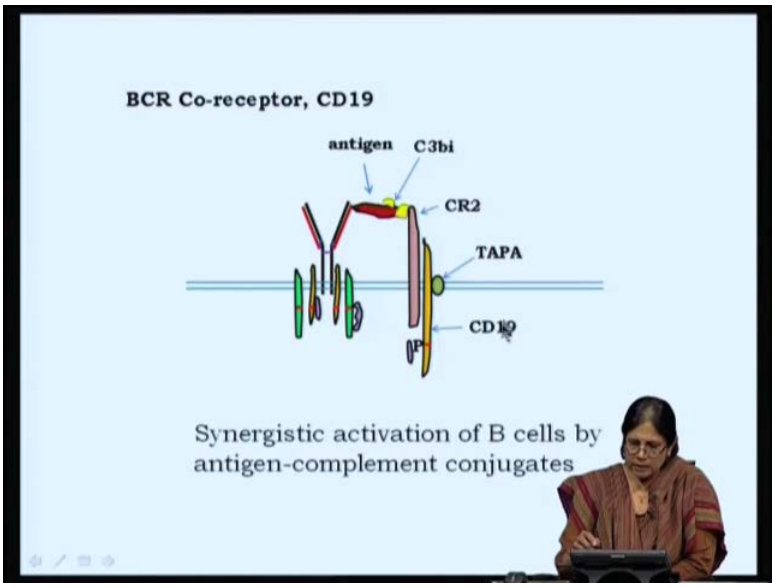

Are there any co-receptors for immunoglobulin apart from the immunoglobulin alpha beta? Yes. B cells also have molecules that can enhance. Now, I must emphasize that initiation of the response or the B cell signaling always happens to the B cell receptor; there is no other way the B cell receptor binding to the cognate antigen. This is what makes the cell go into an activation phase. Once this happens and you have phosphorylation because of which Src and Syk kinases get activated, after that, there can be recruitment of certain receptors such as CD19.

Now, I have told you CD19 is the mark of a B cells, B cells have CD; all B cells have CD19 and these are these are molecules that are in close proximity. In fact, there is a complex of three membrane-bound molecules called CD19, CR2 which is complement receptor 2 and TAPA. CD19 is the one that you can see in yellow and what is important is that CD19 bears the ITAM or the immuno receptor tyrosine-based activation motif. You have an antigen which is shown in red which is the bacteria.

Now, I would like you to remember here that the CD19, it induces this enhancement only for antigens which are particulate such as bacteria and which can bind to C3bi which is complement component 3 and a part thereof; so, the smaller C3b which binds to bacteria cell surface; such a bacterium with the C3bi which is shown here in yellow can bind to CR2 which is the complement receptor.

So, let us look at now the antigen. The bacterium is bound to its cognate receptor, the immunoglobulin receptor, and this has induced the proliferation sorry the activation of phosphorylation of the Src and Syk kinases and now there is phosphorylation all around. at such a during this event, now, if CR2, TAPA, CD19 - these three molecules come in close proximity of the antigen receptor. Then the C3bi is recognized by the CR3. Because it is present very close as a complex with the CD19 induces a conformation alteration in the CD19, such that the ITAM which is present which is shown here as a small red strip gets access accessible. When it becomes accessible, the Srk and Syk kinases which activated in the vicinity can bind to this ITAM and bring about activation and phosphorylation. So, you can imagine, now, in this situation instead of having only 4 ITAMs you have a fifth one which is contributed by CD19. This is known as synergistic activation of B cells by antigen-complement conjugate.

Now, I have not introduced the molecule C3b earlier and I will be talking about it at length when I discuss the complement cascade, but remember that C3 is a complement component and it undergoes a very slow cleavage to C3b and C3bi, and C3b can bind to bacterial cell surface and this is quite a stable conjugate. Now, such a conjugate can bind to CR2 and gets rid of the CD19 in turn gets recruited to this site. BCR co-receptor is CD19. Are there any that inhibit the entire activation process?

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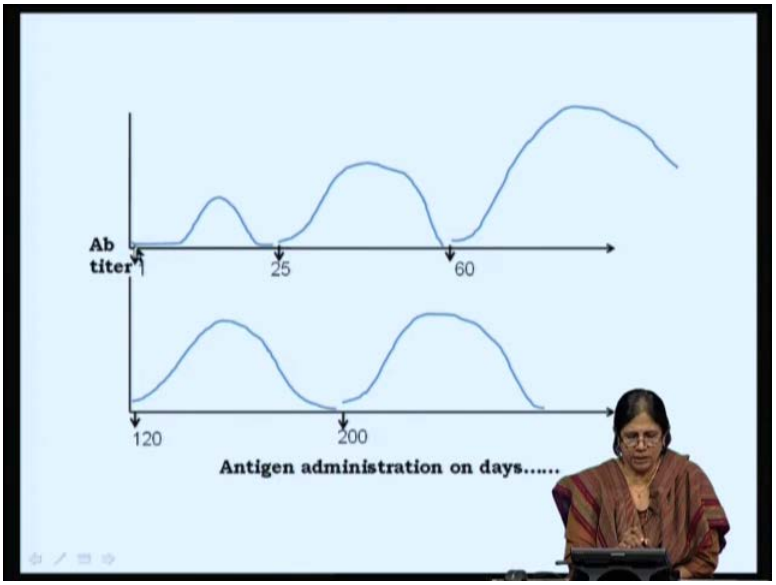

Now, even before I come to the inhibitor molecule called f CR2b, let me go let me explain this with an experiment, and therefore, which will allow you to appreciate the inhibition much better. Let us say, now that there is a rabbit that you have injected with antigen a. This is done on day one. As I have told you earlier that when the animal sees the antigen for the first time, there is a lag phase, only after which there is synthesis of immunoglobulin as seen in circulation. Now, what does the x axis here show? It shows the days after immunization starting with 1, 25, 60, 120 which could have been 19 and this could have been 120. These are actually arbitrary dates; it does not really matter; just to say and what is the y axis? It is antibody titer.

Let us say after injection of this animal with this particular antigen. Serum is collected every other day and the level of the antibody in circulation specific to that antigen is measured and this is what gives this particular curve (Refer Slide Time: 43:11). So, on day one, when you inject you can see that up to something like day 7 or day 6 there is very little, if any, antibody in circulation. The antibodies steadily increase and around day ten there is the peak immune response after the first injection.

Now, after this level comes down, the animal is injected again with the same antigen. You can see very easily that there is an increase in the titer. In fact, there is an increase in the amplitude of the response and even the duration of the response is much longer. Now, again, when the antibodies in the circulation come down, the animal can be given the same antigen; low and behold, you see even a higher amplitude of the response. What does this amplitude show? Since I have said this is the antibody titer, it shows that there

are many more antibodies to the antigen. You can see it is several fold; it could be hundred fold higher as seen in the primary response.

Interestingly, after this response comes down that this can stay on even for two months depending on the strength of the antigen receptor interaction. Now, why is there no… you can see here there is a lag phase and there is almost no lag phase after the second third and fourth and fifth injection. That is because of the presence of memory cells here which are very fast acting. They already have receptor for those cytokines and therefore, they are very well-equipped to recognize the antigen and undergo the proliferation immediately; here, the cells had to wait for the cytokine receptors to appear.

Now, why do you have… you can see that there is an increase in the amplitude. You will agree that – yes, these memory cells are also increasing; therefore, you have many more clones of B cells here which are making antibodies to this antigen than the number of clones here (Refer Slide Time: 45:21). Interestingly, let us say, after the fourth injection, one does not see a greater amplitude; a more robust immune response than the third. In fact, after the fourth injection, you can see that the amplitude of the response and the even the duration does come down and similarly, fifth injection also the same.

Now, what does this mean? That there must be some inhibitory factors which are controlling the cells from undergoing, you know, further and further amplification. Now, this is of course important because the immune response cannot recognize and respond to only one antigen.

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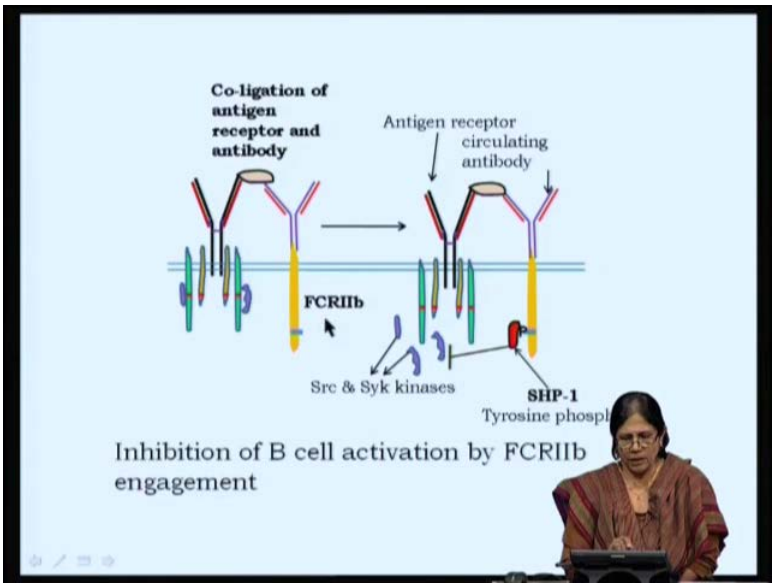

There has to be energy and there has to enough material for being able to recognize several antigens at the same time and this happens in fact by one such mechanism through receptors known as FCRIIb. these are As the name might suggest to you, these are receptors that can bind specifically to the FC region of the immunoglobulin molecule. When does this happen? When does the FCRIIb get activated? This is when there is there is there are circulating antibodies present and these can bind to the antigen as well as to the FCRIIb at the same time, when the antigen is bound also to the activating part that is the cell surface antigen receptor.

First, before even I go to that, let us see how what is this FCRIIb? FCRIIb is a receptor which has recognition sites for the FC region of the immunoglobulin, IgG isotype as well as has intercellularly a motif which is called ITIM - immunoreceptor tyrosine-based inhibition inhibitory motif rather than activation motif. Now, this recruits a phosphatase; anyway, we can just go through the entire process again. We can see that the antigen receptor binds to this cognate antigen which could be a bacterium, let us say. And in this animal, there are already circulating antibodies, let us say, this is the tertiary response and there are already large number of circulating antibodies which to the same antigen.

Now, when the antibody… that is why I have tried to put this in another color. This is circulating antibody and this is the antigen receptor (Refer Slide Time: 48:07). Now, the circulating antibody is bound to the antigen and the same antigen through another

epitope maybe binds to the antigen receptor and triggers a positive signal; phosphorylation signal. Simultaneously, now, the antigen which is bound to the antibody, not the receptor but antibody, the FC portion is free; it is not membrane-anchored. This can now be recognized specifically by the FC receptor and this brings about a conformation and alteration in the FCRIIb, such that the ITIM or the inhibitory motif gets exposed. This is also phosphorylation event.

So, for the ITIM to get activated, it has to be phosphorylated which is done very nicely by the kinases in the vicinity because remember, this molecule is the activation molecule and it is activating or phosphorylating the Src and the Syk kinases. Once these phosphorylate the FCRIIb ITIM, this can now recruit SHP which is a tyrosine phosphatase. The phosphatase, now, this should ring a bell. What would the phosphatase do? It can remove the phosphates from the kinases making them inactive. So, you have inhibition of the B cell activation by FCRIIb, but this requires co-ligation of the antigen receptor and the antibody; only then FCRIIb conformation alteration happens and exposure of the ITIM.

Now, this co-ligation can happen only when the animal has been exposed to the antigen. So, that means it is after the primary response; perhaps even after the secondary response when the circulating antibodies are there, and there is a prolonged duration for the presence of these antibodies. In such situation, if the animal, let us say, is injected here (Refer Slide Time: 50:33) by the same antigen, then the inhibition would be even more profound because there would be engagement of a large number of FC gamma RIIb.

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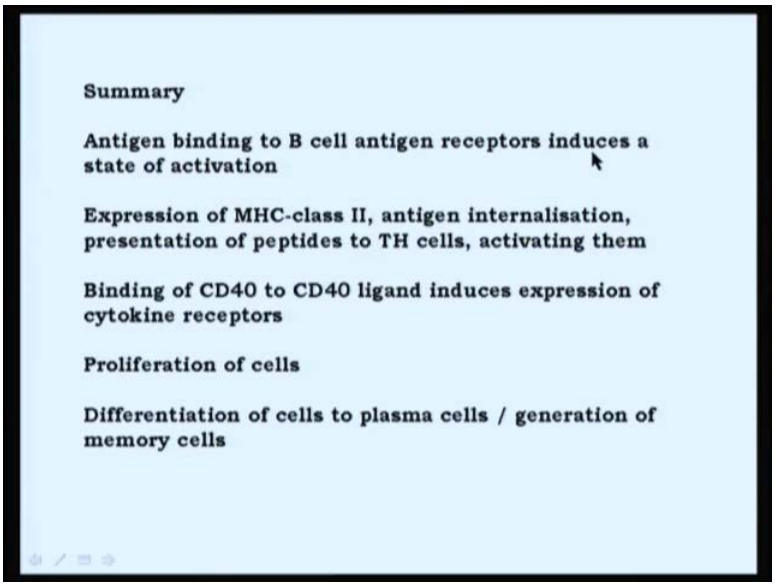

Now, there are apart from just the FCRIIb, there are several other mechanisms by which B cells are inhibited from going berserk and for producing immunoglobulins or antibodies to the same antigen. If this would happen, if there were no checks in place, our bodies would be making antibodies only to those antigens that they see first, and in doing so, would not be able to mount immune response to the other.

I had started my lecture by saying that there are large number of… we are surrounded by a large number of pathogens, large number of bacteria, viruses and we need to be equipped to be able to counter an immune response to all of them. Therefore, I mean nature has it that there are these inhibitory molecules which allow a B cell to be very specific, yet be limited in the way the response can outdo the antigen and not allow too much of the same.

In summary, antigen binding to B cell antigen receptors induces a state of activation. So, the binding of the B cell antigen receptors to the antigen only makes the cell activated or go into an activation state, but such a cell cannot proliferate unless there is a production of the cytokines interleukin 4 and 5 by T cells. Therefore, B cells recruit T cells and helper T cells. So, antigen binding to B cell antigen receptor induces state of activation which now is concomitant with the expression or the MHC class II molecules leading to antigen internalization, presentation of the peptides to TH cells and activating the TH cells, so that the TH cells can start making interleukin 4 and 5. TH cells also make the

CD40 ligand which quiescent TH cells do not. CD40 is a receptor present on B cells recognizing CD40 ligand which now induces the expression of cytokine receptors on the B cell. This cytokine binding to the cytokine receptors induces proliferation of B cells. Subsequent differentiation of cells to plasma cells when the cells start to get receptors for interleukin 6. When there is differentiation of cells to plasma cells, a few cells become memory cells.

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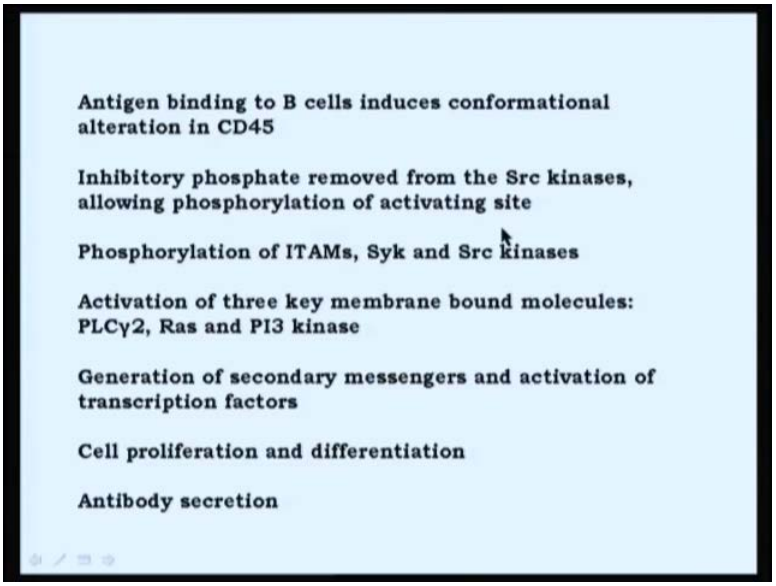

Antigen binding – now, this is what happens in the outside of the cell. So, whatever I talked about, let me get back to the… now what I talked about so far is what happens in the… summary is what happens outside the cell, what happens inside the cell is the antigen binding to B cell induces conformation alternation in CD45; the phosphatase, this removes and inhibitory phosphate from the Syk kinases from the C terminals; you can think in terms of you know you know the Src kinases being closed and the CD the inhibitory molecule is present in the C terminals on a tyrosine. When this phosphate is removed, the molecule now opens up allowing phosphorylation and allowing it to get activated.

Now, the activating site gets activated by phosphorylation, so that the Src kinases can now phosphorylate the ITAM, Syk and in turn Syk and ITAMs can also inter-phosphorylate each other. Now, activation of three key membrane molecules happens by Src and Syk kinases - these enzymes called PLC gamma 2 Ras and PI3 kinase. These, in

turn, allow generation of messengers and activation of transcription factors. The transcription factors are absolutely required. They go from the cytoplasm into the nucleus for inducing cell proliferation and differentiation. The differentiation allows antibody secretion.

So, you can see now that there are so many different events that happen in the small B cell. we also We always have to look at the small B cells as a factory which produces immunoglobulins which produces antibodies. But for the cell to become an antibody producing cell, it has to undergo so many steps of activating molecules, antigen antibody, I mean antigen and antigen receptor recognition, phosphorylation, generation of umpteen number of molecules before they can become… first they start to proliferate and before they can become antibody producing cells.

So, with this I will stop here and my next class will be on the molecular events that take place for the reorganization of the immunoglobulin genes which allows the antigen receptors to be expressed on B cell surface.

Thank you.