Essentials in Immunology

Prof. Dipankar Nandi

Department of Biochemistry

Indian Institute of Science, Bangalore

Lecture No. # 26

T cell Activation

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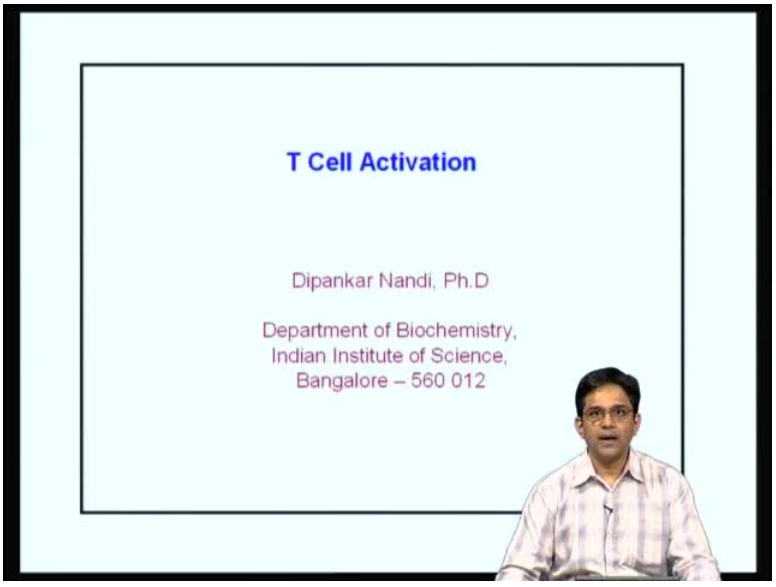

So, today, we will discuss T cell activation. It is a very important topic because T cell activation plays a very important role in the generation and sustenance of an immune response. It is also, it has clinical manifestations because, for example, during transplants, transplant surgeries and all, you want to suppress the T cell activation and therefore immunosuppressants are used. On the other hand, with vaccines and all, you use adjuvants, so that you can enhance T cell activation. So, these are very important aspects and that is something that we need to understand.

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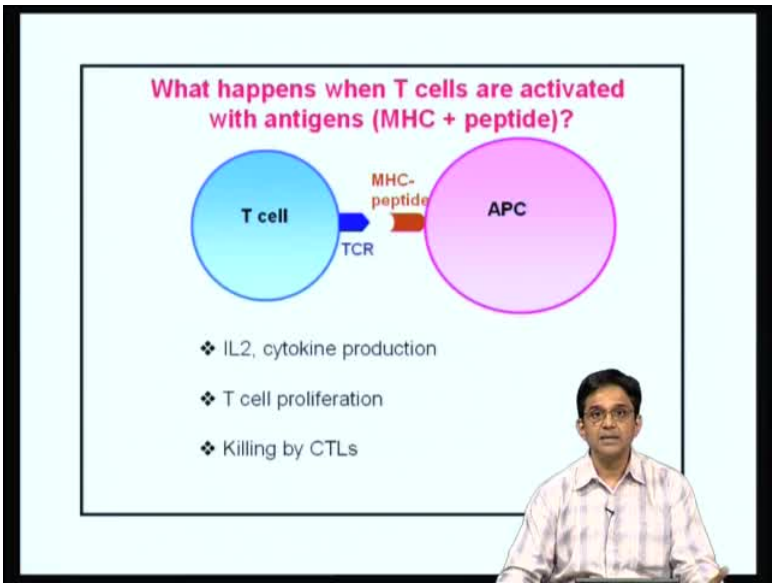

So, we will start off with the basics first. What is shown over here is, here is a T cell with the T cell receptor; remember, specificity for the T cell is via the T cell receptor, which recognizes the cognate MHC peptide over here. So, here you have an antigen presenting cell, presenting a particular peptide on its MHC. This particular MHC peptide complex is recognized by T cells, by the T cell receptor and it leads to T cell activation. So, what exactly happens?

So, once T cells are activated, they produce IL2 and other cytokines. IL2 is an important one because IL2 is the autocrine T cell growth factor, so it is produced by T cells and it helps them proliferate, and that is for, and this process is important for the killing of target cells by, by cytotoxic T-lymphocytes. And we will discuss this in a little bit greater detail in the subsequent slides.

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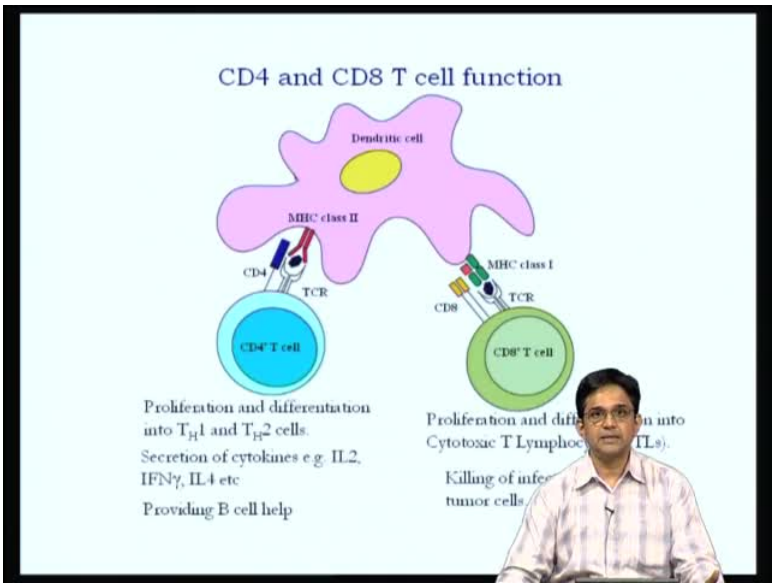

So, this is just to tell you a little bit about CD4 and CD8 function. Here, what we have shown is a dendritic cell, antigen presenting cell is presenting MHC class 2 and here is a CD4 T cell with cognate T cell receptor. So, upon activation, what happens is T cells, CD4 positive T cells are helper cells, so they help macrophages. They help B cells and this leads to proliferation and differentiation of the CD4 helper cells into T H 1, T H 2 types. These are the primary types, results in production of cytokines and so on.

On the other hand, the CD8 positive T cells differentiate from CD8 positive, from CD8 positive T cells into CTL. So, this is a very important point, so what happens upon activation is that these, these CD8 positive cell differentiate into CTLs; so, the CD8 differentiate into CTL. So, there is a difference between CD8, which cannot kill on their own, but CD8 after differentiation becomes CTLs, which can kill. So, it is a very important aspect, and these kill infected cells and tumor infected cells and T cells and tumor cells.

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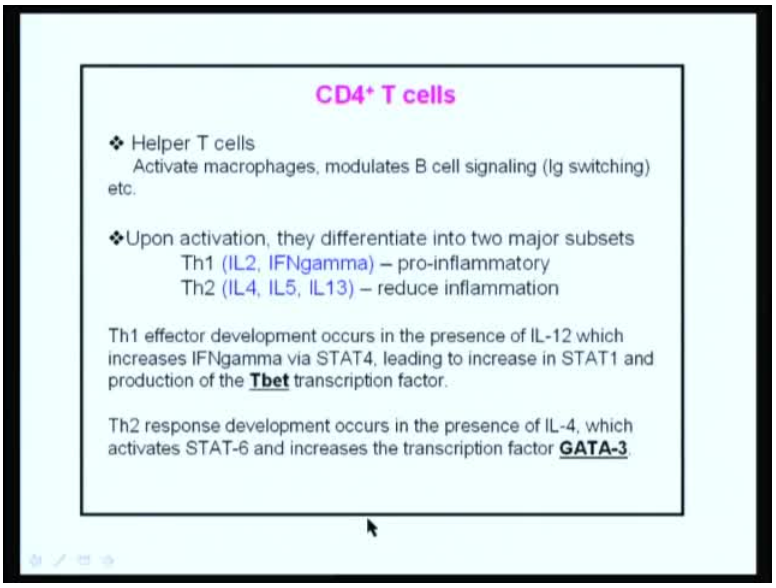

So, this is a little bit more about CD4 positive T cells. These are helper T cells, as I, as I mentioned. They, they, they activate macrophages, they modulate b cell signaling and they differentiate into 2 major sub-cells the Th1, which are, which are more pro-inflammatory and that is because they produce lots of IL2 interferon gamma or Th2, which produce IL4, IL5, IL13, which sort of reduce inflammation.

So, the mechanisms by which Th1 and Th2 differentiate are, are, are well studied. So, what happens in case of Th1, this occurs in the presence of IL-12, which produced by, primarily by the antigen presenting cells. Macrophages are very good produces of IL-12, and this increases, IL-12 increases interferon gamma via STAT4 and it leads to increase in STAT1 and production of Tbet transcription factor.

So, one of the important signatures of Th1 positive cells, are, is Tbet and Tbet is very important because in the absence of Tbet you do not get the Th1 types. Now, similarly, in Th2 development, it occurs in the presence of IL4, it results in activation of STAT-6 and it increases the transcription factor GATA-3. So, you can see, the, there are 2 important transcription factors, that play roles here, Tbet for Th1 and GATA-3 for Th2.

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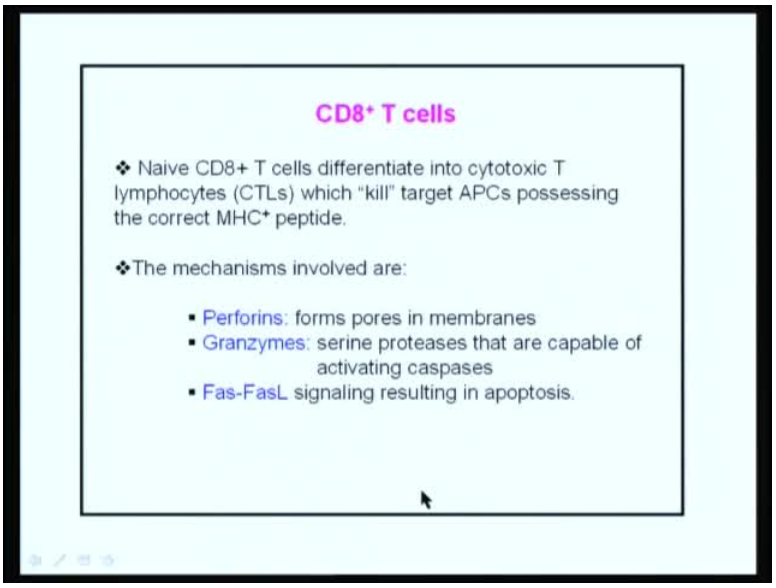

Now, for CD8 positive T cell, I have, I said this, these differentiate into CTLs, now, which kill. Now, how is it, what is the difference between the naive CD8 and the CTLs? What, what gives CTLs the property to kill? And the reason for this is, and the CTLs have molecules known as perforins. Perforins make holes in the membrane of the target cells and then, through these holes you have molecules, that get in, one of which is granzymes. And these are serine protease, which are capable of activating caspase. So, they, so they initiate the dead caspase and then, you have Fas-FasL signaling, which results in apoptosis. So, you have different mechanisms involved over here and CTLs can kill because of these important molecules.

Perforins, which make the holes; granzymes, which are the proteases, which activate the dead pathways and then, Fas-FasL pathways; we will be discussing Fas-FasL and later dead pathways in subsequent slides, but for now, I think this is fairly reasonable introduction into the importance of T cell, activation of CD8, which turn them into CTLs.

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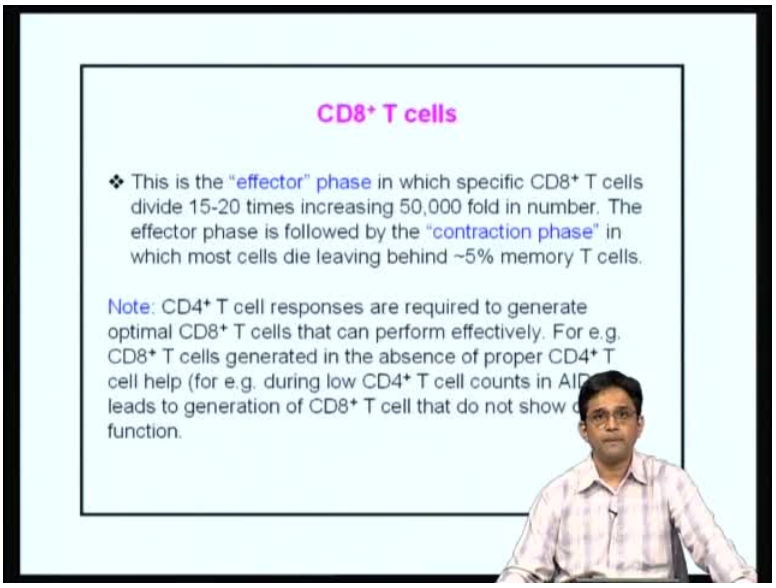

Now, it is also important to remember, that there is a, there is an effector phase in which, you know, the CD8 positive T cells divide several times. So, once you have the initiation of the immune response, these cells divide incurably fast and increase, you know, by in, in some cases 50000 fold in number. Now, if you have that many T cells activated, T cells is bound to cause problem in the body and that is why, we have system by which there is a contraction phase.

So, you have an effector phase, which results in increase in the numbers of the CD8 and CTLs, and they have contraction phase in which most of this cells die and they leave behind small number of memory T cells. So, this is a very important aspect about T cell activation, you need to activate T cells, but after activation you would need to bring them, bring the numbers down or bring lower the activation, because if you do not, if, if the host has to constantly be in the presence of huge amounts of cytokines and is activated T cells, it causes problems, what is known as immunopathology. So, this is again, this is an important aspect and so you, in T cell activation, we need to learn both, about activation as well as bringing down of responses.

So, now, the other important point that needs to be emphasized is that the CD8 positive T cells are required to generate effective CD8 response; the CD4's are required to generate an effective CD8 response. So, if you see AIDS patient, the CD4 numbers are lower, but the CD8's are there, but however, the CD8, the CD8 function in these AIDS patient is

compromised, is compromised because in the absence of appropriate CD4 help, they do not show proper function and that is again, tells you about the importance of the CD4 as master regulators of the immune response. And that is why, they help macrophages, they help B cells, they help CD8's and overall orchestrate this immune response, and as a, you know, this is manifested and can be seen in case of AIDS patient, who have reduced number of CD4. As the result of which, their immune system is compromised and they are more susceptible to infections by, by opportunistic organisms, that reside in our body.

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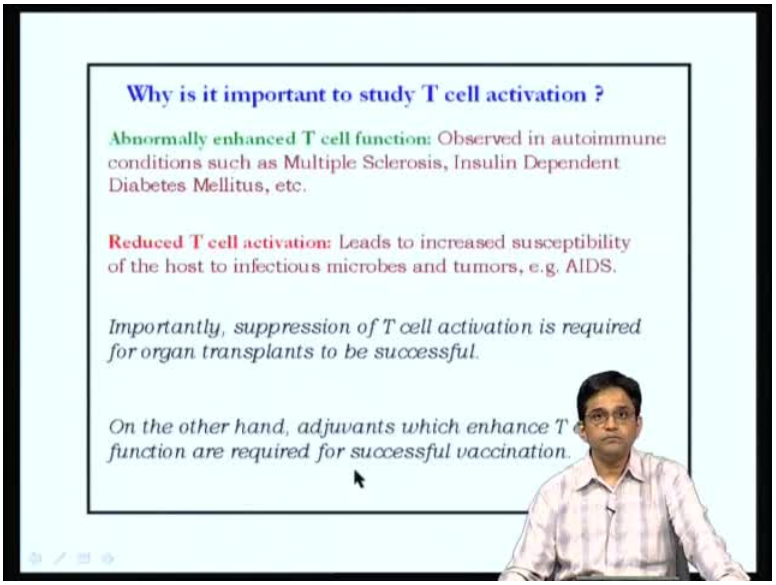

This is a, this is an important aspect, so why it is important to study T cell activation? So, if you see, abnormally enhanced T cell activation is observed in autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, diabetes mellitus, etcetera. On the other hand, reduced T cell activation leads to increase susceptibility of the host to infections, microbes and tumors; a good example of that is, is AIDS and the HIV infection.

Now, importantly, separation of T cell activation is required for organ transplants to be successible. So, this is where we, when in our, in our, when we were discussing MHC and you will recall, that the way Medawar got into scientific research was to help 2 pilots be able to survive skin burns and that is the way how he got interested into, into, into scientific research. But for skin grafts, in order, in order skin grafts to be successful, you would need to do grafts and how, how do you achieve grafts, that have a higher rate of

success? So, not only do you need to match the MHC, but you also need to suppress the endogenous T cell, so that you do not, you do not reject graft very quickly and you have to give a chance for the graft to be successful and therefore, immunosuppressants are used. I have also cyclosporine for example, something that we will study is, is, is, is used, cyclosporine and cyclosporine light molecules are used.

Now, as I mentioned, you know, for vaccines to be, to be, on the other hand, vaccines to be successful, you need to boost immune responses and you need to find out, what are the mechanism by which T cell function can be increased? So, these give you, these are example to illustrate the importance of studying T cell activation.

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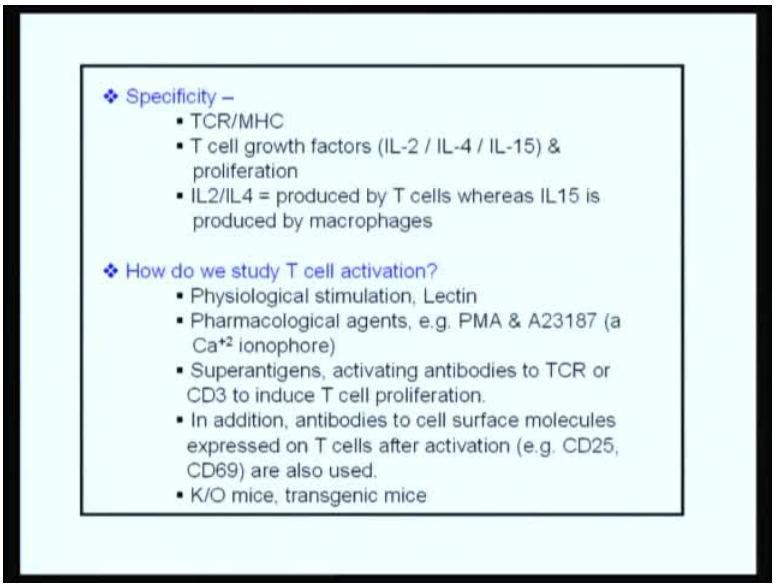

Now, this, this slide tells a little bit about specificity. Now, as was obvious, the T cell activation occurs via the T cell receptor and the cognate ligand for the T cell receptor is the MHC peptide. Now, upon activation of that, you have T cell growth factors, that are produced, that results in proliferation of these, of these T cells.

Now, IL-2 and IL-4 is produced by T cells, whereas IL-15 is produced by macrophages IL-15 is also an important factor, which allows for T cells to proliferate. Now, how do we study T cell activation? If you, if you just think a little bit about it, the T cell receptor is specific for MHC peptide. Now, now, the T cell receptor is a variable and so the chances of finding a particular antigen or cognate antigen is going to be very difficult. So, what researches used were to use some nonspecific mitogens initially, so lectins, for

example, concanavalin A is a lectin, it binds to T cell receptor and activates T cells. So, those, who are once that, that were sort of used subsequently, what was found is that you could use, found pharmacological agents. So, for example, the combination of PMA and a calcium ionophore, ionomycin for example, would result in T cell activation. Now, singly these do not work, but the combination works and this becomes very important because when we try and look at the T cell activation pathway, we find, that there is one part where the phorbol ester activates the dycill glycerol pathway resulting in the PKC activation mode and the other is calcium.

So, these 2 combine to result in maximal activation of T cells and this is something that we will, we will study. PMA for example, binds constitutively to a protein kinase C and keeps it activated. And what this ionophore is doing is it opens up calcium channels, so you have increased amounts of intercellular calcium.

Now, now, we had talked about antigens, which is antigens from MHC peptide, as being the most physiological. Now, however, there are some super antigens, now this antigen is very specific for the cognate T cell receptor. Now, you have super antigens, now super antigens will activate a large number of T cells having diverse, having diverse, having diverse TCR specificities. They may bind to families of T cell receptors, but overall they activate a large pool of T cells and so, that is why, they are known as super antigens because they are not the normal MHC peptide complex antigens.

The other ways of activating T cells is to use antibodies to T cell receptor or CD3, which is, you activate the antibody, binds to a constant region of the T cell receptor or it binds to the CD3 complex and it uses T cell proliferation.

Now, so, that is how you can activate, but in order to study activation, you can take a look at, at surface expression. So, once T cells are activated, they express certain cells of molecules. For example, CD25, CD69, these are expressed upon activation, so you can look at the kinetics of expression of these molecules and find out what percentage of your cells is activated.

In today's world if, for example, you have a molecule, that is important for T cell activation, so we need to use, usually a knockout technology by which you have mice, that do not, that do not express this particular molecule and then see the effects on T cell activation. On the other hand, what people often use is TCR, transgenic mice. So, you

have mice that express a particular type of T cell receptor. So, you can now feed it antigen and you can actually look at cognate TCR, cognate antigen interactions because you have majority of the T cells, that would express this particular T cell receptor.

So, there are different ways of studying T cell activation, I hope this gives you a certain idea about the different ways T cell activation has been studied. And you also need to understand, that it is somewhat difficult to study T cell activation, primarily for the reason, that especially, because with respect to specificity, because the T cell TCR is specific. So, there are ways by which we can bypass it, you can use, you can use molecules like lectins, which will bind to larger pools of, which will bind to, nonspecifically to T cell receptor and activate them or you can use super antigens, which will bind to classes of T cells, T cells and activate them. The other way to activate, you bypass the surface interaction is to use PMA ionomycin and you, it, it, it results in nonspecific activation of all types of T cells, T cells super antigens over here.

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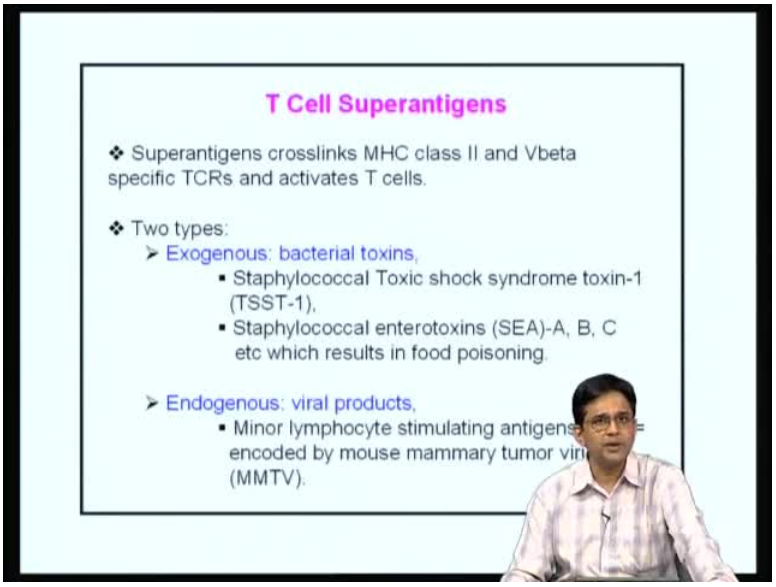

So, superantigens are ones, that cross link MHC class 2 and Vbeta superantigen are specific for certain Vbetas. So, they will bind, so it does not matter what the V-alpha is and does not matter what the antigen is. It will bind to MHC-2 and the Vbeta family, a particular Vbeta family, as a result of which it will activate.

This binding will result in activation of a pool of, of T cell, T cells and there are 2 different types, that is, the exogenous ones, that means, they come from outside and

these would be, examples would be, bacterial toxins. So, for example, the staphylococcal toxins, they have to pass staphylococcal toxic shock syndrome, TSST and then, you have staphylococcal enterotoxins and then, you have also endogenous ones.

Endogenous ones are products form viruses that, that are within mice or maybe, you know, are with humans and they, sort of, get passed down, and this is known as the minor lymphocytes stimulatory antigens and one of which is encoded by the mouse mammary tumor virus, MMTV. And so, they were being actually passed down from mother to litters, and it was being passed down and since, they are now seen as endogenous, so those mice lack certain Vbetas because they are seen as cell, and so, this sort of, this deletion happens during thymic differentiation.

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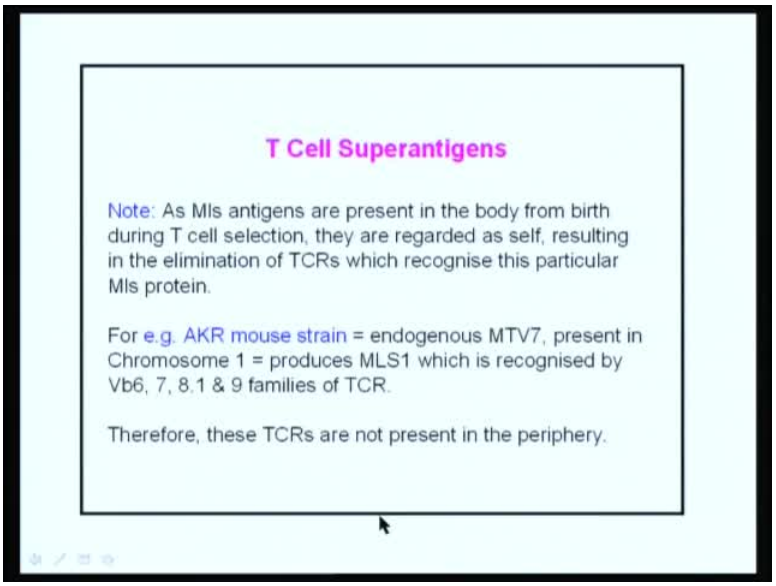

Now, this is what I was planning on saying, that the MSL antigens are present in the body from birth and so therefore, they are recognized at cell and are deleted. Now, an example of this is seen in the AKR mouse strain, where you have this endogenous mammary tumor virus and it produced MLS1 and it is recognized by this certain, by a large number of families of T cell receptor, the Vbeta 6, 7, 8.1 and 9 and therefore, these TCRs are absent in the periphery because they are, they are seen as self and are deleted in the thymus during thymic differentiation.

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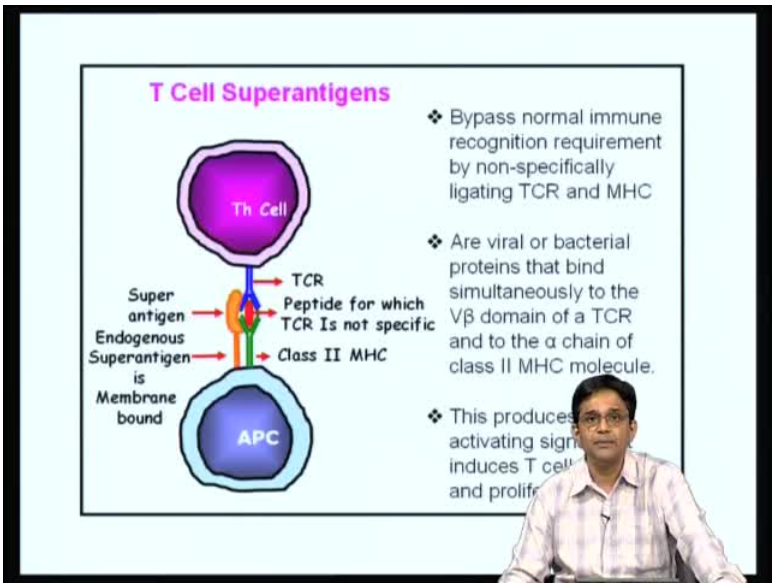

This is an example of antigen. This is, you have the MHC class 2, this is the T cell receptor and here you see, that the super antigen is being presented by the APC and it is cross linking the MHC class 2, as well as, the TCR beta part and so, it results in activation of the, of the T cells.

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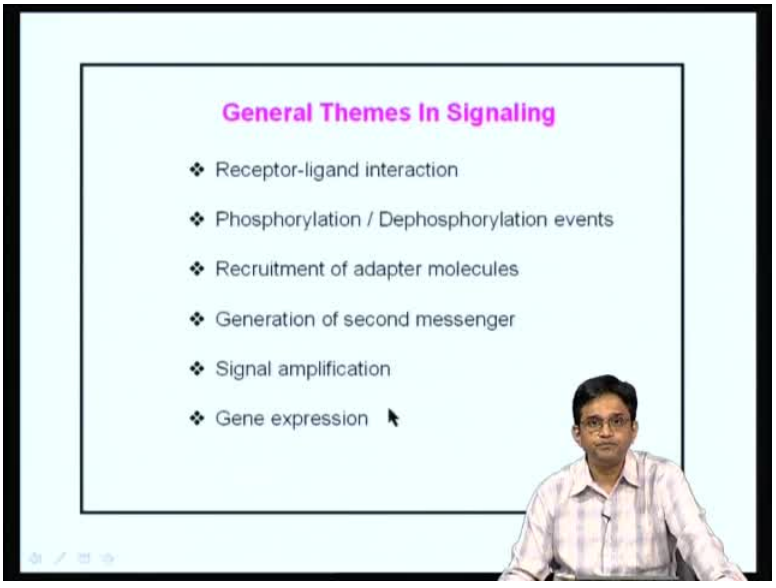

Now, in terms of general T cell activation, what, the way I look at it is, there are some general themes and I think, as long as students understand themes, then they will understand the integrating details. So, in terms of themes, you have 1st receptor ligand

interactions, which bond into specificities. Then, you have phosphorylation-dephosphorylation events followed by, which you have recruitment of adapter molecules and adapters are important because they can recruit other molecules, that would, that would amplify the signal. You have generation of 2nd messengers, then you have the signal amplification and then ultimately, gene expression.

In terms of T cell activation, the gene expression part is, really comes down to the level of cytokines and especially IL2, which as mentioned, is the autocrine growth, growth factor. So, we start off with TCR MHC and then end with IL2, and that is the important aspect and this cartoon, sort of, depicts that.

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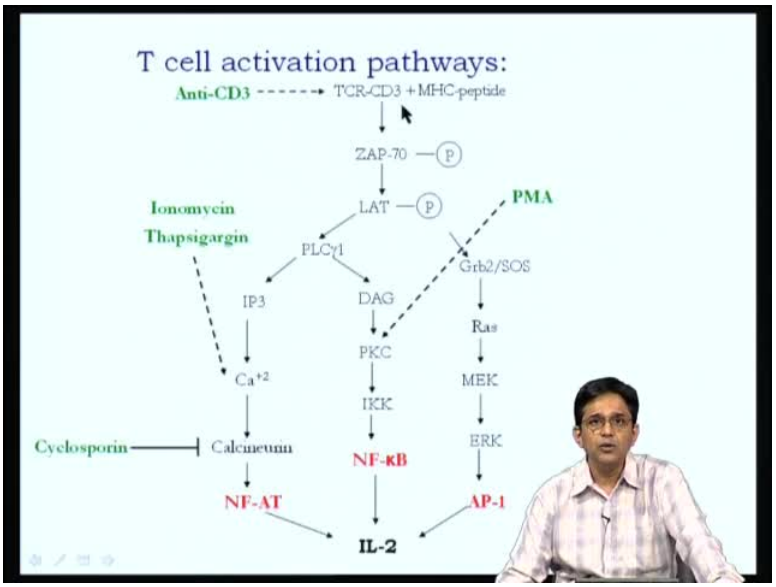

So, here, you have the TCR CD3 complex with the MHC peptide. It gets activated and you can bypass this using Anti-CD3 and then, what we have is phosphorylation-dephosphorylation events leading to activation of the phosphorylation of the ZAP-70 and Lat, a linker, associated in T cells. So, these 2 are adapter molecule and subsequently, you have, what is an amplification of the signal, where you have the PLC gamma getting activated. It results in activation of the dycill glycerol and the IP3.

This result in increase in, in calcium and you can see, this activates a phosphatase known as, calcineurin. This results in activation of transcription factor, known as NF-AT, whereas the dycill gycerol activates the protein kinase c and this results in activation of

the NF-kappa B part, which again contributes to IL2. They also have the activation of the Ras pathway or MEK pathway and you have the activator protein AP1 being produced.

Now, what is important to note over here is that for IL2, for optimal IL2 production, you meet the 3 important transcription factors to be induced and activated. The 1st one is NF-AT or nuclear factor presented inactivated T cells; you have NF-kappa B and then, you have AP-1. So, you have a single, right from here it means to have pathways that would activate these 3. Now, if all the 3 pathways are not activated, what happens is, you do not have proper T cell activation and that is a very important aspect to understand.

So, for example, if you do not have NF-AT then, then again, you do not get, generate IL2 and the T cells will not get proper T cell activation; T cell activation will be compromised. The other important point to note over here, I understand it is a busy slide and we will break it down in the subsequent slides, but for now, there are some important aspects for students to understand.

One is, this is an overview of T cell activation, so it gives you a pretty much the bird's eye view of the different pathways that are involved in T cell activation. We will discuss this in greater detail. 2nd is, there are ways by which bypass T cell activation here, for example, is the TCR MHC peptide, is the is the most physiological cognate interaction. You can bypass it using Anti-CD3 and if you do not have Anti-CD3, you want to activate all types of T cells. You can bypass it by using the combination of phorbol ester – PMA, which binds, which activates PKC constitutively, which binds and activates PKC constitutively.

And the other way you need to do is to open up the calcium or increase inter-cellular calcium and this is a calcium ionophore, ionomycin is a calcium ionophore, and which will result in activation of, which will open, which will increase inter-cellular calcium. Again, what is important is that you meet the combination of a calcium ionophore and a, and PMA or phorbol ester to activate the T cells, again singly, they were, they are not able to do it.

The 3rd important point over here that is shown is, that cyclosporine, the immunosuppressant cyclosporine, that target is calcineurin the phosphatase. These are important aspects, we will discuss this in greater detail in the subsequent slides, but you have a bird's eye view over here of pretty much of T cell activation.

Once again to remind you, you have T cell, you have, you have activation of the T cell receptor, it needs to activate 3 independent pathways over here result to, result in increased production of these 3 transcription factor and to induce IL2.

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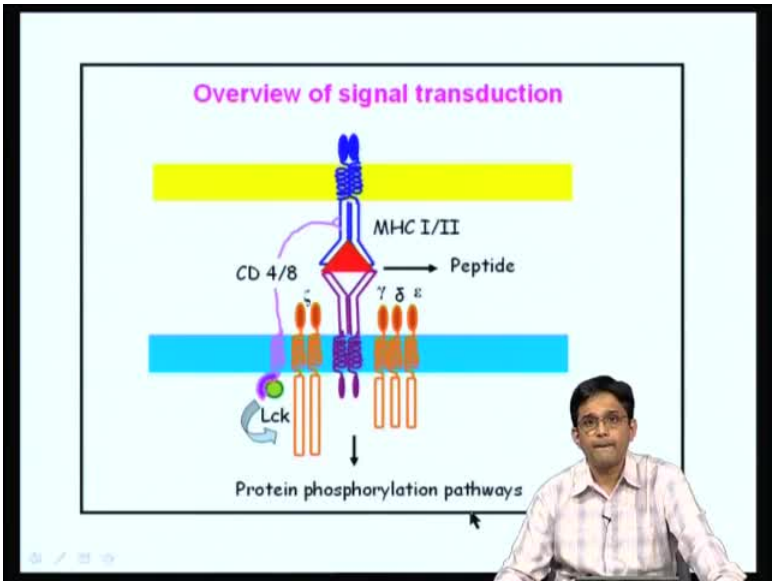

So, now, we will break it down a little bit. So, this is the T cell receptor, which is, which the cognate, the ligand is the MHC peptide and the T cell receptor is associated with CD3, and you have the gamma-delta epsilon and here, you have zeta.

Now, what is, what is important to note is that the gamma-delta and epsilon have a single ITAM motive or immunotyrosine activation motive, whereas zeta has 3 activation motives. So, this is important and we will see that subsequently. What is also shown over here is that CD4, CD8 are present on T cells and associated with the molecules known as LCK. So, this is again something that is going to be important, we will discuss this later.

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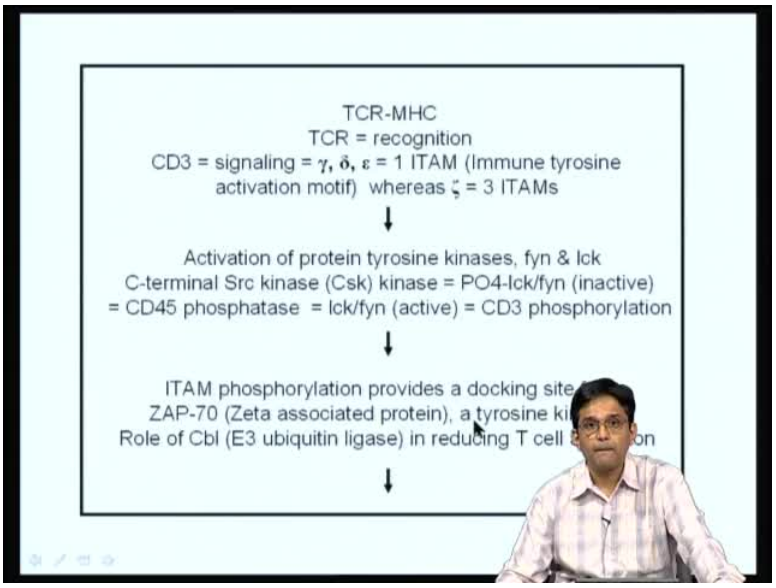

So, what I have shown over here is you have the TCR; MHC-TCR is important for recognition, but the signal is actually passed down by the, by the CD3, which is important for, for this aspect. Now, the activation, now, of CD3 gets phosphorylated. Now, for CD3 phosphorylation, you have the tyrosine kinases, fyn and lck to be important. Now, on the normal circumstances they are inactive. They are inactive because they get phosphorylated with, by a kinase known as cycsk, C Y C S K or C-terminal Src kinase. So, as a result, over which they are inactive and they can phosphorylate the ITAM motives in the CD3.

Now, however, upon T cell activation, what happens is you have, CD4, CD45 being activated, CD45 then dephosphorylates lck-fyn, which in turn results in increased phosphorylation of CD3, increased phosphorylation of CD, increased phosphorylation of CD3.

So, now, what happens is, you have, the ITAM is being phosphorylated. This allows docking, docking site for another protein, known as ZAP-70. ZAP-70 stands for zeta associated protein and this was, this was found, because only upon T cell activation it was found, that the protein associates with zeta and that was identified to be the ZAP-70 and 70 stands for the KDS, so it is a 70 KDA protein.

Now, again, as I mentioned, what happens with T cell activation is, you know, once you have T cell activation, you need to bring it down. So, the cell, as well as, the (( )) are

figured out, ways by which you can do that. And so, one important molecule over here is Cbl; Cbl is E3 ubiquitin ligase, which is important in…, substrate for Cbl is ZAP-70. So, so what Cbl does? It will reduce activation of ZAP-70, reduce amounts of ZAP-70. So, as a result of which you can lower T cell, T cell activation down, but so, in, in conditions where there is no Cbl, what would happen? You would have increased phosphorylation of ZAP-70 and that would go on and lead to problems with autoimmunity and that is something that we will see later.

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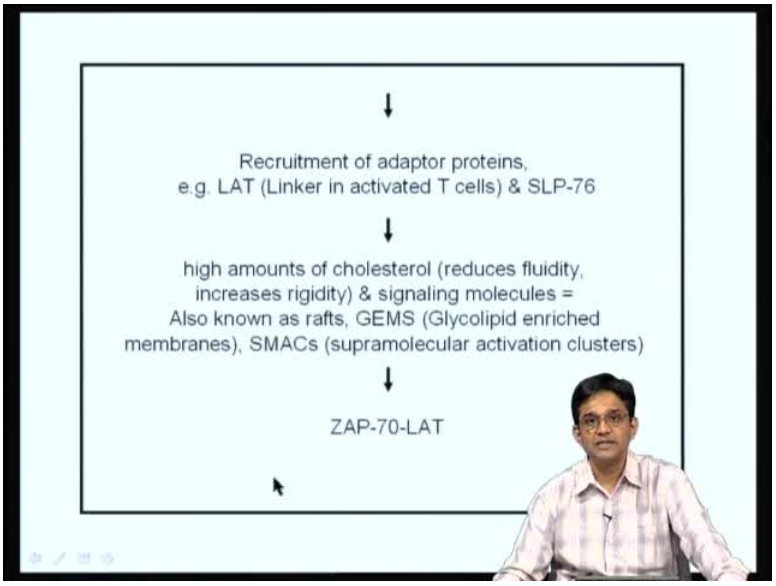

So, once, once you have ZAP-70 being phosphorylated, it recruits other adapter molecules, one of which is important, one which is LAT, which linker in activated T cell and SLP-70. So, these all accumulate in a particular part in the T cell, T cell surface and this part is known as the supramolecular activation cluster. So, what happens is, over here you have the T cell receptor, the CD3 and other activation molecules, all present over here, and there are high amounts of cholesterol over here and so, so membrane fluidity is reduced. So, they are all present over there and they are bound to MHC-peptide complex and so it results in increased signaling. And you have, you have, in fact, molecules coming over here and they form this particular cluster, known as a, known as SMACs.

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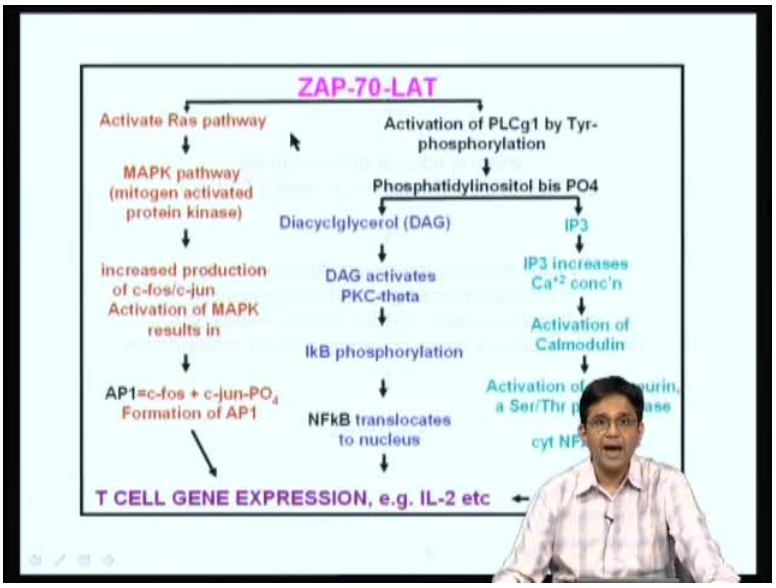

Once you have this, you would activate different pathways and what is shown over here is, you have activation of the Ras pathway. This results in, of the, of the MAP kinase pathway, increased c-fos c-jun and, and ultimate formation of AP1, which is the one of the important transcription factors. So, AP1 is actually c-jun phosphorylated and c-fos.

And over here, you have the phosphatidylinositol-bis-phosphate being cleaved due to activation of PLC gamma-1. It results in diacyclglycerol. Diacyclglycerol binds to, PK, PKC-theta; in fact, PKC-theta is important in T cell activation.

This activates it; it results in, in phosphorylation of the inhibitor of kappa-B. Now, you, you will remember, that the NF kappa-B is bound to the inhibitor of kappa in the cytosol. So, once the I kappa-B gets phosphorylated, it gets degraded and so now, the NF kappa-B is free to move to the nucleus and activate genes and the IP3 increases inositol-bis-phosphate, increases calcium concentrations. It results in activation of calmodulin and this result in activation of the calcineurin, which is a serine threonine phosphatase. Now, the cytosolic NFAT is phosphorylated.

What calcineurin does is to dephosphorylate it. Upon dephosphorylation in fact, moves to the nucleus. Now, you have these 3 transcription factors - AP1, NF kappa-B and NFAT in the nucleus and this result in increased gene expression, especially IL-2. So, again, to reinforce for, for IL-2 to be activated optimally, it needs the 3 transcription factors - AP1, NF kappa-B and NFAT.

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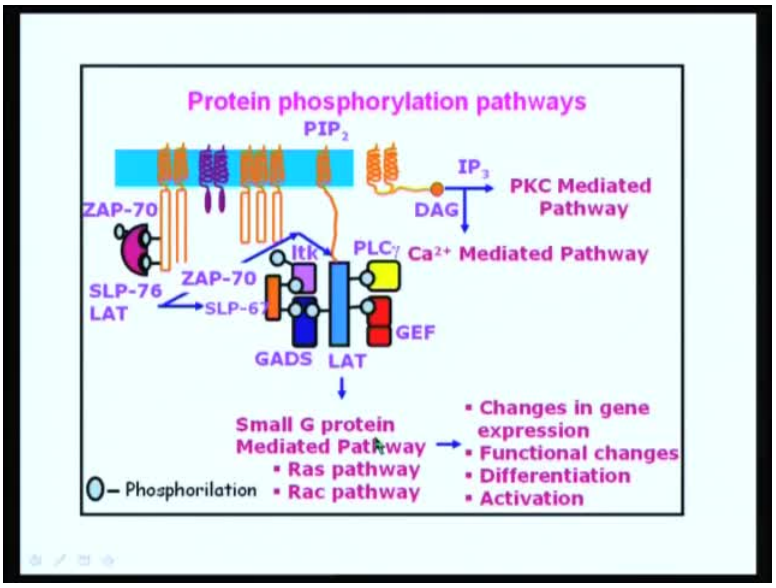

So, this is again to show the different pathways that are involved. This is the ZAP-70 and it allows for binding of the other molecules and the calcium pathway. And then, you have all these different pathways coming together, it results in changes in gene expression, functional changes, differentiation, activation, so on.

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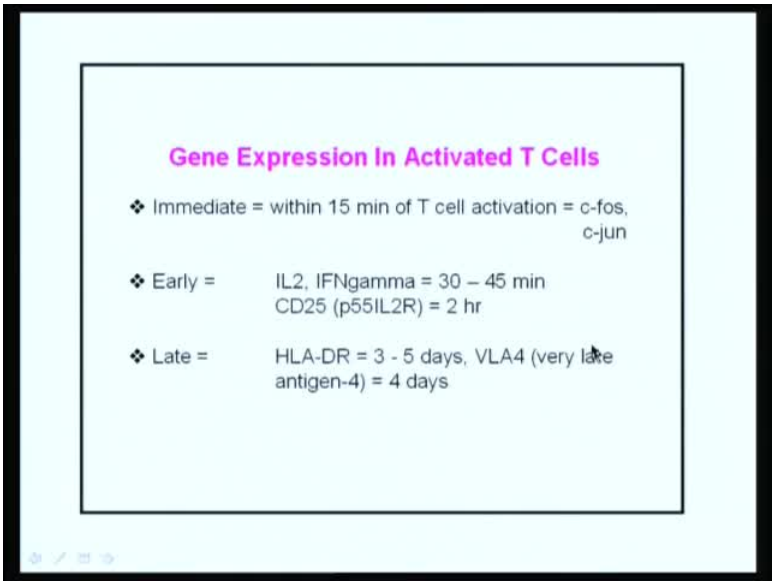

Now, gene expression in activated T cell occurs very quickly. One of the first one to be activated, within 15 minutes is the c-fos, c-jun. Subsequently, the cytokines get activated, IL2, IFNgamma about 30 to 45 minutes and then you have CD25 or the p55IL2 receptor,

which gets increased by about 2 hour. So, IL2, the CD25 is an important marker, a cell surface marker of T cell surface activation.

So, IL2, MRNA maybe getting induced, but it takes some time for the protein to be made and be expressed on the cell surface. CD25 is also expressed very early, reasonably early in T cell activation and is a marker of T cell, T cell activation. There are some late ones, like HLA-DR, which is MHC class 2 in human cells, HLA-DR gets activated and you see, it present, it get, it is induced by 3 to 5 days and then you have other antigens, known as the VLA4 of the very late antigens.

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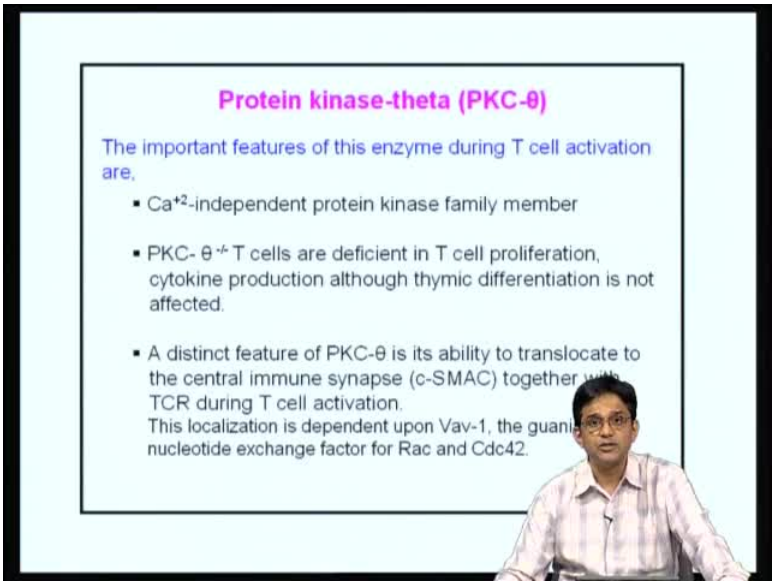

We will now discuss some important molecules in the T cell activation. The 1st one is protein kinase C- theta. Now, there are different protein kinases, the one, that is thought to be physiologically important for T cell activation is the PKC-theta and this one is the calcium independent protein kinase family member and the PKC-theta knockout mice are deficient in T cell proliferation, cytokine production, all those, although thymic differentiation does not appear to be effective.

Now, a key feature, the PKC-theta, it is ability to translocate to the, to the SMAC or which is, where I said all these molecules form together, where along with high cholesterol, during T cell activation and this is, sort of, dependent upon some g protein, like Vav and the exchange factor Rac and CDC42.

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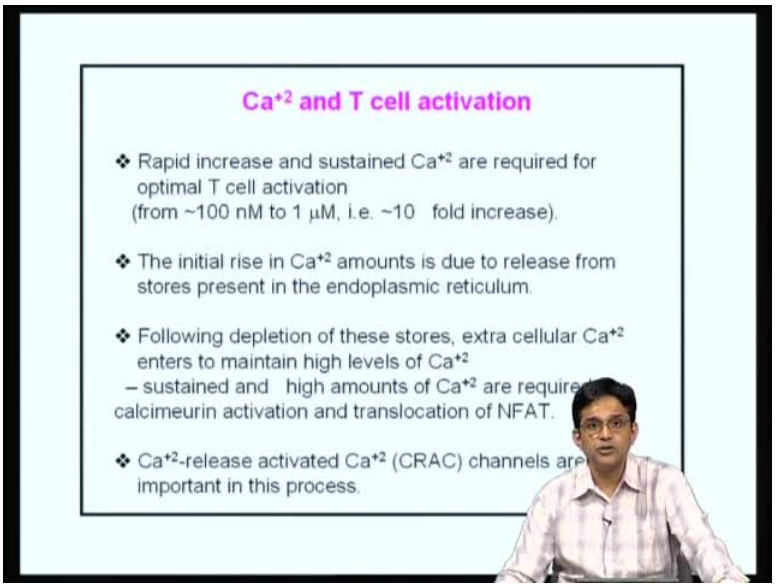

Now, calcium is really the important or one very important component in T cell activation. Now, there is, under normal circumstances the, the amounts of intercellular calcium are about 100 nano molar or so. Once T cells are activated there, they rise very quickly to about 1 micro molar and this is initial rise in calcium, is due to depletion from, from the endoplasmic reticulum, is released form the, from the stores and the endoplasmic reticulum.

However, for sustained T cell activation, you would need to have, you know, rise in intercellular calcium. So, you need an increase in intercellular calcium and which you need to be able to be sustained. If you are unable to sustain it, you would not get proper T cell activation and so therefore, following depletion of these stores, the extracellular calcium for, of the depletion of intercellular calcium store, you have calcium coming in from outside. To maintain high levels of calcium amounts of intercellular calcium are important because they are required to sustain the activation of NFAT, which is important for, for IL2. And the calcium release activated calcium channels are important in this process or the CRAC channels.

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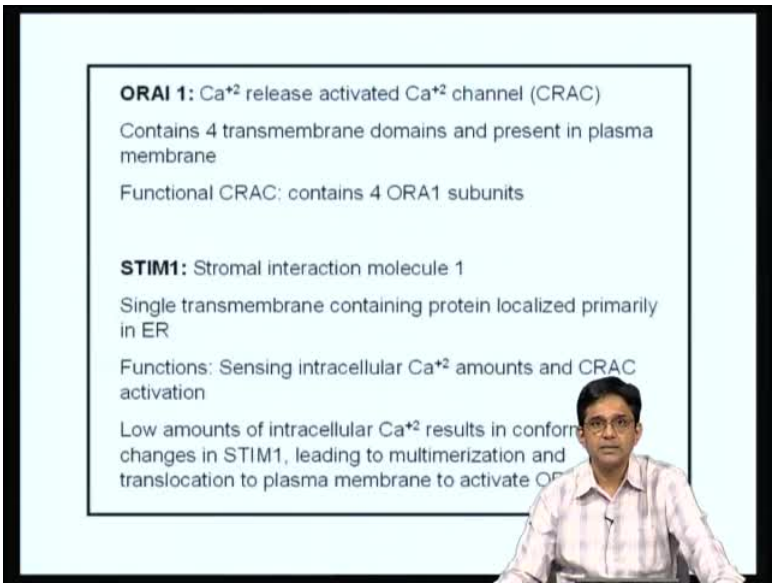

There are 2 important players in this; you have ORAI 1 and STIM1. And ORAI 1 is the calcium release activated calcium channel, it contains 4 transmembrane members and present in the plasma membrane. So, it is a calcium channel, it is a proper calcium channel, it is present on the plasma membrane. And whereas, STIM is mainly a sensor, it, it, it, it senses intercellular calcium and when there are low amounts of intercellular calcium, it, there are conformational changes in STIM, leads to multimerization and translocation to the membrane, to the plasma membrane to activate ORAI 1. So, it is a sensor and an activator of ORAI 1.

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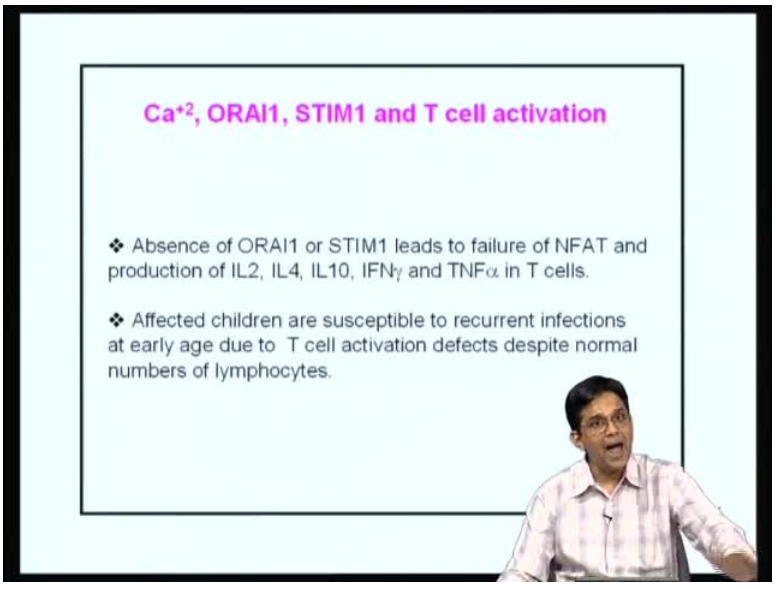

So, what would happen to people who either have deficient ORAI 1 or STIM 1? And so absence of these molecules, these 2 failure of, to activate NFAT and production of different types of cytokines in T cells, and these effected children are highly susceptible to recurrent infection at early age. And this is a, this is, results in, in, in, in aberrant T cell activation and we will naturally have immune defects.

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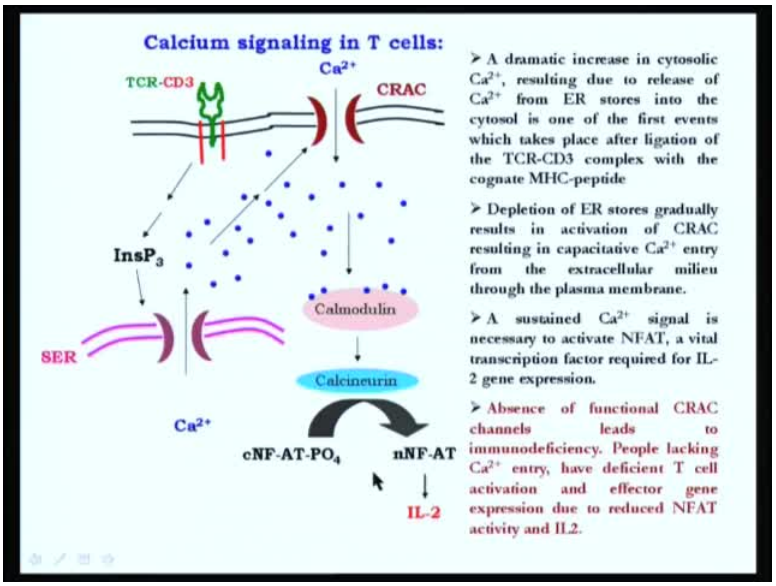

So, this is again a, to a, cartoon to tell you little bit about T cell activation. So, this is the TCR and it results in inositol-bis-phosphate, and this result in depletion of the intercellular in the ER calcium. It goes outside and then, these calcium's intercellular calcium activates the, the, the CRAC channels and once CRAC channels are activated, you have now calcium coming and from the outside coming inside this.

Now, you have sustained amounts of calcium, which results in activation of calmodulin. Calmodulin on the other hand, activates calcineurin, which is the phosphatase and now the phosphatase, it cleaves the cytosolic NFAT and then, so it upon dephosphorization, NFAT migrates into the nucleus and it activates IL-2. So, this is again a cartoon to depict calcium channel signaling in T cells.

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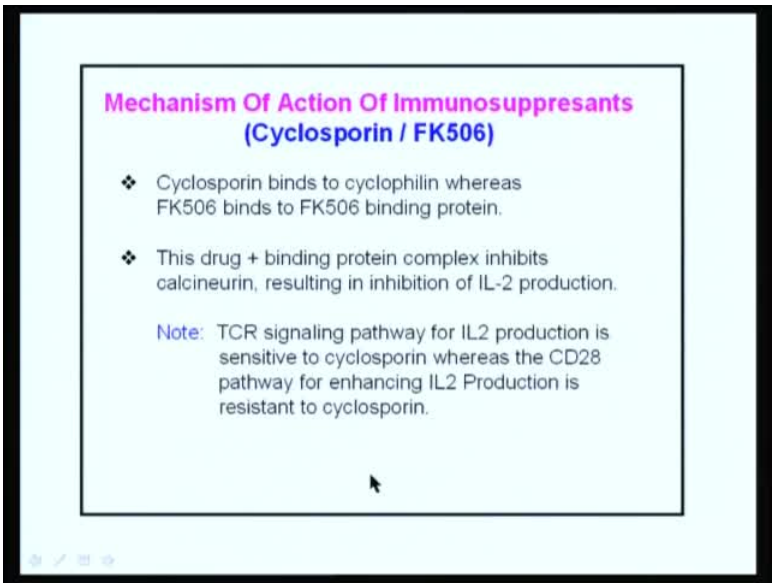

An important aspect over there was the activation of calcineurin. What is interesting is a cyclosporine, which is a very effective and useful immunosuppressant. I mean, cyclosporin has been used for quite some time as the numeral suppress. However, the mechanism was not known and now it is well known, what cyclosporin does. It binds to cyclophilin and you have another molecule known as FK506.

Now, FK506 binds to its cognate ligand, which is FK506 binding protein. It does not matter, both cyclosporin and FK506 are immunosuppressants, they bind to different proteins, but this, this drug and binding protein complex, it inhibits calcineurin. Now, it inhibits calcineurin, therefore, it would inhibit the production of, of IL-2. Because what happens, as a result of inhibit IL-2, inhibition of calcineurin, you have the cytosolic, NFAT cannot be dephosphorylated, as the result of which calcineurin remains in the cytosol, sorry, NFAT remains in the cytosol and as a result, it cannot translocate to the nucleus and to activate IL-2 gene transcription. So, as a result of this, you have you have the inhibition of T cell activation.

An important aspect over here is that the T cell receptor signaling pathway for IL-2 is sensitive to cyclosporine, whereas the CD28 pathway is resistance to cyclosporine. So, there are other ways by which CD28 acts and this becomes important to differentiate, whether a particular T cell activation is going primarily through the T cell receptor pathway or it is going through the CD28 pathway or the costimulatory pathway.

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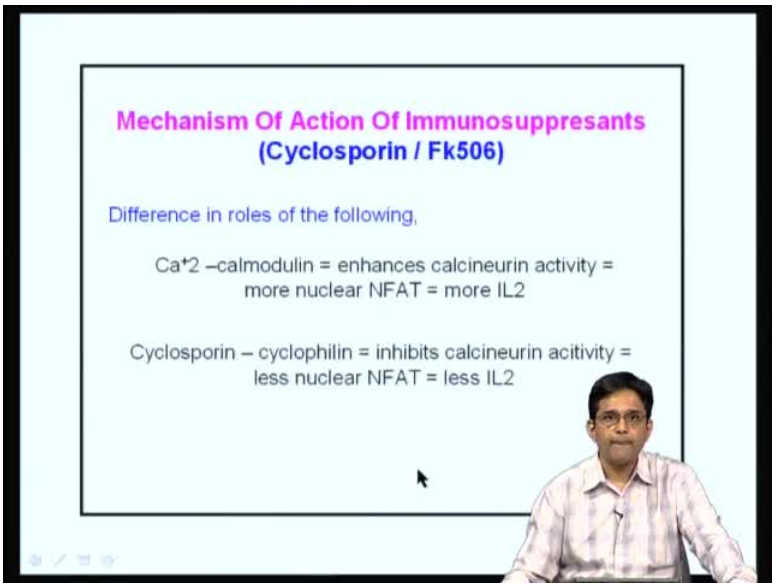

So, this is, this is what is shown, you have calcium calmodulin. It enhances calcineurin activity, so you have more, more phosphatase activity, resulting in more translocation of the cytosolic NFAT into the nucleus. So, you have more nuclear NFAT, therefore you have more IL-2. Now, what happens when you have cyclosporine-cyclophilin complex, this inhibits calcineurin activity. As a result of which you have less nuclear NFAT, you have less IL-2. So, this is important in terms of mechanism of, of a cyclosporin action, which is very important and, and even if you see the newer drugs that come out, these are, all these, these pretty much act on this particular pathway, which is inhibition of calcineurin. So, they may have different binding proteins or they may have different structures, but the mechanism of action is inhibition of IL-2, inhibition of calcineurin, which leads to inhibition of IL-2; very important.

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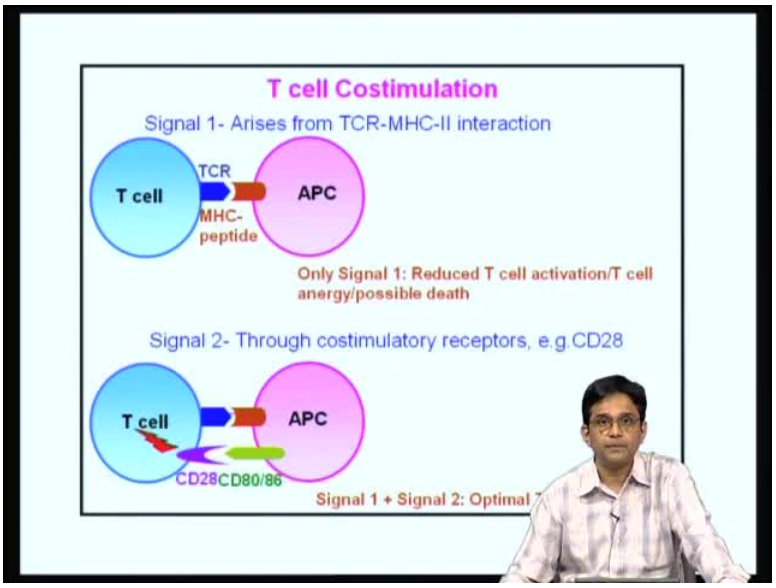

So, another aspect of T cell activation is T cell costimulation and this, the basis for this is as follows. You have, I mentioned, that you have the T cell and you have T cell receptor and the T cell receptor recognize the MHC peptide complex on the APCs. Now, just this binding alone, while it is specific, this binding alone does not result in optimal T cell activation. In fact, it results in reduced T cell activation, T cell energy and possibly death.

Now, for optimal T cell activation, what you need is signal 2 and so this, what is shown over here, signal 2 in the form of CD28. It is a cell surface molecule present on T cells; it binds to ligands on antigen presenting cells, known as CD86. The binding of these 2 together results in optimal T cell activation.

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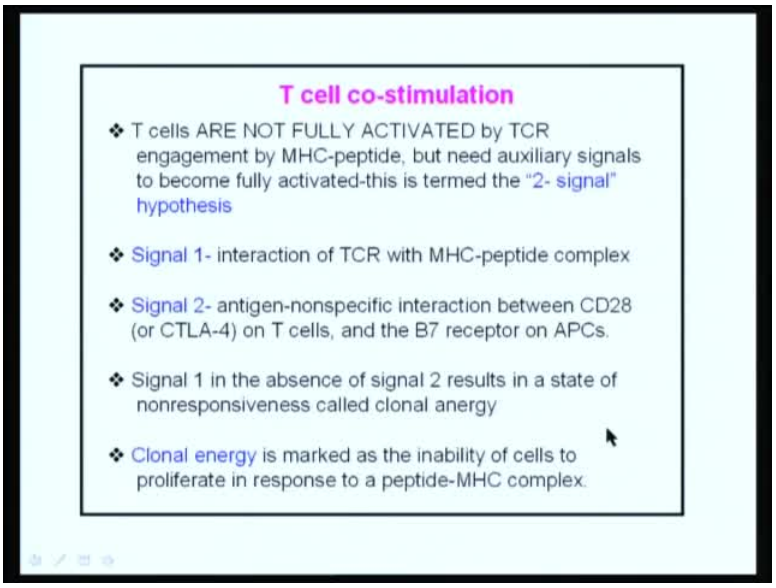

Now, why is it, that you need to have these 2 signals to activate T cells or why, why cannot just single signal activate T cells? And so, this is perhaps because every time the T cell receptor sees MHC lycognate molecule, if the interaction is not all that optimal, it should not activate these cells. So you, you need T cell, activation needs to be in a context where you have, you have, you have, the conditions are right for optimal T cell activation, and we will discuss this part a little bit in greater detail.

So, what happens is, once you have this environment or inflammatory situations, you have the costimulatory ligands being expressed. Once you have these costimulatory ligands being expressed, they will bind to the costimulatory receptors and if the MHC TCR interaction is there, only then, it will activate T cells. And so, if you just have the signal 1 interaction, it is marked by inability of the cell to proliferate in response to the peptide-MHC complex.

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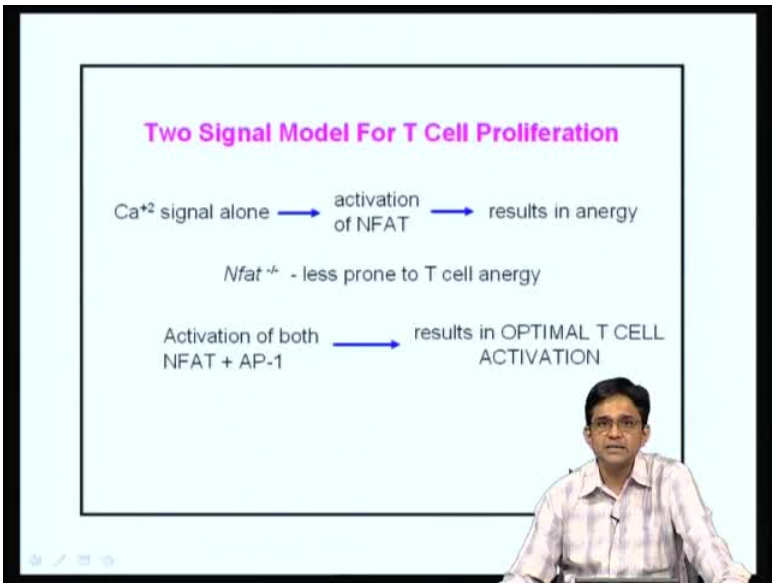

So, this is, and this is shown here in this model, where you, few have the calcium signal alone. It results in activation of NFAT and it results in energy. So, this is why I said, for optimal T cell activation you need different pathways coming together, which would optimally activate your T cells.

So, and if you have just 1 signal alone, for example, what is shown over here is a calcium signal alone, it results in activation and it results in energy because you have NFAT, but you do not have the other molecule. So, you need a combined, combined input of different signals to tell the cell, that look, now, you know, it means to be activated properly.

So, in fact, with NFAT, mice that lack in NFAT are less prone to T cell energy. And therefore, what is shown over here is activation of both, NFAT, AP1 and NF kappa-B are required for optimal T cell activation.

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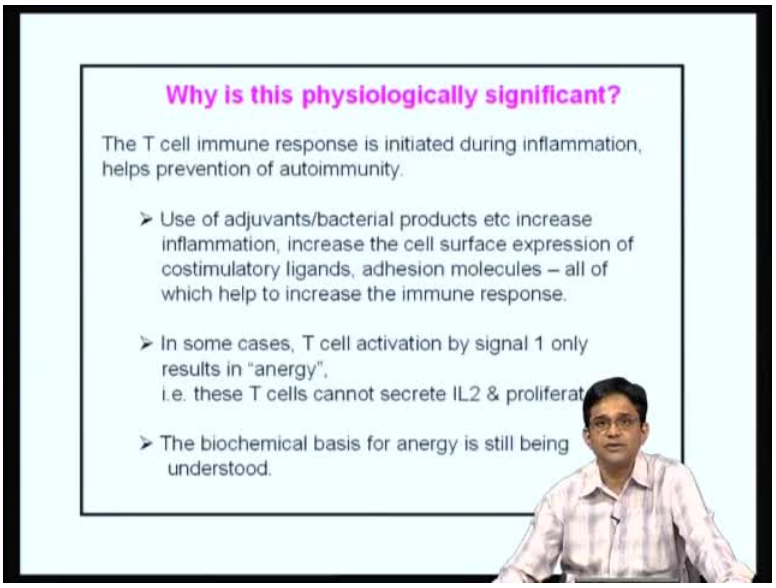

So, this is my, this is the question, why is this physiologically available?

So, the T cell immune response is initiated during information and this helps (( )) because every time, if for every little thing the T cell saw something and then it got activated, it might result in autoimmunity and this is not to say, that autoimmunity is not, is exist in the populations, but perhaps those numbers will be lot more if this different signals, or, or, or the costimulatory aspect was not required by T cells. You would have, you would have much more numbers perhaps about immunity. And so, so T cell activation is, is, is, is done in terms of a context, and when upon, upon the proper environmental cues, for example, the use of adjuvant bacterial product. They increase inflammation, so they will increase the surface expression of costimulatory ligands and all of which would help in immune response.

Now, as mentioned, in some cases, T cell activation, you have signal 1 and it results in energy. In these, in these cases, the T cells cannot secrete IL2 and proliferate. So, the biochemical basis for energy is still being, is still being understood.

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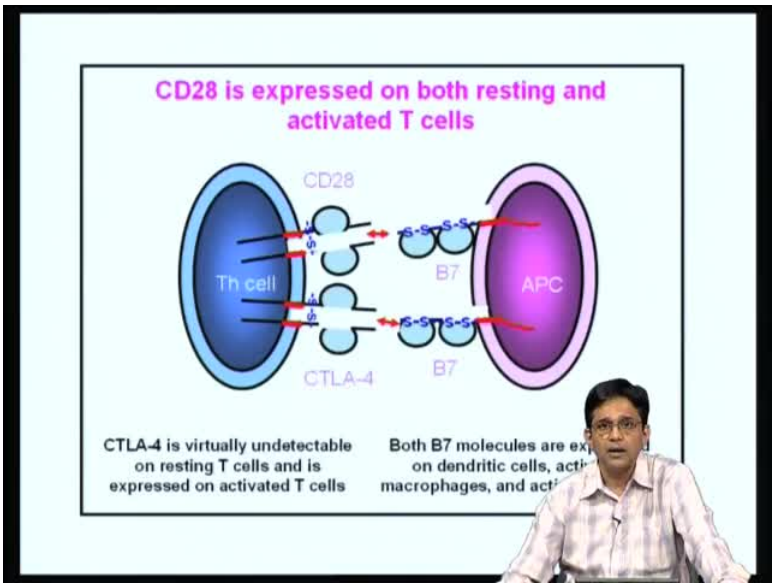

So, as was shown in that slide, an important costimulatory receptor, that is present on T cells is CD28. Now, what is, what is important is that CD28 is present on both, resting as well as activated T cells and CD28 binds to molecules known as B7 1 and 2, also known as CD80, 86.

CD80 and 86 are primarily found on antigen presenting cells, so you have the T cell receptor CD28, which binds to, if the ligands in APCs. Now, remember the amounts of CD80, 86 increases upon, upon inflammatory conditions. So, that is, when the chances of costimulatory are highest and that would allow for T cell activation to occur under physiological conditions.

Now, what is also shown over here is you have CD28; you have another molecule shown as CTLA4. Now, CTLA4 is not present on, on, on the surface of naive T cells. CTLA4 comes up upon T cell activation and again, this is a mechanism by which CTLA4 completes with CD28, and it actually shuts down T cell activation. So, you have CD28, which enhances T cell activation along with the TCR and then you have CTLA4, which binds to CD80, 86 and has the opposite role. So, why would you want that?

Again, as mentioned to you, for T cell activation, once you have T cell activation, you would bring, you would, molecules by which you can regulate this process and bring it down. It is not in the interest of the host to have sustained T cell activation because,

because then it would result in immunopathological conditions. So, this is a very important aspect for students to understand.

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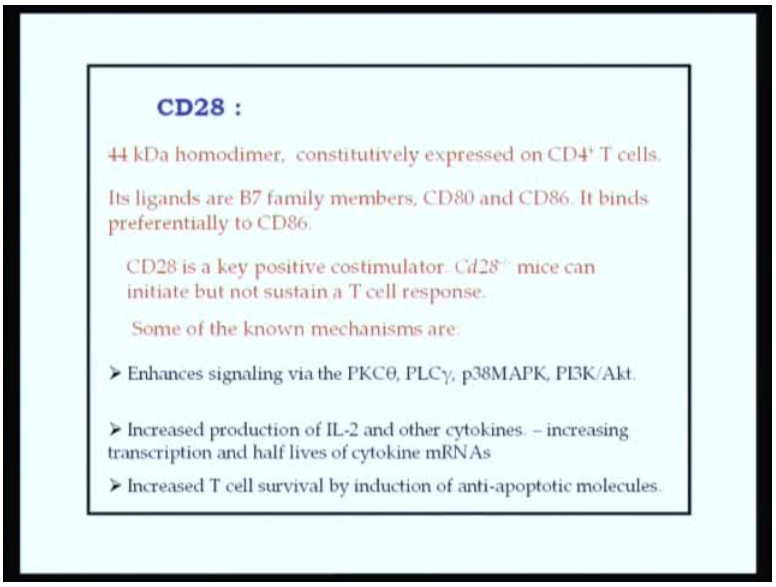

A little bit about CD28. CD28 is a 44 kDa homodimer, so, and it is constitutively present on CD4 positive T cells. Its ligands is, mentioned as CD80, 86; CD28 is a positive costimulator of T cell activation. What is, what is important is that the CD28 (( )) mice can initiate, but cannot sustain T cell responses. So, they will initiate, but sustenance of T cell response needs costimulation, and so what are the mechanisms by which it enhances signaling by the various pathways p38MAP kinase, PI3K, so on.

It increases production of IL2 and other cytokines, this and what is important is that it not only increases transcription, but it also increases the half-lives of the cytokines mRNAs. This is the very important aspect, important aspect for CD28. The other very important role of CD28 is that it, it reduces the T cell dead or, and in other words, it increases T cell survival, and it does so by induction of anti-apoptotic molecules, VCL2, BCLXL are induced by CD28; very important aspect.

So, it, so it uses the combination of mechanisms, it activates T cell, but it increases the production of cytokine mRNAs, as well as, increases the half-live, so you have more amounts of cytokines and it increases T cell survival. So, the combination of these increases proliferation, increases survival, so it plays a very important role in sustenance of T cell response.

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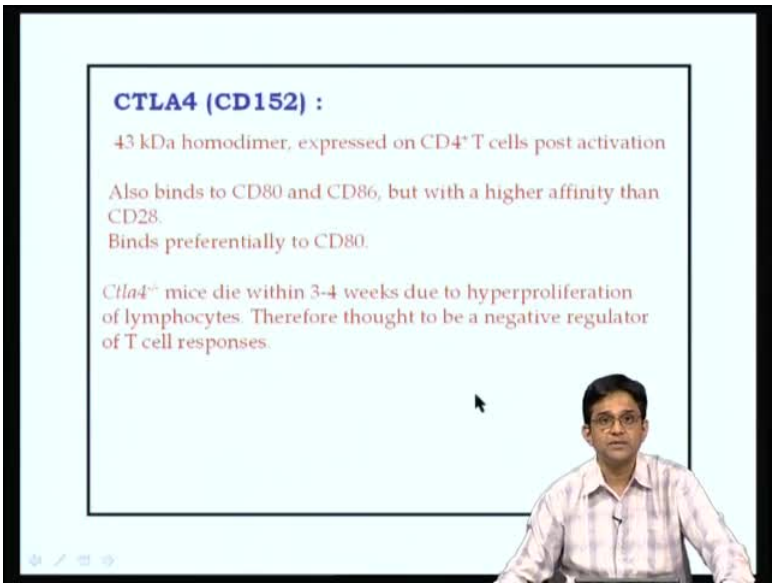

Now, upon activation, what happens is CTLA4, now comes in. Now, CTLA4 is, you know, similar 43 KDa. It is, it is expressed on T cells post activation. Now, it binds to CD86, just like CD28, but it does so with the, with the higher affinity and it prefers to bind CD80.

What the phenotype of the knockout mice is striking, in fact, CTLA4 knockout mice die within 3 to 4 weeks and they die because due to hyperproliferation of lymphocytes, and these are primarily CD4 positive lymphocytes. So, in the absence, so this is what is happening in the absence of CTLA4. You have the CD4 positive cells, but they are now hyper-activated, any small little thing they are getting activated, and, and, and as a result of which, they are producing a whole bunch of cytokines. They are activated and, and it results in immunopathology and so, the host cannot handle this sort of a situation and then, ultimately, the mice die. And so, because of the phenotype of this mice, CTLA4 is thought to be a negative regulator of T cell responses; very important aspect over here.

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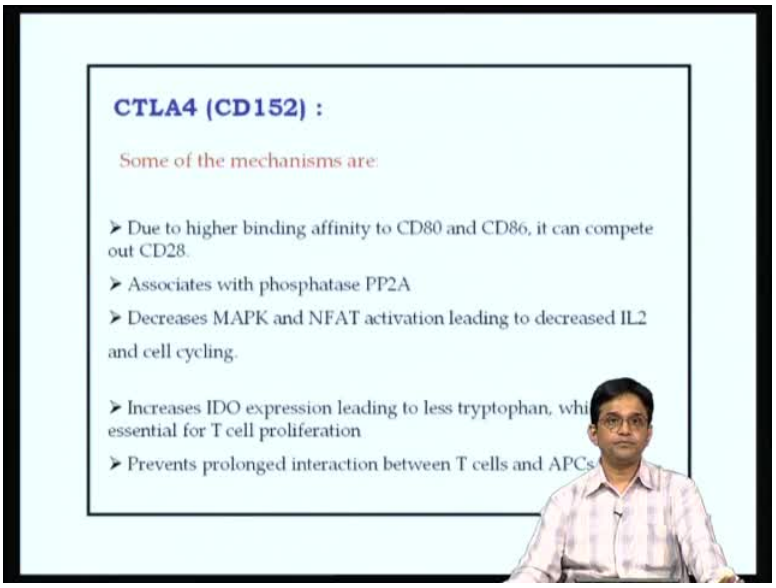

Now, some of the mechanisms of CTLA4. So, how does CTLA4 work? We, we, we had heard the mechanisms by which CD28 works, it increases anti-apoptotic molecules, it increase production of cytokines, but CTLA4 has different mechanisms.

Due to higher binding affinity, it can out-compete CD28 for binding these ligands. It also, there are different mechanisms listed and I will, I will tell these mechanisms and then, we will try, perhaps the combination of these mechanisms are effective. It associates with the phosphatase, so as a result of which, it down modulates T cell activation, it decreases MAP kinase, NFAT activation, resulting decrease in IL2 T cells, cycling it increases IDO expression.

What IDO will do, it results, as a result of which you have less tryptophan, which is essential for T cell proliferation. Also, it prevents prolonged interaction T cells and APCs, so that prolonged interaction by the SMACs, that, that we discussed, that is reduced with CTLA4. So, perhaps, that is less interaction, so less T cell activation.

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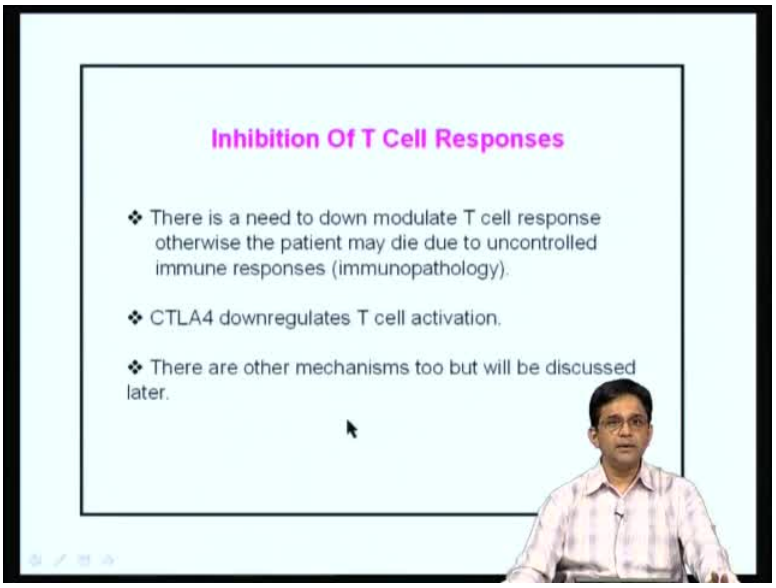

And so, inhibition of T cell response, so we had discussed this aspect, that there is a need to down modulate T cell responses because otherwise, the patient may, may die due to uncontrolled immune responses. And this is what I mentioned by immunopathology and a good example of this is, is CTLA4, which down regulates T cell activation.

So, actually in this class we, we, we started talking about T cell activation. We, we looked at the different pathways, different molecules, that are important due to T cell activation and finally, we are actually coming out innovation of T cell responses. And because, because, because if you activate immune cells, you need to find out ways by which you can bring them down, because having activated immune cells is not, is not in the interest of the host.

One of the mechanisms that we discussed in this class regarding inhibition of T cell responses is, is CTLA4. There are other mechanisms, but I thought, for an introductory class on T cell activation, we will discuss the, the, the simple one and CTLA4 is a very good example of the importance of regulating T cell activation.

In fact, this, this, this interrelationship between, CTL, CD28 and its cousin CTLA4, because remember they are cousins because they are related to each other, they bind to the same ligands, but they have completely opposite functions. CD28 is a positive regulator, CTLA4 is negative regulator and the mechanism by which they function are, are also are very, very distinct and they have differential effects on T cell activation.

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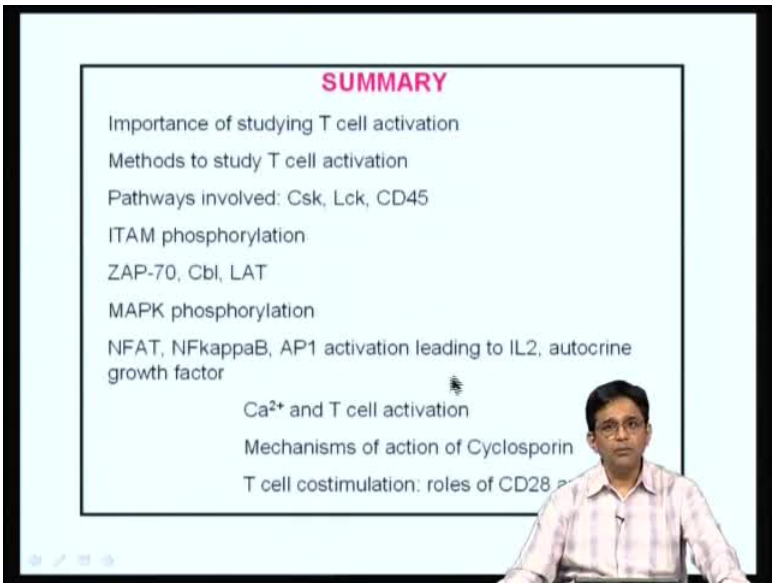

We will now briefly summarize this class. So, 1st is, what is the importance of studying T cell activation? We, we discussed, we discussed, this T cell activation is important in terms, especially in terms of transplants, you want to be able to suppress, so that you would give some time for the graft to be excepted by the host. On the other hand, in case of vaccines, you want to induce T cell activation.

There are different methods of studying T cell activation. Now, in most methods, what people use is the use of antibody to send a signal through the T cell receptor. You can use antibodies, TCR or anti-CD3; anti-CD3 is most commonly used. There are different pathways that are involved. We discussed the whole gamut of pathways, right from the role of, of csk in keeping the LCK in active form and once you have CD45 being activated, it dephosphorylates LCK and then, which, which now activates. The ITAMs get phosphorylated and upon, once the ITAMs are phosphorylated, you have ZAP-70 being recruited over there, ZAP-70 in turn the tyrosine kinase, it recruits other ones.

We also importantly discussed the role of Cbl. Now, Cbl is E3 ubiquitin ligand, which and one of the substrates for Cbl is ZAP-70. So, in fact, in mice that lack Cbl, you have increased ZAP-70 because there is, there are enzymes to bring on ZAP-70 are not that efficient in the, and as a result of which you have again hyperactivation of T cells and which gives you an autoimmune phenotype. So, this tells you about these very important regulators of, of T cell activation.

Then you have, you also discussed the mitogen activated, the MAPK phosphorylation pathway and the Erc for example is a very important, phosphorylation of Erc is, is an important aspect over here. These finally lead to the activation of, of the 3 important transcription factors: NFAT, NF kappa-B, AP1, leading to IL2 and IL2 is the autocrine growth factor. So, that is where it, sort of, comes down to.

So, you start off with the T cell receptor, then you end with IL2 and in this intermediate, you have all these different players, especially the 3 transcription factors leading to IL2 synthesis. What is emphasized or what is emphasized over here is that if you just have a single transcription factor being affected, it does not lead to proper T cell activation. It can have negative consequences, for example, calcium alone increases NFAT, but what it does is that it makes the cells allergic, which means, they are now resistant to T cell activation and, and, and T cell activation and proliferation.

We also discussed the role of calcium and, and T cell activation in this process. Calcium is extremely important, we have an initial burst in calcium and then, followed by sustenance of, of, of, of intercellular calcium. In the absence of sustenance, you do not get proper activation of calcineurin, as a result of which the cytoplasmic NFAT is not dephosphorylated, it remains there and it cannot translocate to the nucleus. As a result of which you, you do not have enough IL2 being activated.

The other important aspect that we discussed was the mechanism of cyclosporin action and the immunosuppressant's very important cyclosporine binds to cyclophilin and this complex binds and inhibits calcineurin, which is the phosphatase. I think the mechanism of action of cyclosporine is very important and student should pay very careful attention to it; these are, are important because of the translational benefits that it has.

Then, finally, we, we, we discussed the role of T cell costimulation, especially the role of CD28 and CTLA4, one being a positive costimulator, one is a negative stimulator. They, they bind to the same molecule, but they have different functions; their expression is different, their mechanism for action is different.

So, overall, this class was on T cell activation. So, I hope, you understand the importance of T cell activation and the pathways and the translational benefits of, of T cell activation. Thank you