Essentials In Immunology

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Lecture No. # 27

T Cell Activation / Differentiation

So, today's class is going to be mainly on T cell differentiation. I will cover some aspects about T cell activation, that remain, but we will cover mainly T cell differentiation.

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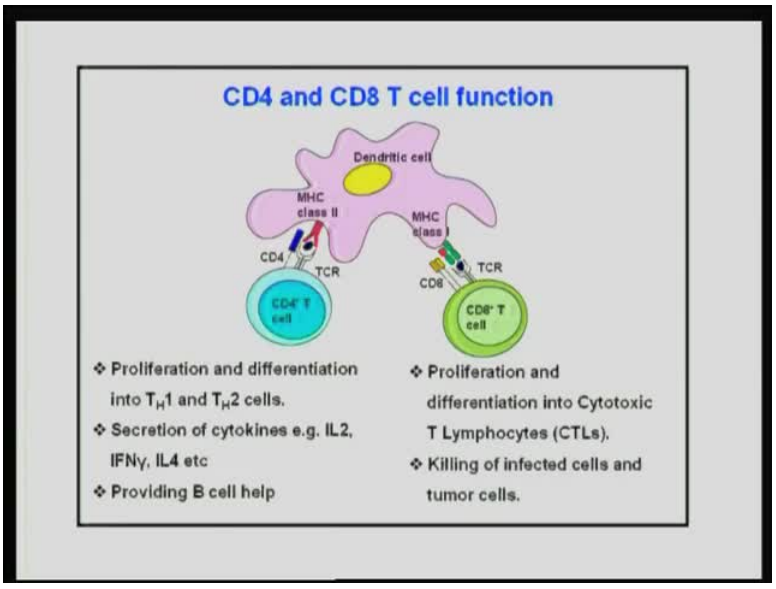

So, this is just a small introductory slide to remind you, that this is the dendritic cell, is shown as an antigen presenting cell. And this is the CD4 positive T cell, which is coming in contact with MHC class 2, which is presenting the cognate peptide ligand to the T cell receptor and this leads to proliferation differentiation of the T helper substance. And they produce, CD4 positive T cell produce cytokines, which give help to B cells, CD8 positive T cell and macrophages.

And what you have shown over here is a CD8 positive T cell in contact with dendritic cells, and it is coming and it is recognizing MHC class 1 or with peptide. This results in

differentiation of CD8 positive T cells into CTLs, which will now kill infected cells, tumor cells and so on.

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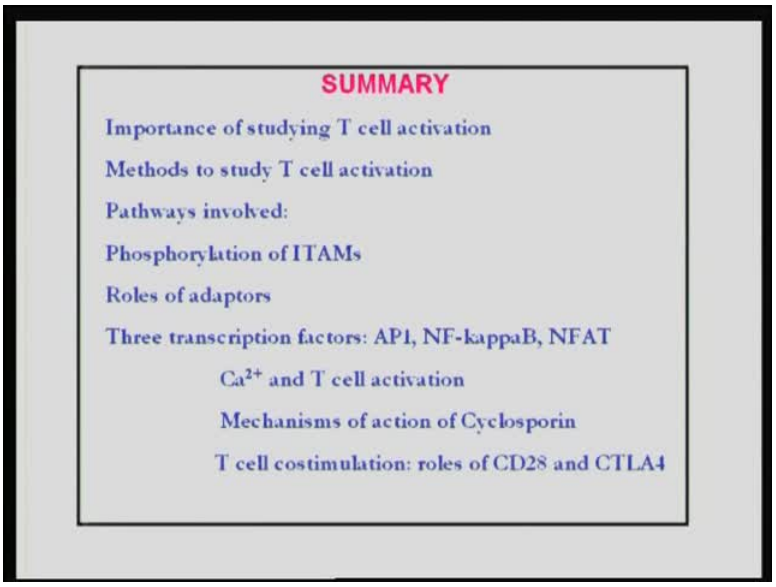

So, in the last class, we had studied different aspects about T cell activation and it is important to do it because in terms of transplantation and all, you know, transplants need to be done under the cover of, of immunosuppressants. Even though there is MHC matching, that is done, it is not possible to take care of, you know, getting the exact MHC. And so, so to, to give some time for the graph to a, sort of, settle down to be accepted, you need to do it under the cover of immunosuppressants. So, it is clearly very important to study this activation.

We also covered different aspects on, on how, what are the methods to study T cell activation? Remember, the T cell expresses a specific T cell receptor, I mean, and it is difficult to find antigens to recognize this and so, therefore, what, there are ways people found to polyclonally activate T cells using lectins or the combination of (( )) and calcium ionophore and specific antibodies to the T cell receptor-CD3 complex and so on.

There are different pathways involved. We had talked about, we had talked about the role of, of a fyn, lck and then the subsequent recruitment of adapters by ZAP-70, then, and then different pathways being activated, such that they activate 3 transcription factors, which is AP1, NF-kappa B and NFAT. Now, remember NFAT is a nucleus factor present in activated T cells, which plays an important role in T cells. So, you need

a synergy coordinated, all 3 transcription factor path is need to be activated for optimal T cell, T cell activation.

If you just have a single one, for example, using calcium, if you activate only the NFAT pathway, often T cells are energic, which means, they can, they can no longer respond to secondary stimulation that is done and this is, these have important physiological consequences, some of which will be, which were discussed in the last class, and it will be reinforced again in this class when we talk about periphery tolerance.

The role of intercellular calcium in T cell activation was emphasized and the role of the ORAI and the STIM pathways in terms of, of acting as calcium sensors and as calcium channels and it is, it is, it is very important because patients who lack these molecules are, are susceptible to, to infectious agents.

The mechanisms of action of cyclosporin, which is one of the very important immunosuppressants was discussed, especially its role in binding and inhibiting calcimine phosphatase. Then, subsequently, we, we discussed the role of T cell activation, the role of costimulatory molecules, especially CD28 and CTLA4.

CD28 and CTLA4 are cousins, but they have different roles. CD28 is a positive costimulatory receptor, which means it enhances T cell activation; CTLA4 is a negative costimulatory receptor, which means it, it lowers T cell activation; both are physiologically very important term. CD28 knockout mice, as shown, that T cell activation, the sustained T cell activation requires CD28 and in CTLA4, knockout mice have shown there are lympho-proliferation of CD4 positive T cells, as a consequence of which, the mice stands.

So, clearly, both these molecules play important roles, even though they bind the same ligands, which are CD80, 87, which belong to the B7 family of molecules.

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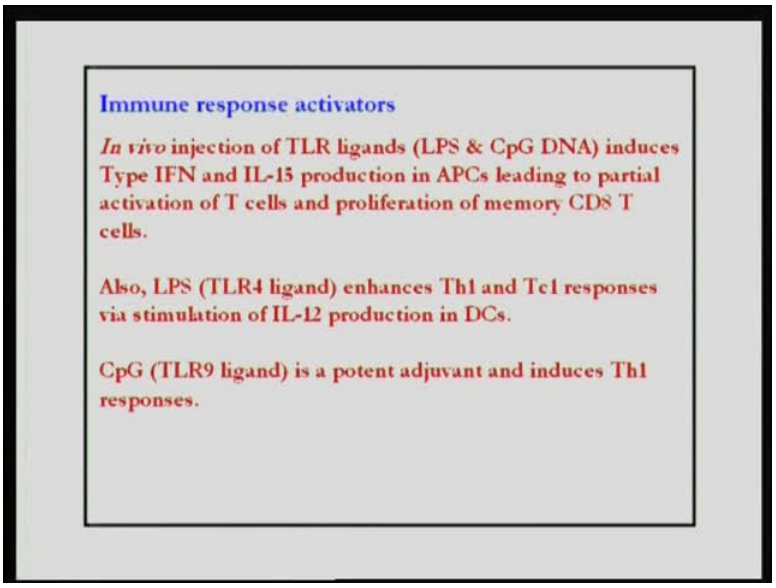

So, right now, we will discuss some other activators. We had talked in our first class on, on innate on, on, on this, this adaptation or this interplay between innate and adaptive responses, and over here is a good example of that because injection of TLR ligands, like LPS and CpG induces type 1 interferons and IL-15 and it leads to partial activation. LPS, TLR ligands, both LPS and CpG have shown to enhances Th1 type of responses and this is primarily via APC mediated production of IL-12.

So, APCs get activated, as a consequence of which they, they, they are able to activate a larger number of, of T cells and it results in increased T cell activation. What has been found in this case is that it is primarily a Th1 type of response.

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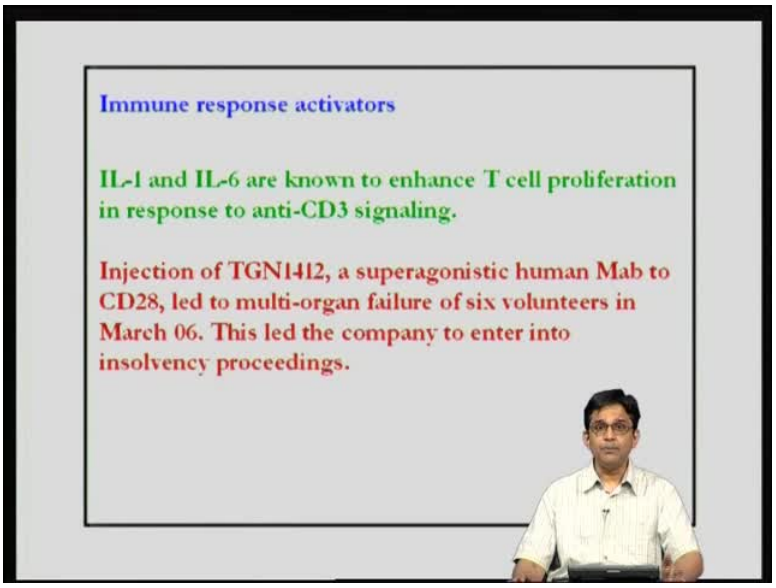

There are other activators, so cytokine, for example, are known, IL-1, IL-6 is known to help in T cell activation. Note, the, you know, they cannot circumvent the TCR pathway, but they, but they help in T cell activation.

What is interesting is that there was an antibody that was found, which is the super agonistic, which means antibody by, by, by itself activated T cells and in fact, when a small trial was done, it led with this particular antibody. It led to multi-organ failure of the 6 volunteers in March 06 and subsequently, the company had to, had to enter into insolvency proceedings. And this, sort of, illustrates the importance of T cell activation and the limits also of T cell activation that if you try and treat the system too much, it can go in the other direction. So, that is why, I say, T cells need to be activated, they are important for immune response. However, you need to bring this down because if you activate them in aberrant ways, like for example, though what was done with TGN1412, then it will result in hyper activation of T cells and it can lead to consequences that are deleterious to the host.

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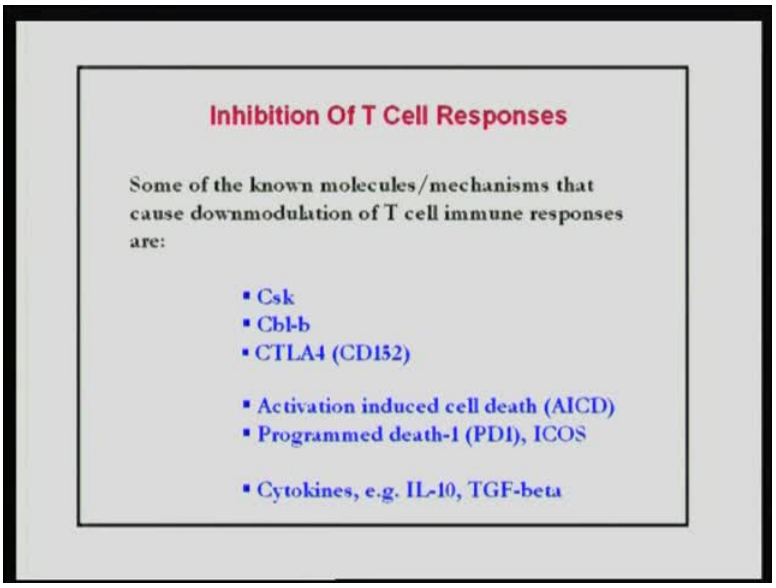

So, with that we will come to some of our points that we had discussed on inhibition of T cell responses. Now, we had discussed several molecules, that were important and we will, we will just briefly go over them. Some of the ones, that we discussed was CsK, which is a kinase, which is involved in phosphorylation of Lck-fyn and keeps it, keeps them phosphorylated and, and as a result of which they are inactive.

Now, once CD45 comes in upon activation, then you have, then you have dephosphorylation and then it takes over. So, in CsK knockout mice for example, you have hyper proliferation because the T cells are activated; the same holds true for Cbl.

Now, I had told Cbl is an E3 ubiquitin ligase; one of the substrates for Cbl is ZAP-70. So, in the absence of Cbl-b, what happens is that you have, once T cells that are activated, there is, you cannot lower the T cell activation because ZAP-70 keeps on being phosphorylated and keeps on recruiting and leads to hyperactivation. So, in these Cbl knockout mice, you have, what is primarily, autoimmune scenario. We discussed about the role of CTLA4 previously, again its role is to act to load T cell activation.

There are other mechanisms involved and activation induced cell death and programmed, programmed death pathways. We will be discussing these little bit later, mainly when we discuss autoimmunity and we will also be discussing the role of inhibitory cytokines, especially IL-10 and TGF-beta subsequently. But they will all come under a path of inhibition of T cell responses and this is illustrated by the fact, that T cells are, are

activated, but they need to be lowered after some time because continuous or consistent activation of T cells is delirious to the host.

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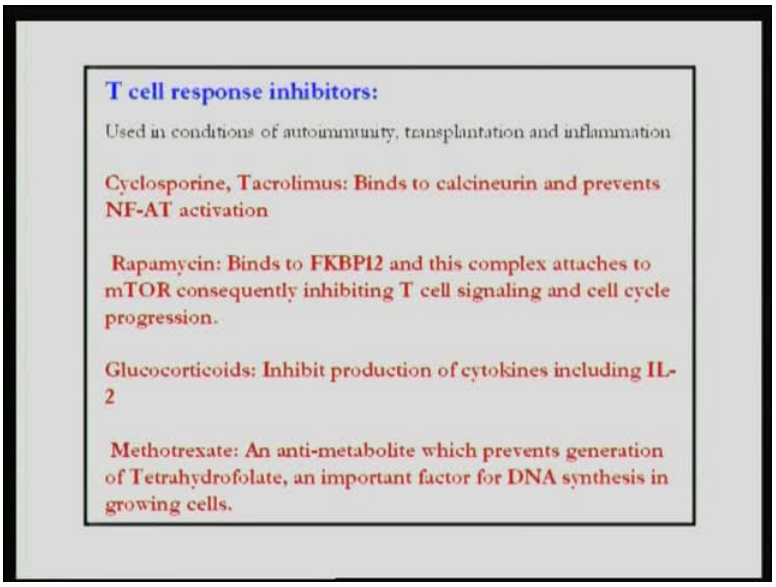

So, in terms of T cell response inhibitors, there are several molecules that play an important role, for example, cyclosporine. We have, this is something, that we have discussed; tacrolimus is another molecule. The mechanism is similar; they bind to calcineurin and, and, and inhibit NF-AT activation; so, that pathway has been discussed.

There is another molecule, known as rapamycin. Now, rapamycin binds to another, to another molecule, known as FKB binding protein-12 and this complex attaches itself to what is known as mammalian mTOR or the mammalian target, or rapamycin, and by binding to mTOR it inhibits T cell signaling and cell cycle progression. mTOR is involved in, in, in, wide variety of effects, primarily leading to proliferation activation. So, what rapamycin does is to inhibit this process.

There are other ways to inhibit T cell activation. Glucocorticoids had been known for, for a long time to inhibit the production of cytokines, especially IL-2. In fact, often in some allergic diseases and all, glucocorticoids are given and in particular of amounts, so as to lower, lower T cell activation.

Methotrexate has a different mechanism of, actually it is an anti-metabolite and it prevents generation of Tetrahydrofolate folic acid and which is an important factor in DNA synthesis.

So what, what methotrexate does is, by inhibiting the production of tetrahydrofolate it results in lesser number of, lesser amounts of, of, of nucleotides, importance of DNA synthesis, as a result of which the cells do not proliferate because of the limiting amounts of nucleotides.

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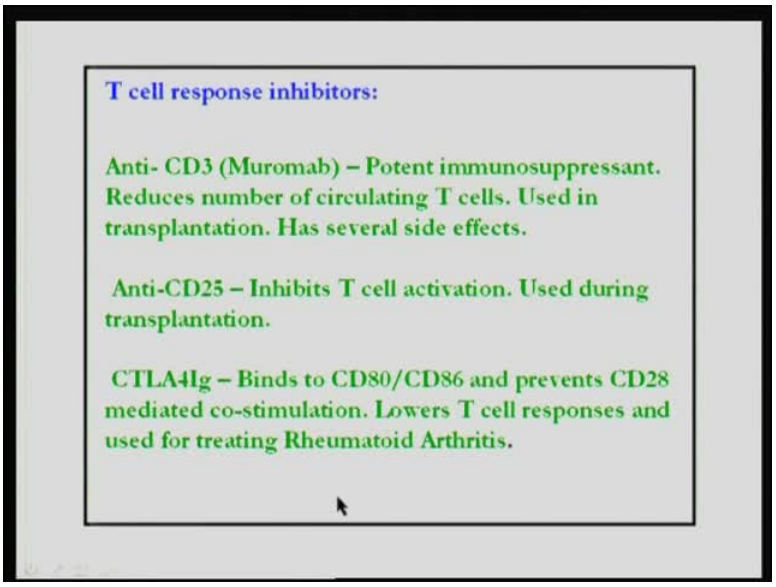

There are other T cell responses inhibitors, one of the first ones, anti-CD3 muromab and now these are, are, are used in terms of, of, of transplants or under some pathological condition. Now, anti-CD3 it is a potent immunosuppressant, it would bind to T cells and reduces the number of circulating T cells, its use in transplantation, but unfortunately, it has severe side effects.

So, clearly, you know, these are, are not the best, these are something that people have used and can use it and are useful under certain circumstances. Anti-CD25 also inhibits T cell activation; it is used again during transplantation.

CTLA4, now CTLA4Ig, this is, this is a fusion protein; it has the binding domain of CTLA4. So, what these does, it binds to the ligand CD80, 86, prevents CD28 mediated

co-stimulation, and lower T cell responses and it is used for treatment of rheumatoid arthritis.

What these molecules, the reason, why I put forth these molecules, is to give an idea about the role of small molecules in T cell activation and the fact, that increasingly people are trying to find newer molecules, that might inhibit T cell activation, because, because this part is a very important aspect in, in our understanding about how to treat T cell disorders. So, the more number of molecules or unique molecules, that begin, come up and understanding the target may be very useful in this process.

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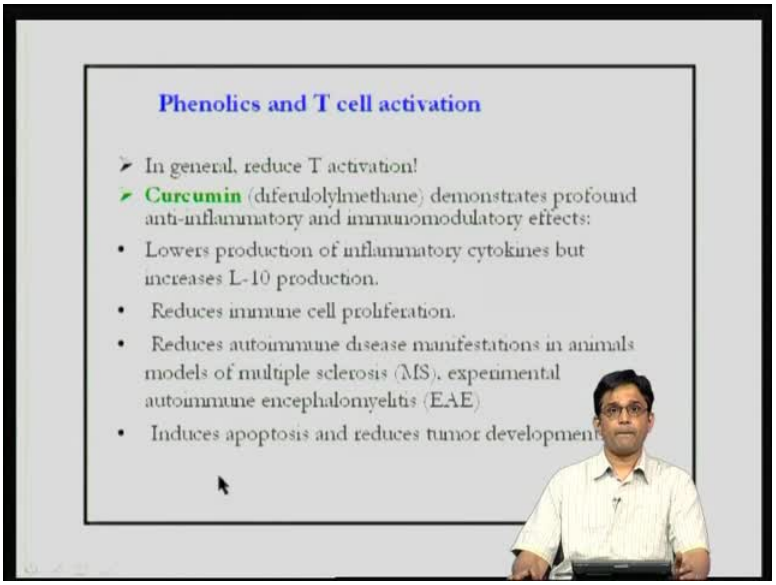

So, among the, the other molecules, I thought I should mention 2 of them come up. These are phenolics and 2 of them very well-known phenolics, one of which is curcumin which is, which is from turmeric, which is isolated from turmeric. In both, curcumin and Resveratrol, these are anti-inflammatory and immunomodulatory.

So, curcumin, for example, lowers the production of inflammatory cytokines, but increases IL-10 production. It reduces immune cell proliferation and it has been used under some condition, basically is anti-inflammatory, induces apoptosis, reduces tumor development.

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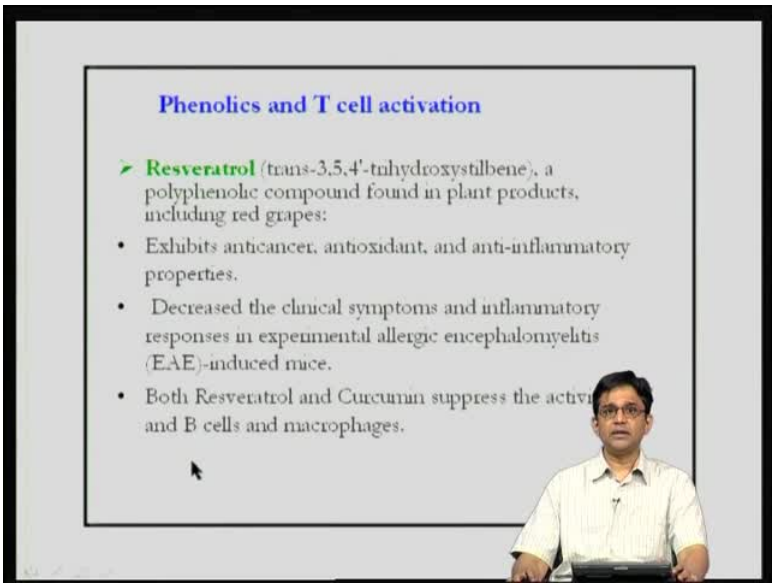

Resveratrol, on the other hand, is polyphenolic. It is found in, in, in plant products, for example grapes, it is an antioxidant, it has several properties. Again, it is being used as an anti-inflammatory agent and they suppress activation of T cells, B cells and the macrophages.

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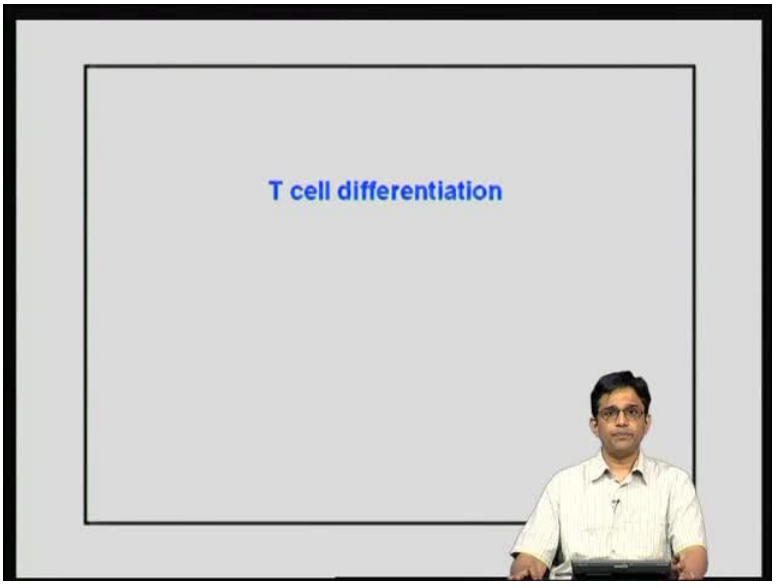

So, with that we will come on to T cell differentiation, which is the main focus of this lecture.

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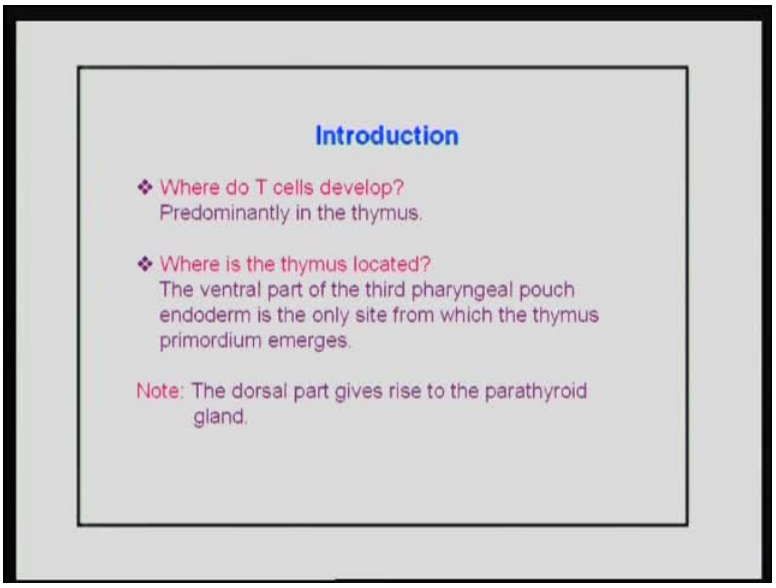

So, one of the main things is where do T cells develop? Now, if I were to ask you this, you say the thymus and, but where is the thymus? So, the thymus is actually, sits slightly above the heart and it is, it is, it, it, it is, starts, of, from the ventral part of the third pharyngeal pouch, where the, this part emerges and the dorsal part gives rise to, to, to the parathyroid.

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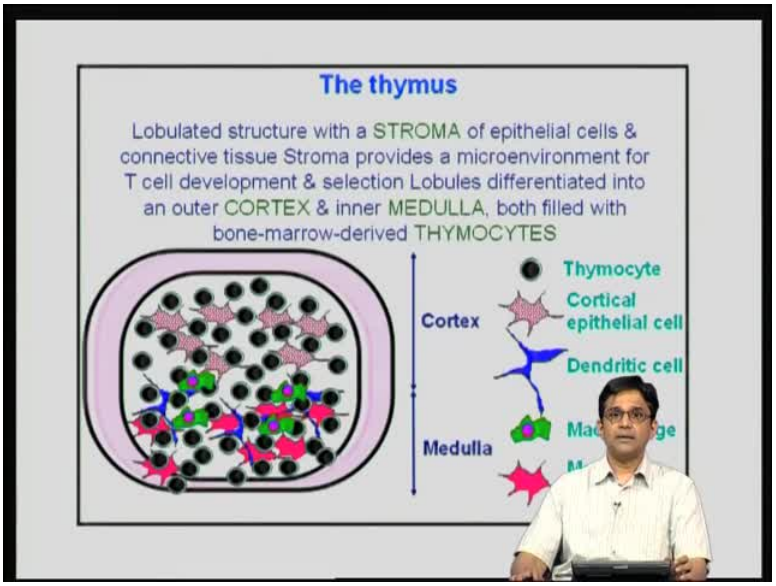

Now, this is what is shown over here. So, you have the thymus consists of 2 main parts, one is the cortex, the outer cortex, the outer cortex and inner medulla. So, this is very

important because the cells, initial cells come in to the cortex and they exit primarily through the medulla. So, and, and there are, the parts of, where different processes occur during T cell differentiation, whether the cortex or medulla is an important aspect and that will be subsequently discussed. So, suffice to say, the thymus is important for T cell differentiation and cortex and medulla are 2 important parts and it is something that students should be aware of.

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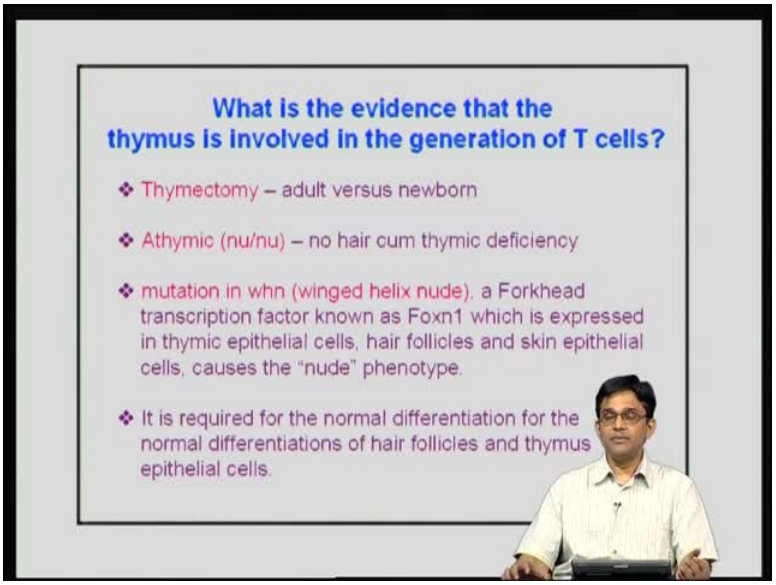

So, what is the evidence, that the thymus is involved in the generation of T cells? So, for example, if a thymectomy is done, which means, you dissect out, you dissect the thymus and you remove the thymus and ask, what happens? If you do that with an adult, then there is no effect, the T cell are there because the T cells have already been generated by and enlarged; they are reasonably long live. So, you do not see a major effect.

The major effect is seen when thymectomy was done in a newborn. So, that is before you have populations of T cells that have a reason and seeded the periphery, that is, when the effects were seen. So, if you, if you, if (( )) thymectomy was done in a newborn, then you have very lower amounts of T cell. So, when thymectomy is done, where in adults and newborns, is a very important aspect and that is something that students should be familiar with.

Now, there are other evidences, for example, you have a nu mice, the, which is shown over here, nu nu, and they do not have, they do not have hair and they do not have

thymus. So, in other words, they are deficient in T cells, but their B cell part is just fine. And these are useful because, because they do not have T cells. They are, in a sense, immunocompromise.

So, nude mice are often used for transplants or to study the effects of, or growth of tumor because usually, what could happen, especially human tumors, especially if you take these tumors and put them in, in mice, they are usually rejected and they are rejected primarily, by the T cell mediated arm. Now, a nude mice, because they do not have T cells, these will, will allow for the tumors to grow and so these are very useful in terms of tumors studies.

Now, what is the reason for nude mice to lack hair as well as the thymus? Studies showed, that the mutation was in a winged helix nude protein, which is a forkhead transcription factor, known as Foxn and it is expressed finally in epithelial, thymic epithelial cells, hair follicles. Consequently, absence of Foxn results in a nude phenotype, that is, no hair and also, no thymus. Now, Foxn is required for normal differentiations of hair follicles and thymus, which is why, you have this particular phenotype.

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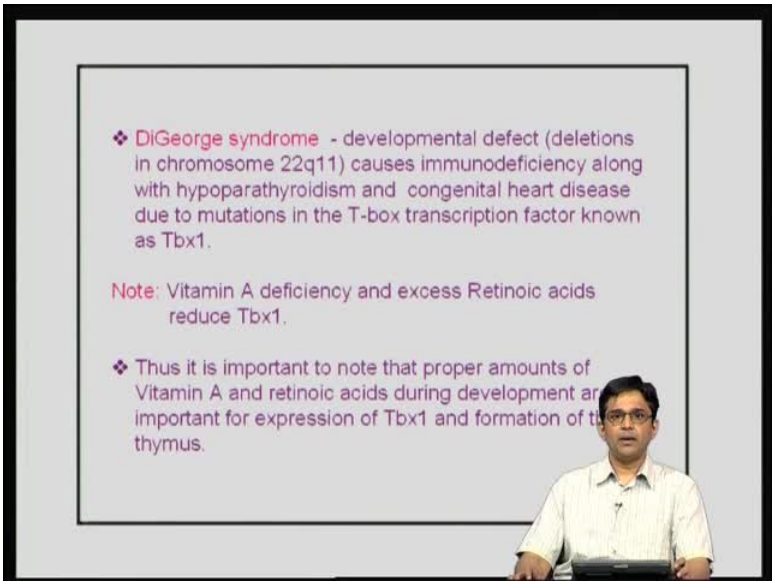

The other evidence comes from DiGeorge syndrome. Now, in DiGeorge syndrome, this causes an immunodeficiency along with hypoparathyroidism. Remember, you saw, we

had discussed, how the thymus and parathyroid rises and, and this is due to the DiGeorge syndrome, is finally due to mutations in T-box transcription factor known as Tbx1.

What is interesting over here is the vitamin A deficiency and excessive Retinoic acids reduces, Tx, Tbx1 and therefore, it is important, that proper amounts of vitamin A and Retinoic acids are important during development and for the formation of, of, of the thymus.

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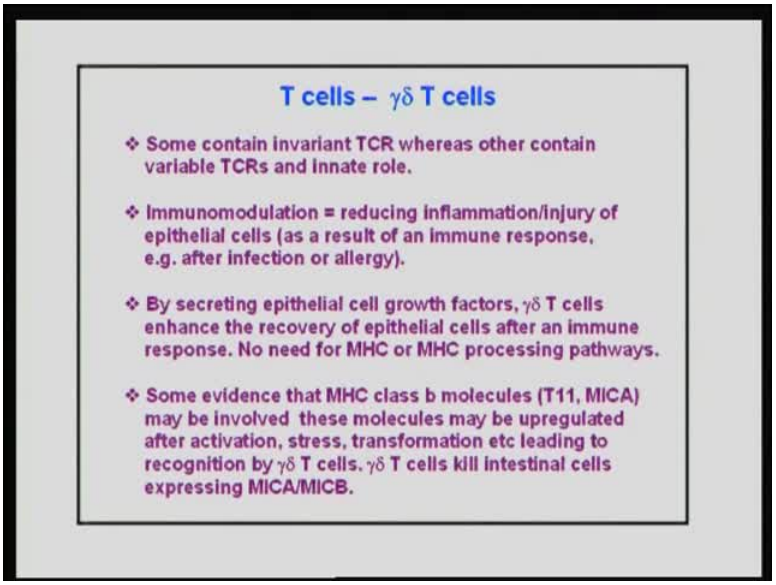

Now, you, this is a part, that must have been covered. So, with respect to T cells, you have 2 broad categories of T cell, one is those expressing the alpha-beta T cell receptors. Then, when we talk about T cells or T cell activation, we are talking mainly about the alpha-beta type of T cells, which are, which are the majority.

There is a smaller group of gamma-delta T cells. Now, these gamma delta T cells express they have, they have different genes, gamma and delta, as opposed to alpha and beta and so they express the alpha-beta T cell receptor. Now, the function of alpha-beta cells is, is not clear, but they play, play certain roles in, in stress, secretion of, of, of TCR specific growth factor and these are smaller in numbers and they are found in specialized initials, especially epithelial tissues.

What is also interesting about gamma-delta T cells is that they arise early on during on turgene and it is thought, that they might be a part of the innate system, and because you see them arising early during T cell, T cell development.

Now, in the gamma-delta T cells, some of the gamma-delta T cells are, are invariant in nature and I have given you some evidences, that gamma-delta, some reduce inflammation or injury of epithelial cells, they secret epithelial growth, growth, growth factors and there are evidences, that they may be involved in killing of epithelial cells, that expresses stress molecules.

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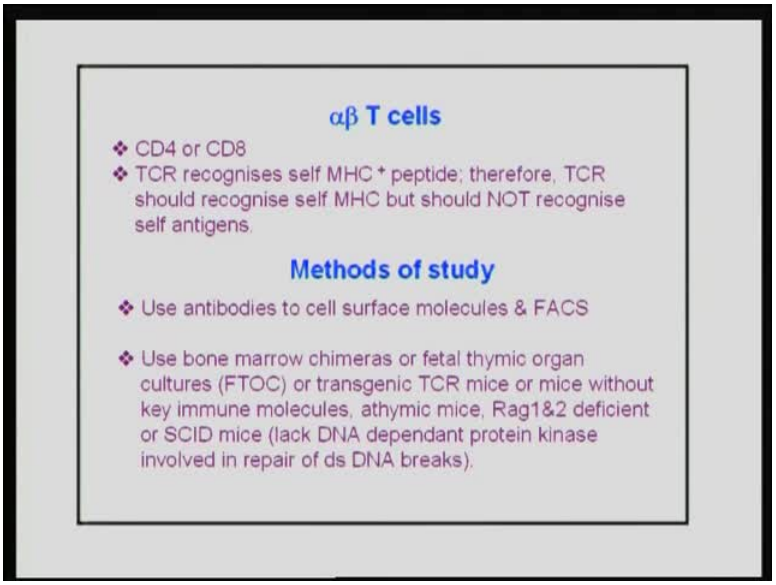

And this part is going to be important because once we look at the way T cells arise in the thymus, we will see, that the first T cells, that arise in the thymus are the gamma-delta T cells. And so, therefore, we, we need to know a little bit about them before I, sort of, introduce you to this part of the topic.

Now, now, now, the alpha-beta T cells are the majority and they express CD4 or CD8. So, they are basically single positive in that sense. So, the alpha-beta cells are either CD4 or CD8, so CD4 is primarily C 2 restricted, CD8 are MHC class 1 restricted and that is something that has been discussed earlier.

Now, T cell, alpha-beta T cell receptors recognize self-MHC with peptide. So, now, as, as you know, it should be clear, that the T cells should recognize self-MHC, but should

not recognize self-antigens because it is the self-MHC, which is presenting these antigen. So, clearly, you do not want T cells, that see self-MHC a whole lot or bind to self-MHC whole lot, because that will result in activation of self-T cells, resulting in autoimmunity. So, these are aspects that we need to consider while we are studying thymic differentiations.

Now, terms of methods of study. So, the methods of study had been, usually, the use of antibodies to cell surface molecules and fluorescence activated cell sorting analysis. So, you have antibodies to different cell surface molecules and then, you can study the expression of different cell surface molecules using the cell sorter and this has been, you know, of great use in the study of thymic differentiation.

The others are the models for study. So, for the models of study, often bone marrow chimeras are used, so that, you know, which cells belonging to which lineage, sort of, give rise to what kind of cells. You also have the fetal thymic organ cultures, where thymuses are dissected from, from fetal and acute in culture.

And then, if you have the proper culture conditions, these thymocytes in then will differentiate and give rise to, to, to, you know, to that would, sort of, mimic thymic differentiation and if you have that going, now you can put in certain chemicals or antibody and see, what are the effects that these have on the normal differentiation process.

Subsequently, you also have transgenic mice and mice lacking key immune molecules, for example, the Rag 1 Rag 2 or SCID mice, which lack DNA dependent protein kinase, which is involved in double strand DNA break repair.

So, these are, these are important in that, that respect, so there are different ways of studying T cell differentiations. I just wanted you to become a little bit familiar with the different processes.

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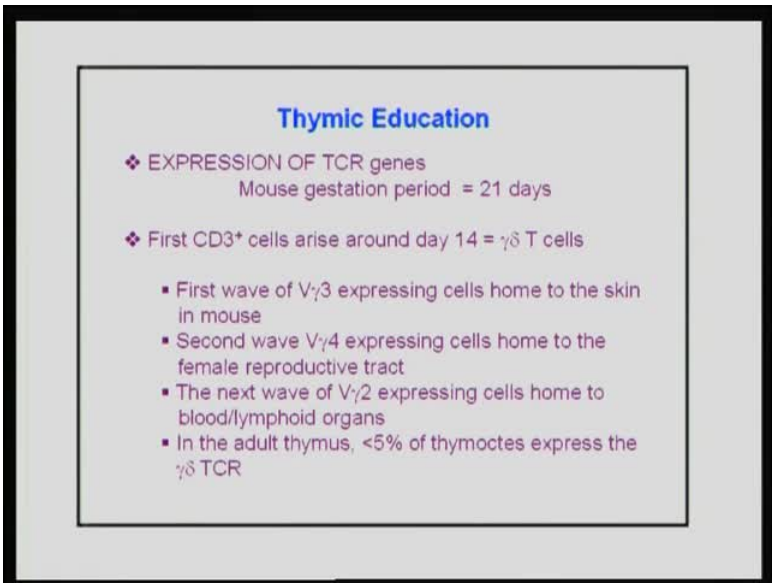

Now, I am going to introduce thymic differentiation in, in actually different ways. And so, the 1st way we need to look at is, in, in terms of the gestation period of the mouse; so, gestation period is about 21 days. So, right from the time it starts to, to the time pups are born, it is about 21days.

Now, with the 1st CD3 positive T cells to arise are around day 14, so it takes about 2 weeks to 1st to be able to dissect of, you know, fetal thymus and find out, ask, what are the T cells, that arise and it turns out to be the gamma-delta T cells. In fact, the 1st wave of T cells express this particular, particular gamma or the V gamma 3 expressing cells, so in the mouse they are the 1st response that arise and the home to the skin.

The 2nd wave comes in the form of V gamma 3 and they are home to the female reproductive tract. The next of V gamma 2 expressing cells home to the blood lymphoid organs and in the adult's thymus, less than 5 percent of thymocytes express the gamma-delta T cell receptor.

So, very few of them, but however, the importance of it, of gamma-delta T cells is that the 1st T cells to arise in the thymus are the gamma-delta cells, they arise and they quickly go into the SCID, and the 2nd wave also goes to the female reproductive tract and, and, so the 1st ones are ones of the gamma-delta cells, that arise and they go to the, to the, specific tissues, that they are destined to go.

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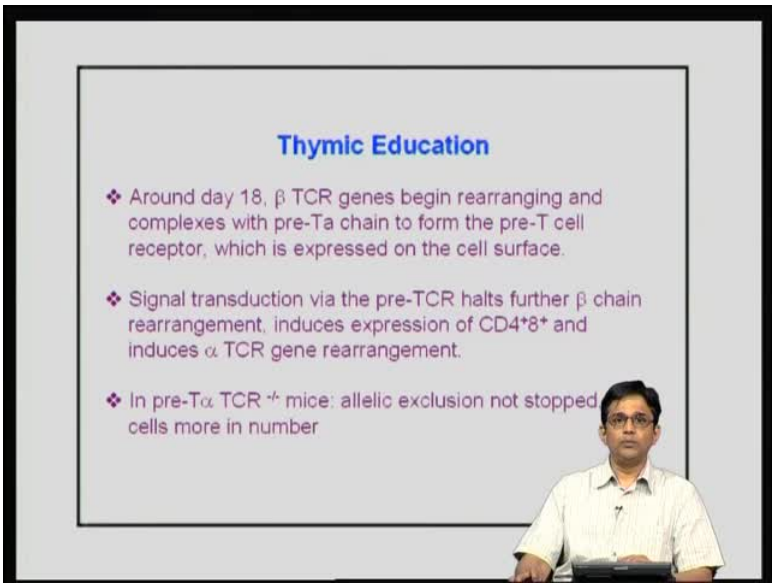

Now, we said, that the first gamma-delta cells arise on day 14. By about day 18, you have the beta T cell receptor genes begin rearranging. Now, remember, you will have to rearrange these genes before they can combine and come up on the surface. Now, what is interesting is that the beta T cell receptor complexes with, with pre-alpha T cell receptor. So, it is not actual alpha T cell receptor, but it is the pre-form, which is actually an invariant form and it complex with that and goes to the cell surface.

Now, this is important because the moment it goes to the cell surface, the initial, the double negative thermocytes proliferate a lot. So, once it goes to the cell surface and you have a productive, productive expression of it, the proliferation stops and the other alleles of beta, that are trying to rearrange, that is also stopped over here. So, once you have, have this going up, so there is the signal for these other processes to stop and so that is what I have said over here, signal transduction via the pre-TCR halts further beta chain rearrangements. It induces and from double negative, it induces the expression of double positive, which is the CD4 minus 4 minus 8 minus, now start expressing 4 plus 8 plus and subsequently, it induces the alpha TCR gene, gene rearrangement.

So, then, you will have now productive formation between a beta, between the rearranged beta and the rearrange and a rearranged alpha to now express the proper alpha-beta T cell receptor. Now, in, in, in the pre-TCR alpha knockout mice, you have, an allelic exclusion is not stopped and these cells are more in number.

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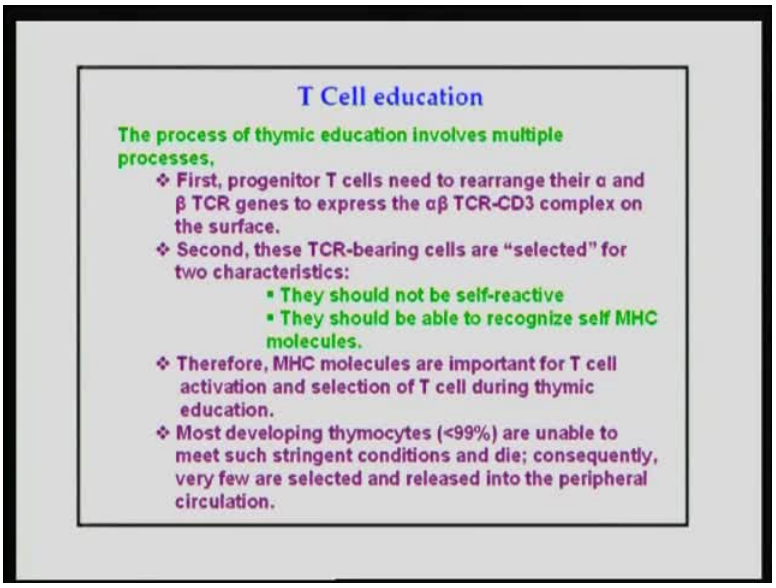

Now, with respect to thymic education, so there are several processes, 1st is that the T cells need to rearrange their TCR beta genes and then, subsequently, rearrange the gamma genes to express the gamma-beta TCR complex and then, once they are expressed on the cell surface, they need to be selected for. So, and this selection is done based on 2 characteristics, one is, they should not be self-reactive. So, because if they see MHC, self-MHC molecule, which too much affinity, then you will have activation of these and it will result in autoimmune phenotype.

The 2nd is, they should be able to recognize self. So, so they, while they should be recognized self-MHC molecules, they should not do it with, with very high affinity. So, 1st is ability to recognize self-MHC is, so you have to positively select for the T cell receptors, that are able to recognize self-MHC, that is known as positive selection. However, once having, once having done that, if they recognize self-MHC with very high affinity, something you need to delete them and that is known as negative selection. So, you have 2, 2 main processes, may be 3 main processes over here.

1st is that they need to express the alpha-beta T cell receptors and they need to bind MHC. So, whatever alpha-beta T cell receptors are expressed, they need to bind MHC. If they do not do it, then they die by what is known as, dead by neglect that is the first thing. So, upon binding of self-MHC, they are selected, they are rescued from death and

that is known as positive selection because you are positively selecting for those TCR, that can now recognize the self-MHC.

Now, having done that, you need to ensure, that these TCRs do not recognize self-MHC with greater affinity and you need to get rid of them. And so, though that is known as negative selection, so let me introduce the 3 terms, one is dead by neglect, positive selection and then and negative selection.

So, now, this process is highly stringent and in fact, you know, almost 99 percent of the thymocytes, most developing 99 thymocytes are unable to meet such stringent conditions and they die. So, as a result, very few, this whole processes is very, is very stringent and only very few are able to survive this stringent conditions and go out and see the periphery. Remember, while, while T cells develop in the thymus, develop and differentiate in the thymus, very few of them are able to get all these conditions together, rearrange the TCR, you know, be positively and, and undergo negative selection in proper way.

And only those ones are that, are able to fulfill of the criteria and are selected, are able to, are able to enter the periphery. So, from the thymus, then they need to enter the periphery because then they are supposed to act in terms of guarding and protecting the host.

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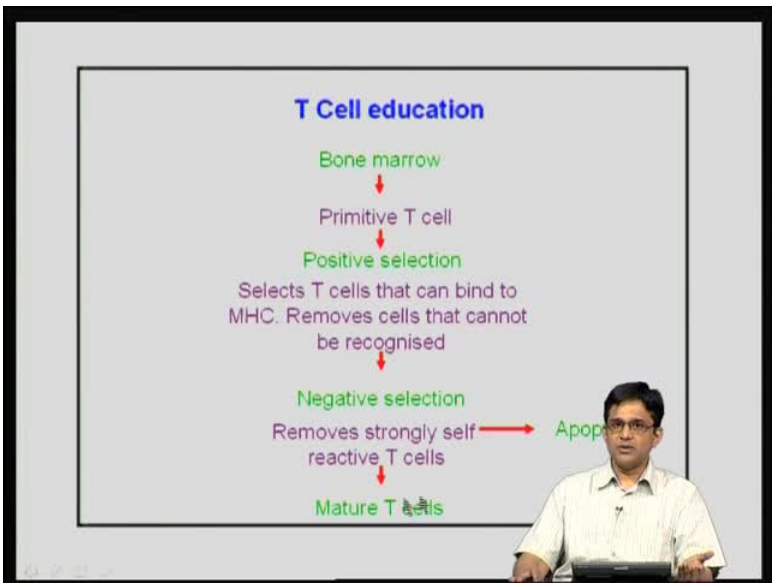

So, that is what is shown over here, you have bone marrow cells and this is the primitive primordial T cells that enter the thymus. Then, as you know, they rearrange their T cell receptors and they are positively selected.

So, you select for those T cell receptors, that can bind to MHC molecules and those that cannot be, that cannot bind, that do not express T cell receptors or cannot bind self-MHC molecules, they die by process known as death by, death by neglect.

Subsequently, those that bind MHC are negative selected. So, you remove any strongly self-reactive T cells, so then you have these mature T cells that are, that seed the periphery.

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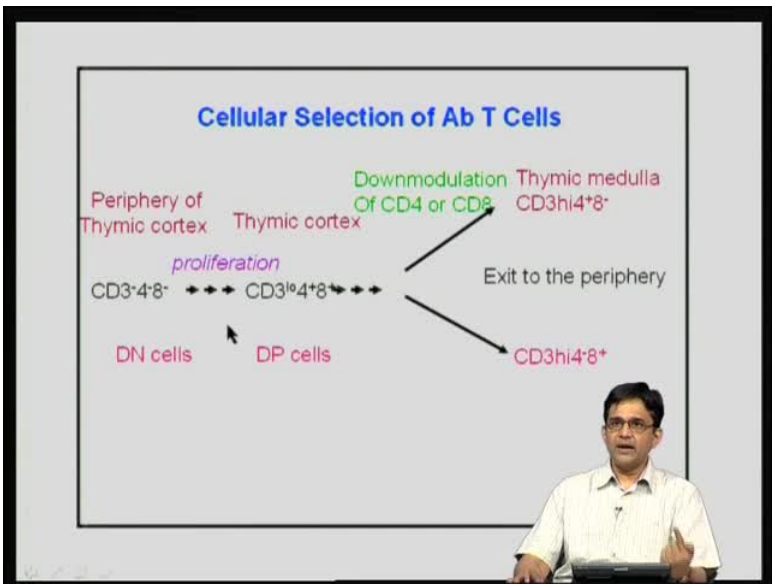

And so, in, the other way to look at it is, is over here that is shown here. So, this is CD3 negative 4 negative 8 negative, so these are the double negative cells, which, they proliferate and they go through different stages DN1, DN2, DN3, DN4, so on.

So, they proliferate and they go through different stages and then, as was mentioned at the very end of this stage, they express the rearranged beta and together with pre-TCR alpha and they, and then they stop proliferating and then they become double positive.

And now in the double positive here, they initially start over CD3 low because now they are expressing the T cell receptors, but there are selection conditions, that are occurring, you have positive selection, you have negative selection. Subsequently, depending on

whether they bind MHC class 1 or MHC class 2, there is down modulation of either CD4 CD8, and so, you will have in the medulla. Finally, you will have CD3hi, these are 4 plus or 8 minus or CD3hi 4 minus 8 plus, and they exit into the periphery.

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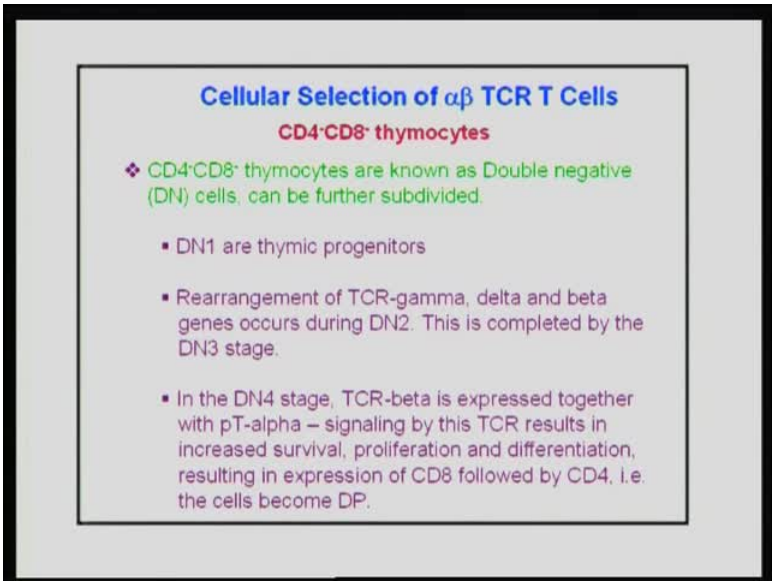

So, an, as must have been obvious to, you have the double negative cells, which are CD4 minus 8 minus, that is how they start off and then, you have double positives and then, you have the s p or the single positive ones, which seed in to the periphery.

So, this double positive population is a unique population, that is found only in the thymus and I talked about the different stages of double negatives. So, the double negatives are thymic progenitors and they rearrange the TCR genes and they proliferate these. Double negative stages are highly proliferative ones, they proliferate and they also rearrange it in different stages, and as I mentioned here, by about, that the latest stages TCR beta is expressed together with the pre alpha, with pre alpha TCR and signaling by this results in increased survival, proliferation and differentiation. More importantly, then they, from double negative they become double positive.

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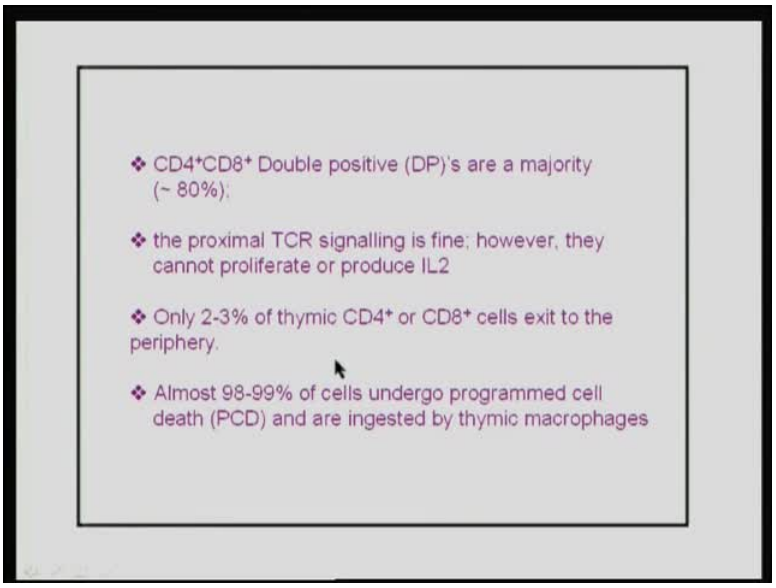

And in the thymus, primarily, majority of the cells are double positive. What is interesting about the double positive is that the proximal T cell receptor signaling is fine, but they cannot proliferate or produce IL-2 and there are very few of them, that, that are selected and they exit to the periphery. And this is what is known, is that majority of the cells, about 98 to 99 percent of the cells undergo program cell death and are ingested by thymic macrophages.

What is, you know, for so much, so many of the cells die over here, if you dissect and open up a thymus, you will not be able to tell, that there are all these processes going on and that is because it is a very efficient way to reorganize tissues, and that is done by a process known as apoptosis, and that is again something, that we will discuss in a subsequent class, about the role of apoptosis and the T cell survival, a very important aspect.

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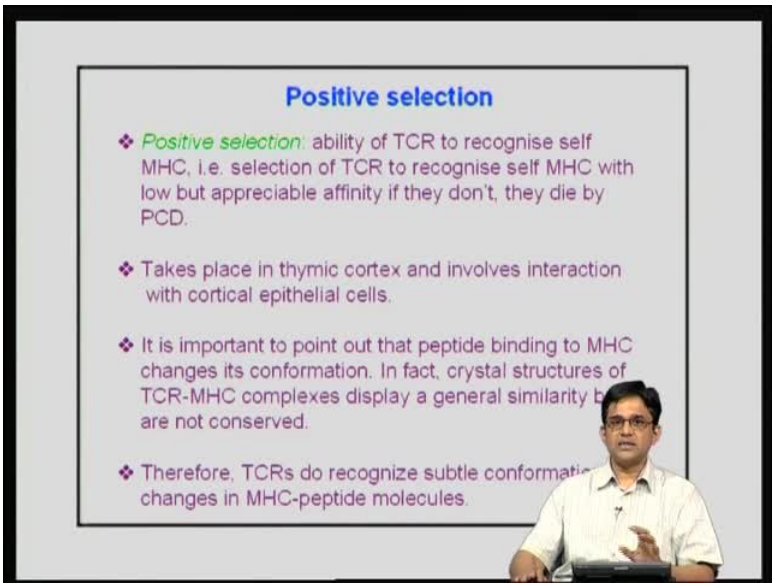

Now, I talked about 2 processes, 2 main processes, one is positive selection and the other is negative selection. Now, positive selection is the process by which you select for T cell receptors, that recognize the self-MHC and any, if they do not, then they die. Now, the positive selection takes place in thymic cortex and involves interaction with cortical epithelial cells.

Now what is, what is important to point out, that, bind, peptide binding to MHC molecules, you know, changes the conformation. In fact, what is shown is the crystal structures of the T cell receptor to MHC complexes display a general similarity, but they are not conserved. So, the binding is not, you know, you do not have just 1 structure or 1, 1 way of binding. So, there are different structures or different subtle conformation changes that occur between the binding of T cell receptors and self-MHC molecules. So, there are, so T cells do recognize subtle conformation changes in, once they recognize MHC peptide molecules.

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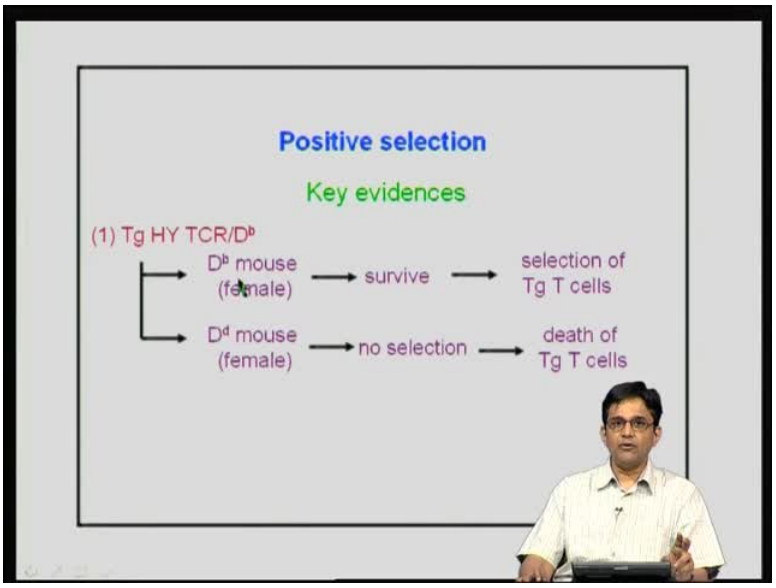

What are the evidences for positive selection? So, what is shown over here, I have, I have selectively, I am going to show some of the, some of the ones, that I consider are better evidences, this is a transgenic HY. So, HY is a particular antigen, that is presented in, in its encoded by the y chromosome and so it would be present in males. And there is a T cell receptor, that recognizes this antigen and so that is why, it is, it is a T cell receptor against, they recognize HY, but it does so in the context of D of b.

So, transgenic, so this was the transgenic mouse and once you see the, the, the development of this particular of transgenic in D of b mouse, which means, it is the same one and D of d mouse and here, both are female, so because they are female, they would be selected, selected fore.

What is shown over here is, in this particular one, the T cell receptor is actually selected in the D of b mouse and it survives under the selection of this. However, in the D of d, which means, the wrong MHC, there is no selection because the right self-MHC is not present and it results in death of these cells.

So, this is a good evidence to show about the role of self-MHC in selecting T cell receptors. So, clearly, this particular, even though it is transgenic, it needs to be selected and if the appropriate MHC is not there, it will not be selected and that is what it shows.

There are other evidences for it. Slide at 37:38 is not included here. Pls include.

So, for example, CD, CD4 and CD8 play a role. Now, we had discussed beta 2 microglobulin deficient mice. Now, if you remember, MHC class 1 requires the binding of, of beta 2 microglobulin for stable cell surface expression. Now, beta 2 microglobulin deficient mice, there is no MHC class 1 and therefore, no CD8 positive cells because MHC class 1 expression is, is deficient. But the CD4 are present because CD4 are dependent on MHC class 2.

Now, similarly, in a MHC class 2 deficient mice, there are no CD4, but CD8 positive T cells are present. So, this shows you about the role of MHC molecule in positively selecting TCR that are able to recognize the self-MHC.

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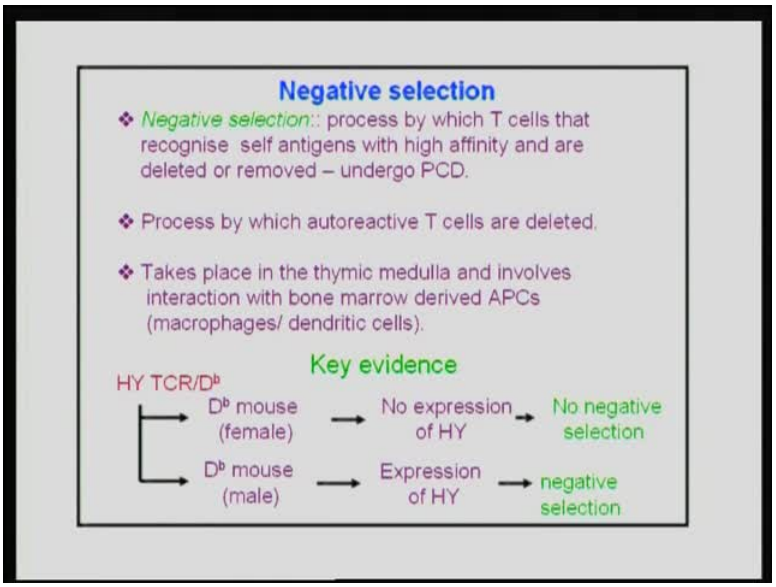

Now, now, what about negative selection? Now, negative selection is a process by which, those, that recognize, self-antigens with high affinity are deleted or removed or they undergo program cell death.

Now, it takes, negative selection takes place in thymic medulla and the evidences over here was the use of the same TCR transgenic HY mice. Now, here was in the D b, in the D b mouse in the female one, there is no expression of HY because it is for a mice, it is encoded only by the y chromosome and therefore, there is no negative selection, that means, they will be, there is no negative, there will be, there, because there is no negative selection.

So, whereas, in the male mice, it is a self-antigen and there is an expression of HY. So, since it is there, you have negative selection, as a result of which these transgenic do not develop. So, I particularly showed this because I think, it is a very nice use in terms of experimentation, where one transgenic mouse was generated and to show the role of both, positive and negative selection, and if you do the proper causes, you are able, you are able to show it. So, very elegant experimentation to be able to, to illustrate these complex processes known as positive and negative selection.

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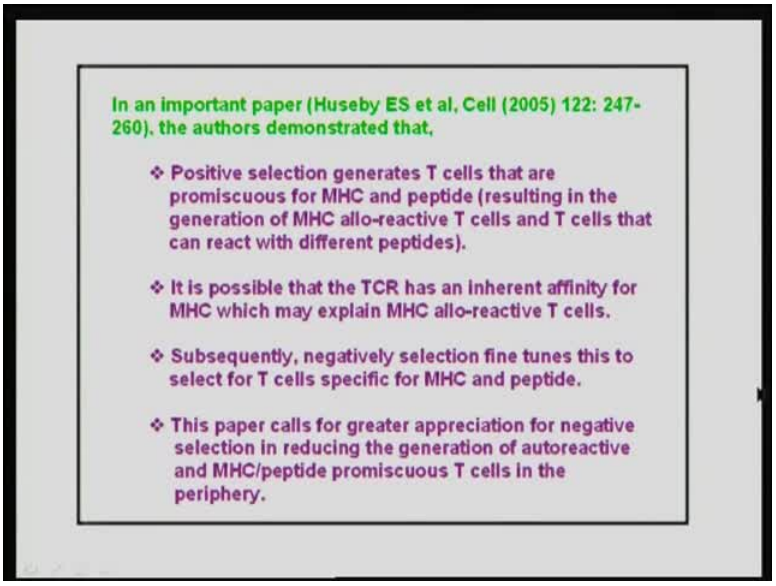

So, I just wanted to mention again, that in, you know, during positive selection, there are, there is promiscuous MHC peptide. So, ones that are selecting for these T cell receptor, they select for wide range of, of, of MHC molecules are selected.

Now, in this particular paper, that is what was shown, you know, the number, the thymocytes, that undergo positive selection, you have a wide range of them and they are able to recognize MHC with different affinity and you have different types of TCRs, but the ultimately, the ones that are going in the periphery, you, you do not have this wide range and so therefore, negative selection does take care to ensure, that you do not have all types of T cell receptors, that are entering into the, into the peripheral system.

And it calls this study, sort of, calls for a greater appreciation of negative selection in reducing the generation of auto-reactive T cells, that is one of the main roles of this processes. So, while positive selection will allow for, for, for a broader panel of T cell

receptors, finally you have negative selection, that sort of you know, that fine tunes this, this selection to ensure that those, that are highly, that bind self-MHC very strongly are not going into the periphery.

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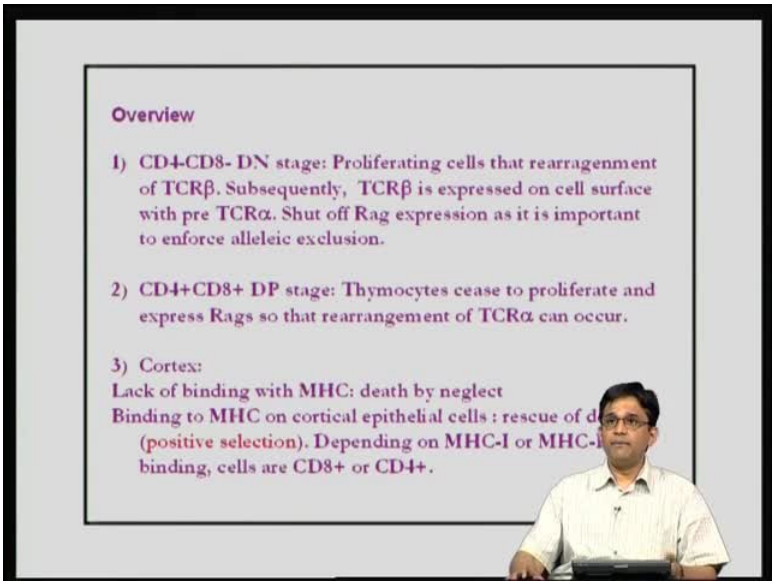

So, we will just briefly, again have a little bit overview on, on this part on, on, on the, on the thymic differentiation path. So, the first one is the fact, the double negatives, that is, the CD4 minus 8 minus double negative cells, these, these proliferate a lot and the proliferative cells, they rearrange their TCR beta and TCR beta is expressed on cell surface along with the pre-TCR alpha. So, this shuts off the Rag expression and as it is important to enforce allelic exclusion, because once you have the beta, you do not want other betas trying to rearrange. So, that, that, that is what is meant.

And then, this is expressed on the pre-TCR alpha, so and then, what this does is it goes on, allows the next stage, which is the double positive stage. And here, they cease to proliferate and their expression of Rag is turned on again, so that it allows, now for the TCR alpha chain to rearrange.

So, ultimately, you will have a proper TCR beta and alpha on the cell surface. Now, this is where, now selection comes in to play and in the thymic cortex, you have initially positive selection, where you are selecting these different TCR. You will need to find out, which of these TCR can bind to MHC. Now, select for those and now depending on

whether they bind to MHC-1 and MHC-2, they are going to be CD8 positive or CD4 positive.

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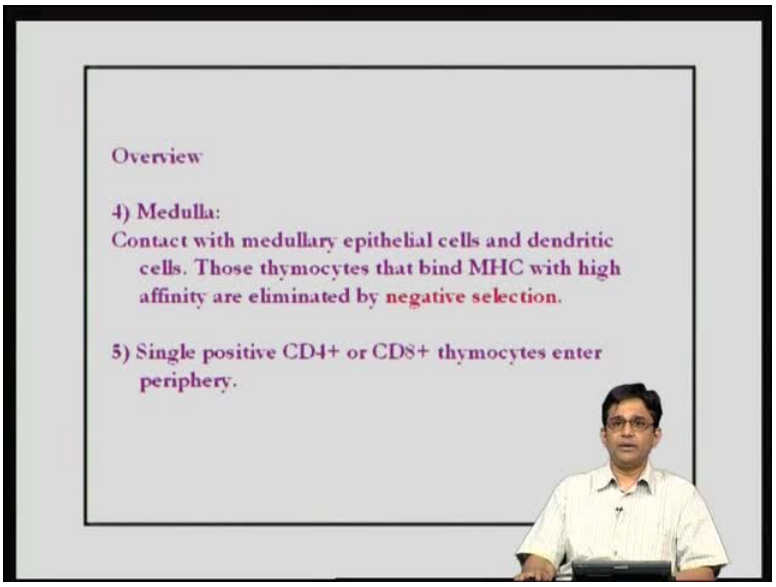

Then, finally, they go to the medulla and in the medulla they interact with medullary epithelial cells, dendritic cells and those that bind MHC with very high affinity are eliminated by negative selection and then subsequently, the single positive CD4s and CD8 thermocytes enter the periphery.

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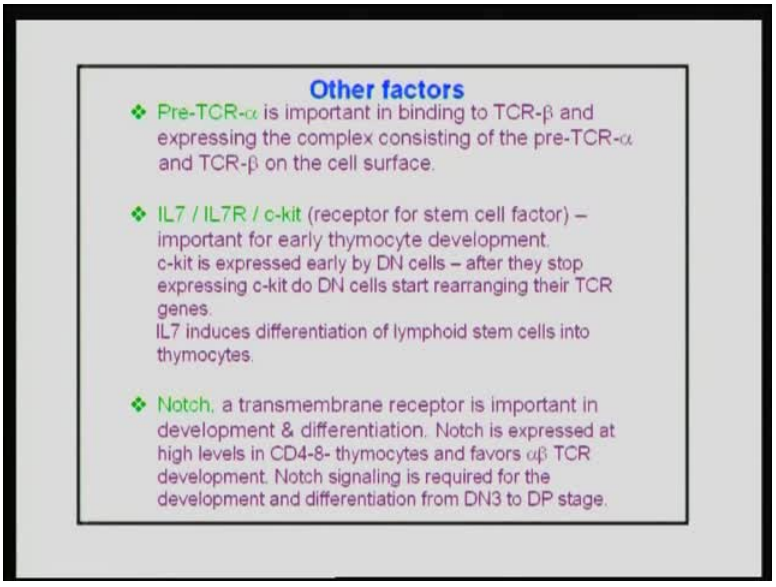

Now, apart from the CD4, the CD4 CD8 expression, double positives, single positives, positive selection, negative selection, there are other factors, that are also important in this process and we have, we have initially, understood the different processes of MHC of thymic differentiation, but we need to understand some other factors. One of the key ones, as was discussed, is the pre-TCR alpha, which is clearly very important and it is important for binding a TCR-beta and expressing it together in the initial T cell receptor.

And we have discussed the importance of it, you know, it ends at the, at the very end of the double negative, the kit, then it results in the double positive. It is important for stopping allelic, for other, the rearrangements of the other betas and it allows for the, the initial T (( )), which and then, this signally is important to stop these other events.

You have other molecules, they are important for example, c-kit, for example c kit is actually a receptor for stem cell, stem cell factor. It is expressed by early double negative cells, after they, after they stop expressing c-kit do double negative cells start expressing the TCR genes. Now, IL-7 is very important for thymocytes, induces differentiation of lymphoid stem cells into thymocytes.

A notch is a transmembrane receptor, which is very, which plays important roles in development and differentiation. Notch is expressed in very high levels in double negative thymocytes and it favors alpha-beta T cell receptor development and notch signaling is required for the development and differentiation from the double negative to the double positive stage.

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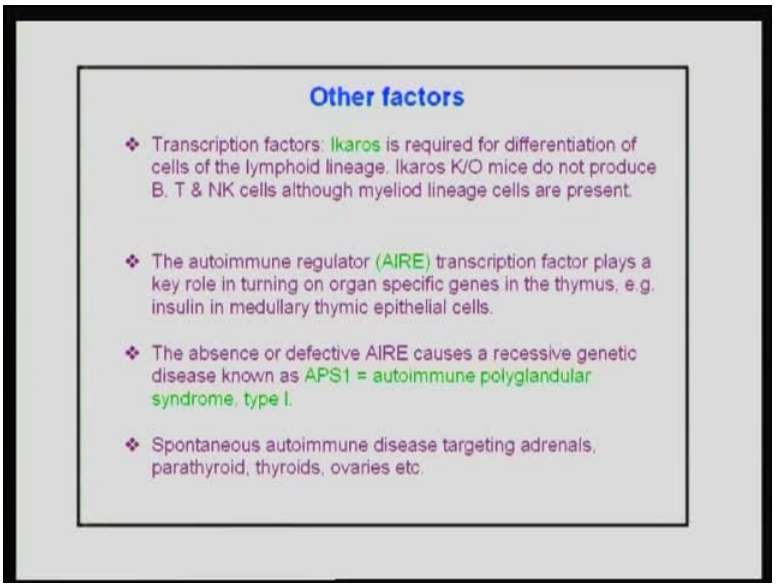

You have transcription factors also, that play an important role. I am sure there are much more and as time goes back by, we will be able to appreciate the roles of, of different molecules. And Ikaros, for example, is important for the differentiation of cells of the lymphoid lineage, and mice, that lack Ikaros, do not express B, T and NK cells, although myeloid cells are important.

Now, with respect to thymic differentiation, 2 molecules are, are extremely important and their importance has come into being in the past few years. The 1st one is a regulated, known as AIRE. Now, AIRE is an autoimmune regulator and, and it is a transcription factor. Now, it plays an important role because it allows for the expression of tissue specific genes in the thymus.

So, one of the big questions was, since the thymus expresses is important for self, for, for selection of cells, that do not see self-antigen, how is it, that you have all these different tissue specific antigens being expressed in the thymus? And over here, this is its importance because AIRE is important in the expression of tissue specific, for example, insulin is expressed by patriotic beta cell.

There is, but it is also found to be important because it is expressed in medullary thymic cells and in, and its expression is controlled by AIRE. So, as a result of which insulin T cell receptor, that you have to see, insulin will be negatively selected and so, you will not, you will be preventing these autoimmune TCRs from entering the periphery.

Now, the absence of AIRE causes a recessive genetic disease, known as APS1 or autoimmune polyglandular syndrome type 1. What happens over here, it is a spontaneous autoimmune disease and it targets different organ, for example, adrenals, parathyroid, thyroids, ovaries, etcetera.

So, AIRE place a very important role in, in, in preventing autoimmunity and it does so, because it allows for the expression of tissue specific antigen in the thymus. As a result of which those TCRs, that recognize these particular antigens, are eliminated and they are not allowed to seed the periphery.

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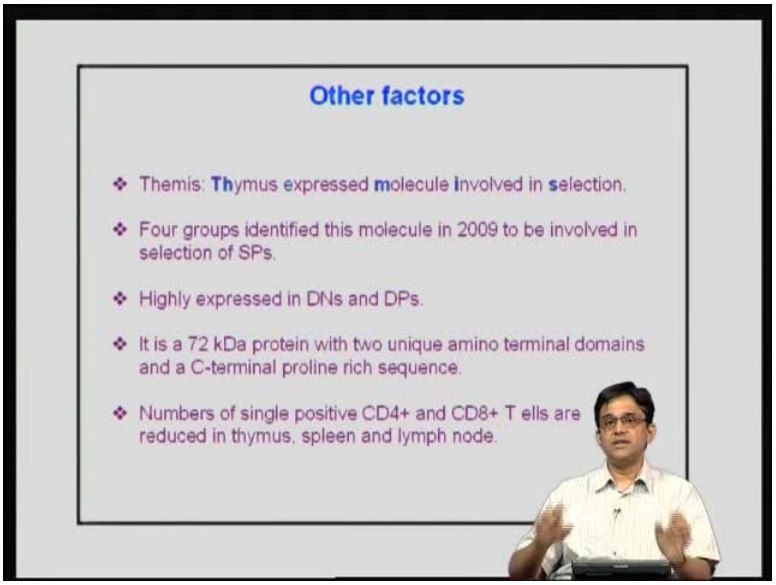

The other factor, AIRE is one important factor, the other factor is Themis. Now, Themis was discovered fairly recently in 2009. Now, it is, it is, Themis stands for Thymic expressed molecule involved in selection, and from here, you can see, these parts were taken in to make the name Themis. Now, it is highly expressed in, in double negative and double positive cells. It is important in selection of the single positives, so both in, in, in its absence, you have very few single positives or highly reduce single positive, which is CD4, CD8 positive, CD3 positive in the, in the thymus, as well as in the periphery, which is lymphoid and spleen.

So, it, so it does not prevent double positive, but it prevents this, the selection from double positives into the single positive, CD4 positive, CD8 positive.

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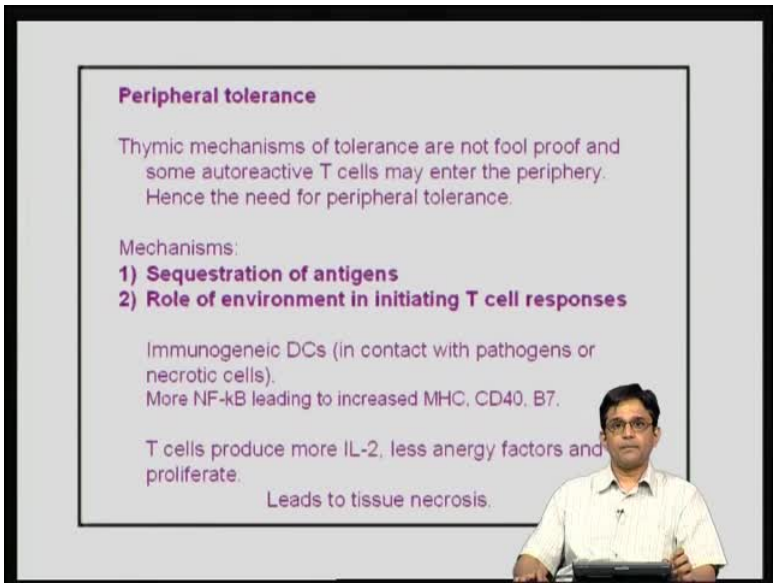

Now, we need to understand, that no matter how good our thymic selection processes are, that a few, few autoimmune cells are able to bypass and they are able to seed the periphery, and what is the evidence for it? The fact, that there are autoimmune diseases that are prevalent at large is evidence, that thymic selection is not fool proof.

But therefore, we need to understand, what are the processes by which tolerance occurs in the periphery? So, there are 2 types mainly, that the one that we discussed today was thymic tolerance, which is responsible for the major amounts of, of the prevention of autoimmune diseases and for particular selection of, of T cells, that recognize self-MHC.

So, so, thymic, thymic tolerance is important for that part because it allows for differentiation of T cells and not only differentiation, it allows for selection of T cells, that recognize self-MHC, and ones that do not recognize self-antigen. So, thymic part plays an important role, but the 2nd important role is peripheral tolerance. That means, you must, there must be some mechanism that sort of, takes care of peripheral tolerance because every time there is some cross reactive antigen, you do not want an autoimmune disease, but you know, by and large, these are, these are, these are controlled. So, we need to understand, what are the mechanisms involved in this.

The 1st one that is involved is sequestration of antigens. So, often, what happen is while T cells are, are, are using different parts of the body, some of the tissues, they are, they

are sequestration from these antigens, so, they do not come into contact with these antigens.

So, as a result of which, they do not see these antigens, so they are not able to see, for example, eyelids; eyelids is sequester from it. However, if there is damage to the eye, then T cells would be coming into contact with it and then we would have a, a, a reaction, but otherwise, they are sequestration tissues; sequestration plays an important role in this.

The 2nd is and I have tried to emphasize, this is the role of environment in initiating T cell responses, and there are 2 types over here and especially the role of APCs. So, the 1st is the role of immunogenic dendritic cells. Now, now once you have the dendritic cells, which are in contact with pathogens or necrotic cells, these APCs or necrosis presenting cells, there is more NF-kappa B activation in these, which leads in increased MHC, CD40 for the coast MHC ligands B7.

As a result of which, T cells now produce more IL-2, there are less anergy factors and they proliferate. And when this happens, it leads to tissue necrosis because what you are happening is you are turning on the T cell pathway and then, it leads on to a T cell response. So, this is an immune gene dendritic cell, which lead to T cell response and which is what you want in terms of, when pathogens are, are coming in contact with the host and so on.

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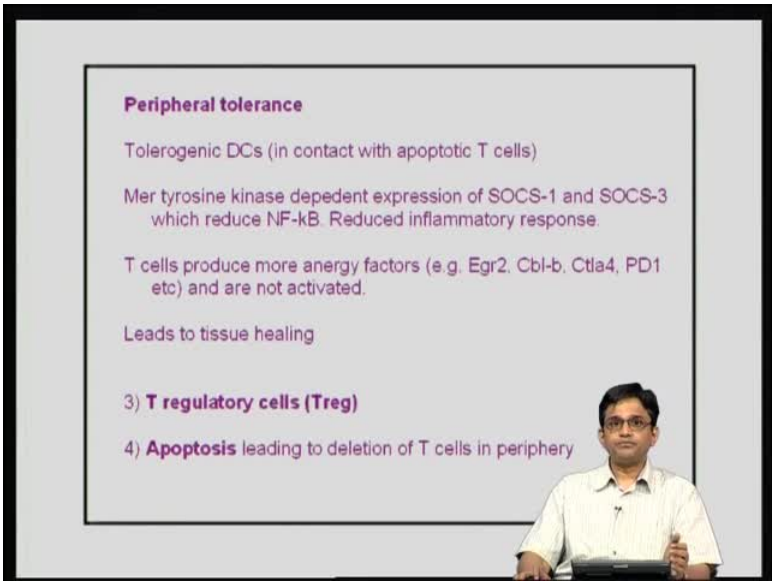

The other part comes in when they are in contact with tolerogenic dendritic cells. Now, tolerogenic dendritic cells often, are in contact with apoptotic cells and these, because of apoptotic cells you have the activation of the Mer tyrosine kinase pathway and this results in expression of molecules, known as SOCS-1 and SOCS-3. These are initially, stands for suppressor of cytokine signaling-1, 2. These genes, sort of, they bind to certain kinase, they are inhibited, they are cellular inhibitors of these and they inhibit the activation. And these result in reduction of NF-kappa B and you have reduced inflammatory scenario.

Now, T cells, when they come in contact with these tolerogenic D cell disease, they produce more energic factors, for example Egr2, Cbl-b, CTLA4, PD1 and as result of which they are not activated and this leads to tissue healing. A good evidence of this is actually in the thymus, where you have a lot of, a lot of death occurring, lot of the, most of the majority with thymocytes are dying and they are, sort of, taken away by, by, and they are phagocytosed and taken away. These apoptosis cells of phagocytes are taken away, it is an excellent case, but there is no inflammatory scenario over here and it is, it is, it is an example, it is an excellent example of good mechanism. So, this is part of it, that is, that may be occurring also.

The other mechanisms are regulatory T cells and these regulatory T cells are important because they suppress T cell activation, they suppress autoimmunity and this is something that we will be discussing in subsequent class. The other mechanism is apoptosis, which leads to deletion of T cells in the, in the periphery and this is again something, that will be discussing in subsequent classes.

So, it is very important for students to be able to appreciate the different types of tolerance, one is thyme tolerance. The thymic tolerance is primary form of tolerance, but however, some aberrant T cells may seed the periphery and you must have mechanism by which you take care of, of peripheral T cell tolerance and these are the different mechanisms by which it is, by which it is taking care of, so you have, you need to have done in, redone in mechanisms, that take care of this.

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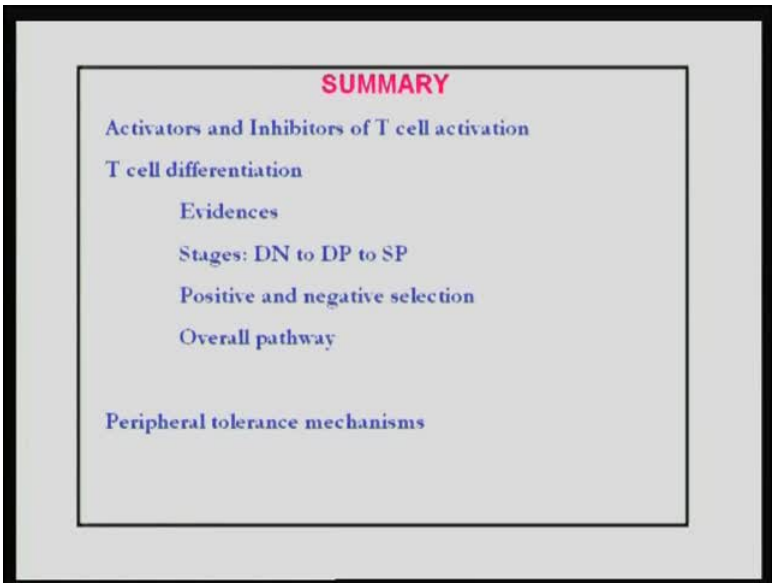

So, I will summarize this part of, of the class. So, what initially, what we had discussed were activators and inhibitors of T cell activation and this is actually in context with what happens in, sort of, in view of scenario. Let us say, you have pathogens attacking a TLR, TLRs, TLRs pathways being activated. The TLR pathways will activate the APCs, which will generate a greater T cell activation and what was shown is the TLR ligands, LPS, CpG. They activate primarily Th1 response and there was the main, main part that was, that I tried to illustrate.

The others are inhibitors of T cell activation. These are very important because in terms of transplants and all, you want to inhibit or lower T cell responses and that is why, it is important. And we discussed some inhibitors of, so we discussed cyclosporine, but there are many other mechanisms (( )) they are belonging to the same family by which they bind to calcineurin phosphatase and they inhibit T cell activation.

But there are other pathways we talked about. Rapamycin and its role in binding to the mammalian, talk it of rapamycin and again inhibiting T cell activation. We talked about glucocorticoids, which suppress T cell and glucocorticoids are important, because in terms of allergies and, and in terms of arthritis, often doctors prescribe glucocorticoids, corticoids, these are steroid treatments to reduce the T cell activation.

We also talked about another class, which is the methotrexates, which will result in low number of tetrahydrofolate, which will result in reduced number of nucleotides, which are important for DNA since the.., so there are different ways by this functions.

In terms of newer molecules, you have anti-CD3 used in transplants. It again, these have side effects, but these are important; anti-CD25, remember CD25 is a part of T cell receptor. CD25 rapidly increases the T cell activation to form the high affinity IL-2 receptors and antibodies. Again, this have been also used to lower T cell responses and CD25 was known as the tac antigen, so this is known as the anti-tac, TAC, mediated therapy.

Then, we subsequently discussed the T cell, T cell differentiation; we talked about the some evidences of it. The best evidence is the nude mice with mutation and foxine, which results in transcription factor, which plays an important role in the differentiation and proper functioning of hair follicles and in the, in the thymus, as a result of which, they lack a thymus, lack T cells, also lack hair.

We talked about the different stages of thymus differentiation from the double negative, which is CD4 minus 8 minus to double positive 4 plus 8 plus to the single positives 4 plus and 8 plus and these express high levels of CD3 and they seed the periphery.

We talked about the mechanisms over here, positive and negative selection. You want to positively select for self-MHC and you want to negatively select, you do not want to cells, that recognize or vary. They bind to your self-MHC with very high affinity.

And we talked about the overall pathway and the evidences, both positive and negative selection in particular. I will again emphasize the use of the HY TCR transgenic mice, which if you properly breeded with either the different types of MHC molecules or male female, which illustrates positive and negative selection; very nice evidences of it. There are other evidences too, that were, that were discussed.

Finally, we come to peripheral tolerance mechanisms. Now, despite the great role of the thymus, there are some, some autoimmune T cell receptors, that might go out and the periphery has taken care of it by different mechanisms. One is tissue sequestration, the 2nd is, the T cell activation is done in the context and if you do it in the context of pathogens, you will initiate a T cell response. If you do it in the context of tolerogenic,

tolerogenic dendritic cells, you suppress that T cell responses, you have the role of T Rags and finally, you have the role of apoptosis. The Fas-fasL pathway and there are other pathways, the icon PD-1, which you will discuss. There are also roles for in cytokines and that is something we will be discussing in subsequent classes.

Thank you.