Essentials in Immunology

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Lecture No. # 28

T cell synapse, motility and subsets

So, in today's class we will be studying actually different aspects of T cell responses following activation, and in particular, we will focus on T cell synapse, motility and subsets, so that is what this class is about, and hopefully, we will start off with some aspects of T cell activation which will go on to T cell motility migration, and then subsequently subsets, and these are going to be important, because they are very important in terms of regulation of T cell responses.

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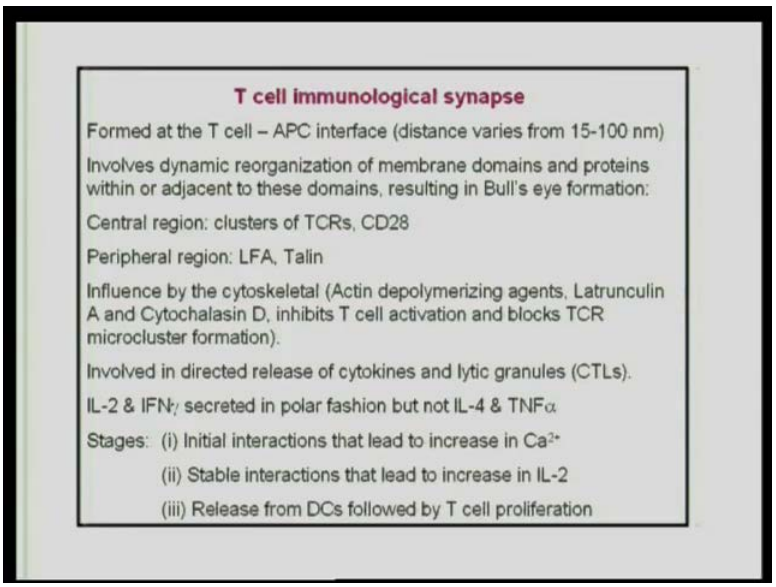

So, if we go to the part on a T cell activation, we see that the T cell APC junction is the most important part, and now because that is the site, where the T cell receptors bind MHC, what is interesting to note over here is that, the T cell receptors from all the other parts also coalesce to form micro clusters; this is a very important principle, you see, so what is happening is cell surface receptors are sort of migrating.

And the coalesce set 1 point and that point, and these are different clusters, that they form, and this is known as the immunological synapse, and the T cell receptors along with certain cell surface molecules, they form in fact the organization is quite clear, and they bind to the MHC molecules on the APC; so, it is a very important aspect. Now, what is interesting is that, the T cell APC interface the distance varies from 15 to 100 nanometers, that means, in some parts, you know, they are very close and in some parts they are not so close, and a diagrammatic representation of this, we will see in the subsequent slides. Now, what the cluster involves a central region, and this in the central region, you have the T cell receptors CD28, CD4, CD8, so on, and in the peripheral region, the peripheral region you have LFA Talin which is important and recruiting the integrin members.

And so you can see that, because you have all these migration of cell surface receptors into these clusters, obviously it involves cytoskeletal components, and actin cytoskeletal actin, and both tubulin are important in this process; so, in case you inhibit actin polymerization, for example, if you have these agents that will inhibit actin de-polymerization, you have inhibition of T cell activation, and you block this micro cluster formation.

Now, why is this important, now the question then is, whether this T cell, APC there is a direction to this, and what are the consequences of it in terms of CTL, lysis, it is quite clear, because the cytotoxic T lymphocytes need to kill the targets, and the targets are over here, so if you have formation the toxic granules, the (( )), the granzyme, and all are (( )) towards your target cell, now (( )), what is interesting is, it is observed that (( )) is released in a polar manner, that is towards the APC, (( )) Il4 and TNF, which means, they are not released in a particular direction or manner, they are released everywhere.

And of course, this interaction between the T cell and APC involves initial stable interactions, that lead to increase in calcium; these will sort of lead to stable interactions that lead to increase in IL2, because that is, ones the T cell is getting activated the program is on, you also need a sustain calcium for that, and that is something, that we had studied in the previous class on T cell activation; subsequently, there is release followed by T cell proliferation.

And these newly divided T cells can go on and bind to other dendritic cells, and then get activated, and this process can continue on. So, the T cell immunological synapse is a very important aspect, and one of the newer, this has been the use of imaging; so, imaging, (( )) really lead to, you know, local connection and documentation of the different regions, that are involved in this formation, and subsequent information on T cell motility, how T cells move within compartments.

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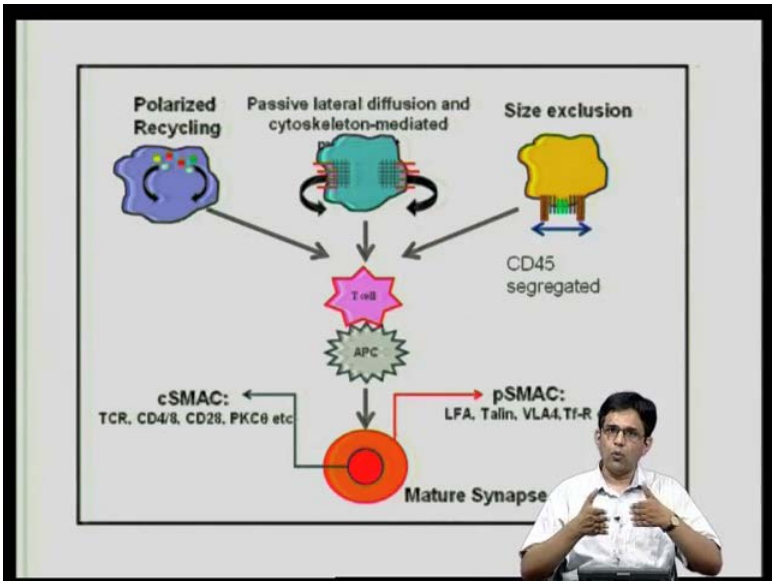

Now, how is this formation done; now, we are talked about this is the T cell, and this is the APC, and here you can see, this is what I talked about this is the central supramolecular activation cluster and this consists of TCR, CD4, CD8, CD28, PKC delta, which is the important PKC involved in T cell activation; so, the central part is over here, and the peripheral ones contain LFA Talin, a very late antigen, the transfer in receptor etcetera.

So, this is what we are seen is actually the mature synapse, this is, where there is reorganization as I mentioned of cell surface receptors, and they form different clusters. So, it is not a random generation of clusters, it is a ordered formation, because all the T cell receptors coalesce over here as is shown over here; now, how does this happen. Now, what is shown over here is you see polarized recycling, that means, you know, you have different processes that are involved over here, you can see these molecules are sort of all coming together over here.

You also have lateral diffusion and cytoskeletal mediated changes, and then, you have size exclusion, for example, molecules that are larger in size, for example, CD45, they are excluded over here, you will recall that CD45 is a phosphatase, and you know, it is primary job is to inhibit T cell activation; so, once you want to initiate T cell activation, it might be a good idea to have the phosphatase away, and size exclusion is used; so, you have different processes that are involved, and that is sort of come here to form this T cell synapse a very important part of T cell activation.

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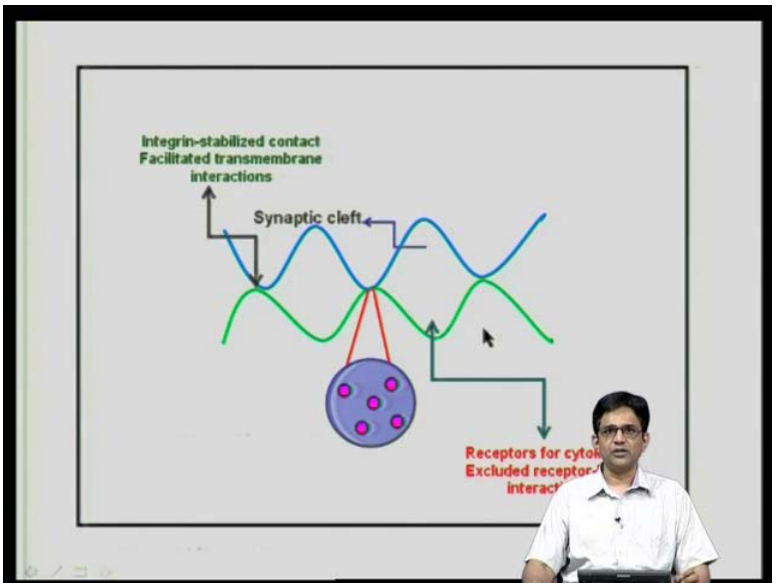

This is a closer detail of what is shown, the T cell synapse is very much like the synaptic cleft found in neuronal cells, and what is shown over here, this is the APC, and this the T cell, and you can see these interactions, so these interactions are very close the membrane are very closely linked over here, and whereas in others, you know, they are quite far off, so the difference between the APC over here, and the T cell over here, there is a big difference, and what is also shown over here is that, this part is known as the synaptic cleft.

And this is the localization, and here you can see a visual representation of the micro clusters that are formed; now, there are some important aspects that need to be kept in mind; first is this integrin mediated stabilization, this part is integrin mediated, so integrins obviously plays an important role in the previous slide; you had seen LFA being part of the peripheral cluster, the VLA, and so on.

And then over here, you have receptors for cytokines, and may be the other molecules that are excluded from these micro cluster formations; so, this is a sort of a diagrammatic representation, it gives you an idea of what the APC membrane would look like what the T cell membrane would lo like at the region of forming the synapse.

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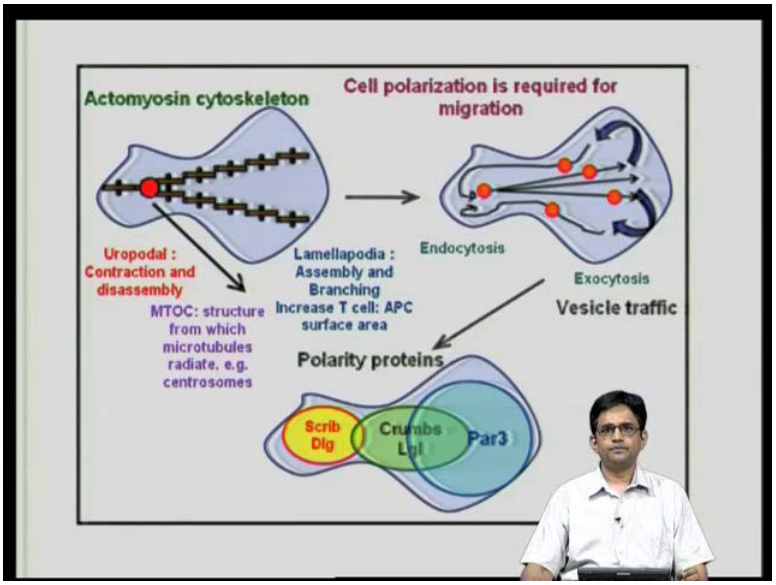

Now, in terms of activated T cells, T cells you have a leading edge that is shown over here, and a lagging edge, and the lagging edge is actually the uropod which is shown over here, and this leading edge is characterized by what is known as the lamellapodia, and the lamellapodia, you can see this broad surface area, and you have the actin, the lamellapodium is supported actually by a network of actin fibers over here, and what this allows for a broad surface contact with the APC.

So, you are maximizing the surface contact, maximizing the chances of your clusters to come together, and to result in T cell activation, what is interesting to note over here at the distill end of the leading edge, you have the lagging edge which is known as the uropod the structure that is shown over here is the microtubule organizing cluster which is important or this is the structure from which microtubules radiate, for example, centrosomes and all.

So, that is what is shown over here, and over here what is shown is vesicle traffic, you can see that vesicle traffic, they come in, and they go around, and they come back; so, that is what the arrows are showing, what is also interesting is that, in these T cells, you

have a distribution (( )) proteins, so for example part 3 is found more towards the leading edge over the lamellapodia compared to the uropod, and whereas scrib and dgl are more towards the uropod, then the leading edge or the lamellapodia; so, you can see that there are distinct structures, that that play an important role, there is a distinct organization of these T cells, and you have these different protein found in different locations which play an important role in this process.

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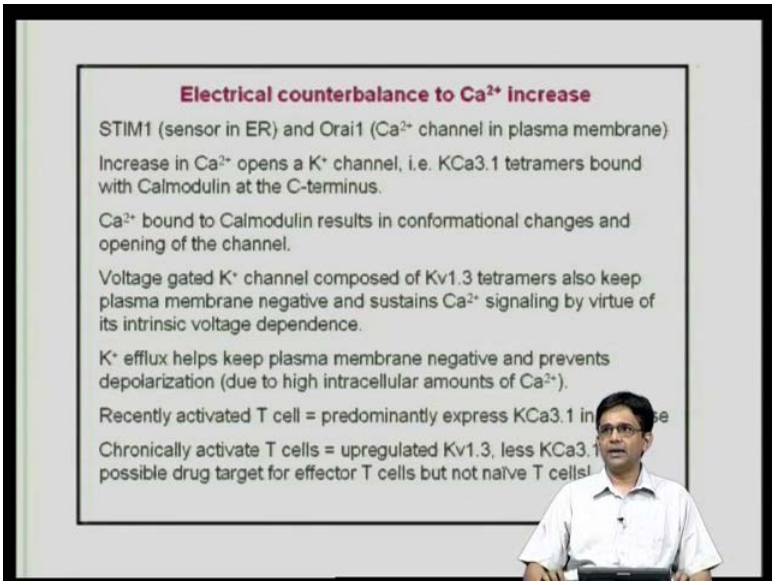

We will start off with T cell activation, and you know, we had shown that the calcium plays a very important role in T cell activation, actually once the TCR, MHC comes together, one calcium, intracellular levels of calcium increase very quickly, and so, initially, you have the calcium stores in the ER releasing calcium to give this burst, and then, subsequently you have calcium levels need to be maintained, because they (( )) calcium from outside.

Now, how is this done we had discussed the role of two molecules, one is the STIM which is the sensor in the ER, and the Orai1 which is the calcium channel in the plasma membrane, and once the calcium levels start dropping, that is sensed by STIM, and STIM goes to the plasma membrane, and activates the Orai1 calcium channel, so that calcium, now from outside can sort of come in; now, you can understand, that if you have too much calcium, it would lead to a positive charge in the cell, because you have a vast increase in the net positive charge; so, this has to be counter balanced.

So, for the increase in calcium CA plus 2, you would need to throw out some plus irons; so that, you maintained the electrolyte balance, and the iron, that is thrown out is potassium; now, how is this done and this is an important aspect. So, what happens is as calcium comes in, the increase in calcium leads to opening of a calcium channel which is known as KCa3.1, and what happens over here, these are tetramers that are bound to calmodulin in the C-terminus.

Now, calcium bound to calmodulin results in a conformational change, and opening of the channel, so that you know, as calcium levels increase, you have potassium being thrown out; now, apart from KCa3.1, you have another calcium channel known as the Kv1.3, which also plays an important role; now, what is important to note over here is that the recently activated T cells predominantly express KCa3.1, whereas the chronically activated ones show higher levels of Kv1.3; so, this is an important aspect, because especially in terms of chronic activated disease like arthritis and all, where you have these chronically activated cells, you can target molecules for Kv1.3, so in which case, what you will be doing is your targeting, and (( )), T cells (( ));so, you are trying to shut down these chronically activated cells, but you do not affect the naïve T cells which would express primarily KCa3.1; so, this is an important aspect, a very important things for students to understand in terms of targets and modulating D-cell responses.

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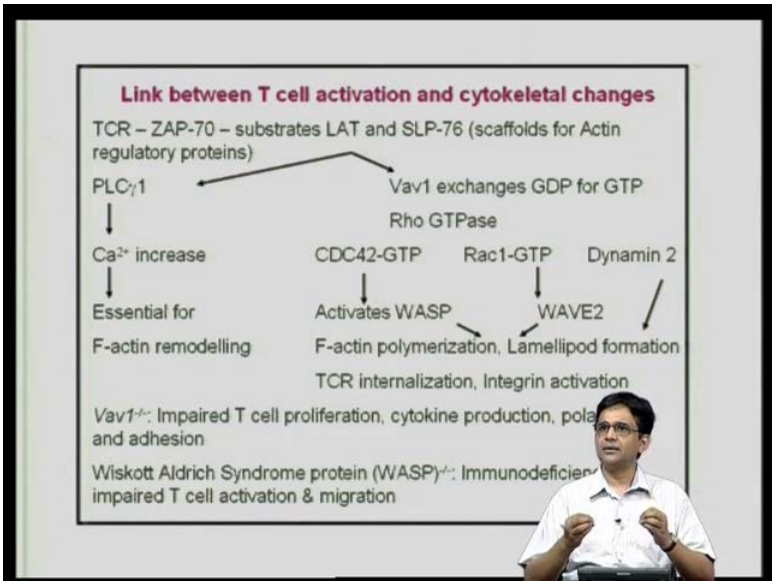

There is also a link between T cell activation and cytoskeletal changes, we are now coming back from calcium, now we are coming back into the cytoskeletal changes, we had discussed on the T cell receptor activating the zeta activated protein 70, which is a tyrosine kinase, and which phosphorylates the linker activator in T cells, and the SLP-76; now, LAT and SLP-76 are scaffolds, and they recruit several other proteins among the proteins, they recruit are the phospholipase gamma 1 which results in increase in calcium.

And this is important for the filament actin remodeling; also what is this, what the, these molecules do is they activate Vav1 which is the G-protein exchange factor. Now, G-protein exchange factor is important activating CDC42-GTP, which activates the WASP and WASP is the Wiskott Aldrich syndrome protein, which is important in terms of actin polymerization, it also activates Rac1-GTP, which activates WAVE2; now, WAVE2 is a molecule that is related to the WASP family of proteins.

WAVE also activates a Dynamin2, and all together, these are important in terms of cytoskeletal changes in F-actin polymerization, Lamellipod formation TCR internalization integrin activation, no wonder cytoskeletal changes effect T cell activation, and finer results of this have been shown in the Vav1 knockout mice, where you have impaired T cell proliferation cytokine production, polarity is effected, and adhesion also in the WASP knockout, you have immuno deficiency, and impaired T cell activation and migration. So, these are evidences a of mechanistic links between the T cell activation, and the link to the cytoskeletal changes, and also the consequences or what happens if these are compromised.

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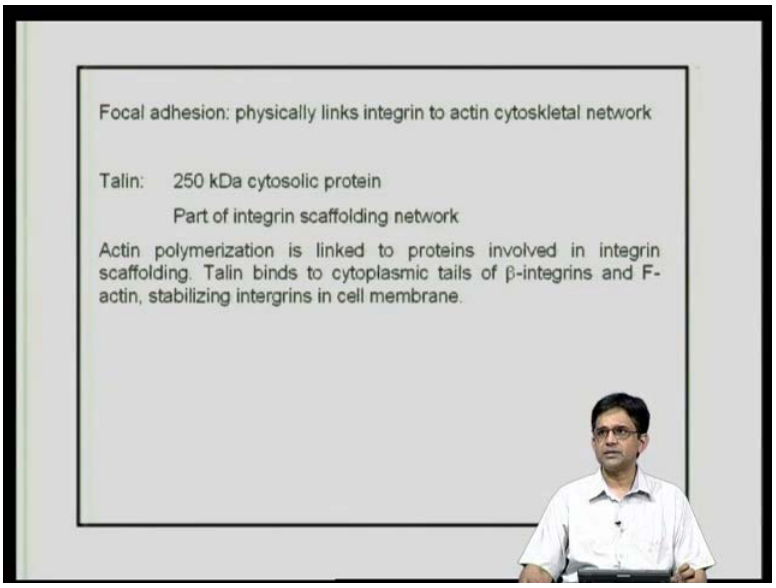

Over here as I mentioned that focal adhesion is physically links the integrins to the cytoskeletal actin network, and Talin which was present in the peripheral smac, if you remember a few slides earlier on, and this is a part of integrin scaffolding network, and so it holds on to the integrin, so basically what you are doing is, you are stabilizing the structure in which T cell receptors and other molecules are important.

So, overall immunity of interactions is increased, and because you need certain amount for T cells to be activated every small tickle, does not result in T cell activation, it needs other molecules to be in there, and to hold the membrane together to have proper formation on the immune synapse.

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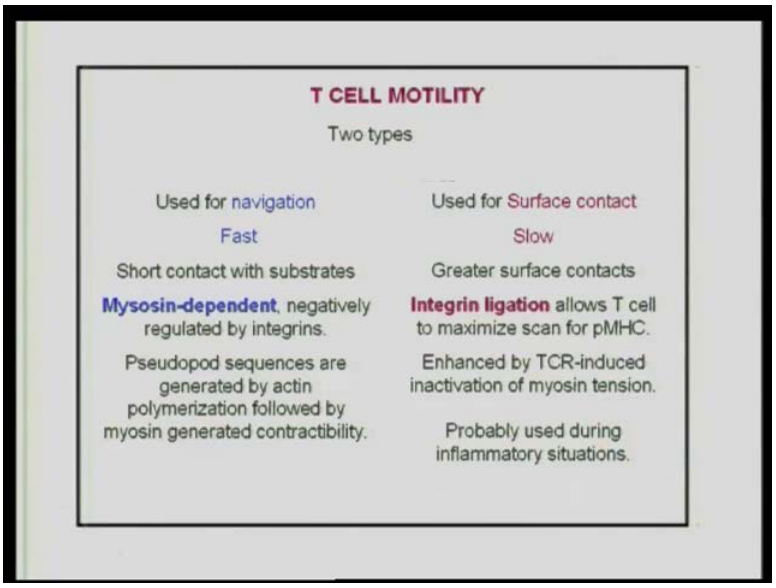

Now, in terms of T cell, motility T cells are motile, and there are two types of motility changes that are observed, one that is fast, now T cells move around trying to look for places, where maybe there cognate MHC is, but they do not want to waste a whole lot of time, and so, one is a fast movement, and this fast movement is usually myosin dependent, and in fact, it is negatively regulated by integrin, and what happens over here is a pseudopod sequences are generated by actin polymerization, and it is followed by myosin generated contractibility.

So, the fast ones are some myosin dependent, whereas the slower ones are important over here, because now there is some possibility that the T cell receptor might get activated might find its cognate, and then get activated; so, therefore you need a slower contact, and it needs to be able to look at it carefully, and this is integrin mediated, and integrin ligation plays a very important role, and this probably is important during inflammatory situations, and whereas in vivo, in general for perusing through different structures maybe the myosin dependent is what is playing an important role.

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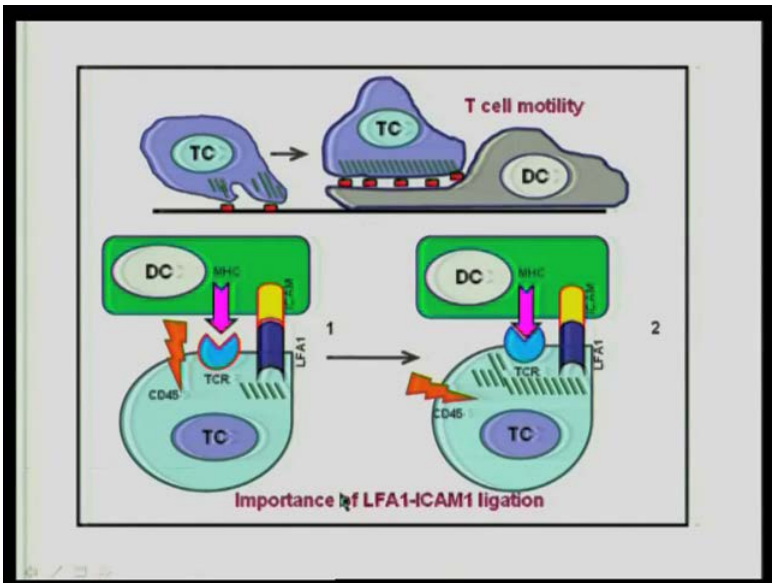

And this actually shows you, so the fast movement is depicted over here; so, in fact, over here it looks as, if the T cells are walking, you know very very quickly, and then as it meets a dendritic cell, it slows down, and you can see the surface area has increased dramatically, that is the lamellapodia has increased dramatically, because, and you can see these clusters, where you know chances of formation, and this immunological contact points are established over here.

And so, while this would be myosin dependent, this would be more integrin dependent, and that point is sort of shown over here in a more clear cut manner, and what is shown over here is what LFA does, this LFA ICAM binding sort of tethers it, and then allows for better MHC TCR interaction, and what is also shown over here is because of that CD45, the phosphate is excluded from these sort of complexes, and so that T cell activation can initiate, you can see this is the actin cytoskeleton are the basement of the lamellapodia formation that is shown.

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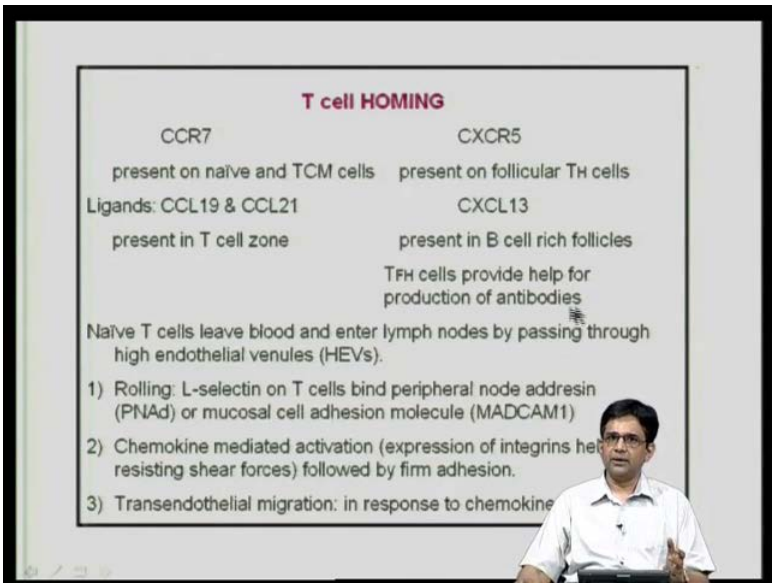

The other aspect is now T cell homing; now, in terms of T cell homing, there are two main kinds, one that is CCR7 dependent, the other is CXCR5 dependent; now, the CXCR5 dependent is rather specialized, it is present on what is known as follicular T helper cells, and CXCR binds to its ligand CXCL13, and this is present in B cell rich follicles, and so follicular T cells are very important for providing help for the production of antibodies; so, this is a little specialized the CXCR, CXCL13 is somewhat specialized.

And that is something that we will be studying a little bit later, because follicular T cells are extremely important, but they are specialized, so the one that we will be studying primarily is the CCR7, and now what is CCR7 is present on naïve, and a population of cells known as the central memory cells; now, CCR7 binds to 2 ligands CCL19, and CCL21 which is present in a T cell zone; now, what usually happens is naïve T cells, they leave blood, and enter lymph nodes by passing through a high endothelial venules.

So, what are these high HEVs are structures in which the T cells are or lymphocytes are floating around in blood, how do they enter lymph nodes; they enter lymph node through these HEVs, and how does that happen, because initially you have rolling these cells roll, and what happens over here L-selection on T cells binds to these peripheral node addresin or the mucosal cell adhesion molecules on the HEVs, and once you have this binding, this is a sort of a sign, and then, you have chemokine activated mediated

activation, you have expression of integrins to that resist the shear forces, and this is followed by firm adhesion.

Subsequently, you have trans endothelial migration, so that the T cells can now enter the lymph nodes to the HEVs; so that, that is what helps them, and over here clearly, CCR7 plays a very important role in this process; the reason why the CXCR positive T cells enter into these B cell rich follicles is, because the CXCL13 is present in the B cells; so, these cells with CXCR5 are attracted to it, because of the presence of the particular ligand, so and over here chemokine gradients play a very important role in this process.

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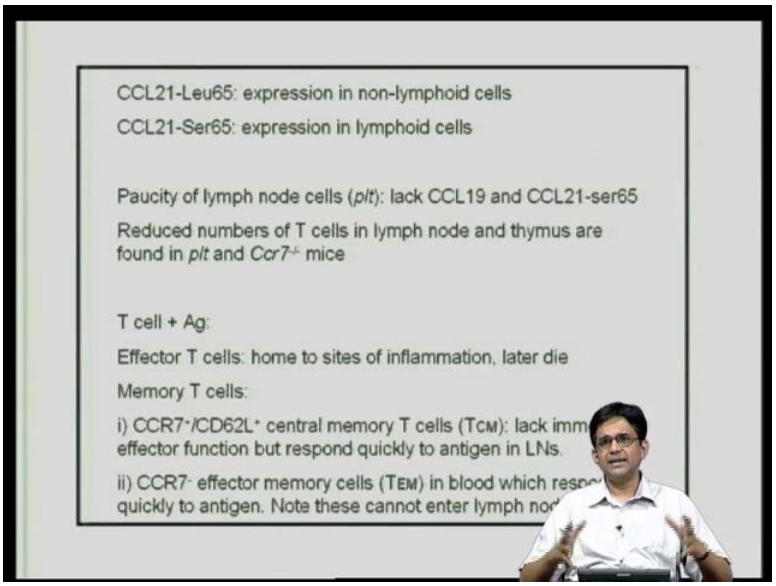

Now, a little bit more about the CCR7 ligands, there are as I mentioned CCL19, but in terms of CCL21, there are actually two different types, one that has lucien in position 65, and this one is expressed in non-lymphoid cells, and CCL21 with serine 65, which is expressed in lymphoid cells; now, what is interesting is there are mutants, in fact, there is natural mutant spontaneous mutant, that was isolated, and the name of it is interesting,

because it has possessity of lymph node C-cell, and these lack CCL19, and the CCL21-Ser65, because remember this is where it is expressed in lymphoid cells; in fact, there is a deletion as a result of which these CCL19 and CCL21-Ser65 are not expressed, and you write from the name of the mutant which is possessity of the lymph node cell at the time the mutant was generated, they had no idea of you know which gene is responsible or which gene or genes are responsible for it, but the phenotype was apparent.

You have possessity of lymph node T cells, why is they are possessity of lymph node T cells, it is not that the T cells or not the T cells are there, but they do not enter the lymph nodes, because the ligands that are necessary for attracting these T cells to the lymph nodes is missing. Similarly, you have CCR7 knockout mice, in which you have reduced number of T cells in lymph node, and thymus, because either you lack the receptor or the ligand, or so they are not able to come in.

So, in terms of entering lymph nodes or thymus or like pears patches, where in which entry is dependent on the high endothelial venules; if you do not have the either the ligand or the receptor, then you will have the less number of these cells in the organs, and this sort of demonstrates the importance of these molecules in a T cell homing and function; so, that is why I thought, I would sort of bring this, in, so at least students are sort of get necessary information on this.

Now, a little bit about the T cells, once you have the T cell that activated with antigen these effected T cells, they home on to the sites of information, they do what they are supposed to do, and they subsequently die, and that is a process of activation followed by death; so, that you can regulate T cell numbers, but however a small population of T cells do remain, and you have, in fact, two types of memory T cells, once that are CCR7 positive, and these are known as the central memory T cells, these are present in lymph nodes.

And while they do not have immediate effecter function, but they respond quickly to antigen, once the antigen comes into the lymph nodes; so, the other population you have is the CCR negative effector memory T cells or Tems, and which are present in blood, and which respond quickly to antigens; so, basically, there are two different types of memory T cells, ones that are in the periphery in the blood, with which can respond quickly to antigen, but these lack CCR7, that means, they cannot enter the lymph nodes, and by not having these CCR7, they are excluded away from the lymph nodes, where you also have another group of memory T cells, in which have CCR7 are present in lymph nodes, and they respond to antigen. Once, they come to the lymph node, why do you think, we have this, and you in fact, if you see the immune system is full of redundant mechanisms, that is because if the one mechanism does not work, you need a backup mechanism, and the immune system is excellent at this, because I guess it has evolved that way, because it is not clear how the antigens are going to evolve, and so it is

best for us to have many redundant mechanisms to take care and protect to the host to the best possible ability.

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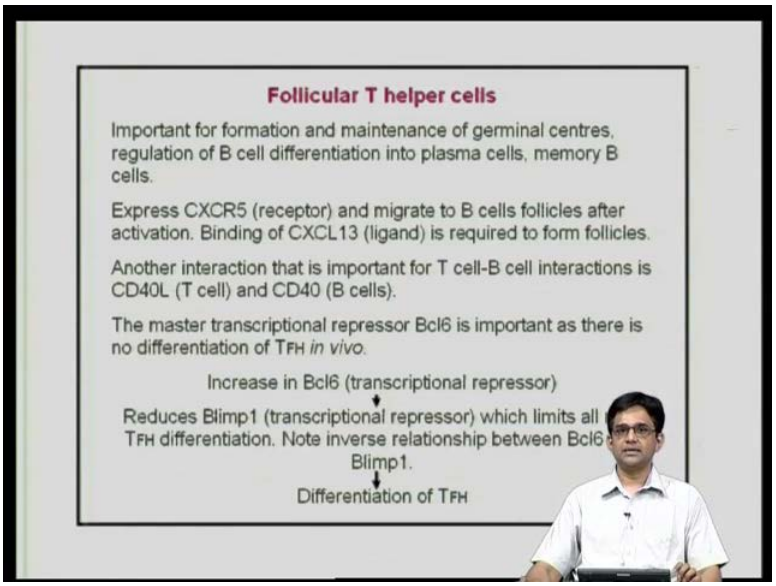

So, we will start of a little bit on follicular T cells, now I mentioned that we would deal with this a little bit, before the characteristics of follicular T cells is that, they are important in germinal center formation, and the very important in increasing the antibody production by B cells; now, how does one distinguish follicular T helper cells, they are distinguished by the expression of the CXCR5, and what happens is they migrate into the B cell rich follicles.

And they do so, because they are attracted to it, because of the presence of the CXCL13 which is the ligand for the CXCR5 receptors; I said chemokine gradients play an important role in this, another set that is important or molecule receptor set that is important for T cell B cell interactions, where lead to germinal center formation is your CD40, which is CD40 ligand which is present on T cells, and CD40, which is present on B cell.

Now, coming back to follicular T helper cells distinguishing transcription factor for follicular T cells is the presence of Bcl6, and what Bcl6, in fact, there is no Bcl6, there is no differentiation of follicular T cells in vivo. What is important to understand is that, Bcl6 expression results in reduction in a Blimp1, which is a transcriptional repressor;

now, the absence of Blimp1 ensures that these cells will differentiate only as follicular T helper; in fact, there is inverse relationship between Bcl6 and Blimp1.

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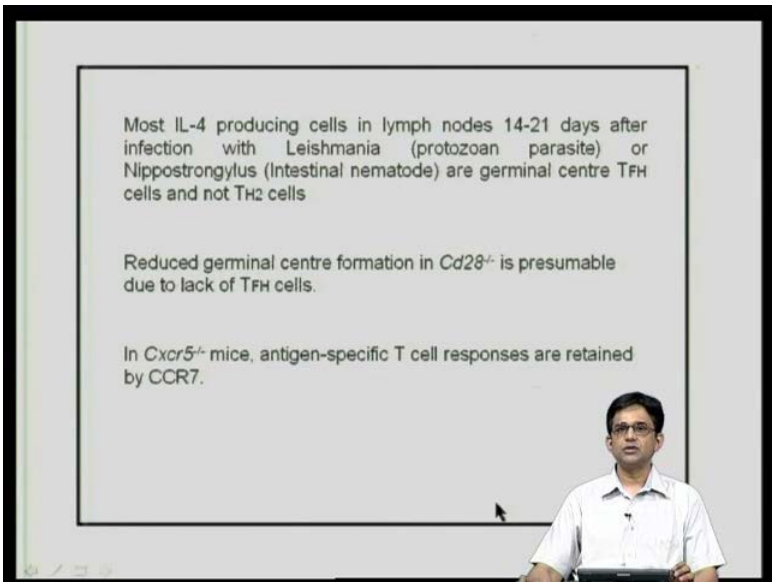

And so, ultimately leading to differentiation of these follicular T cells, so where are these follicular T cells playing an important role? In fact, most IL4 producing cells in lymph nodes after infection with Leishmania or the intestinal nematode Nippostrongylus are germinal center T follicular cells, and not in fact, your T helper 2 cells, germinal center formation in CD28 knockout mice is presumably, due to lack of T follicular helper cells in CXCR5 knockout mice antigen specific responses are present, and they are done by CCR7.

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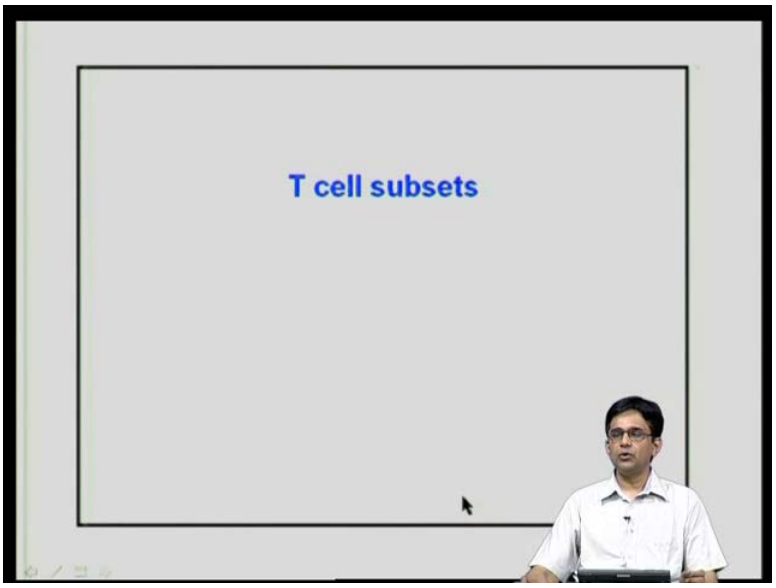

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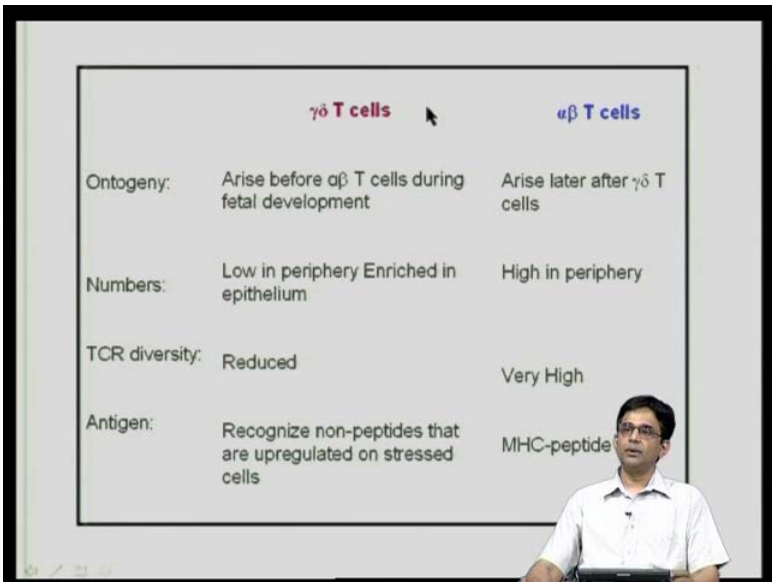

Now, we will talk about some other T cell subsets, and we will first start of, now though in general, when we talk about T cells, we usually are referring to the alpha beta T cells, now, but you have learnt, that there are two main types of TCR receptors, you have the alpha beta, and then you have the gamma delta. The alpha betas are the ones, they are predominant, they arise much later after the gamma deltas, they are the numbers are much higher in the periphery, their diversity in terms of TCR usage is very high and they recognize MHC peptides.

So, the gamma delta cells, they arise early on, remember ontogeny recapitulate phylogeny, and this case it appears to be showing signs of that, they are present the numbers are somewhat low in the periphery, but they are enriched in the epithelium; so, they probably have some role in protecting epithelial surfaces, that something we will, we discussing. The TCR diversity is reduced, they do not have as big TCR diversity as the alpha betas, and they recognize non-peptides that are up-regulated on stressed cells; in fact, they recognize different kinds of cells and their functions may be consequence of different aspects.

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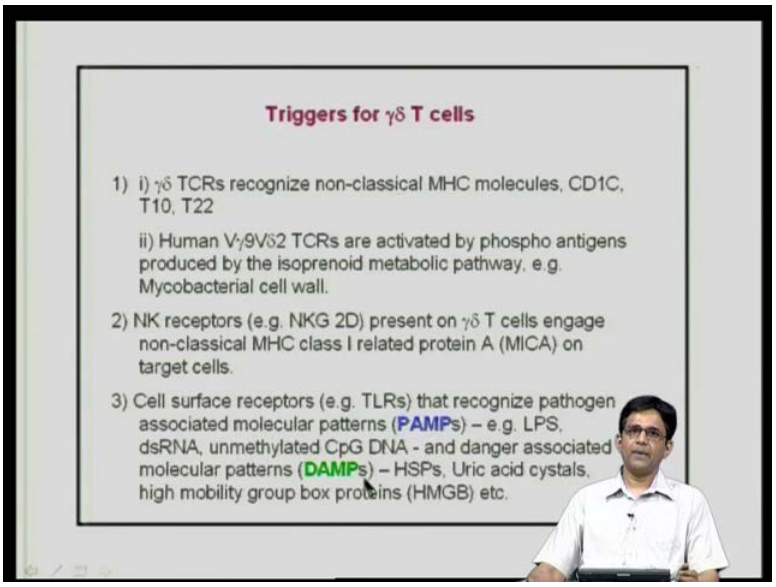

And that is something that we will be seeing here; so, what are the triggers for the gamma delta T cells, the gamma delta T cells recognize non-classical MHC molecules, remember when we were discussing MHC, we had discussed the classical ones like k d l and i a i e in mouse, and so you have the other set that is non-classical which are expressed less in different tissues; so, over here, for example gamma deltas have been shown to recognize CD1, T10, T22, now gamma deltas especially in humans, the peripheral gamma deltas, the v gamma 9s in the v delta 2s are activated by phospho antigens, and these are produced by the isoprenoid metabolic pathway.

And often a lot of these phosphor antigens are present in mycobacterial cell walls, and so you have activation against these, now apart from the gamma delta receptors, gamma delta cells also have NK receptors, which are presence, so for example, NKG2D engages

non-classical MHC class 1 protein present on target cells; so, that is also playing an important role.

Apart from this they possess TLRs, and TLRs may recognize the pathogen associated molecular patterns, for example, like LPS, double stranded RNA, unmethylated CpG or they can recognize danger associated molecular patterns, which are the endogenous signs of the tissue activation or tissue damage like heat shock proteins, uric acid crystals, high mobility group proteins, so on.

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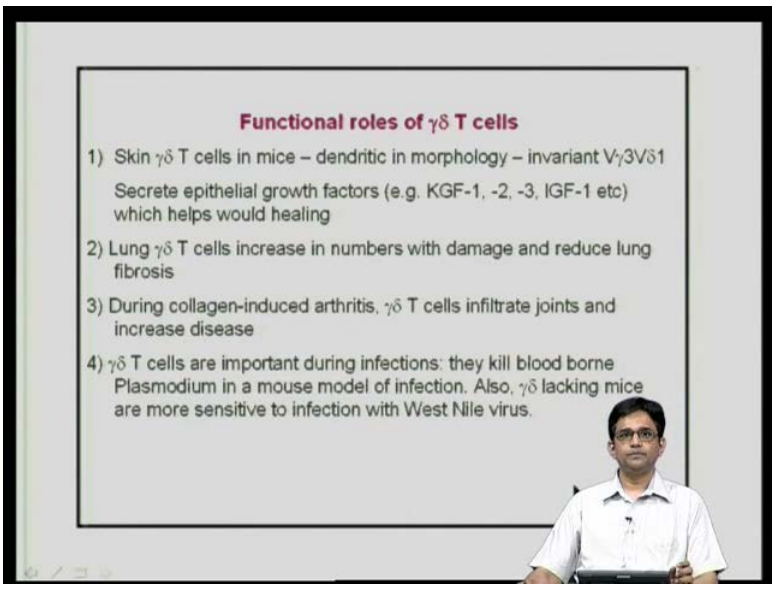

Now, what are some of the functional roles of the gamma delta cells; now, in the skin, in fact, especially in mouse, the skin shows the presence of this invariant v gamma 3, v delta 1 T cells, and in fact, if you recall that the v gamma 3 v delta 1 are the first ones to arise during T cell differentiation, and they arise in the thymus, and they depart, and because they go and reside in the skin, the v gamma 3s are followed by the v gamma 4s, which go into the reproductive track and so on. What has been shown in terms of the skin gamma deltas, that they secrete epithelial growth factors, and especially site growth factors 1, 2, 3, insulin like growth factors, and which help in sort of wound healing.

So, this makes actually biological sense, the T cells are there, if the epithelium is damaged, then the T cells secrete these factors to try and help wound healing; now, gamma delta cells have also been shown to increase in numbers, and reduce lung fibrosis during collagen induced arthritis, which is a model for autoimmune diseases gamma

delta T cells infiltrate joints and increase disease. Gamma deltas cells are also important during infections, they kill blood borne plasmodium in a mouse model of infection, also gamma delta lacking mice are more sensitive in infection to viral infection, for example, West Nile viruses.

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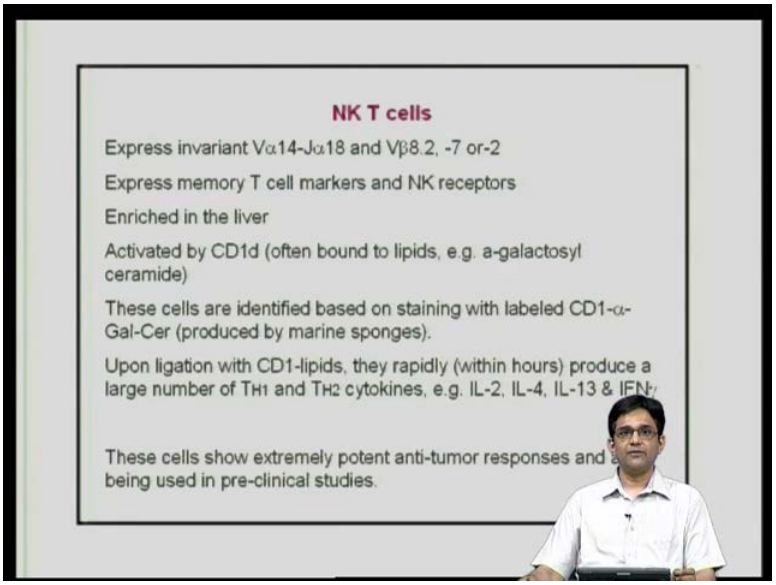

The other type of, so we will talk mainly about the broad different types of, we talked about gamma delta cells and their functions. We will talk a little bit about an animatic group of cells known as the natural T cells or the NKT cells; now, these cells are ones, in which they have sort of components of both the natural killer cells as well as T cells, and their T cells, because they express CD3, in fact they express in invariant T cell receptor where you have the v alpha 14, the j alpha 18, and linked with v beta 8.2 7 or 2, so the their alpha chain is invariant.

Now, as I said these are interesting, because they express memory T cell marker as well as NK receptors, they are enriched in the liver, they are activated by CD1d, which is also a non-classical MHC molecule, and what is interesting was CD1d is that, it is associated with lipids, and in fact, the lipid that is often used for detecting, these are the alpha Gal-Cer; so, you have CD1 linked to alpha Gal-Cer, and that is used for detecting NKT cells, because the alpha glacier will bind to the receptor, because that is what the receptor recognizes. The functionally, these cells are very interesting because upon ligation with CD1, and the lipids, they very quickly produce a whole bunch of TH1 and TH2,

cytokines, and both types of cytokines are produced, and these cells are very important during cancer; in fact, there are preclinical studies going on to study of these natural T cells as in anti-tumor.

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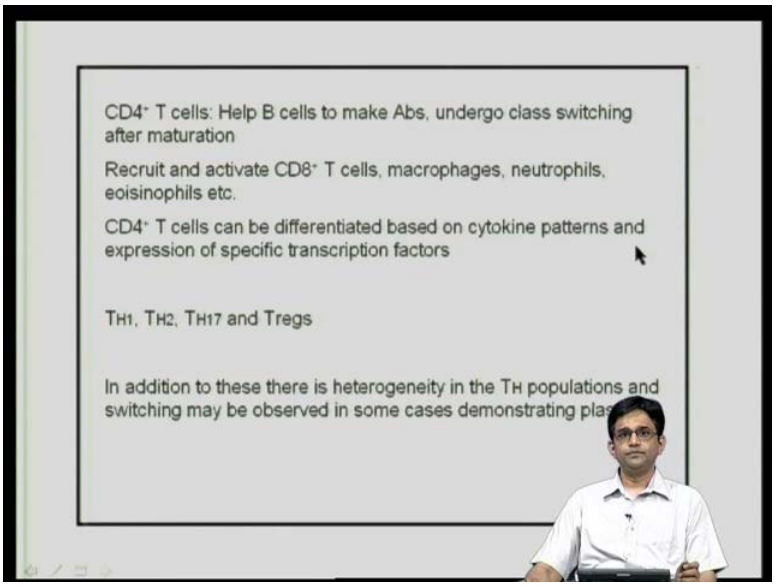

Now, we will come to the broad role and subsets of T helper cells, now we had discussed that you know T cells CD4, positive T cells help B cells to make antibodies undergo class switching, they recruit and activate CD8 positive cells, and but they can be subdivided into different subsets based on cytokine patterns, and expression or specific transcription patterns, in fact most of you sort of aware, that at least the main sub routes will be the TH1, TH2, TH17, and the regulatory T cells, so we will discuss these in a little bit greater detail.

Now, in addition to these four main subsets, there are other groups, in fact some we had discussed, for example the follicular T cells, and that is a different group, because of its specific expression of the chemokine receptor, and its affinity for the ligand, and it has a separate function also, and we also saw the role of Bcl6, and Blimp in that cell, but in these, this subdivision is based on expression of cytokines as well as transcription factors and that is very important.

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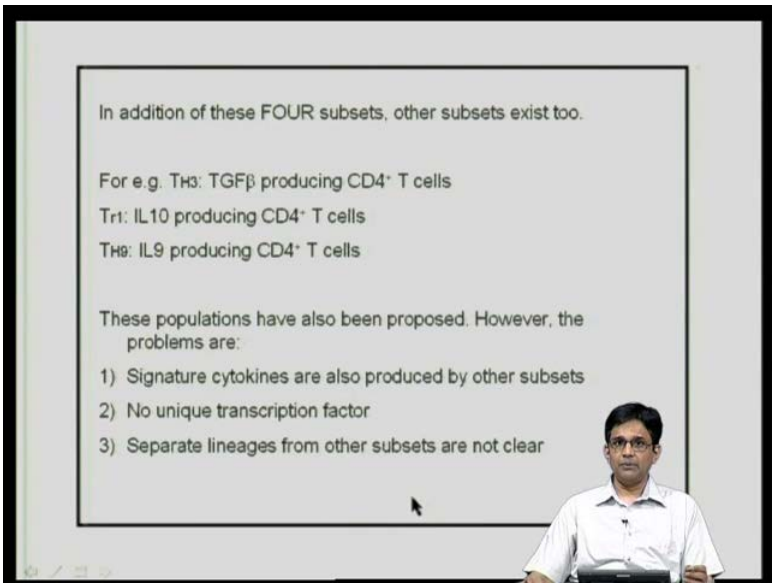

Now, in addition to these, there is heterogeneity as people have shown, in fact, you have, you have other ones like TH3 are the TGF beta producing CD4 positive cells, you have TR1, which is the IL10 producing CD4 positive cells, you have TH9 which is the IL9 producing CD4 positive cells; these populations have been proposed, however in terms of clear subsets, there is a problem, because the signature cytokines are produced by other subsets too, and there is no unique transcription factor, that is associated with them, and they separate lineages for these are not yet clear, and perhaps for the studies are required, but for now we will study the main four sets.

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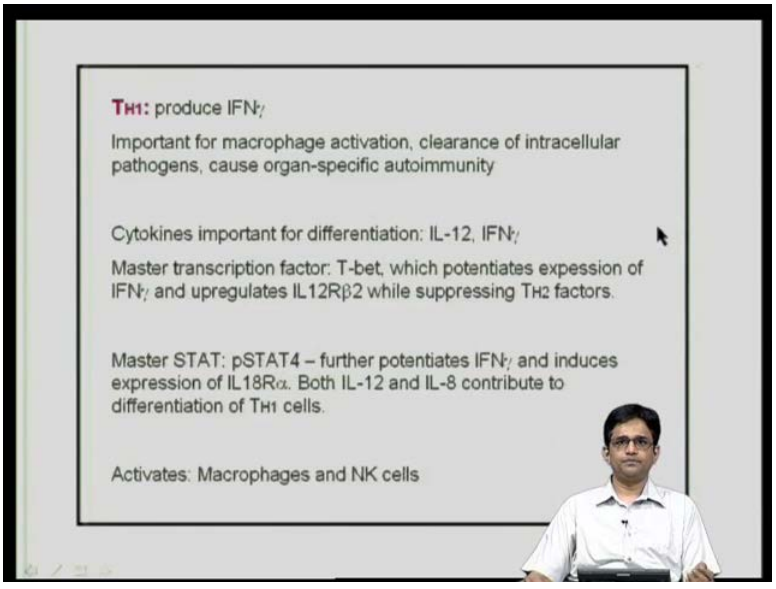

So, TH1 as you are familiar with these produce interferon gamma, they are important for macrophage activation clearance of intracellular pathogens, they also too much activation of TH1 causes organ specific autoimmunity. The cytokines that are important for differentiation are IL12, IFN, gamma, the master transcription factor is T bet over here, and which potentiates expression of IFN gamma up regulates the IL12 beta receptor, the master STAT or the signal transducer an activated of transcription is STAT 4 which is very important, and what TH1 does it activates the macrophages and natural killer cells.

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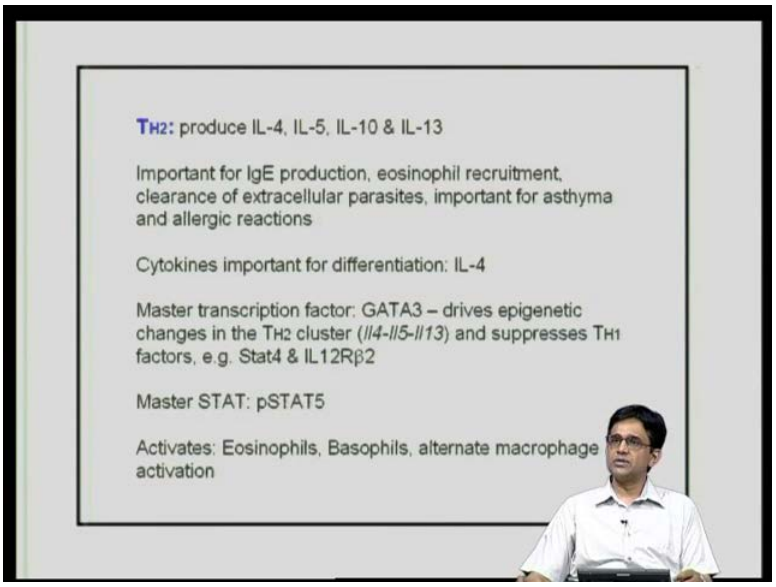

TH2, on the other hand, it produces IL4, IL5, IL13, these are the most important ones, they are important in IGE production, eosinophil recruitment, important for asthma, allergic reactions; so, clearly TH2 has a different set the cytokine that is really important is IL4. The master transcription factor in this case in GATA3, and over here, there is a locus in fact the IL4, IL5, IL13 locus which is important over here.

And the GATA3, you know shows epigenetic outdrives, epigenetic changes such that this cluster is activated, while suppressing the TH1 factors the master STAT, in this case is the STAT5, TH2 cytokines activate eosinophils, basophils alternate macrophage activation; so, that you have suppression of inflammation.

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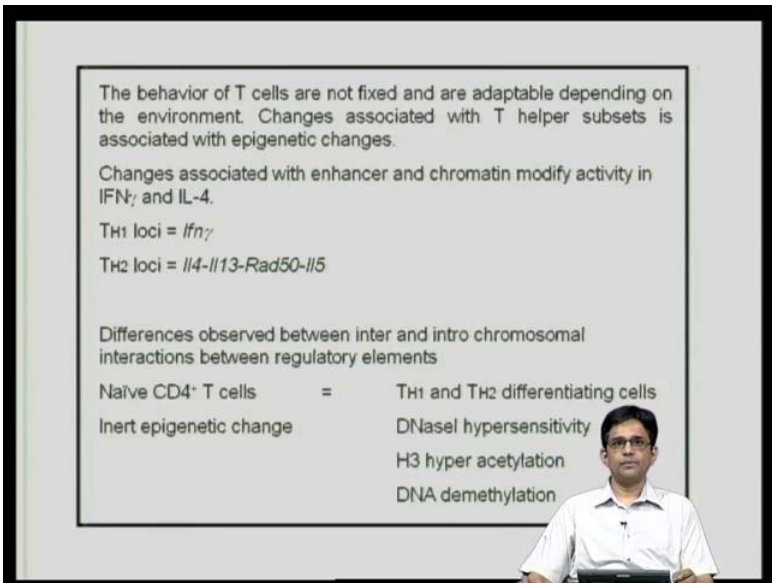

What is important over here is to understand that the behavior of these T cells is not fixed, and adaptable depending on the environment. Now, how do these finally differentiate, and this is followed by epigenetic changes, by epigenetic changes means, they are not firm changes, they are somewhat dependent on the environment, and so it is very important that you understand, what is the meaning of epigenetic changes, what has been shown is that, along with differentiation of these subsets, their enhancer and chromatin get modified.

So, especially studies have been mainly done in the TH1 loci, which is the interferon gamma, and in the TH2 loci, where you have IL4, IL5, IL13, that particular loci, and how does one study epigenetic changes, and you can see that the chromosomal changes take place, and these have been studied using DNase hypersensitivity histone modification, DNA demethylation changes and so on. So, TH1, TH2 are very closely associated with epigenetic changes, and that is something that people or students should be a little bit familiar with.

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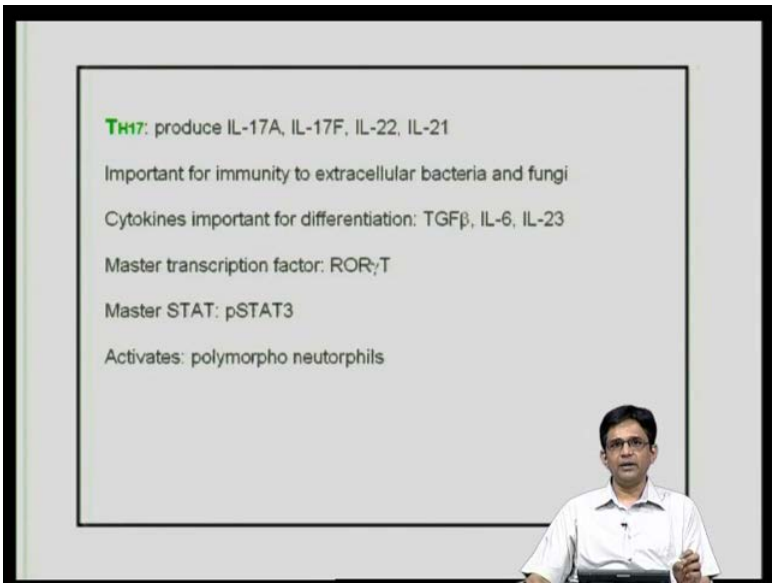

Apart from TH1, TH2, there are TH17 has recently come into prominence over here. The TH17 cells produce IL-17A, IL-17F, IL-22, IL-21, they are important for immunity to extracellular bacteria and fungi. The cytokines that are important for them, you have a whole bunch of them, you have TGF beta, IL-6, IL-23, the master transcription factor over here is ROR gamma T, and the master STAT is the STAT3, what these do is they activate the neutrophils.

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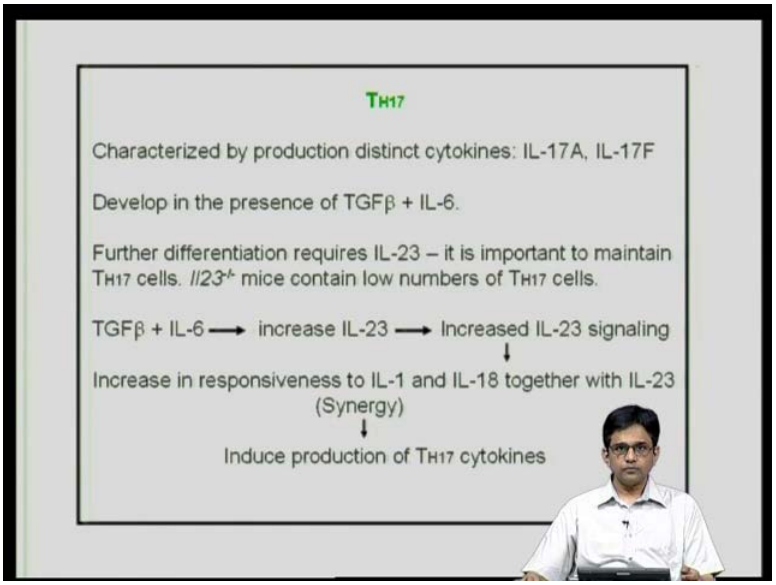

Now, TH17, as I said that, they are characterized by production of distinct cytokines, especially IL-17A, IL-17F, they develop in the presence of TGF beta and IL-6. Now, what is important over here is that, development, they need TGF beta, and IL-6 for subsequent differentiation, they need IL-23, and now IL-23 is very important for maintenance of this, in fact, IL-23 knockout mice contain very low number of TH17 positive cells. What is known is that TGF beta, and IL-6, what this does is, it increases IL-23, and you have increase IL-23 signaling, now this increases responsiveness to IL-1, IL-18, together with IL-23, in fact, there is a synergy and this results induces production of TH17 cytokines.

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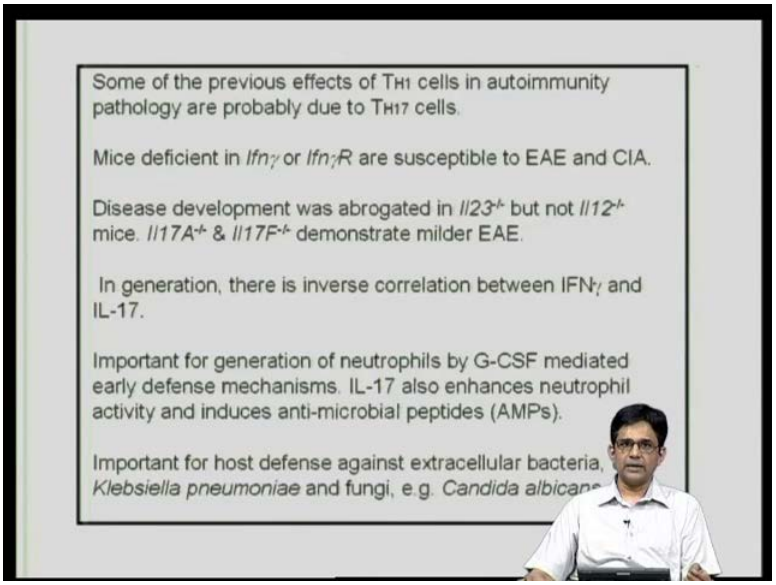

Now, previously a lot of the effects that people thought you to TH1 positive cells are now known to be due to TH17 cells, in fact, and this was shown, because mice that are deficient in interferon gamma or interferon gamma receptor, and these are the classic TH1s are susceptible to EAE, and collagen induce arthritis. So, clearly, there were other cytokines that were playing an important role, because even in the absence of interferon gamma, you had development of these.

Subsequent studies show that the disease was abrogated in the IL-23 knockout mice, but not in the IL-12 knockout mice. So, clearly showing very important role, also IL17A and IL (( )) knockout mice demonstrate milder forms of encephalomyelitis, which is shown in EAE, which is basically means information of the brain, and in general, there is a

inverse correlation between IFN gamma and IL-17, so that means, as there is more IFN gamma, there is tendency to shutdown TH17.

On the other hand, if there is a less IFN gamma IL-17 is usually more, and so you have much more of TH17 response. Now, what is known is that, IL-17 enhances neutrophils, they are primarily work on these neutrophils, and induce antimicrobial peptides, they are important in host defense against extracellular bacteria, Klebsiella pneumoniae and fungi is specially Candida albicans, and there are had been studies to show this.

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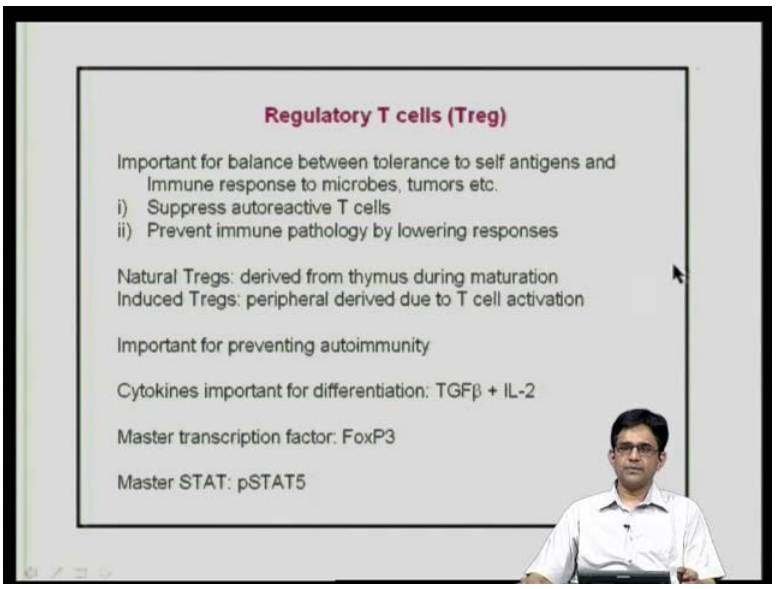

Finally, we will come down to population of cells known as the regulatory T cells. Now, T regs are very well known, and it is important to understand over here, that while thymus is important in terms of differentiation. And so that it teaches cells that we need to recognize self the auto reactive T cells are eliminated during thymine selection. The fact of the matter is that, it is very difficult for all possible auto reactive T cells to be completely eliminated; so, you would need some peripheral tolerance mechanisms.

So, peripheral tolerance, the primary driver of peripheral tolerance is thought to be the T regulatory T cells. These are extremely important these T regs, and what they do is they suppress auto reactive T cells. They prevent immune pathology by lowering responses. These are the primary roles, so every small little bit, you know of activation by T cells does not result in a major immune reaction. So, one has to be a little discriminating about this, and so every time a T cells sees antigen, it does not go crazy.

There has to be an environment built, by which T cells would know, this is the timing how is that environment built, that is something we need to look into, but primarily the role of regulatory T cells is as follows is to suppress autoimmune T cells, in fact, that is how they would discover. So, what happens, if you do not have T regulatory cells, if you do not happen, then you have these mice or humans are highly prone to autoimmunity and that is something that we will be studying.

There are two main types of T regs, one is the natural T regs, and these are derived from the thymus during maturation; the other is induced T regs, which are generated in the periphery upon T cell activation. So, you have the thymic ones which are naturally produced during T cell differentiation, the other is during activation. The, cytokines are important for them, and TGF beta and IL-2 is very important, the over here, the master transcription factor is the FoxP3, and a FoxP3 is very important, and this is something that we will be studying a little bit in greater detail, and the master STAT is the STAT5.

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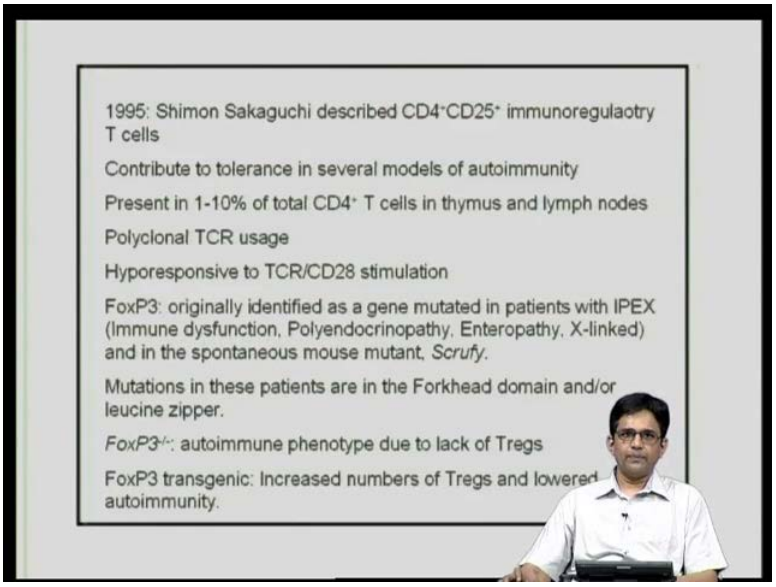

And the STAT5 because of IL-2 and IL-2 sort of activates the STAT5 pathway; these were discovered in 1995 by Sakaguchi and colleagues, and this is a classic sort of work, where they identified a population that was had immuno regulatory properties, and they were primarily involved in suppression of auto reactive T cells, and in fact, they defined the population, and the population was defined by expression of CD4 and C25; C25 is

the alpha chain of the IL-2 receptor, CD25 is also produced very quickly upon T cell activation.

And you have, so just the expression of CD4, C25 is not sufficient, you also need FoxP3, but at the time they discovered it, they showed that there was a population of CD4 positive, C25 positive cells, which would present without activation, and it was this population that was important in suppression of autoimmunity. Now, what has been shown is that, they contribute to tolerance in several models of autoimmunity, they are present under normal stage, I mean, it is without activation or 1 to 10 percent of your total CD4 positive cells in thymus and lymph node express it.

They have a polyclonal TCR usage, that means, they would be recognizing different sorts of antigens may be some self-antigens, but it is not because of proliferation of only one type of cells, that these have come up or 1 type of antigens, and their polyclonal TCR usage, in fact, that is what it shows. What is interesting about T regs is that, they are hypo responsive to TCR/CD28 stimulations, you can activate them, but they do not respond very well; in fact, they are very good at inhibiting responses, but by themselves they do not get activated.

The signature transcription factor which is FoxP3 is important, because it was originally identified in patients with IPEX disease, and IPEX disease is immune dysfunction Polyendocrinopathy, Enteropathy X-linked, and so you can see that these patients suffer from multiple diseases, and these multiple diseases are, because they lack a certain population and this is the T reg population.

What is also important is that, they have this is in the spontaneous mouse mutant scruffy, you have this in mutation. So, the mutations in these patients are in the Forkhead domain or in the lucien zipper, so the FoxP3 knockout mice obviously show autoimmune phenotype, because of lack of regulatory T cells. On the other hand, you have FoxP3 transgenic mice, you have increase number of T regulatory cells and they have a lowered autoimmunity.

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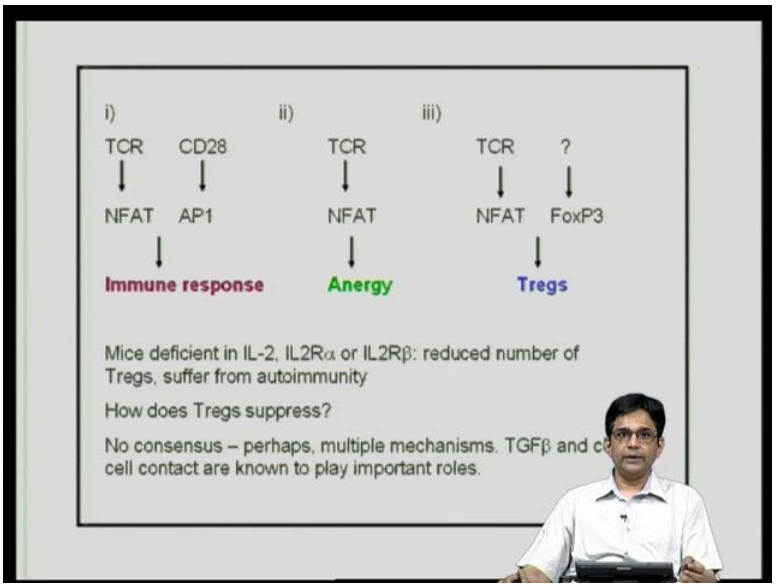

What is shown over here, and this is something that we had deal in the T cell activation class. You have the T cell receptor, it activates the NFAT, the CD28 pathway activates the AP1, and together you have the immune response. Now, if you activate T cell through the T cell receptor only, then you will activate the NFAT, and these results in T cell energy, however now what has been shown is both NFAT and FoxP3 are important over here, so the T cell receptor activates NFAT.

The signals that are important in activation of FoxP3 are not known, but together these sort of give raise to your T reg regulatory T cells. So, there is a correlation between NFAT and FoxP3, but FoxP3 is the primarily, is a primary signature transcription factor in these regulatory T cells. What is important about the T regs is that, mice that are deficient in IL-2, the IL-2 in CD25 or the IL-2 receptor, beta, they have the phenotype is that of autoimmunity, they suffer from autoimmunity.

And the main reason for it is they have also reduced numbers of regulatory T cells. So, this is an important aspect. So, IL-2 is very important in regulating T reg cell numbers, now the question is how do T regulatory cell suppress immune responses; so, there is no consensus on this perhaps by multiple mechanisms TGF beta is thought to play an important role, also cell contact is important and that is how suppression occurs.

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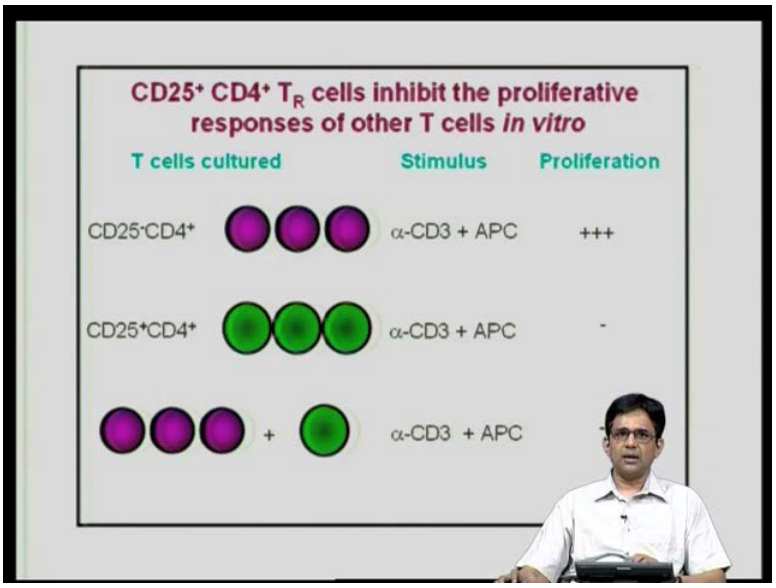

Now this sort of shows you what is an assay actually; so, these are CD25 negative, CD4 positive cells, and you stimulate them with CD3 plus APCs in the proliferate a lot. So, this is actually an assay for T regs, and if you take regulatory T cells, and you activate them, they do not proliferate this is what I said, this is one of the phenotypes.

Now, if you add a very few regulatory T cells, and you have a whole bunch of your normal CD4 positive cells, and you activate them, and what you find is that, there is a less proliferation. So, this is an actually what is showing is an assay for T reg function, that is you have very few regulatory T cells, they will somehow manage to suppress the proliferation of your other T cells, so this is an important aspect.

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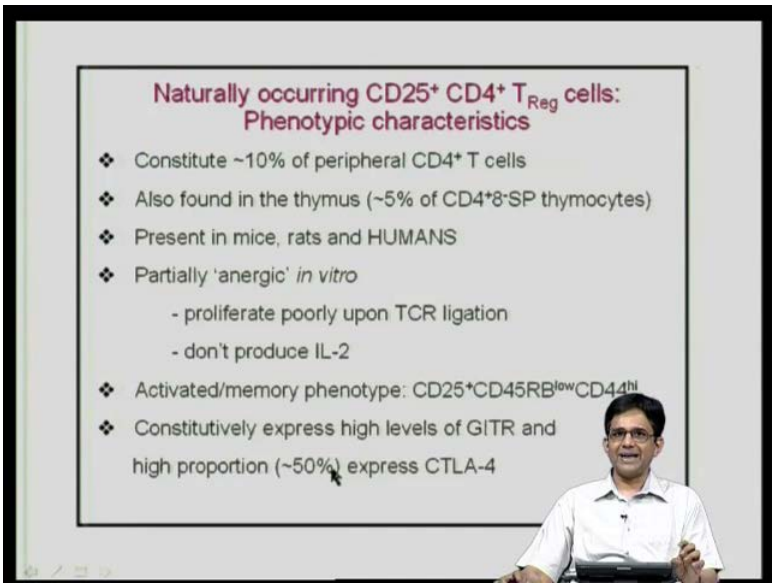

And some of these characteristics of regulatory T cells are shown or again over here, and just to show that about 10 percent of them have it, they have activated memory phenotype, they express high levels of glucocorticoid induced TNF receptor, they also some of them almost 50 percent of them express CTLA-4.

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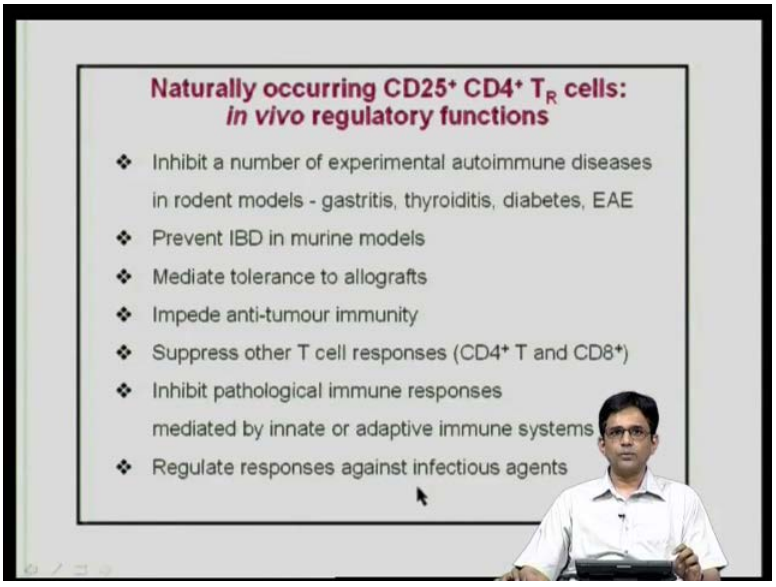

And they have been shown to play a wide variety of roles, they inhibit a large number of autoimmune diseases in several models, they prevent inflammatory bowel disease mediate tolerance to allograft, they impede antitumor immunity, because they suppress

immune responses, so including antitumor immunity; so that, it is not particularly good for it, they regulate responses again infectious agents.

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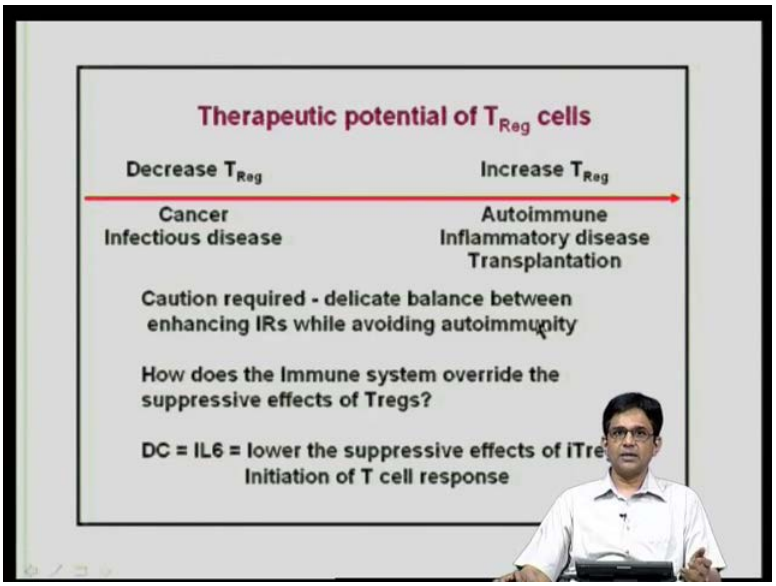

So, that is precisely what is shown over here. For, autoimmune diseases, it might be a good idea to reduce immune responses, if you want to reduce these T cell responses, you can do that by increasing regulatory T cells. On the other hand, during cancer on infectious disease, you want to increase your immunity responses, and in that case, a good way to do that would be to decrease T regulatory function. So, by modulating regulatory T cells, you can modulate immune function; so, this is a very important thing. So, if you have regulatory T cells, and you want to initiate immune responses, how you are able to do that, how are you able to germ start the immune response part.

What has been shown is during dendritic cell activation often IL-6 is produced, other cytokines are produced, and some of them, they lower the suppressive effects of the inducible T regs, and so then, there is a balance between T regs and immune responses, if these factors that are produced they suppress, the regulatory T cells, then the balance is tilted, and the T cell immune response will take over; otherwise, the regulatory T cells will suppress immune responses; so, it is this constant balance between the regulatory T cells and your other immune responses, that together will determine actually what happens in terms of immune responses.

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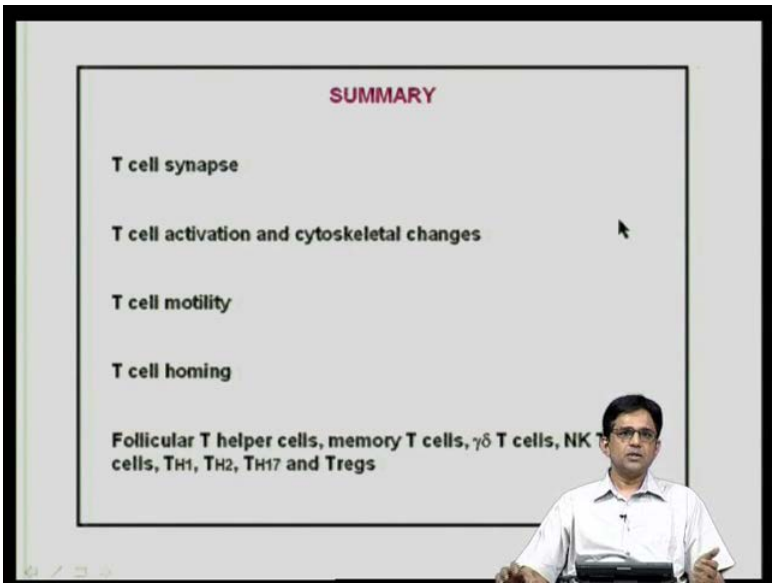

So, finally, I will briefly go over I will summarize this class, you will recall that we discussed the T cell synapse very important part, because you have cell surface receptors coming over, especially some cells surface receptors, the T cell receptors, obviously important one CD28 and so on. They form a central core, and then you have a peripheral core consisting of LFA Talin and so on; all this involves cytoskeletal changes, cytoskeletal is very important, because if you inhibit cytoskeleta, inhibit if actin depolymerization and so on, you inhibit T cell activation.

T cells are motile, there are two main mechanisms, by which one is they use in terms of, there are two main types of T cell motility, one is fast, the other is slow; the fast ones are myosin dependent, and that is just for regulate scanning, the slower ones are integrin dependent, where they will need to study that dendritic cell properly to see, if there are T cell receptors that are there, and over here LFA icam interactions are important, because if icam is induced on dendritic cells, LFA gets activated.

And so, it will help keep the T cell dendritic cell intact; so, while there is reorganization that is occurring, and the T cell receptors scans for possible binding to MHC molecules, then we also discussed T cell homing, and in T cell homing, there are two main types the CCR7 and the CXCR5. The CXCR5 is important in terms of follicular T helper cells, the CXCR7 is very important in terms of getting entry into the HEVs. We also saw about the

role of memory T cells over here; you have two different types - the central memory cells and the effector memory cells.

The central memory cells have CCR5, the effector memory cells do not have, so you have again different types of memory T cells, one that will come quickly in contact with the antigen, the other one waits for it to come to the lymph nodes; so, you can initiate you have redundancy we also discussed different types of T cell subsetst from the follicular T helper cells, the memory T cells.

We talked about the gamma deltas, their role in protecting epithelial surfaces, the NKT cells their anticancer role, especially with galcer, their ability to be very quickly activated produce both TH1, TH2 cytokines very quickly, they have anti can tumor properties. We also discussed the other main sub types the TH1, TH2, TH17, and the regulatory T cells, the regulatory T cells are really important in terms of autoimmunity, we will discuss more in subsequent classes.