Immunology
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Lecture No -33
Development of T Lymphocytes (Contd.,)

Hello everybody, today also we are going to continue the development of T-lymphocyte. So in continuation to previous lecture we are continuing.

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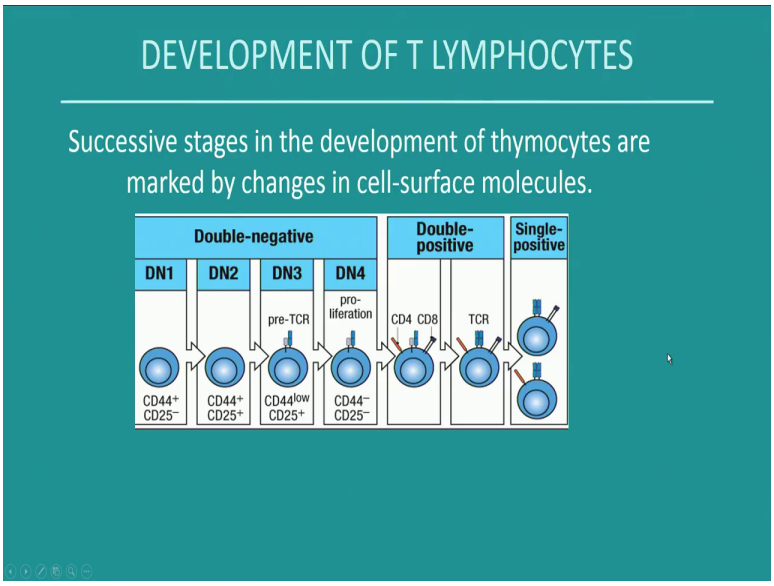

In last slide you are discussing about this figure right different markers at different time for like DN 1, DN 2, DN 3, DN 4 and then double positive then double negative. So having different markers some are appearing disappearing so it is kind of complicated. So first we should remember I mean in general we should remember there are three stage one double- double positive single positive that is the final stage. So to make our life little easier we will go to next slide.

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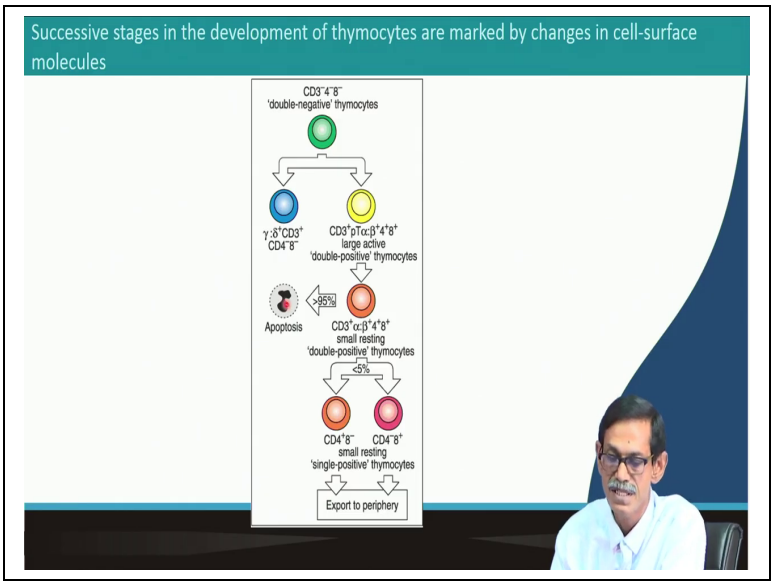

And we will see next slide what we are seeing that the same thing what we have seen in the last slide or in the last lecture this is a similar thing slightly represent slightly different way.S what we are showing this is double negative time asides which is not yet mature like see the see also negative 4, 8 negative from there both alpha beta type T cell and Gamma Delta type T cell both originated from the same progenitor.

So I am going to come up with the Gamma Delta little later. So what happened initially what we discussed in last lecture also I am just quickly repeating again it becomes DT plus CD 4 plus ETA plus that means double positive and the T alpha receptor is preliminary. So beta is form and pre T alpha that means alpha chain is not yet. From at this stage because I mean after that which we have seen in the last class that then it becomes CD 3 plus both alpha beta plus then 4 plus 8 plus this is kind of double positive cells.

In this stage what happened it is looking for self MHC recognition. So in because T-cell receptor is already made it has a variety of diversity to identify or recognize most diverse pathogen possible like can infect us right. So, but as T cell receptor cannot recognize without MHC so force recognition this alpha beta receptor should recognize the T cell. And we already call this is called positive selection you can see at this stage 95% of the cell dying by apoptosis what does it mean?

That means they are not selected in positive selection. So they do not interact with our MHC neither MHC 1 nor MHC 2. So normally what happened in at man adult thymus there are 10 to the to the power 8 to 2 times 10 to the power 8 number of cells are there and party 10 to verse 6 to 10 to the power 2 times 10 to power 6 cells generally get matcher this now no one can tell the fixed it is a variable number.

But generally say 10 to power 8 is a normal number of time aside present in adults thymas and 10 to the power 6 cells get matured per day. So 95% of the cells dying and then from here also that double positive 4 plus and 8 plus 5% cell contribute to either 4 CD4 or helper cell or CD8 or cytotoxic T cell, clear. So this is how they are been only of the total thymocyte that enters into thymes from bone marrow only 5% cell are maturing into CD4 and CD8.

And out of few of them are also going to make this Gamma Delta and another I already told in variant NKT or natural killer type T cells which is has very less variability in their receptor they do not bind directly with they cannot recognize. So many pathogens so if they have certain other roles mostly the peripheral tissue they handle the infection.

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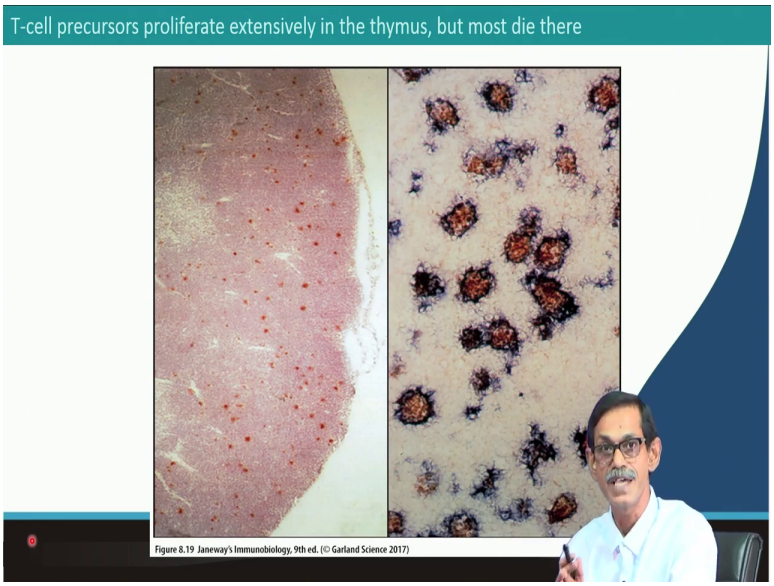

So when 95% is dying this is just a picture like just to show you like what is happening. So these death if you see the previous slide this death is written apoptosis. There are number of our process marker that we can identify by immunofluorescence or immunostaining so this is the

experiment. So in this is a cross-section of thymus if you see all these red dots that is localized many way we can localize the apoptotic cell.

I am not going to detail in that so you see all these red dots you see these are apoptotic that means this cluster is there a lot of apoptosis is going on and if you see this is a cortex region. In medullary part the number of apoptotic zone is not much. If you enlarge it you can see even so many cells are dying 95% of the segments huge number of celestine right. But thymus is not increasing if you keep on dying the dead cells the size should increase.

But it is not increasing because I already told who clear or miss inside the body macrophage. Macrophages just are eating all the dead cells here in the second point is just enlarged you can see the blue is the macrophage one macro body and there are lot of dying thymocytes inside. So this is the bigger version of this. So this is also I mean it is just not written like 95% cell is dying counting you can count by flow cytometry if you isolate the thymus ID or you can see visually that the cells are dying.

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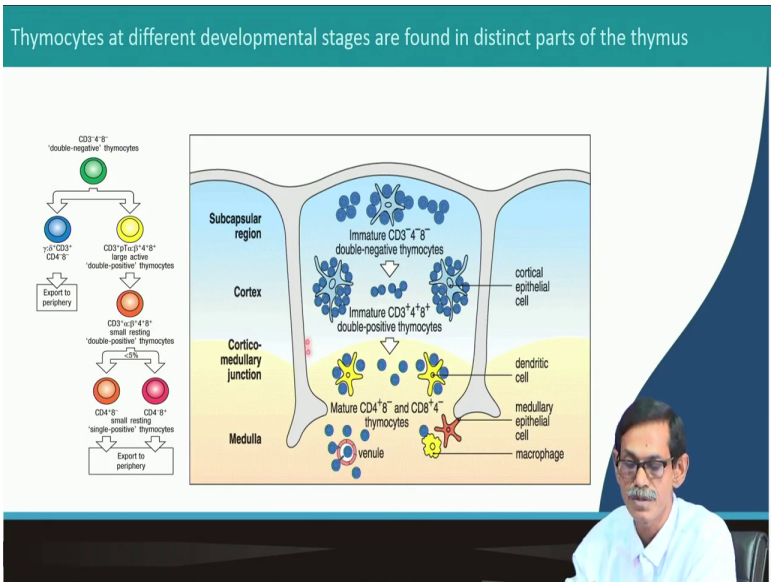

And thymocyte at different development stages are found in different parts of thymus different developmental stage means that double- double positive single positive they are not distributed all over the thymus. They are distributed they are distributed hope you remember this low view

structure where we see that this is a cortex and this is a medulla. And these are again I kept this slide just double negative, double positive.

This is the double positive complete like CD 4 alpha beta 4 CD8 CD 3 all are present. And this is single negative or single positive cells. So, how; they are distributed at the top of the cortex there CD 3 minus 4 minus 8 minus. So these are the immature thymocyte; who starts their journey from top and then gradually they are going down. You remember the previous yesterday's lecture or previous lecture actually that this particular area is not having much dendritic cells or macrophages.

If you remember correctly the bone marrow derived macrophages dendritic cells are mostly populated here. So they are going here by epithelial cells so that positive selections are happening by MHC presented by the epithelial cell of the cortex. Then it is coming in the middle region see the 3 plus 4 plus 8 plus that means this is the double positive cells. From here they undergo the selection which is called negative selection.

So here the dendritic cells macrophage they are presenting our own antigen and because whether it will be CD 3 on CD 4 will be decided so that is already there so they become single positive here after that they also die again why, because if they interact with our own protein or cell protein they should not survive. Otherwise it will be a real problem for that individual so that die. So, negative selection happen and ultimately the match yourself pass through this manual to the peripheral blood.

If I see even more if I see more detail like of what is next? Next is if I see this double negative cell again slightly more detail so it enters from here the first coming from the venue DN1 you know what is DN 1 then is going this way DN 2 DN 3 DN 4 so what we saw in the last slide double negative cell here this is DN 4 actually. Then DN 4 to match your double positive that is in this part is same. So if you combine both the slides you can understand how these immature thymocytes get matured in the thymus.

And each lobule it is happening it is not single and if you see DN 2 is here DN 2 is also here so it is not one-sided it is going from both side and coming like this. So going this way double-double negative positive double positive then single positive and again it is going out in the periphery. So that we will see again I mean what is happening who brings them how it is going when you will see there are T-cells mediated immunity.

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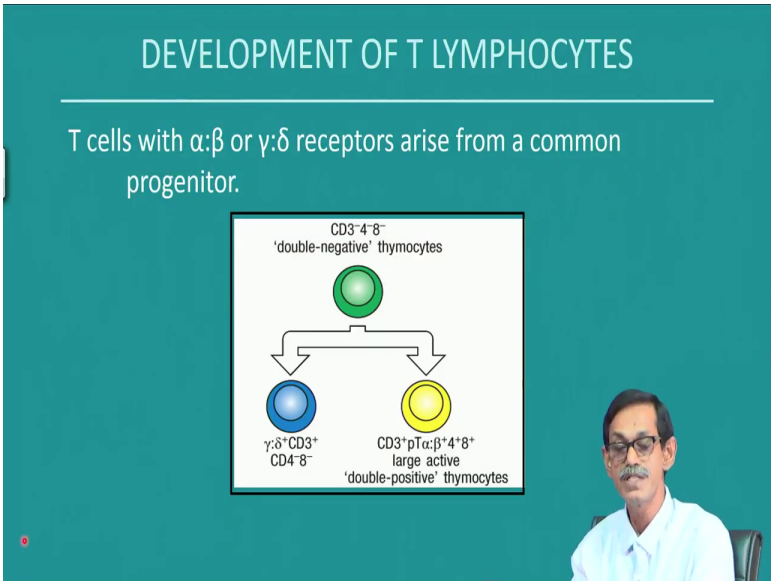

So if you remember this part of the picture like when we are showing the development of T-cells the CD 3 minus CD4 minus CD8 minus double negative thymocytes that T-cells with alpha beta or gamma delta receptors arise from the common ancestors or common progenitors that we already mentioned. So here this is the part of the image that we already sold. So some will go to alpha alpha beta and some will go to Gamma Delta how this thing happened I mean why suddenly some cell will make alpha beta some cell will make Gamma Delta receptor.

Because we are studying now the development of T lymphocytes so as much as possible we should discuss.

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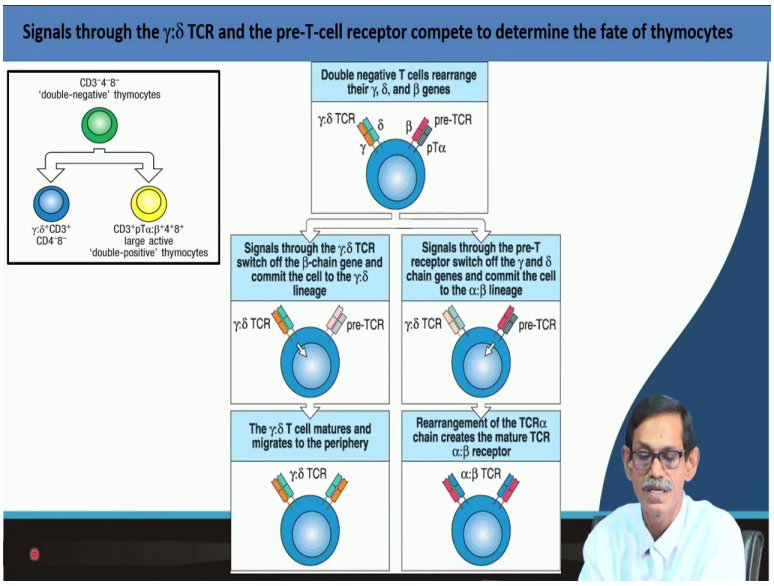

So this again I mean this is the same slide from the same image so what is happening this one how it is happening initially what happened. Now one particular CD 4 minus 8 minus 3 minus L expressed both Gamma Delta as well as complete beta and pre T alpha, so both are expressed. So these signals if this wind this is again it is a kind of black box or mystery not clear properly. So if this arrived gum I if the Gamma Delta receptor of TCR can give signal or have Windows rest then it gives signal which turn off the expression of beta J.

And if beta chain turned off then automatically alpha chain will not. If there is no beta chain expression then alpha chain expression will not happen. So what happened they will win and ultimately this beta chain and pT alpha will disappear and this particular cell will be Gamma Delta type. But if the signal goes through pT alpha receptor then it stops the Gamma Delta receptor expression. So this Gamma Delta receptor will gradually slow down and not express anymore and ultimately alpha beta receptor is whether it is there is an instructive model or it can be a stochastic model like random either this one or that one.

So one goes slow and then this can also explain about this type of mechanism is also there whether it will go CD4 or CD8 which were having from double positive which whether it will go to CD4 type or CD8 type that also is not much clear but of course a lot of research is going on and we have little information but that information is not yet in the textbook. So this is how that one common progenitor goes either to Gamma Delta type or alpha beta drive.

And we will see but sometimes like how this delta is region is deleted if alpha region is V D J recombination is happening in alpha beta type receptor T-cell expressing Gamma Delta receptor.

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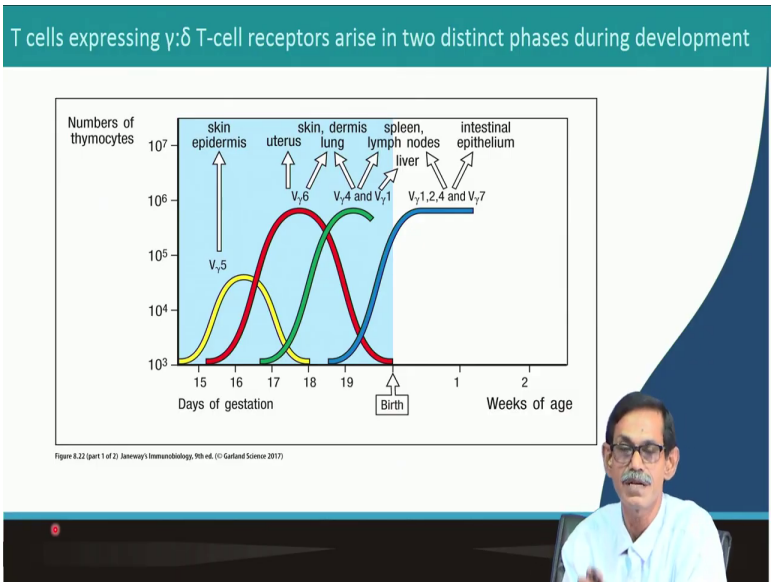

So we talk a lot about the; because whole T cell immunity we are going to talk about the alpha beta receptor. So let us start little bit like morality receptor that they are making from the same progenitor but they express in two distinct phases during the development what is that? So the progenitor cell from where both Gamma Delta and alpha beta receptor containing T-cell develop is already discussed. Now what are going to what I am going to tell you is that the T-cell expressing Gamma Delta receptor has two very distinct phases during development.

What is that? So if I see that there I told while we are discussing the T cell receptor generation there are multiple number of variable region in or the delta delta chain right variable region of delta chain there are 1 2 3 4 5 6 7 ok. So the gamma del gamma chain is but the delta chain is fine but varieties of gamma chain is pairing with the single type of delta. So if you see, that see V gamma 1 become a 2 V gamma C X 5 like that so the expression of different gamma chain has two distinct space why.

Because if you see the shaded area this is before birth and the white area is after birth. If you see then V gamma 5 type starts expressing before 15 days same a 10 days and then go up and then

go down within 18 this is definitely for Mouse. And the red one that is B 6 it is going up going now whereas this B 4 1 and 1 2 4 and 7 they continue after birth also. Green is going down but it is going to continue it is not like completely 0.

So at adult age this green one I am not telling the number so you can understand from the slide what the numbers are. So green one and the blue one mostly the blue one exist in the adult stage and other one just express do something go away. So before bar up it is it is function is over.

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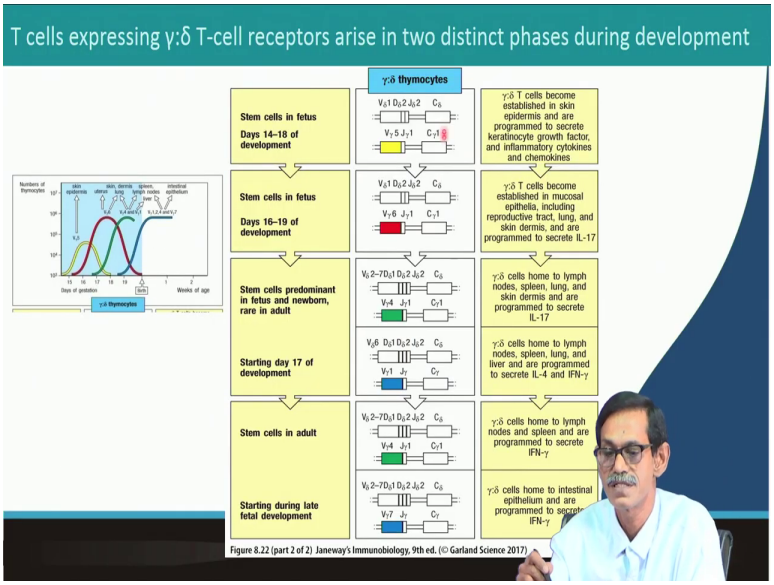

What they are doing actually this is a series of least like what they are doing. What they are doing is, so if you see I am not going to go and read all this thing it will be really monotonous and boring you can definitely understand. Say for example the yellow one I am keeping the graph also in the side so the yellow one you see what they are doing they are 14 to 18 days actually of the day during development they are there.

What is they are doing in the fetus they are the gamma delta T cell become established in skin and epidermis because that is a more; they do I mean at that stage body is not developed fully. Skin epidermis are programmed to secrete keratinocyte growth factor. So that means the hair or the carotene is going to form. So you see these particular proteins which is normally we think it is not only do the immune system protection or do definitely it is doing something for protection but it is also helping the growth.

So there are many proteins in immune system involved in immune system or immune activity they are not only doing immune system during development it has a major role. And how this is discovered is very interesting because when transgenic mouse develop or gene knockout mouse develop in late 90s what happened we will started, so the gene knockout is discovered. So we can specifically knock out or delete one gene from an organism we can make Mouse say without one particular cytokine.

Say IL 2 or IL 4 so one mice they cannot produce aisle two one mice they cannot produce rag1 one mouse they cannot produce rag 2 or both. So when this system or this technique develop a big scientists those who are working in this field work extremely excited and set thought that will explore everything. But the problem is when the knockout very important gene for an immune system they saw that the mice is not growing or the development is incomplete.

So there are a lot of developmental defect when they are knocking out the immune gene responsible for immune system in adult. So that actually tells us that these immune system genes are not only doing the protection only but they also have some other role in development or maybe some more or throughout body there or they are doing but we do not know. So here is the least so which time which gamma-delta cells are performing what and at the end this blue one is the final which we see in the after bar the Gamma Delta cells come to intestinal epithelium and program to secret interferon gamma.

So that epithelial cells where there is no direct antibody the T cell because microfusion interaction is not there that case they are helping. So this is the list you go through I hope there is no big deal here and you can understand there is very simple and straightforward.

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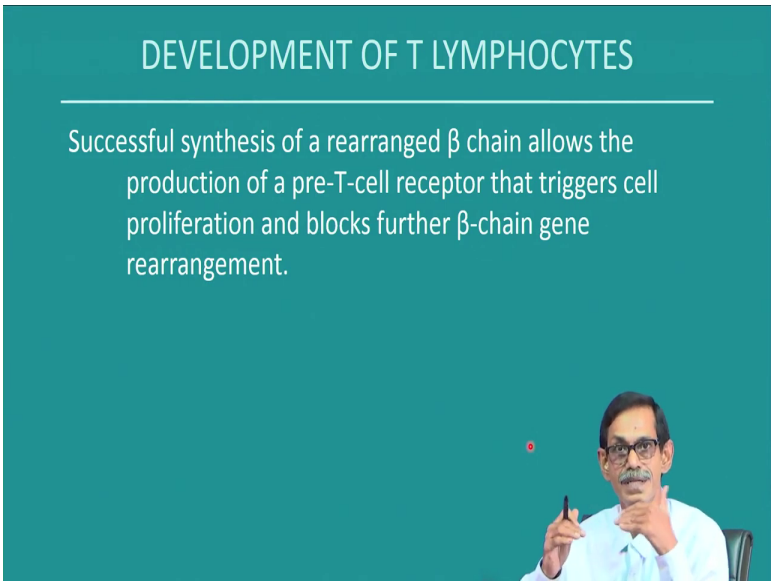

And now I am coming back to again alpha-beta T cell receptor. So if what it is written here successful synthesis of a rearranged beta chain allows the production of pre T cell receptor and triggers a cell proliferation and blocks further. We have to remember that in beta chain what happened first D J and then V D J recombination right. So D J and V D J recombination makes a complete beta chain same way the heavy chain of antibody.

So what happened in during b cell development also you will see after this it will use study that part. So, once the heavy chain rearrangement is complete in antibody or the beta chain rearrangement in bit T cell receptor complete then only alpha cell star alpha chain start. Not only that and once one beta chain is formed like one successful V D J recombination happened no further V D J recombination is going to happen because then there will be a mixture.

You want to see if you see that that same clonal selection hypothesis same thing is saying like one particular lymphocyte will be a particular type of or specific receptor throughout their life. So it is not that one T-cell will express multiple type of receptor. So while the T-cell receptor will be same. So it is not possible if there are multiple beta gene in there are multiple alpha gene so there should be something there which stops that once it is done no further recombination is going to happen.

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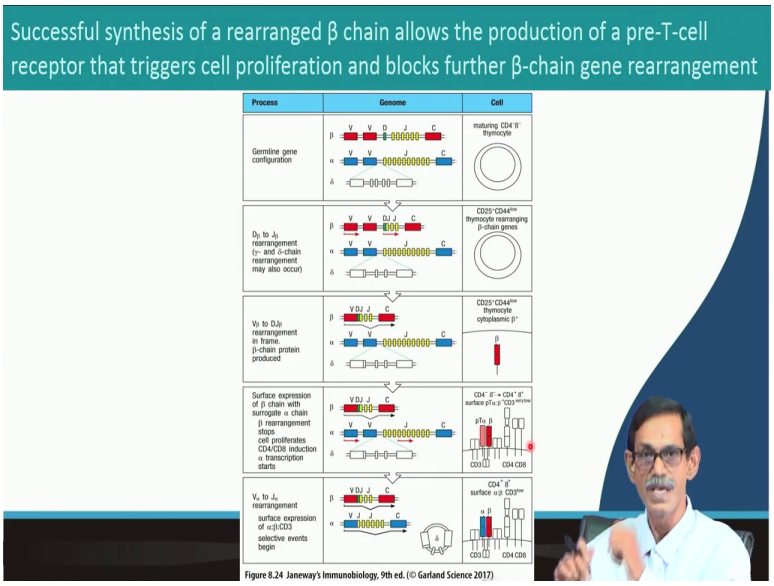

And this is the what I just told this is a graphical representation of that here I am not going detail here because same thing you know whenever you have time go to any book or this lecture see slowly pause this see it otherwise it will be I mean if I keep on repeating the same thing it will be boring. So here the D J recombination is going on here the this D J recombination is coming with V who is V? We do not know it, will continues try so C is there.

So V D J recombination is complete beta chain formation complete and then pT alpha we need to support this beta chain because only beta chain will not survive. And then alpha chain coming when alpha chain rearrangement happened I already told you during this T cell receptor generation the delta part of the delta chain actually in between this V and J. This V and J Delta is there as soon as VJ recombine in alpha this J part deleted.

So this rearrangement of beta chain once this rearrangement is done and pT cell receptor trigger the I mean procedure T cell receptor synthesis starts that immediately stop the further beta chain reaction. So it actually specify that in one B cell there will be only one type of beta chain as it is already scepter after that what happened that V D J recombination is over pT so I mean pre T alpha stops beta chain rearrangement.

Then alpha chain rearrangement happens and once alpha chain it happened then what happened there will be no rag1 and rag2. So, rag 1 and rag 2 stops, if you remember the last lecture

regarding T-cell lymphocyte that stops. So if there is no rag one rag 2, so further recombination happen. But it is not that simple and straightforward.

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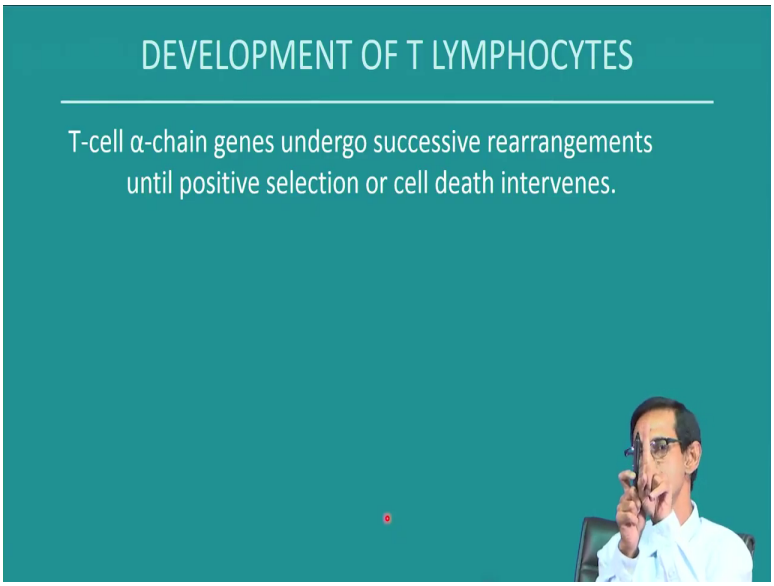

What happened the Alpha chain undergo successively rearrangements. So beta chain is done pT alpha supported that I am telling so pT Alpha supported that which is slightly smaller I am saying just because it is not completed one then alpha chain starts but what alpha chain it is not like that one alpha chain one DJ recombination will happen.

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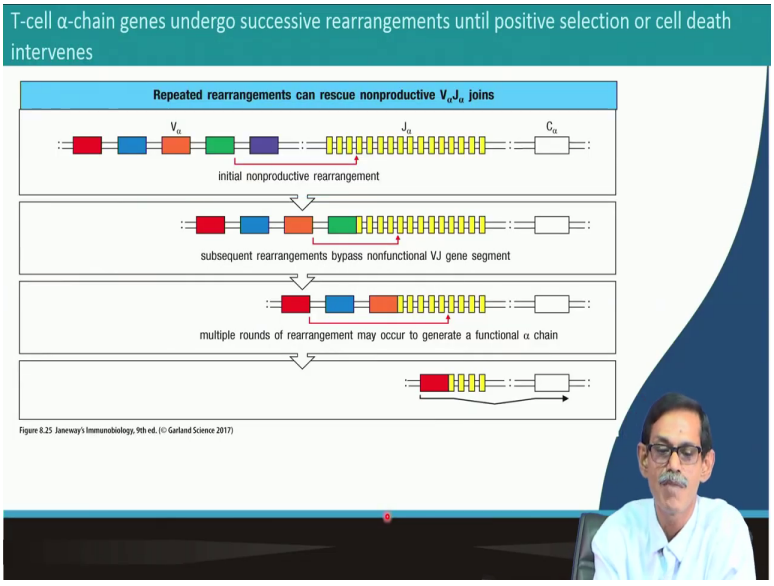

What actually happen is there are multiple V there are multiple V alpha you the number you can get from your previous lecture or any book and there are a lot of J here. What happened suppose

the first this green and one of the J it is random any we can join any J. So one V say suppose for this according to this picture one V is the green one join with fourth J and somehow this recombination is not functional.

This joining is not functional why this is not joining function because recombination can I mean after joining in the recombination it can happen there are a lot of deletion or addition can happen particularly in this case what happened there are T DT is there. It is going to add random nucleotide that may make this recombination not fruitful not fruitful means the product is not going to the right because if the addition is not in multiple of three so what will happen if the because you know codon is three nucleotide right.

So if there is something then there should be something so it should be in frame if there is either 2 or 4 or 5 what is happen there will be a frame shift. So the right gene the right gene is not going to happen. So if right gene is not happening then that T cell or alpha chain is not going to be complete. So that is tested at this moment I cannot tell you how it is tested but there is a I can I mean the temporary transcription happened and they figure it out that this recombination is not a fruitful recombination.

Well fine if this is not fruitful they go for second one so another joint and this kind of continually DJ recombination is going to happen as long as it is getting a fruitful one. So it is continuously happened in each cell. So it happens in the first time the first recombination is fine no further recombination, clear. So if the first one is not right then the second is again and how long it will try very simple answer all of you know.

As long as either V or J is getting exhausted particularly the V see the recombination is happening suppose this one the red one join with this J 1 and this recombination will delete everything right. So this B the red one if join was the last J segment what will happen the whole thing will be deleted recombination that loop formation it will be deleted. So there is no scope of harder recombination.

But if as I mean that is also a kind of exertion are not all exhausted. So if possibility is there like if there are 5 yeah according to this picture 5 variable regions are there break 5 different color you can see and there are multiple J. So one at a time it will try and how long it will try as long as it is not making a fruitful positive selection.

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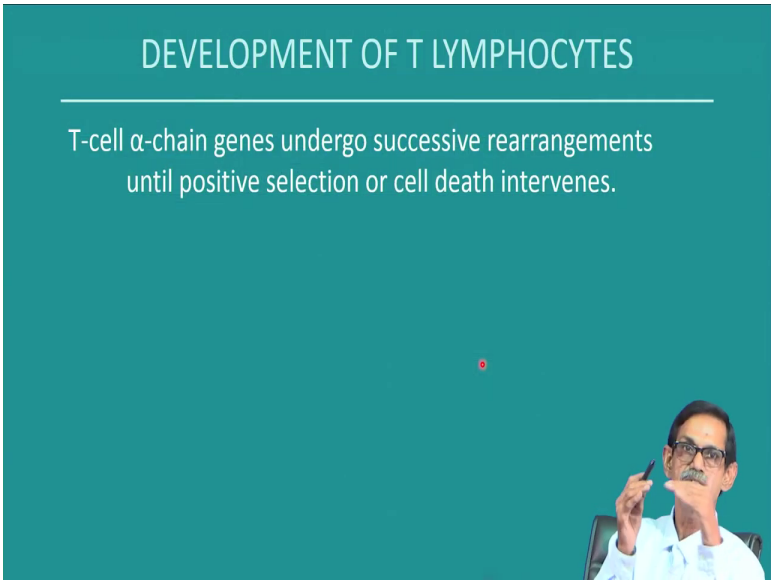

So T cell alpha chain genes undergoes successively arrangement like in the last slide what you said that beta cell rearrangement happen and it is stopped by pre T cell because once it is done successfully there is no more beta chain. Alpha chain also undergoes successive rearrangement and until the positive selection or cell death intervals. What does it mean? It means like what happened beta chain is done pT alpha supported it, now alpha chain reals mean like V J is going to happen.

So there are multiple V chain and there are multiple J chain that we already know. So suppose this one or the green one according to the picture let me say. So the green one recommend with the 4 J 1 and this recombination somehow is not fruitful what does it mean not fruitful means that recombination is not going to give the right protein because of deletion or I mean frameshift maybe for deletion or addition right.

In recombination here what will happen no deletion because T DT is going to add a lot of nucleotides. So if this addition is not making the protein in frame so that is that means it is not a

fruitful recombination. So if this recombination fails what will happen then the second one there I mean the B cell will try with the second one if not the third one then fourth one and it will come continuously try as long as the T cell alpha receptor is complete.

And when it will stop it will stop in the negative selection or positive selection. So if this cell failed I mean this thing is going on if the positive selection is failed to recognize any MHC then only it will die. So B cell T cell receptor gamma delta we discuss alpha beta we discussed. In alpha-beta T cell receptor is formed in T cell developmental stage what next?

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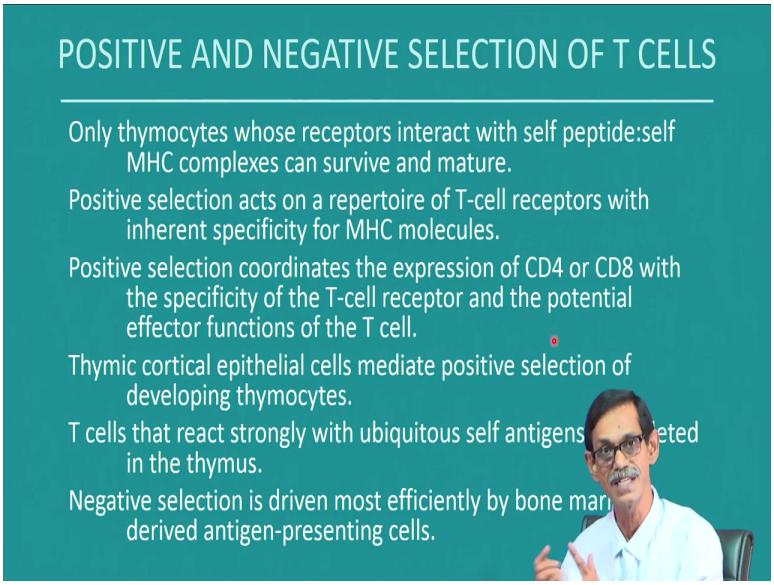

Then it should go for positive selection. And negative selection. What this positive selection means positive selection means these thymocytes bearing a receptor should recognize our cell peptide why I already told few times I am repeating again. So the self peptide and MHC complex should interact with the T cell and that put is positive selection. And positive selection actually meant I mean brings how what will be the repeater of T cell receptor.

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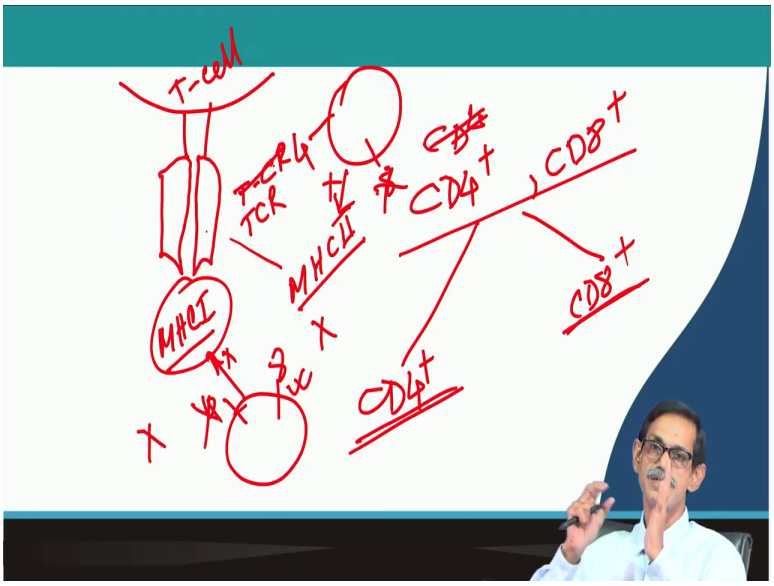

What happened suppose the T cell receptor is like this T cell receptor is like this say alpha chain orbital or I can make so there may be one like this and another is like this. So this is the T cell receptor T-CR. This is T-CR T cell receptor if this T cell and this is the T cell, if these T cells recognize MHC one possible and this T cell also can recognize MHC 2 because receptor generated. So it can fit either a MHC 1 or MHC 2.

So the thing is either one should interact. If it does not interact with neither up all possible a based on that present in say if I am talking about my immune system. If my T-cell receptor does not interact with the MHC 1 possible MHC 1 all variety or MHC 2 present in all all variety of ABC to present in my body that means they cannot survive. So if they do not interact with this first thing is no not with MHC 1 and not with the MHC 2 then they will die.

So to survive, they either they have to interact with MHC 1 or MHC 2 clear. So if they do not survive they die by apoptosis and that is the majority of the cell die and the very beginning during their development. Then now the question is when I am saying the double positive that means CD that means CD sorry CD4 plus and CD8 plus from there they become what CD 4 plus or CD8 plus. How this thing happening there are two things two possibility.

You can find that in many books but I can tell it is nicely written in or with a good picture with the cuvee mean ology you can go and check. So in CD4 plus and CD8 plus what happened T-cell

expressed both 4 and 8 I am just making simple. So what happened double positive cell if they interact with MHC 1. If they go interact with MHC 1 then 8 survive if double positive cell 4 and 8 interact with MHC 2 ok interact with MSC 2 then it goes out.

It interacts with MHC 1 this is called instructive model. So this is how the so positive selection is happening that means it is telling that it interacts with MHC 1. So it will survive then this interaction with what kind of MHC is interacting it is telling which way it will go whether it will make a CD 4 or it will make CD 8 this is called instructive that is also a random or stochastic model is there.

That means what happened, what it is suggesting that in CD4 CD8 double positive cell without another unknown reason some CD4 goes down or CD8 goes down but that it is this theory is much more acceptable at least to me, I mean I do not know exactly what is that, if it is interacting at MHC 1 then it will survive, if it is interacting with MHC 2 that will survive ok. So that is how that Gamma Delta receptor is also kind of model instructive like one chain is inhibiting other and then survive or win the race or randomly something is going slowly down that is 1-1 in same way CD4 CD8 is also going to happen.

So this positive selection is this then it is coming negative selection what is that after positive selection if it interacts with our wound antigen strongly initially also it is interacting with this. So during positive selection also it is interacting with MHC this is also self-antigen but it is not dying but these interaction supposed to be it is not very clear supposed to be very weak interaction. In during negative selection this interaction like the selected positively selected receptor if interacts with T-cell receptor and MHC binding is too strong this should definitely die.

So interaction pattern is different there are nice experiment many nice experiment. So if time permits at the end of the course we will discuss some experiment of this. We will come back again and see some experiment how they have come into this point. So primary interaction during positive selection is weak and during negative selection same self interaction is very

strong. Otherwise positive selection if the past negative selection they should fail we should not have any T cell.

But same interaction cannot happen so one is weaker during positive and similar weak is very strong and if it is very strong during negative selection they will be eliminated. Finally we have or 5% of the total cell which is positive. So this is the end of the story for T cell development. There are many things left but for the basic course I think this is good enough and anything you keep continuing the study the book I hope you understand.

And if you have any problem definitely after this session we will have an online interaction or live interaction that time we will discuss, thank you very much.