Immunology
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Lecture No -32
Development of T Lymphocytes (Contd.,)

So in this lecture we are going to talk about the T cell lymphocyte development. T cell lymphocyte development we already discussed the introductory part of the T cell development lymphocyte what is the difference between B lymphocyte development and T lymphocyte development they are different part.

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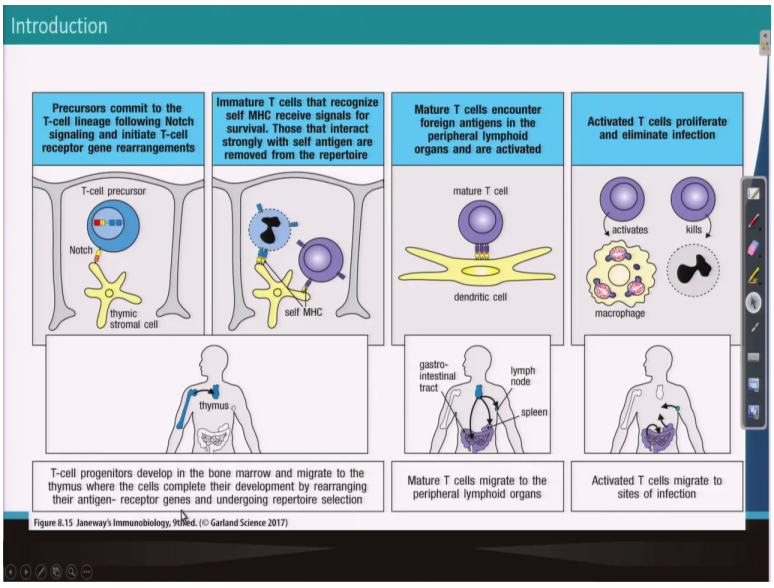

So now just we are going to see the basic outline of whole T cell development you know most of the thing what happened T cell progenitor developed or synthesized in the bone marrow and then migrate to and if you see this migrate to thymus where the cell complete their development by rearranging their antigen receptor, detail you know already. Receptor genes and undergoing repertoire selection like who will survive will not.

So initially what happened T cell receptor developed and interact with that thymic stomal cells that I will come later. What is this you see this kind of structure it is called lobule that will come in the next to next slide we will discuss that. So T cell receptor interact with that and that is gives a signal and T cell development mostly happened through a specific kind of signaling

calleDNotch signaling NOTCH notch signaling. Let me get so this is not signaling and after this signal what happened if it interact with self MHC then it survive. If it is does not interact with self emergency they dies or if the interaction is too strong they also die.

So here in fact in this panel we are showing both positive anDNegative selection we will see in much more detail. And mature T cell when they pass both the positive selection anDNegative selection. Then from thymus so here first bone marrow to thymus and thymus they developed after development when they are finally pass all the test they go to peripheral blood. In peripheral blood they see dendritic cells or macrophage presenting the foreign antigen they interact the maturity cell they convert to the effector T cell in lymph node or spleen the secondary lymphoid organ.

And activated T cell you know what they are doing either they are killing the cell the virus infected cell like cytotoxic or the T helper cell help B cell to develop antibody or help macrophage to kill the intracellular parasites or the pathogens like bacteria also. Mycobacterium tuberculosis in case of path parasite the lash mania. So they kill it so this is the general outline of the T cell development from bone marrow synthesis to development or the training period then peripheral blood where it interacts then do its own job some of the cell convert to memory T cell memory.

T cell maintained in our system so if there is any infection second time or the secondary infection happen then it makes the immune reaction much quicker than the primary reaction. So the question is how that we are discussing in the last class how suddenly we came to know that thymus is the place where this thing happening.

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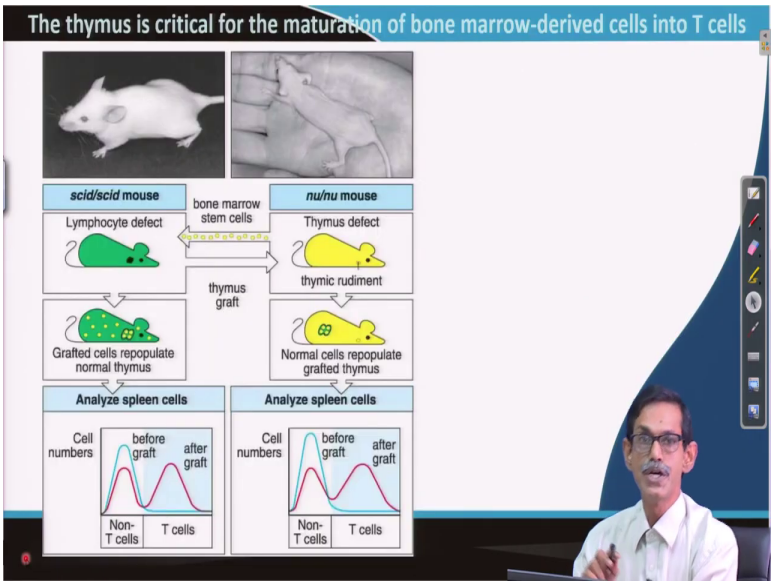

So to do that this is an experiment ok this is an outline of an experiment I am going to tell you what is that. So before that so it was I told already that before B cell T cell identified it was known that if you remove the thymus then lymphocyte population is very low and immune system is highly disturbed. So it found in the mouse calleDNude mouse nude mouse here is nuanu nude mouse is actually possible that if if the thymus is not formed.

It is possible by a particular mutation it is I am not telling you the name of the mutation you can go and check it otherwise do not want to put you in burden like you are already remembering. So many names, so due to mutation it can happen or just at the neonatal stage or when the mouse is in mother's womb that time we can operate out the thymus. So why we call nude mouse then because as soon as you remove the thymus they lost their skin hair.

If you see this mouse there is no hair in the skin thats why it is calleDNude mouse so there is nose here in the skin. There is another kind of mouse is also available it is called scid mice or scid mouse, scid is severe immuno combined severe combined immunodeficient, severe combined immunodeficient mouse. What happened they cannot make lymphocyte neither be lymphocyte nor T lymphocyte.

Here thymus is I mean the mouse where the thymus is absent or in the nude mouse they can make lymphocyte their bone marrow is perfectly all right but as they do not have thymus they

cant do something at this moment. Suppose we do not know that thymos is doing the T cell development what happened there is one more thing you see this is the irradiated mice. So this is this particular mouse they do not have any lymphocyte.

Because it is deficient in that if you take the bone marrow cell from this mouse, this mouse has bone marrow is perfectly all right. So you take bone marrow stem cells transplant into this mouse what will see that they are developing the T cell nicely. Listen one more time this mouse cannot develop any lymphocyte neither B not T because they do not produce that so their bone marrow is defective for some reason.

Actually combine not one reason multiple reasons they cannot make B and T lymphocyte match your ratio. But this mouse they do not have thymus they do not they cant make T cell it is known. Now if you take bone marrow from this mouse inject into this mouse or the bone marrow transplantation into this mouse they have a thymus you see this black part is a thymus. So with time it is shown that they are making the T cell nicely.

Similarly where the thymus was not there if you transplant thymus into this mouse they also can make T cell nicely. What is that is means so this thymus deficient mouse if you put thymus they can make T cell a mouse which is defective in lymphocyte production. But thymus is active if you transfer the lymphocyte then also T cell can produce. Hw they measure? They measure by the flow cytometric analysis that we discussed in when we are discussing the different techniques.

So you see so blue line is before graft so when you transplant the bone marrow cells before that blue line and they measure T cells. How they can measure T cell? We already discussed all T cells both CD4 and CD8 one marker is common that is CD3. So if I use anti CD3 antibody with some green or red color say here it is red i am saying CD 3 red color and label all the lymphocyte and measure initially what happened before transplantation if you see the blue line there is no T cell blue line is flat.

Non T cell they have enough this is a histogram plot they have enough non T cells but no T cell. But after bone marrow transplantation T cell population or after graft same thing T cell population is happening. So what? That means lymphocyte was not there thats why it is not happening as soon as you transfer lymphocyte thymus is doing its own job. But that one experiment cannot prove that the thyme must deficient mouse which cannot make T cell if you put thymus similar result happen.

So this experiment actually telling us that when nude mouse if you put back the thymus they can also make T cell. Both the experiment if you combine you can understand or it is clear that thymus is responsible for mature T cell development. So these experiment actually tells us because digest syndrome which is a complicated syndrome is not only immunology they are cardiac they are physical facial. So complicated thing it is not a single reason so diges syndrome also they cannot make T cell properly.

Nude mouse mutation also cannot make T cell properly. So both the cases thymus was the reason. So thymus is not formed properly that is why T cell was not there. So all this information actually made us believe that thymus is the place otherwise suddenly with all this organ why this thing um thymus will be responsible and certainly how come someone can tell us. These experiment tells that thymus is the place where T cell mature.

There are certain before birth so see when from single cell a complete baby is born right cell division. So during differentiation 1 cell 2 cell 4 16 32. At certain point it is divided like which cell is going to make the heart which cell is going to make the brain which cell is going to make other part kidney and so on. So so and it diDNot happen at a time so during this development when the bone is not formed but still the immunity is required then there is some peripheral kind of T cell or the T cell producing ability some stem cells are found in liver.

Those cells are making a kind of preliminary kind of T cells not the T cell that we are talking about CD4 CD8 or helper there are some T cell which produced in bone marrow produced in liver and then go normally in the peripheral region or the skin region of the baby or the mucous region because that time only this part is required. They mostly do the inflammation and check

the infection from outside. Because body is not formed completely so the if there is any infection still the protection starts.

So immunity partly starts before the total differentiation happen. So what I want to actually say here is that our immune system is protecting us as early as possible not even the whole body is formed ok. Gradually the full all the organ developed, bone developed, bone marrow developed, thymus developed. So they started the preparation and in particularly in case of T cell it grows up to pure body after that the T cell maturation it was gradually slowing down and in adult age it or in old age it is almost none.

I shoulDNot say zero but it is almost none and as a result even after puberty or in adult if the thymus is gone for some reason its nothing much happen in our immune system.

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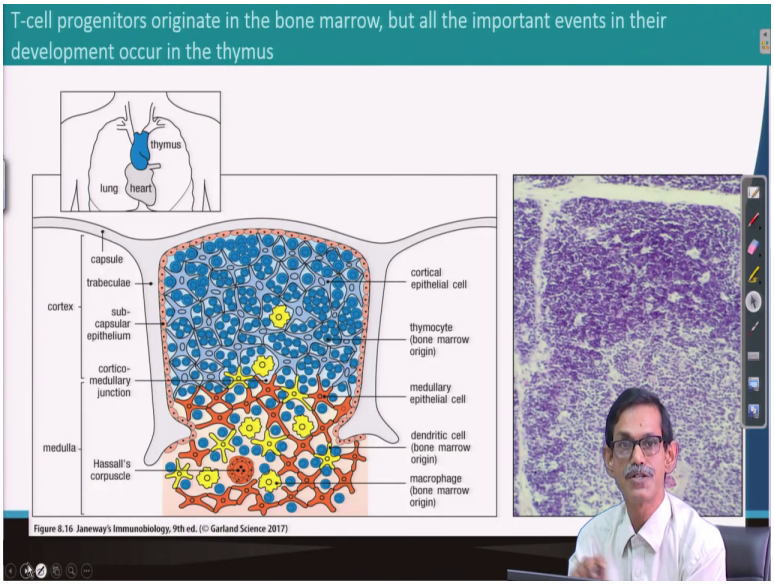

So here what we are saying T cell progenitor originates in bone marrow but all important event happen and development occur in the thymus. This is the outline of whatever we discussed in last class last slide. So now what we are seeing is this is how the thymus cross section look like this is very important because here the development is occurred. Thymus is located where this just above the heart this blue part is the thymus.

It is behind heart but it is above little above the heart. So there are some common zone here. This there are some common so this is the this is thymus. So if you cross section the thymus you will see multiple lobules. So this this part is the lobule so there are series of lobe like that. So there is another one then gradual and so on. And if you see this this part and if I go to next slide lets see here this little bigger version of this.

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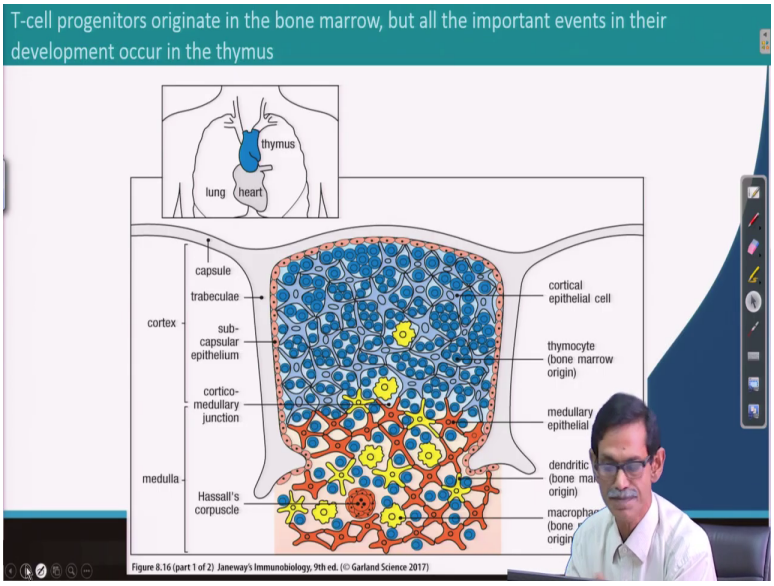

So in this part what we are seeing is there are 2 distinct part here one is cortex this is up to here there is no strong boundary like that but this is up to here. And then bottom part is medulla. If you see this cortex and medulla the population of cell as well as the variety of cells are different. So this is one lobule here you see all these blue circles are the image your T cell we call it thymocytes. Thymocytes are packed and this epithelial cell we call because this this particular these cells is cortical epithelial cell.

Epithelial cell is common and because wherever epithelium is there epithelial cell is here. Here also same way we have medullary epithelial cell. So in cortex we call cortical epithelial cell in medulla we call medullary epithelial cell here just to understand better the colour code is different. So this is the medullary epithelial cell it is like more brown this one is identified. Here very few macrophages are there you see the yellow one this is very few macrophages are there and dendritic cell is also very little or no.

So here mostly what we have we have mostly cortical epithelial cell and blue circles are the immature T cells this is packed here. But just this the scenario is little different in medulla where you see more number of macrophage more number of dendritic cells and definitely if you really medullary epithelial cells are there and T cells are also there. But their number is less it is not as many as that.

There is another thing which is important is Hassell's corpuscles. This one function because through that actually T cell enter from blood to the thymus because T cell synthesized bone marrow they have to come to thymus. So this region actually is very very important because all this one when I say not signaling I am on in this course we diDNot have any scope to discuss the signaling system or the signal transduction part there are variety of signaling system is there.

Some signal tells you to die like apoptotic signal there are not signaling there are wind signaling many TLR based signaling. All signaling process are different but the common any signal transduction pathway normally work like one after another it is not one step. Most of the process in our cell is multiple step because it is gives more control on the cell regulation or the expression of gene regulation.

If there is a single step one to final if there is anything wrong whole thing will be gone. So if I stop it everything will stop but here if you have multiple or cascade kind of system then the control will be much more better. So that is why most of the signal transduction pathway are multiple multiple step. Even if you remember the most simple pathways in your biochemistry I am sure that you must have studied the adenylate cyclase pathway which is a membrane mound the receptor is there and ligand bounds then ATP converted to cyclic MP.

That cyclic MP by the adenylate cycles that cycle can be go and activate the protein kinase this is one of the simplest. If you are not aware of that go and study in any biochemistry book adenylate cyclase pathway. So this is a simple one but notch is not not signaling is not simple. But most of the; not most many developmental part of our system wherever development is there either tissue or organ nausea learning is not very uncommon you will find presence of not signalling in many case and T cell is also.

So all this signaling and initial part of the maturation happen in this region. So here most of the cells are immature neither have positive selection nor have negative selection. So that is why this cell is. So many and after that most of the cell will die that is why the number of population or the mature T cell population is not as much as before. And this particular area is also contain some stromal cells stoma you know which is give the base where all T cell are going to present.

So that stomal cells is making the local environment or micro environment where T cell get all the information all the material like there are any cell development needs growth factors nutrition nourishment. So to do that nourishment by growth factor or many other small protein or release of chemicals and these that I mean its not thats simple and straight forward complicated but you just remember stomal cells are there in this region.

In this region stomal cells are there. So stomal cells are going to supply the all this nutrient anDNourishment material and growth factors so that T cell can survive proliferate and get the chance to mature. And this image is actually the real image cross section of a thymus and stained and seen on the microscope you see. This region this region is so dense ok and this region is not. So this is just represented in the cartoon and this is the Hassell's corpuscles, clear.

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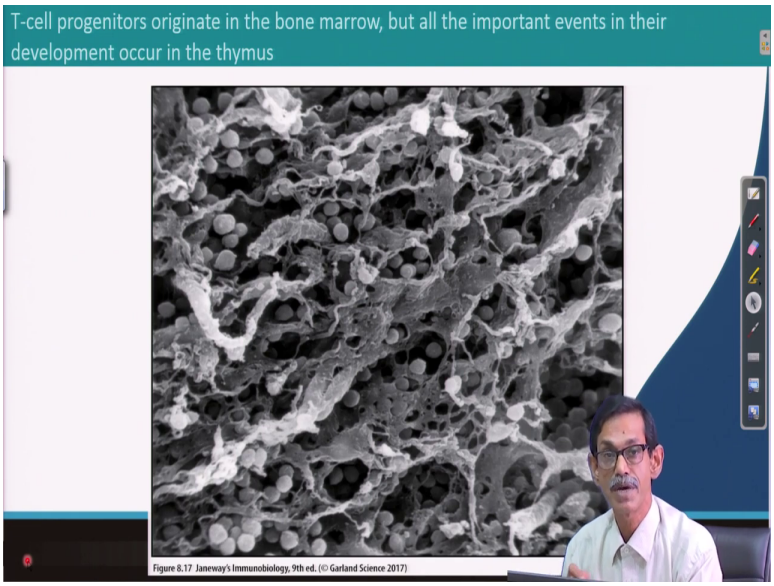

And this is a electron micrograph of that what I was talking about the stomal cells and this all this messy area is the stomal cells and all these spheres like or circular things are the T cells which are embedded into stoma and getting the environment where they can survive proliferate and mature.

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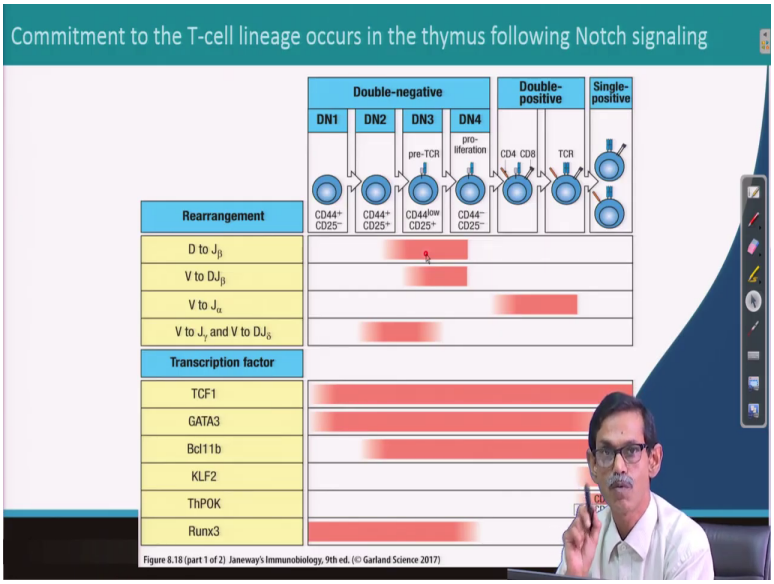

So what it is telling commitment to the T cell lineage occurring thymus following not signaling will not go in detail of non signaling. But we will see. So see do not scare by this image there are so many colors so many names we are not going to go in all this detail what I can tell you why I am showing this picture even we are but something you know here if you see this you know that in beta chain D J recombination happen you know after D J recombination is complete VDJ recombination happen in where you it is happening in beta chain of the T cell receptor when it is done.

Then VJ recombination happen in alpha chain of the T cell receptor. So V to J gamma and V to D J delta also happen. So any T cell that will come later any T cell be either alpha beta receptor type or gamma delta receptor type. This thing we already discussed in the receptor generation or the diversity of receptor generation. This thing I will come later. So we know this but when I am saying T cell is maturing how do you know because T cell looks like does not look like all this blue circle under the microscope.

So many different cells are there how do you know that which one is the just the thymocyte came from bone marrow and which one is in progress so it comes from here and when it mature it gets CD4 or CD8. So this is the final product so in between different stages how can some scientist or some person can figure it out which one is what stage. Again this in this differentiation process or the different steps are also identified by surface receptor.

What is CD I hope I told you right CD4 cluster of differentiation CD stands for cluster of differentiation that means CD is basically in easier way if I can explain CD is the surface protein marker of particular cell. Different cell has different protein instead of giving them 100 different names ok we just designated by number CD1 CD2 CD3 CD4 CD5 something like that there are series more than hundred.

What CD present where you can get a list in end of most of the book there is no point to remember all this. We only remember what the major one we just I mean we discussed the same thing in during the flow cytometry and many classes like CD3 present in all T cell CD4 present in helper cells CD8 in cytotoxic CD19 in B cell see there are many others will come with time. But for the timing CD3 CD4 CD8 and CD19 is fine.

Now we are going to add little more what happened at the very beginning of the thymocyte when it enters into the thymus they have only 1 CD44 plus ok CD44 let me hear it says CD 44 plus and you see there is another thing CD 25. So we are going to add 2 more we already know CD 3 CD4 and CD8 we are going to add 2 more CD 25 and CD 44. So at the very beginning CD 44 is there CD 25.

Next step both are positive positive means both are present in the surface both are present in the surface. Here you see DN1 DN 2, DN 3, DN 4, DN stands for double negative what does the double negative? Means actually this double negative representing neither CD4 nor CD8. Because we understand T cells it will have either CD4 or CD8 remember there are few subset which is not belongs to this general alpha beta category regular T cells that we are not discussing here.

We are mostly considering alpha beta T cell receptor which is helper or cytotoxic. So when CD4 and CD8 both are negative we call it double negative. So double negative also has 4 different stage how this 4 is identified depending on the presence of CD4 T 4 and CD 25 and something else will come slowly. So DN 1 means 44 + 25 - DN 2 both CD 44 CD 25 plus, so if any cell if you take from thymus you find that both are there but neither CD4 not CD8 is there that is DN 2 just for example.

Then what happened CD4 gradually going down CD 44 low is going down but what happened this thing D J recombination star this color is showing that it starts here and gradually going down and ends here. So when this cell is in this stage that means indian 3 stage it is maximum and it stop at DN 4. So D J recombination start at DN 2 and stop at DN 4 V D J recombination starts at DN 3 stop at DN 4.

So when V D J and D J and V D J recombination is complete that means what our beta chain is complete you see this beta chain this TCR beta chain the blue one tcr beta chain the blue one is already completed this blue one is completed. But only beta chain cannot stand alone they need some support that support is given by a temporary light chain it is a temporary light chain is synthesized it is not going to last for long.

And one small you see there is another small chain is next to it this is called pre alpha chain or supporting the beta chain. In DN 4 it is still there pre alpha chain and V alpha J where it is happening that means the light I mean alpha chain when it is synthesizing in this region. So when beta chain complete alpha chain is not yet completed only a pre alpha chain or the supporting temporary support chain is there to support that.

So there are 4 stages of double negative DN one DN 2 DN 3 DN 4 after that what happened more interesting. Then this cell expressed both CD4 and CD8. So it was not there then suddenly both 4 and 8 come you at this stage also you see alpha chain is not there still it is pre TCR that means alpha chain is not there then CD4 and CD8 both are there alpha chain complete. So that means complete TCR form because it is complete here so double positive.

So initially it was double negative now it is double positive what is there at the final stage of double positive CD 4 and CD 8 both are there that means T cell not here decided whether it will make a helper cell it will be helper cell or it will be a cytotoxic T cell. They have both they will go either this way or that way. But TCR is already completed this is the double positive one. Then single positive that means one turn off then TCR will be there then it will be either CD 8 or CD4.

This is CD 8 and this is CD 4 this is the development stage that means T cell developed maturation is not happen maturation happen when they get the training like selection is happen now they just spawn double negative 4 stage DN one DN 2 DN 3 DN 4. Then double positive 2 stage what are the 2 stage both CD4 CD8 there in one case alpha beta is not complete only beta is completed and next is alpha beta both completed both CD8 CD4 there so double positive complete TCR.

Then it goes CD8 and CD4 either one it is called single positive. So this is just the cell is get ready. Now training will happen what the maturation will happen when it will happen it will happen in the next lecture. Now we will discuss in the next lecture till then, see you bye.