Essentials in Immunology

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Module No. # L1

Lecture No. # 25

T cell receptors

Hello and welcome to this lecture on the T cell receptor. In the past lectures we have covered some of the properties of the major histocompatibility complex and how the MHC molecule is involved in self-recognition.

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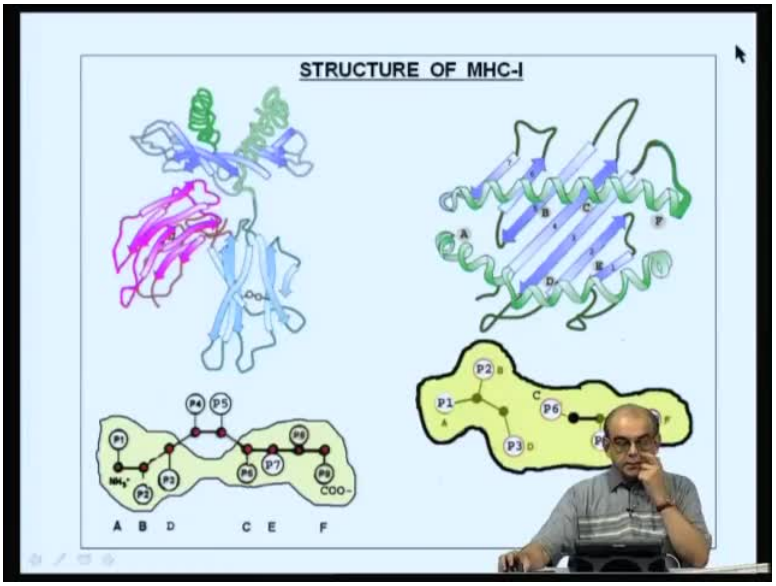

Just to go over that aspect a little bit, we looked at the structure of the MHC molecule and we saw, how the MHC molecule is made up of 2 chains, called as the heavy chain. And beta 2 macroglobulin in the case of the MHC class 1, and this MHC class 1 structure was characterized by the presence of 2 alpha helices on the top, as you see over here, which is also seen over here. These are the 2 alpha helices and the beta pleated floor, which forms the groove, in which the peptide antigen is bound. And this peptide antigen is derived from various pathogenic proteins, which have either infected the cell

or which are found outside circulating in the blood or pathogen derived proteins, such as toxin molecules, which are taken up by antigen-presenting cells and presented to the T cells.

We saw, how the T cells get activated when they are presented with these pathogen-derived peptide antigens that are bound in the groove of the major histocompatibility molecules. Now, let us remember, that the peptide antigen, that is shown over here can be derived not only from pathogens, but also can be derived from self-proteins, which are proteolysed, and then find their way into the, into the MHC binding groove during the antigen-presentation process.

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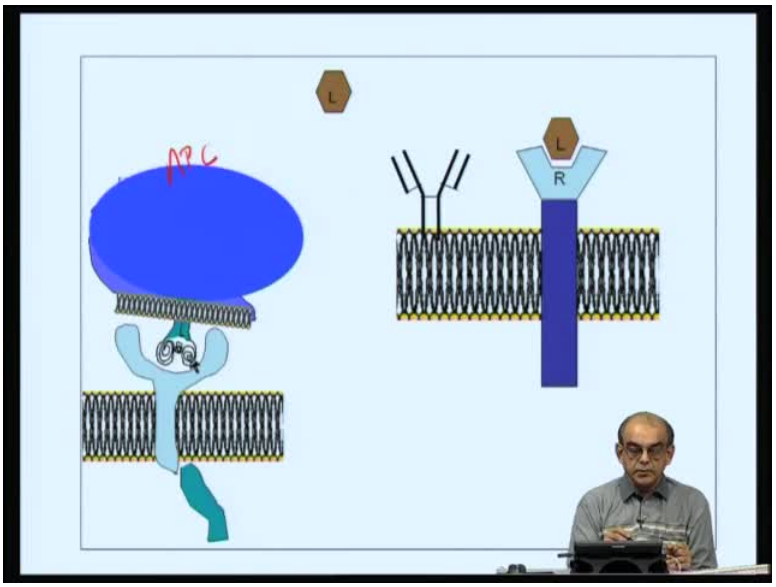

So, to go through the aspect of MHC restriction and recognition along with cell, let us go through some of the aspects of how different cells interact. Cell surface interactions basically, involve binding of proteins to other proteins. For example, if there is a receptor on the cell surface, such as the one that you are seeing over here and a ligand, a free floating ligand, has to bind to this receptor in order to cause downstream consequences, such as cell membrane signaling, which leads to other kinds of events. So, you have to have the binding of the ligand to the receptor, which then causes these consequences down, with, within the membrane. Similarly, for the MHC complex, which is designated over here, these crudely being represented as the alpha helices and this being the peptide

binding groove where you have the peptide, that is bound inside over here, this is the one, that has to be recognized by a T cell in order for T cell activation to occur.

So, you see, that this MHC molecule, whether it is class 1 or class 2 is present on the antigen-presenting cell. This is your antigen-presenting cell, which is presenting the antigen. In the case of MHC class 2, it has taken up the antigen by a process of endocytosis, proteolysed the antigen and by the complex process of antigen-presentation derives peptides, which find their way back into the groove of major histocompatibility molecules.

In the case of endogenous antigens, such as viral viruses, that are replicating within the cells or for that matter, cell proteins of the cells, of the cells, themselves are proteolysed and they are presented along with MHC class 1. This peptide loaded MHC class 1 or class 2 has to be perceived by the T cell and the structure that is present on the T cell is called as the T cell receptor, that is found or represented over here and this interaction is basically, what causes the T cells to activate. So, when the major histocompatibility complex is recognized by this T cell receptor and binds to it and when this antigen-binding groove is complexed with the antigen, that is, that is the event that causes downstream membrane signaling of the T cells, in order for T cell activation to occur. This is the reason why the self also has to recognize because the T cell receptor recognizes the self-MHC molecule. In other words, this entire MHC molecule, especially these alpha helices along with the peptide, that is bound, the T cell receptor are unable to see only the peptide. The peptide has to be bound in the groove of the major histocompatibility complex basically because the T cell receptor recognizes these alpha helical portions, as well as the peptide, that is sitting within the groove.

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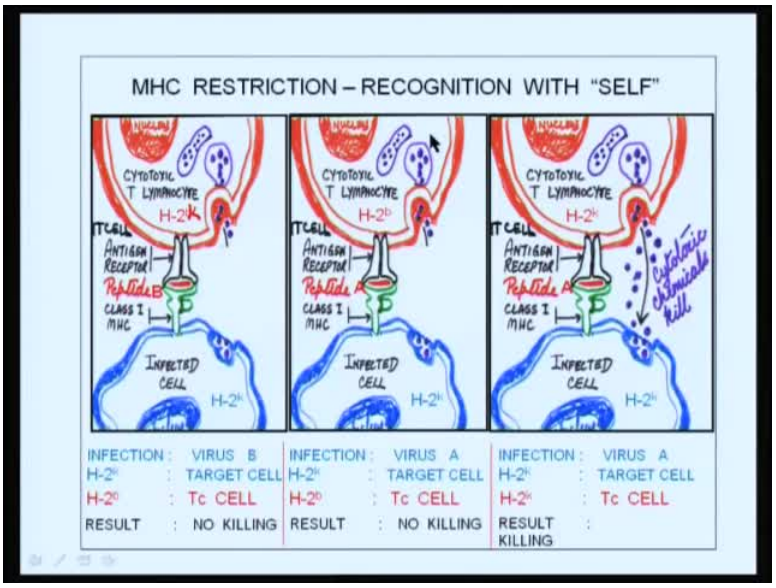

So, going on to some aspects of how T cells recognize and the phenomena of MHC restriction, here is a slide that shows you this MHC phenomena of MHC restriction in the case of cytotoxic T lymphocytes, which are basically killer cells that have the ability to recognize MHC class 1. And class 1 MHC molecules are comprised of a heavy chain, which is complexed with another chain, called as the beta 2 macroglobulin, which is found outside the cell surface and stabilizes the heavy chain by hydrophobic interactions. This class 1 MHC molecule is, in turn, bound to the viral-derived peptide and in this case, we will come to the right of, right of the slide over here, which you call as one virus A, just for an example, it could be any virus, like influenza virus, japanese encephalitis virus and so on.

When this virus A is replicating within the infected cell, the proteins derived from this virus is presented along with this class 1 molecule and that is the red one, that you see over here, occupying the groove of the of the MHC class 1, which is shown in green and this we call as peptide A because it is being derived from the virus A here. This is the structure, that is entirely recognized by the T cell antigen receptor, which we saw in the, in the, in the previous slide, which is expressed on the cell surface of T lymphocytes or for that matter, cytotoxic T lymphocytes or Tc cells or CTL.

When the CTL recognize this peptide A and this CTL shares the same MHC haplotype or the same MHC genotype, then the CTL recognizes the infected cell as self and in this

case, this has been designated as H-2 k and we saw, how it was, how these representations can be done in the MHC lectures. So, these are 2 cells, that match at the MHC complex and therefore, they have recognized their self. The T cell receptor recognizes this class 1 MHC molecule as a self, as self, which is presenting a peptide, that is derived from the virus, that has, that it, that has infected this cell, which is basically an infected cell or we can also call it as the target cell because the CTL are recognizing these cells as targets. This target cell recognition results in the disgorging or degranulation of granules, that contain materials or protein molecules, that have the ability to kill the infected cell, especially, perforent molecules and various other types of proteins called as granzymes, which not only have the ability to punch a hole into the target cell, but they also have the ability to cause this target cell DNA destruction by a process called as apoptosis. Therefore, in this particular slide, it has been represented as cytotoxic chemicals, which are released, which will kill the target cell.

Now, on the other hand, going to the middle panel, you see that you have the same virus infecting the cell, but the cell that has got infected is a cell, that, that is different from the one, that the cytotoxic T lymphocyte has been generated against. The cytotoxic T lymphocyte bears a different MHC haplotype and therefore, it considers this target cell as a non-self-target cell and therefore, even if the virus is the same virus, that is infecting this target cell, which is now recognized as a non-self-cell by the CTL over here, which is basically H-2 b in contrast to the H-2 k target cell, these CTL will not recognize this infected cell and there will be no killing and there will be no degranulation, as you will see, the absence of the cytotoxic chemicals, that are released from this CTL.

Now, going back to the extreme left, you have a situation where you have a peptide, that is derived from a different virus and in this situation, we have a situation where instead of the b, let us have the k haplotype itself, which is, which is self-compared to the MHC haplotype of the infected cell. Despite this match at the MHC complex, if the virus peptide is different, in other words, the virus being different, then also there is no killing of the target cell, unless of course, when the peptide is cross reacting, cross reacting with peptide A. So, this is the phenomena of MHC restriction, which we have alluded to even in the past lectures. So, let us see, what is the nature of the T cell receptor that is recognizing this entire complex?

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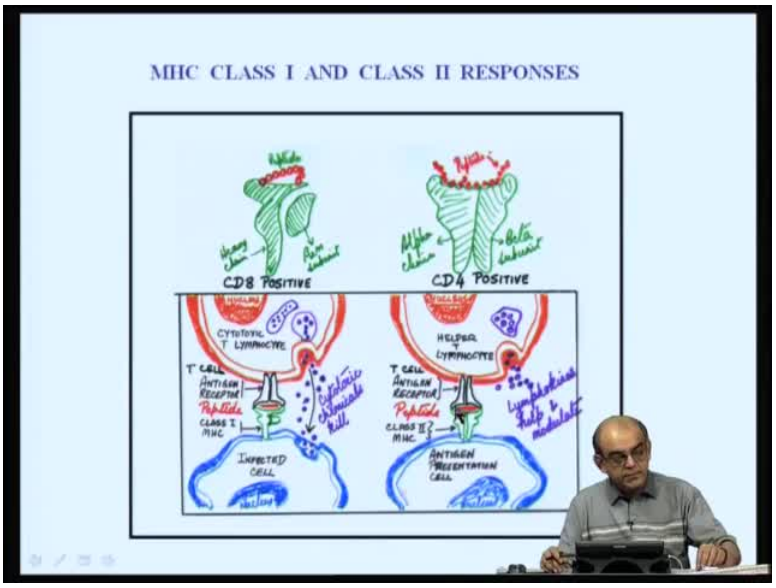

Here is an example, that I have depicted using CTL and therefore, recognizes an MHC class 1 molecule. And the MHC class 1 molecule is recognized by CD8 positivity cells, and this MHC class 1 molecule is different from the MHC class 2 molecule, in that, while the class 2 molecules has 2 chains, both of them embedded in the membrane, the class 1 has got 1 heavy chain, which is embedded in the membrane and the beta 2 macroglobulin, as we alluded to earlier, is the, is the chain, that is found outside or above the cell surface, which stabilizes the class 1 molecule by hydrophobic interactions. In contrast to the CD8 positive cells, which recognize MHC class 1 molecule, it is the CD4 T cells, that recognize the MHC class 2 molecules, which basically presents endocytosed antigens, which are processed and presented within themselves.

So, you see over here, the peptide is bound to the MHC class 2 groove and in contrast to cytotoxic chemicals being released by the cytotoxic T lymphocytes, the CD4 positive, CD4 positive T cells are basically helper cells, in that, they help by the secretion of various kinds of lymphocytes, which help to modulate the proliferation or function of b cells and various other kinds of cells. (( )) as opposed to CD8 positive T cells, which take part in killing, CD4 positive T cells take part in the synthesis of lymphokines upon activation, resulting due to the recognition of the MHC 2 molecule by the T cell receptor. In other words, whether it is MHC class 1 or class 2, it is the T cell receptor, that recognizes this MHC molecule along with the peptide that has been processed and presented; so, what is the nature of this T cell receptor?

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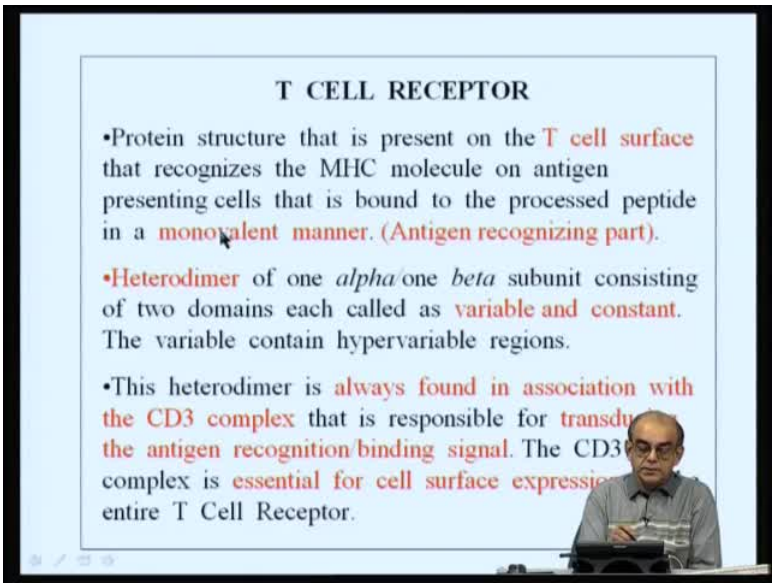

This T cell receptor, it is a protein structure that is present on the T cell surface basically, because these are cell surface interactions, that recognizes the MHC molecule on the antigen presenting cells. And in the case of MHC class 2, these are professional antigen presenting cells, such as dendritic cells or macrophages that take up the antigen, and in the case of MHC 1 molecule, basically MHC 1 being present ubiquitously on most cells; most cells can present MHC class 1 molecules. So, these MHC class 1 molecules are bound to the processed peptide in a monovalent manner, so the antigen recognizing part of the T cell receptor is recognizing the antigen in a monovalent manner.

It is basically a heterodimer consisting of 2 chains, called as the alpha and the beta subunit. There are 2 different kinds of T cell receptor, called as the alpha-beta T cell receptor and there is a 2nd type of T cell receptor, called as the gamma-delta. Basically, these are 2 different cell types that have different functions in the immune system. So, the cells that are bearing the alpha-beta T cell receptor are called as alpha-beta T cells and the cells, that bear the other kind of T cell receptor, called as the gamma-delta T cell receptor, are called as gamma-delta T cells.

This heterodimer, the alpha and beta subunit consists of 2 domains each, one called as the variable and constant. So, you see, the words variable and constant rings a bell, in that, these are similar to what happens or the chains, that are present in immunoglobulin molecules and immunoglobulin molecules are molecules that recognize antigen, millions

of antigens and the variable region of immunoglobulin molecules are associated with this recognition.

Similarly, since the T cell receptor is also engaged in perceiving the antigenic peptide bound to MHC molecules, they also have a variable region. This variable region is juxtaposed or combined with a constant region and the variable region also contained hypervariable regions, just as what was seen in the immunoglobulin molecules.

Heterodimer, that is, this alpha and beta subunit is always found in association with a structure, called as the CD3 complex. So, this alpha-beta heterodimer has other subunits surrounding it, which take part in other kinds of events, like trans-membrane signaling events, which we will come to little later on. This CD3 complex is responsible for transducing the antigen recognition signal, once the T cell receptor or this alpha-beta T cell receptor binds to the MHC plus peptide. The CD3 complex is also found to be essential for the cell surface expression of the entire complex of T cell receptor.

So, if you have knockout animals or knockout cells, that do not express the individual components of the CD3 complex, the alpha-beta subunit of the T cell receptor does not come out on the cell surface and therefore, the T cell cannot recognize the MHC molecules, that is presenting the peptide and as a result, cannot respond to viral pathogens or virally derived peptides, that is presented by the MHC molecule even if, even though the MHC is successful in presenting that pathogenic derived peptide.

Going on, further, because the immunoglobulin molecules as well as the T cell receptors possess these variable regions and constant regions, let us compare these 2 structures and see, what are the structures that are common between the two?

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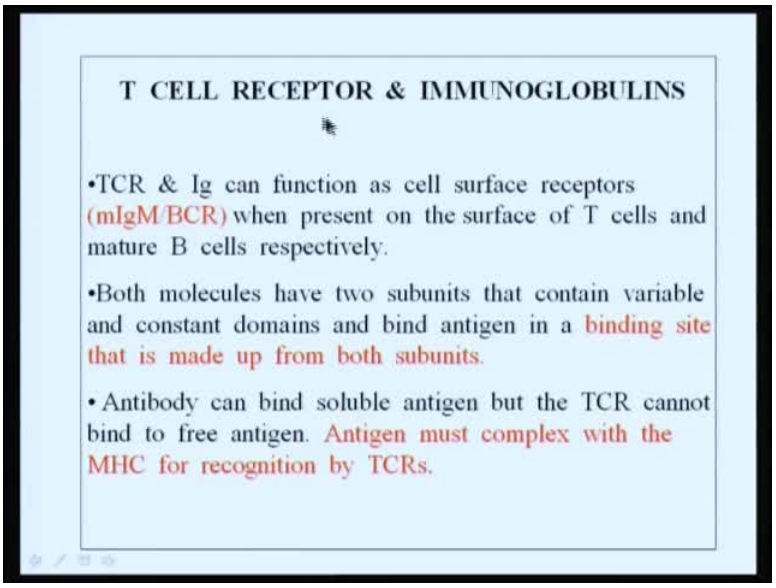

So, if you look at T cell receptors and immunoglobulin molecules, the T cell, as well as, immunoglobulin molecules can function as cell surface receptors. The antibodies that are found floating in the serum, are actually derived from a membrane bound immunoglobulin receptor, to begin with. That means, mature B cells, before they differentiate to plasma cells, that secrete a particular type of antibody, they bear membrane immunoglobulin receptors on the surface, which are activated by the incoming antigen; this also called as the B cell immunoglobulin receptor or BCR, as opposed to, the TCR or T cell receptor. So, both are present on the cell surface - BCR being present on the B cell surface and the TCR being present on the T cell surface; so, these are present on the surface of mature T cells as well as mature B cells.

Both the molecules have 2 subunits that contain variable and constant domains. They also bind antigen and there is a binding site that is made up of both the subunits. Now, in the case of immunoglobulin molecules, it is actually a dimer of dimmers. So, this dimer has a heavy chain and a light chain and 2 heavy chains and 2 light chains come together to form 2 binding sites. So, therefore, the immunoglobulin molecule are supposed to be divalent, so the antibody can bind soluble antigen because this complex of heavy chain and light chain can bind free floating antigens, floating around in the serum. In contrast to, as already mentioned, the T cell receptor cannot bind free floating antigen; the antigen has to be complexed with the self-MHC molecule for recognition by the T cell receptor.

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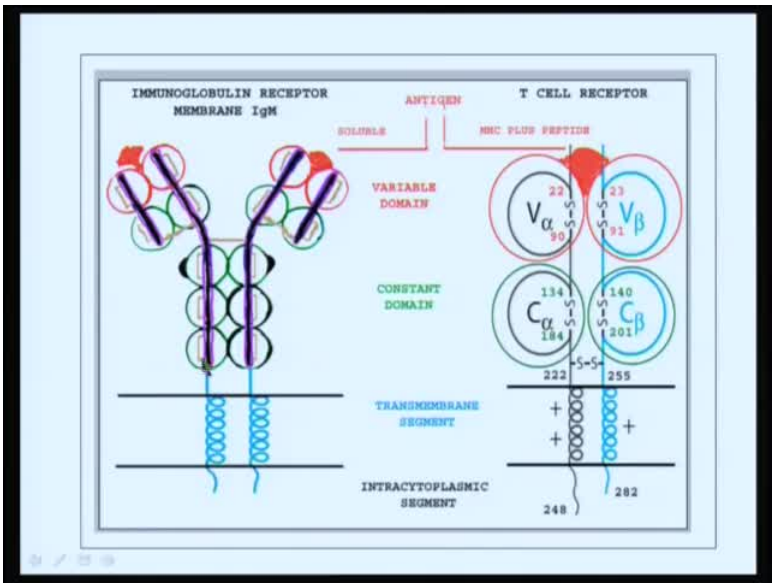

Now, looking at the structure of these subunits, so you see over here, normally, the membrane immunoglobulin receptor on the surface of B cells is usually IgM. So, this is the surface bound immunoglobulin receptor, it has a transmembrane portion or a segment and it has a short intracytoplasmic segment. This IgM molecule has got in the constant region of this molecule, there are, these are the different domains that are present. There are 3 domains, as opposed to 2 domains present on immunoglobulin G. All these have got a characteristic structure, called as the immunoglobulin fold. So, you see, that many molecules, both within the immune system, as well as, outside of the immune system, when they share this particular domain or so called immunoglobulin fold. They are said to belong to the immunoglobulin super family of molecules.

So, this is the domain, the constant region domain, these are held together by disulfide bonds between the 2 heavy chains and the heavy chain and light chain are bound together by another disulfide bond, which is shown here in brown. In addition to these disulfide bonds, there are intra chain disulfide bonds, as you see in brown, indicated in these different domains. More importantly, the red circles, that you see here, represents the variable domains of immunoglobulin molecules, both of which are required, one on the heavy chain and one on the light chain to make up a binding site, that binds to this soluble antigen, which is present on, in a divalent fashion in this particular molecule.

Now, the same thing, when you look at the T cell receptor, it is also membrane bound and as opposed to the immunoglobulin molecule, which is, this is the immunoglobulin molecule, that is then secreted out by the plasma cells. Once the mature T cell, mature B cell is activated, as opposed to this, a T cell never gives out or secretes out its T cell receptor molecule; it is always membrane bound and the actions, that ensure due to the transmembrane signaling, never results in the final release of this T cell receptor molecule into the media, as what happens in the case of B cells.

However, just like the immunoglobulin molecules shown in the red, you have a variable segment over here. There are 2 chains here, called as the alpha and beta. The alpha chains have the variable segments and the green one represents the constant regions, as seen over here, which is seen over here also. So, both these molecules, these 2 chains are bound by disulfide bonds and you have a variable alpha and a constant alpha and a variable beta and a constant beta, both of which have to come together in order to see the MHC plus peptide. And these numbers here represent the amino acids at that particular position. In addition, you see that these transmembrane portions are actually charged; they have charged residues, we will come to that in a little later on, in this next slide.

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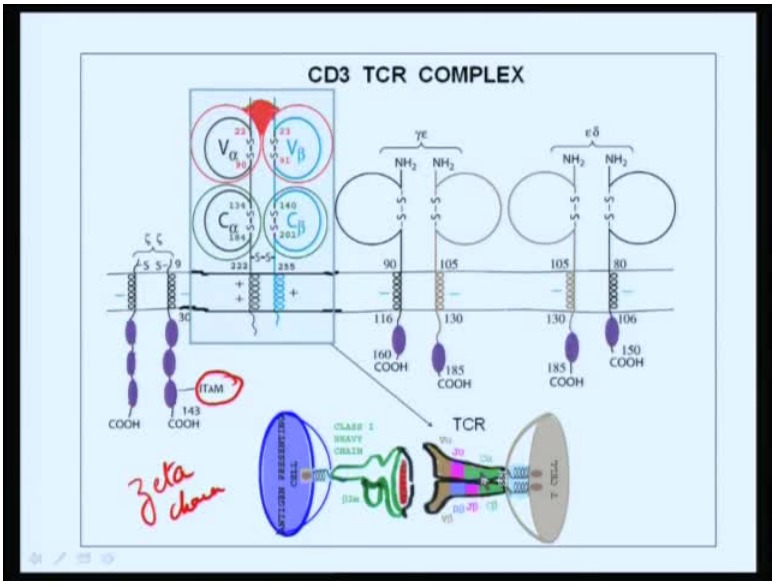

Now, this alpha-beta T cell receptor is found in association, as I told you earlier, with what is called as the CD3 complex. This CD3 complex is made up of different subunits, it is called as, these are the antigen binding portions of the T cell receptor. Note, that they

have a short intracytoplasmic segment. You have the other subunits, called as the gamma, gamma over here, and the delta. There is another subunit, called as the epsilon, represented in this figure in brown. All of them have disulfide bonds, therefore, belonging to the immunoglobulin super family. The amino terminal being outside the cell surface and the carboxyl terminal being within the cell surface, just like, just like in the case of immunoglobulin molecules and this epsilon subunit always pair one with gamma or with delta. So, you always have on the cell surface the gamma-epsilon or the delta-epsilon subunits. And note that these transmembrane segments have opposite charges as that is present on the antigen binding portion of the T cell receptor. Now, this causes these subunits to come together because the plus and minus attracts to each other, so they are all present as a complex.

In addition to that down below the, in the intracytoplasmic portion, which is longer than, what is found in the antigen binding alpha-beta subunit, you have these purple structures called as the ITAMs or immune receptor tyrosine activation motifs; so, immune receptor tyrosine activation motif. So, these contain tyrosines, that get or that gets phosphorylated in response to various signaling events. And phosphorylation is one of the most important transmembrane or modification events, that actually result in the transmembrane signaling, that results in the modulation of genes downstream events, that modulate genes and therefore, give rise to different kinds of protein products derived from those genes; we will come to that a little later on.

Now, we will see, that these motifs immune receptor tyrosine activation motifs are not only present on these molecules; they are present in even other molecules of the immune system, such as IGE molecules. So, you see, that they actually, there are many different molecules in the immune system, that possess these ITAMs; we will come to that in a little later on.

So, if you see these different subunits, like for example, gamma-epsilon or the delta-epsilon, you find that there is 1 ITAM each in these subunits. So, these 2 subunits have 1 ITAM each, as shown by the purple ovals over here. These are the different numbers of the amino acids that are in that position, as opposed to these 2 subunits. There is another homodimer that is also associated with this antigen binding portion of the T cell receptor, that has got 3 ITAMs present in each of the subunits and this is called as the zeta

subunits. So, this is called as the zeta chain or the zeta subunit. This is always found as a homodimer, they do not associated, they do not associate with these subunits at all.

So, basically, then you have these different motifs, that can get phosphorylated and the more number of residues, the more number of ITAMs that gets phosphorylated, then more is the strength of signaling; the more the phosphate, the more the strength of the downstream signaling event, as you will see in the next few slides.

So, basically, if you look at this, this antigen binding portion of the T cell receptor, it is represented over here. And this is the T cell shown in brown and this is the transmembrane, transmembrane portion, and the short intracytoplasmic segment, that is found over here and just for simplicity, I have represented it as a structure, that can recognize this entire complex of MHC-1, that is presented by the MHC of the antigen-presenting cell and that is bound with the peptide at the antigen binding groove of the MHC. So, you see that these brown portions are the variable portions coming from the variable alpha or the variable beta. So, this is the, this red portion here is the peptide plus MHC.

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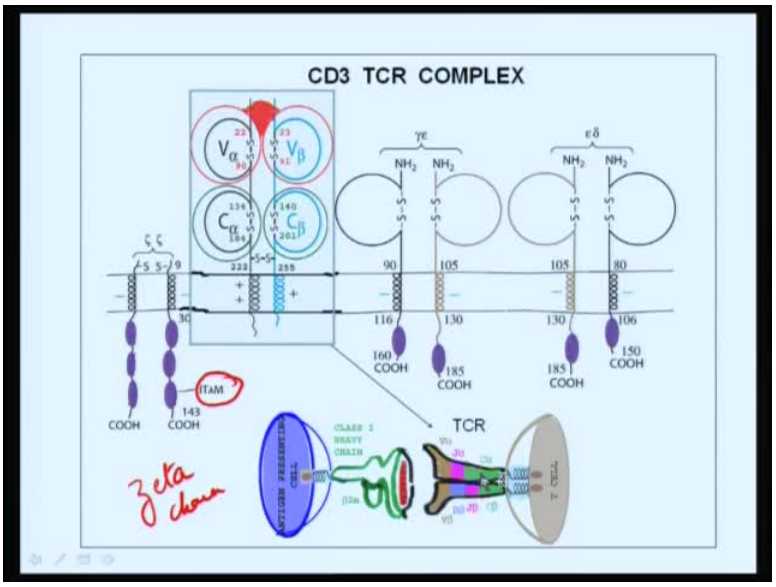

So, in addition to these variable portions, that are found in these 2 subunits, whether they are alpha or beta, you have, just like in the heavy chain of immunoglobulin molecules, you have the participation of diversity segments and joining segments, corresponding to the genes that make the diversity segments, as well as, the joining segments. In addition

of course, there is a constant segment, as you saw here, the constant domains. And in the case of immunoglobulin molecule, these constant regions are made up by the immunoglobulin classes, like IgG, IgM, IgE and so forth.

So, you have these constant portions that are juxtaposed with the joining segment in the alpha, which is similar to that in the light chain, which does not have the diversity segment; it has only the j segment or the joining segment. So, you have VDJ and C for the heavy chain of immunoglobulins, as well for the beta chain of the T cell receptor, and you have the VJC for the light chain of immunoglobulin molecules and the alpha chain of this T cell receptor. Note the comparisons, how similar they are in terms of the heavy chain and the light chain for the immunoglobulin molecule being the presence of diversity or the absence of diversity in the light chain.

So, this is the entire structure of the T cell receptor, as I told you. So, let us see, how this T cell receptor participates in signaling events that are consequent of this structure binding to the entire MHC complex.

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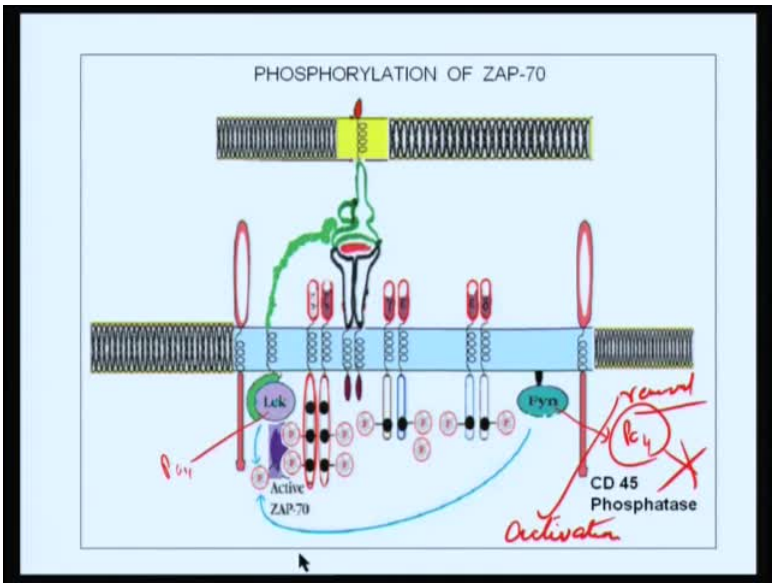

So, going on further, so you see, how these different various molecules participate in downstream signaling events, that cause the activation of genes, that could for cytokines and cytokines are an important process of T cell activation, as we saw earlier. IL2 is an important cytokine that has to be secreted, in order for T cells to proliferate; so, what is it, what are the events that lead to the activation of the secretion of IL2? Now, in T cells,

that do no activate their T cell receptor, in other words, now in T cells, that have not seen the antigen via the T cell receptor, have not, are not activated and therefore, they do not synthesize this IL2 or other kinds of lymphokines.

So, going again to the T cell surface, you find, that you have the antigen binding subunits, this is, this being the alpha and this being the beta, which is recognizing the class 1 molecule over here in the slide. And this class 1 molecule is recognized by a CD8 T cell and the CD8 molecule is found over here in this curved portion. The CD8 actually binds to the alpha 3 domain of the MHC class 1 heavy chain, the alpha 3 domain being the closest to the, to the cell membrane of the antigen binding antigen presenting cell.

So, this CD4, in the case of MHC2 molecules and in this figure, if it is the MHC2 molecules, you have 2 subunits, that are anchored into the membrane, that is basically the difference. And the one, that is binding to this molecule over here would have been a CD4 molecule. Basically, it is the presence of this CD8 or CD4 molecules, that enables a CD4 positive or a CD8 positive T cell to recognize the self-MHC, that is bound to the peptide and therefore, you can say, that the CD4 and CD8 is what imparts the MHC restricting ability for the T cells to recognize self-MHC molecules. So, this is somewhat similar to a rider that is sitting on a galloping horse. If the saddle is loose, the saddle is tightened by putting something beneath the saddle, so the saddle is actually sitting tight and the rider sits tight on the horse.

So, basically, that the, that same function is provided by the CD4 or CD8 molecule, which interact with this class 1 or class 2 molecules, in order to increase the affinity of binding of this T cell receptor to this peptide. So, if the affinity of the peptide that is bound within the groove is not very high and therefore, the peptide is sitting loosely when the T cell receptor and T cell comes close to binding and recognizing this complex. The CD4 or the CD8 molecules funnel like the tunnel in order to make this affinity stronger and therefore, the T cell activation process is enabled in a much more efficient fashion. So, what is this T cell activation process?

So, if you look at the T cell surface, in addition to these are the different subunits that you saw in the previous slide, this is the alpha-beta subunit of the T cell receptor, then you have the zeta subunit followed by the other subunit, all of them represented in this black ovals as the ITAM motifs. Now, you see, they are, they can be phosphorylated,

which is indicated by this white ovals over here. Now, you have specific kinases, like the Fyn and the lymphocyte-specific kinase that is found beneath the cell surface in the T cells. You also have, what is called as the CD45 phosphatase. To begin with, these molecules, like the Fyn, on the surface are already phosphorylated; they have some phosphate already on their residues.

So, this being a phosphatase, this being a phosphatase, the CD45, once the antigen is bound by the T cell, this CD45 phosphatase comes closer to the T cell receptor. When it comes closer to the T cell receptor, this phosphate is removed. This removal of the phosphate on these kinases, that results in activation. So, because they are kinases, they are going to add phosphates to these ITAM motifs and what happens is that all these motifs get phosphorylated. Once this gets phosphorylated, there is a protein called as the zeta activating protein - ZAP70. This ZAP70 has the ability in it to bind to SH2 domains. So, these have SH2 domain that have the ability to bind phosphorylated residues. So, the inactive ZAP70 now comes together in close association with these ITAMs that have been phosphorylated. This close association actually triggers the lymphocyte-specific kinases to phosphorylate the ZAP70. This phosphorylation event of the ZAP70 molecule, as you see over here, actually results in the kinase ability of the ZAP70. It itself is a kinase and the function of kinases is basically phosphorylation.

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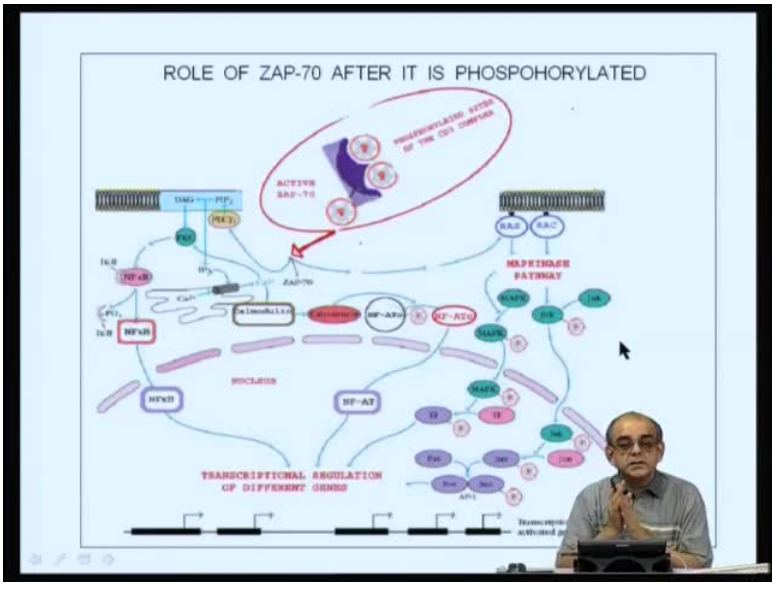

So, when you look at the active ZAP70 over here, which is phosphorylated, it has a kinase activity. It goes and phosphorylates other downstream molecules, like phospholipase C gamma, which results in the degradation of PIP2 to diacylglycerol and diacylglycerol in turn activates PKC, which then phosphorylates important transcriptional factor, which is found in the cytoplasm in an inhibitory form bound to inhibitory I kappa b. This phosphorylation of I kappa b releases the NF kappa b, which has nuclear localization signals to go into the nucleus and bind to its cognate genes. And in different situations, different transcriptional factors bind to different transcriptional regulatory elements and thereby activating different kinds of genes in different situations.

So, you see, these are the different transmembrane signaling events, that are triggered off by phosphorylation and the ZAP70 goes and phosphorylates other molecules in addition, like RAS and RAC, which, act, activates the map kinase pathway, in addition to what is called as the NFAT, which is found specifically in T cells. These are transcriptional factors that are found in T cells for the NFAT, has the ability to regulate different, genes that are different, in addition to what can be regulated by an active NF kappa b.

So, basically, you find, that these phosphorylation events actually activate, cascades or pathways, that finally lead to the activation of transcription factors, such as NF kappa b, NF-AT or for that matter AP-1, which is basically a complex of Fos and Jun, these, which can go and bind to regulatory sites transcriptional regulators on various kinds of genes and therefore, results in the activation of genes, which finally results in the secretion of various kinds of lymphokines into the supernatant of T cells and these lymphokines then help out B cells to make antibodies and so on and so forth. So, this is the basic phenomena of transmembrane signaling, that is resulting due to the activation of the T cell receptor, recognizing this MHC plus peptide complex. A naïve receptor, naïve T cell is not recognized its cognate antigen, but when there is a viral pathogen or a viral replication within a target cell, that finds its way into the MHC class 1 or for that matter class 2 molecules, which then activate either the CD8 T cell receptor or the CD4 T cell receptor and result in these various consequences or downstream consequences of T cell activation.

So, the, T, CD8 follows a different T cell pathway of activation and the CD4 has a different pathway of activation in terms of what sort of lymphokines, that they secrete and therefore, different arms of the immune system are activated.

So, the T cell receptor having variable portions and constant portions was discovered in a very, very unique way. The immunoglobulin genes by that time had been discovered and they had found, that the nature or the mechanism by which the variable portions of immunoglobulin molecules are put together with constant regions in the immunoglobulin molecules, involves a process of gene rearrangement. Many genes coded for the variable regions in immunoglobulin molecules and remember, the T cell receptor also has variable segments or variable portions to the alpha-beta T cell receptor, and also has constant portions, just like immunoglobulin genes, as we covered just now. So, again in the T cell receptor, the variable region, the variable regions had to be put together with constant regions, just like in immunoglobulin genes.

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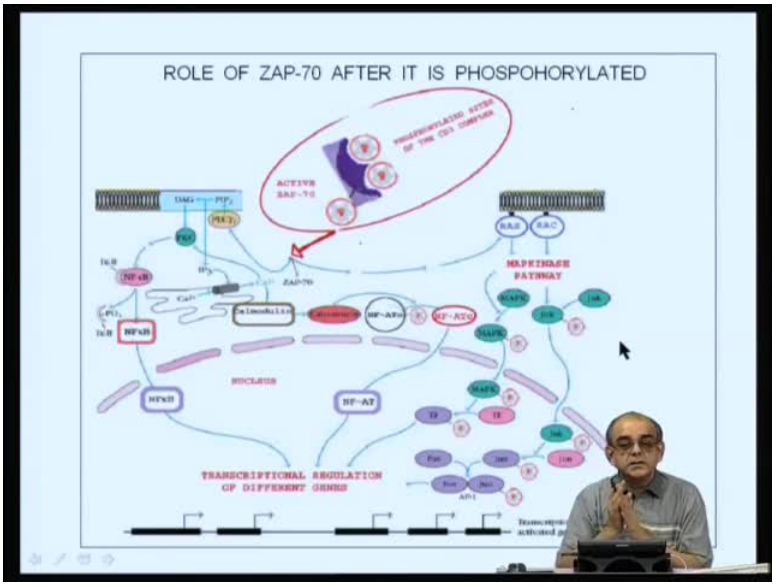

However, to the helplessness of many of these immunologists, they found that by using cDNA probes that they had prepared for immunoglobulin genes would not pick up anything in T cells. So, the availability of these cDNA probes from immunoglobulin gene studies or cloning studies was not helpful in, in detecting these, the structure or the genes that are coding for the variable genes and the constant genes in the T cells, basically because they were quite different from what was present in immunoglobulin

genes; the sequence being quite different. Remember, sequence could be very different because the T cell receptors recognize the complex of MHC plus peptide, whereas the immunoglobulin genes recognize only the free soluble antigen that could be the complexity that resulted in this helplessness in trying to clone the T cell receptor genes, using B cell variable gene probes. So, how was this T cell receptor genes discovered? So, before we go into that, let me cover just as an additional aspect about avidity effect or so-called avidity effect in T cells.

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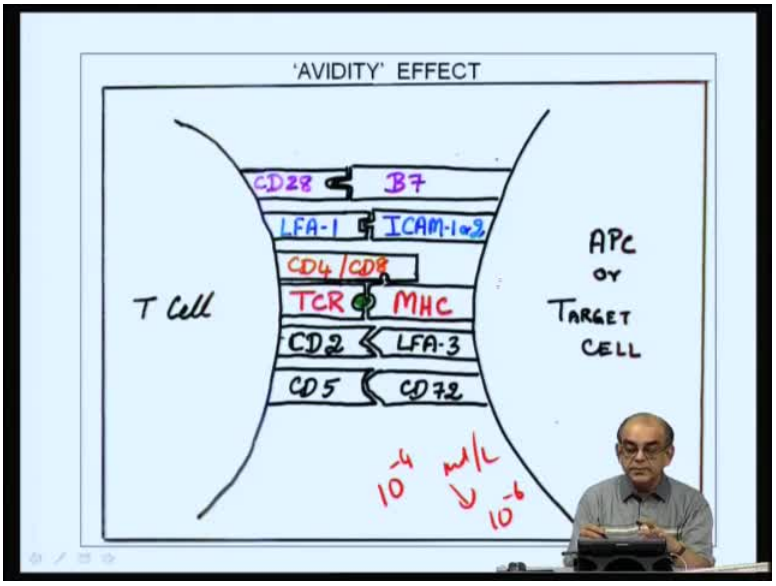

In addition to the alpha-beta T cell receptor binding to the MHC and the CD4 and CD8 participating in that, there are other molecules, these are called as addition molecules, that we covered in the first few classes, called as LFA-3 CD2 and LFA-1 and ICAM-1 or ICAM-2, the co-stimulatory molecule B7and the CD28. So, all these molecules actually participate during the recognition of the, of the antigen presenting cell by the T cell. It is something similar to a kabaddi game, where a person comes into the opposite court who is first coming in and then, once a person catches the shirt, of the, of the person coming in, then all the rest of the people pile in to prevent him from going back. So, it is similar to that, in that, the initial interaction of the T cell receptor with the MHC complex is stabilized and increased. The affinity increases because all these various other kinds of addition molecules take part.

So, the affinity constant for the, for the T cell MHC interaction could be something, something of the order of 10 to the minus 3 or minus 4, which can be increased several orders of magnitude to the moles per liter, can be increased by several orders of magnitude to almost 10 to the minus 7 moles per liter by the, by the participation of these various addition molecules.

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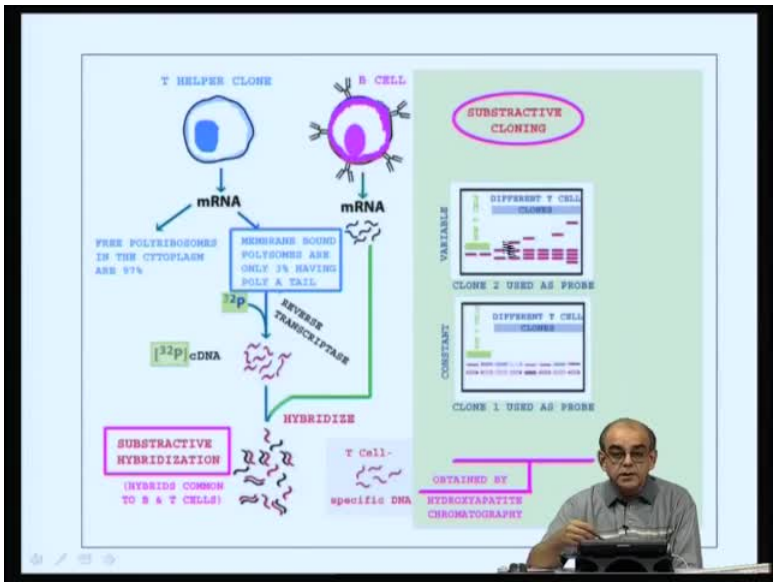

So, coming now to the discovery of the T cell receptor genes. So, what was done was they have clones that had been established, as I told you earlier, Hybridoma clones. These are called as T helper clones that were cloned from T cells. What they basically assumed was, that if you prepared mRNA from T cells and you took the mRNA from B cells and somehow removed the component, that are present in B cell, that are common between T cell mRNA and B cell mRNA, then you would end up with a mRNA population, that is very specific for T cells and therefore, increase the probability of finding the genes, that are coding for the T cell receptor because the T cell receptor is specifically found only on T cells.

So, what they did was to take this mRNA, they prepared the mRNA and in the, this mRNA consists of 2 types, one that is bound to polysomes or polyribosomes, which can be actually purified by using Oligo dT columns because they have a poly A tail, so this these RNA or mRNA as that have A poly, A tail are the ones, that are actively being processed and synthesizing and coding for the proteins being translated to give you

proteins. So, they are polysome or ribosome bound as opposed to several other mRNA, that are not bound to, bound to the ribosomes at a given point in time which make up 97 percent. So, the polysome or membrane bound polysome's RNA is only about 3 percent. So, basically by isolating this mRNA, that is bound to Oligo dT, which have a poly A tail, they got rid of 97 percent of the mRNA. Then they took this mRNA population and used reverse transcriptase in order to make cDNA using labeled P32.

So, this labeled cDNA was then used to hybridize mRNA that had been isolated from the B cell; total mRNA that was isolated from the B cells. So, whatever was common, because this is complementary DNA, whatever was common between B cells and T cells would hybridize together because this is RNA and this is complementary, so they would come together and you will see some of this hybridized population shown over here. So, this hybridized population can actually be separated by hydroxyapatite chromatography to release those, that have not been hybridized, and what are those molecules or mRNA that would not hybridize? Those are the molecules or T cell specific DNA, that, that are derived from this cDNA, which had not hybridized was that, one that would have been unique to T cells because these are RNA, that was derived from your B cells.

So, using this approach, they got this unique population and they used these as probes. So, they took each one of these things, labeled them as probes and they probed the T cells. So, when they did this, they found, you, when you use different clones over here, clone 1 and clone 2 for example, and they had different T helper clones, that had been isolated against, which would recognize different kinds of antigens. They found certain probes giving you only 1 set of bands, meaning, not varying in size when separated on Agarose gels by electrophoresis as opposed to the other probes detecting different sized bands. This is actually because these, these different clones have different T cell receptor, the variable regions are different and therefore, these clones were recognizing these variable segments. We will come to that as to why, why you have different sized clones?

So, basically these are different T cell helper clones that were detecting the signal or being detected by using a different clone as a probe that was detecting the variable region, which was different from clone to clone and therefore, the size being different when electrophoresed, as opposed to the constant region, which would not change in

size. So, they had a probe, that would detect the constant region and they had probes that would detect the variable regions.

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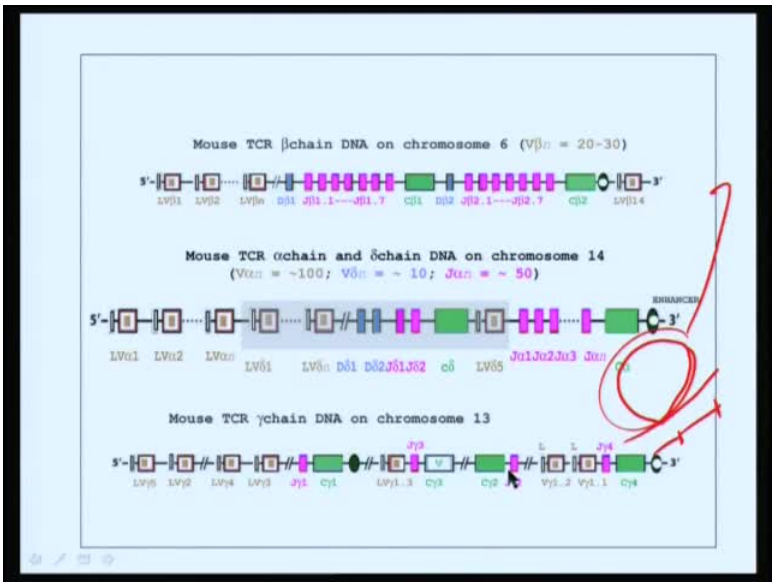

So, putting all this story together, if you look at the mouse T cell receptor DNA, we will come to the actual properties on which chromosomes, that they are found in the next few slides, and if I am not able to cover this due to lack of time, you can read it up because the slides are present. You have basically, just like immunoglobulin genes, you have a number of variable regions accompanied by the leader, leader portions or leader segments, so you will see them in brown over here and you have a diversity segment for the beta chain of the T cell receptor, similar to what is found in the heavy chain of immunoglobulins and you have several joining segments, that are found in addition to 1, 2 constant beta segments over here. So, you see, the organization, you have the variable region genes, you have the joining segments in one lot and then you have the diversity in joining segments in another, in another bunch followed by a constant region with this is the, this is the enhancer.

Now, as opposed to this, the important point, that one has to look at when you are looking at the alpha chain gene segments, you have the delta gene, which is the gamma-delta T cell receptor positioned between the variable region genes of the variable alpha. Now, alpha gene and the joining alpha, now this is something that has evolved during evolution because when, when there is juxtaposing of this variable alpha with the joining

alpha, you find, that when this comes together with the variable region, this delta region gets looped out and this is cut out and these 2 come together and therefore, if there is a gene rearrangement, that involves this alpha region, this delta is removed. This is a mechanism to ensure, that an alpha chained rearranging cell will never have the delta and therefore, it cannot become a gamma-delta T cell, it can only become an alpha-beta T cell. So, this is a very important part of T cell gene rearrangement, which is kind of different from the immunoglobulin gene rearrangement.

Coming to the now the gamma chain; you have the similar set of rearrangement, that you have the variable genes and the constant genes and these are the ones that rearrange, just like in immunoglobulin genes. And the rules followed, the heptamer sequences that are there, the nonamer sequences on either side of a particular variable segment or a joining segment or the diversity segment, are also present in the T cell receptor segments. And the mechanism, which involving, which involves the recombinase activating genes or the rag genes, is similar in both, immunoglobulin genes as well as the T cell receptor genes. And therefore, this mechanism of recombination involving the 12-23 base pair rule or the octamer-heptamer sequences will not be covered in this lecture. They will be covered in more detail in the immunoglobulin gene rearrangement lectures.

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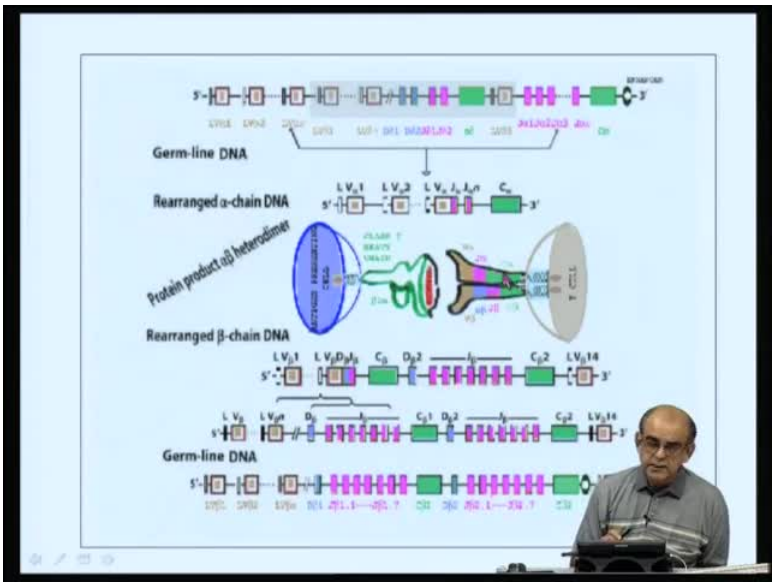

Going on further, then to show you, that you have a gene, that the rearrangement occurring over here and you have the variable alpha coming juxtaposed with the joining

alpha and you will find, that this is the way you have the variable 1, the variable 2, along with the leader segments and it is put together with the joining alpha over here. So, you have some n number of segments over here and is put together with the joining, you still have the other joining n segments, which are represent upstream of the constant alpha segment.

So, after the rearrangement, this alpha chain DNA is actually undergoes splicing in order to give you the mature product, which is found even in the previous slide and this is the beta segment, that is resulting from here and here you have the diversity. So, you have the first, the rearrangement with the diversity segment to one of the joining segments and then this rearranged segment is then put together with the variable segment.

So, basically, then you have this gene rearrangement and the rearranged beta chain giving you the diversity in the joining segments over here, and this resulting in the variable joining and constant region for the alpha subunits. So, this is the process of gene rearrangement, that occurs, that is similar to the immunoglobulin gene rearrangement.

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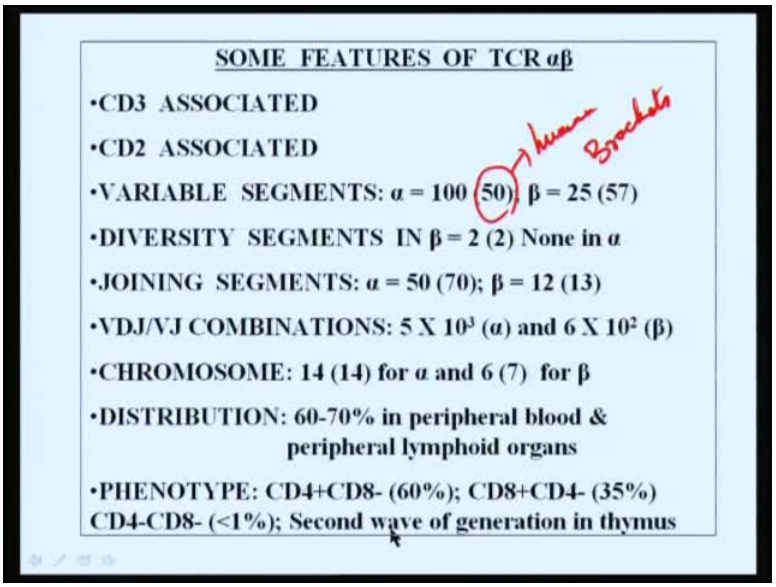

So, going on further, to cover some aspects or some features of this alpha-beta, which is the gene rearrangement, that gives rise to the diversity or the different kinds of combinations is listed in this slide. So, both of the alpha-beta, as well as, the gamma-delta T cell receptors are CD3 associated and CD2 associated. They vary at the number of variable segments; over here, the alpha in the mouse, this is for the mouse and

whatever is found in brackets is for the humans, so brackets are giving you the numbers in humans, so this is 100 for the humans. And in the immunoglobulin genes, you find, that the variable segments are upwards of 100, much more than 100, and then for the beta chain, beta chain genes, it is 25 for the mouse and more in the case of humans. The diversity segments in beta are much less over here – 2, both for humans as well as mouse and in the alpha there is none, because it is similar to the light chain of immunoglobulins, anyway. The joining segment alpha, having 50 in the mouse and 70 in the humans; beta, 12 in the mouse and 13 in the humans.

So, the possible type of VDJ and VJ combinations, you have 5 times 10 to the 3 just for the VDJ for the alpha and 6 times 10 to the 2 for beta. So, this is the one that gives you the diversity in the case of, T cell, T cell receptors and in the case of immunoglobulins, it is the light chain and heavy chain, that corresponds, that gives you the diversity. So, the chromosomes, that code for these subunits are chromosome 14, both in mouse and humans, for the alpha subunit it is 6 in the mouse and 7 in the human, for the beta subunit more importantly, the alpha-beta, the distribution is 60 to 70 percent in the peripheral blood, as opposed to very little for the gamma-delta T cells, we will come to that in the next slide. And these various alpha-beta T cells go into the peripheral lymphoid organs because they are the main effectors for the adaptive immune responses and the phenotype. We will see, that for the alpha-beta T cell receptors, they are either CD4 or CD8 positive T cells and then percentages are given here in brackets, basically this is a general figure which is applicable both to humans as well as mouse.

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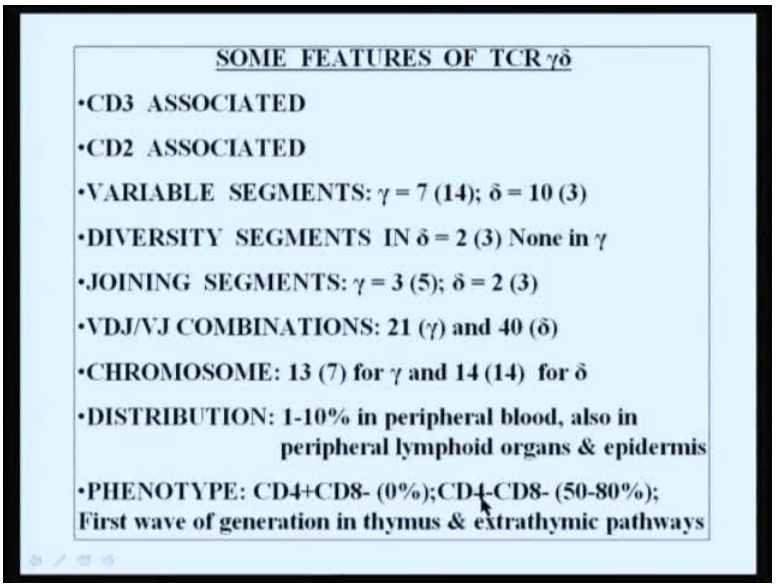

More importantly, the naïve phenotype or the CD4 negative and the CD8 negative phenotype is less than 1 percent, and these are actually coming together, they are generated in the thymus as a 2nd wave and actually the 1st wave are the gamma-delta T cell, T cell, that are made in the thymus. And these are basically 50 to 80 percent are CD4-CD8 negative, as opposed to the alpha-beta. And the nature of antigen, that is recognized by these gamma-delta T cells are actually more phospholipid in nature, as opposed to the other kinds of antigen, that are protein-derived peptides, that are recognized by the alpha-beta T cell receptors and some of the differences in the same features chromosome locations are listed in this particular slide.

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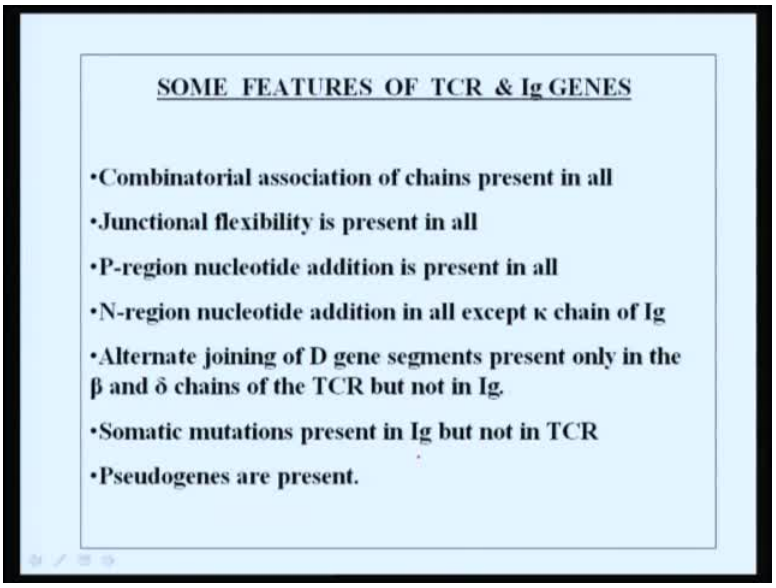

So, some of the features between T cell receptors and immunoglobulin genes, just to recapitulate, combinatorial association of chains are present in all. Junctional flexibility is present in all, that is, the T cell receptor, as well as, the immunoglobulin genes. The P-region nucleotide addition is present in all and the N-region nucleotide addition in all, except the kappa chain of immunoglobulins and there is also alternate joining of D segments present in the beta chain and the delta chains of the T cell receptor, but not in immunoglobulin molecule. More importantly, you find, that the somatic mutations, they are present in immunoglobulin molecules, which is what results in affinity maturation of immunoglobulin molecules, but they are absent in the case of T cell receptor molecules. It is a very important point and of course, for both, pseudo genes are present. Pseudo genes are genes that do not code for functional product.

So these are the principal features of the T cell receptors and they have been compared with the immunoglobulin, immunoglobulin chains. And I leave you with that and this is the T cell receptor class that tells you, how a T cell receptor is made up of and how it recognizes an antigen and some of the preliminary aspects of transmembrane signaling, that results from a T cell receptor getting activated.

I will leave you with this and thank you very much.