Immunology
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Lecture - 12
Structure of Antibody and T – Cell Receptors

Welcome to this lecture. So, we are going to continue the structure of antibody and T cell receptor. So, at the beginning we are going to talk about structure of antibody.

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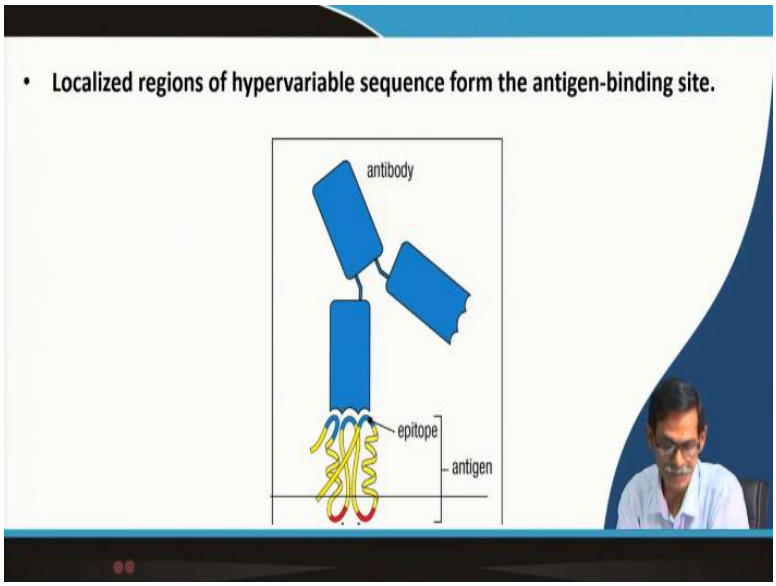

And I will start with a slide which we discussed in my last lecture. Just to remind you as, like what we are studying, actually what happened the antibody molecule, if you see the antibody molecule that this whole molecule you already know that the N terminal part which is a combination of both heavy chain and light chain are basically responsible for interacting with the epitope of antigen. So, if you see carefully only this region is interacting, rest of the part is not taking any part in the interaction of the epitope.

So, we will mainly focus like all the structure some part we already discussed. So, we will mainly focus how this region is formed and what is the structure and why this is, how this can help in the determination of the diversity, because it is not possible as we have already discussed that there are so many variety of antigen presenting the nature and if every antigen is different that means, we need different antibody for each antigen.

So, if it is possible if suppose there are for example, 10 to the power 8 different antigen, say so that means, there should be 10 to the power 8 at least 10 to the power 8 different antibody. So all these variety all over the antibody molecule we do not need, we just need rest of the part maybe the same only difference we need to interact with the different antigen is this region. So let us see.

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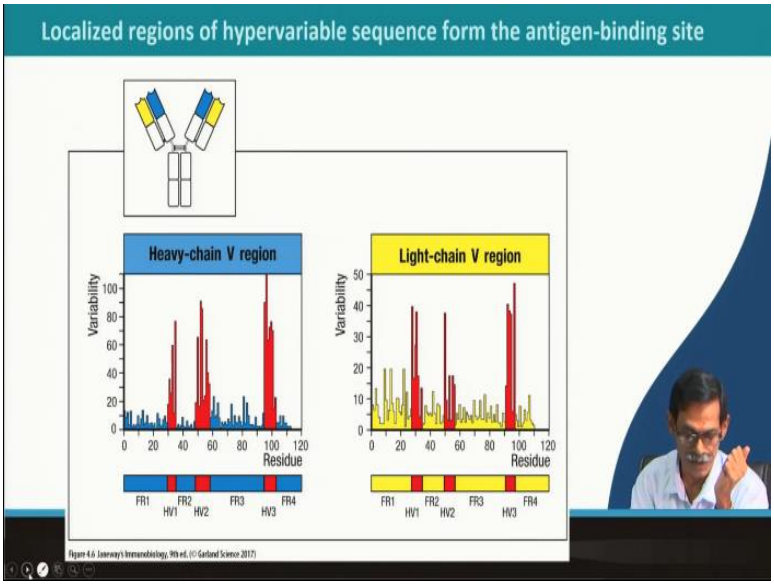

So, again this is the antibody molecule, this yellow one is the light chain, the blue one is the heavy chain, yellow and blue region is a variable region. So, now after the discovery of bioinformatics and genetic sequence on all the sequence is known, are many sequences of this variable region of antibodies available. So what can be done is that simple we can use simple bioinformatics.

What we will do? All the possible variable region of sequence available suppose you just take that so variable region just for your information what I can tell each domain of this heavy chain or light chain, say light chain has 2 domain, heavy chain has 4 domain, each domain is generally consists of very close to 110 amino acid, it is not exactly 110 maybe it may be a little 112, 115 or 108 but very close to 110 residues. So, that 110 residue if I consider that means 110 residues of amino acid.

So that means it is 330 nucleotide. So, if you take that 110 amino acid sequence of as many as variable region of heavy chain possible, then what we can do is using bioinformatics tool we can align them, which is called multiple alignment. There are free software's available so, if you want to do that by your own because sequences are available in the database and the software is clustal W or clustal X that you can try what we can see?

If you take different sequence and align them one by one, what you can see if there are some conserved sequence if there is a variability we can see that and we can calculate the percentage of variability among the sequence. Suppose, for example, we take 10 sequences of heavy chain V region of 100 sequence of a heavy chain V region or 1000 sequences of heavy chain V region then multiple align them and calculate the variability among these sequence which region or which part of the sequence is really variable.

So, if you plot that, like, see the x axis is showing the number of residue and y axis is showing the percentage of variability. So, if you go from 1 to 110 approximately 112 you can see the blue part blue region is not much variable maybe say for the first 30 amino acid, the variability percentage is approximately 20 to 25% but suddenly you see some residuals are highly variable okay.

Similarly, again there is a small region which is not that much variable, again there is a variability and this time the variability is even higher than the first one, then there is a big range of residues or a good number of residues which is not as valuable as this red region. Again, the third region of super variability or hyper variability coming and which is even maximum more than 100. So, it is very very I mean number of variability's very high.

So, if you see, so I will just consider this blue region. So, we multiple align the blue region and calculate the percentage of variability and this blue region is the heavy chain and if we do that, what we can see, we see that there are certain region is not that variable, but there are certain region or only 3 out of this 110 or 15 residue these specific regions are highly or heavily variable.

This region, if you make this say this is a cartoon or the state sequence, if you the linear sequence of the primary sequence if you see and if you mark in the same way the color code the blue and red, you see there is a region blue, then a small red, then again little bigger red region again blue and then red blue, which means any sequence some in the whole variable region because whole blue region we named already the previous variable region, some part is highly variable.

And this region was named HV; the first one is named HV1. So, within this variable region there are 3 joules which are hyper variable, HV stands for Hyper Variable clear, so if you see this, then hyper variable 1, hyper variable 2 and hyper variable 3 and FR stands for Framework Region. We will see what this framework means and in fact, if you remember, I am going to the next slide but if you remember, most of this domain is constituted of the beta sheets.

And there are 3 loops between these beta sheets. There are many loops where 3 loops are within this beta sheets that we will see. So this blue part is mostly constitute the beta sheet structure. And this is I am talking about the heavy chain variable region and if you do the same exercise for light chain variable region, we will see the same phenomena, but here if you see the light chain, the yellow part because we are talking about the yellow light chain.

These framework regions 1, 2, 3 and 4 same both are very much similar that if I want FR2, FR3, FR4 same FR1, FR2, FR3, FR4 also present in light chain but here, the variability in the framework region is a little higher in comparison to heavy chain. So now, so light chain also have 3 hyper variable region HV1, HV2, HV3 and heavy chain also have 3 hyper variable region HV1, HV2 and HV3. So let us proceed.

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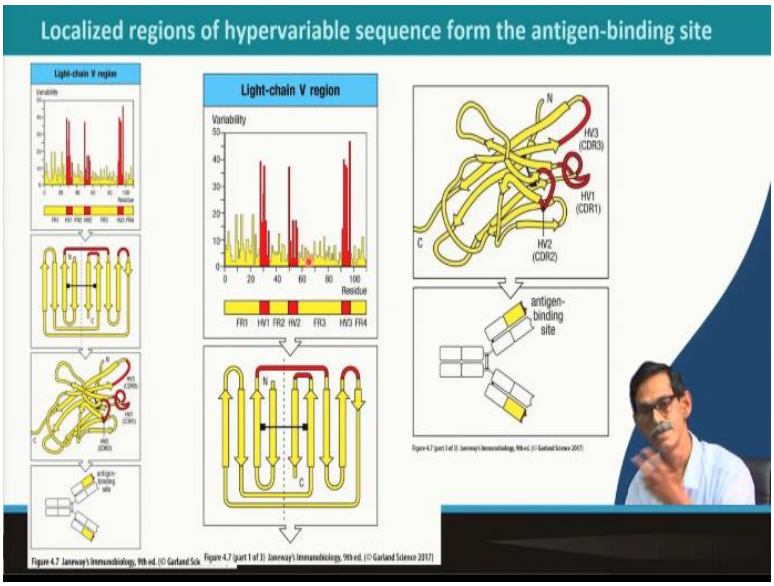

So, this is the same figure, I will come in little more detail same figure. If you see this one, these are corresponding one. So, this is we are showing or I am showing you just the light chain region and whatever I will say this is almost equally applicable for the heavy chain region also and heavy chain variable region. So, this is the light chain variable region which is the same picture what we have seen in the last slide. So, if you see, actually the yellow corresponds the beta sheets and 3 hyper variable regions HV1, HV2 and HV3 or 3 different looks.

I am repeating again. So, these framework regions are basically or mostly the beta sheets structure and the hyper variable regions are the loop structure. And if you what next these say, these picture if I represent like this what you have seen before, this is the same picture, we have seen this before, but only differences here, this hyper variable region are marked, so HV1, HV2 and HV3. This is also called CDR1, CDR2 and CDR3.

CDR stands for complementarity determining region HV1 and CDR1 are same or similar region either you can tell HV1 or you can tell CDR1 that means same thing. So, these 3 loops of this hyper variable I mean the variable region are basically the part which is interacting with antibody antigen molecule. So, antibody if you see this figure, if you see this figure I am just zoom little bit if you see this figure that 3 loops are contributing this the tip of the antigen binding site or the variable region of the light chain.

Similarly, the tip of the heavy chain variable region is also constituted by same HV1, HV2 and HV3. So, out of this whole antibody molecule, a very little segment, not many amino acids, you can count them from here that it is not many; very few amino acids are really responsible for interacting with antigen. So, now, the thing is if I say there are 10 different amino or 10 different sorry, there are 10 different antibodies that means most of the part will be very very similar.

They do not need much difference, but 10 antibodies, if the 10 antibodies interact with 10 different antigens. That means they are HV1, HV2 and HV3, these 3 things are different. So, genetic sequence wise if we go because all antibody is a protein molecule, and there are 2 chain light chain and heavy chain if it comes from 2 different gene, we do not have to worry about the whole thing should be changed. So each antibody only HV1, HV2, HV3 or CDR1, CDR2 and CDR3 need to be different.

And that is also not many amino acids or many nucleotides. So in fact, if we consider or if you compare other 2 different antibodies with 2 different antigens or specific for 2 different antigens, then we will see the difference is only or mostly rather in CDR1, CDR2 and CDR 3.

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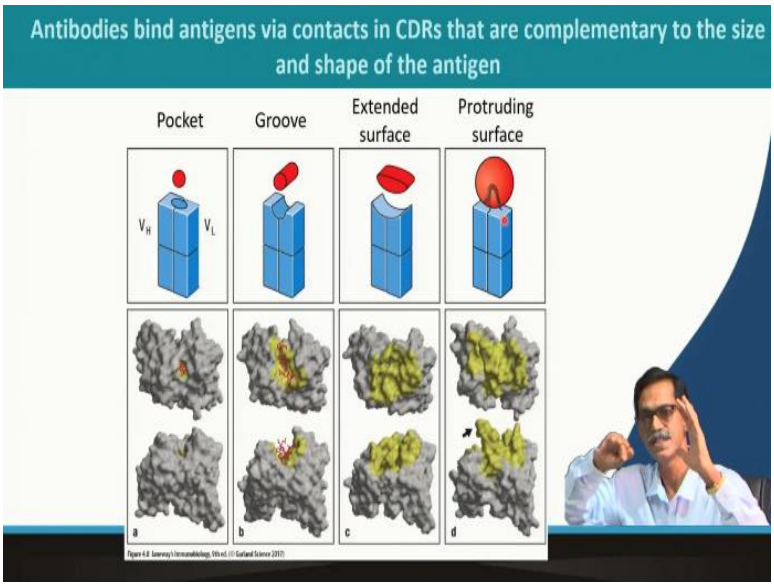

So what they are making they are forming these heavy chain and light chain. So, if you consider the heavy chain and light chain one more information I should give you or tell you and you should remember that heavy chain and light chain the combination makes a structure and both

the heavy chain light, chain combination because antibody has 2 arms or 2 valency both are identical, it is not that in one antibody one can pick one antigen another one can pick another antigen.

No both are specific to the same because it is a combination of same heavy chain and same light chain. So, same heavy chain light chain combination, what all what kind of formation they do so that they can interact with antigen. So, antigen maybe different size, different kind, different shape. So, this is here very general cartoons that predicting like, so V H and V L can make a pocket. Pocket where is small molecule can fit, this is actually real crystal structure presentation.

So this is an antibody molecule crystal structure. And you can see the red part the red taking out this laser because it is so for radius matching. So that the red part actually the antigen most of the time it is too small it is like hapten, hapten is a small molecule which cannot produce immune response but antibody can bind. So, these molecules this heavy chain lighting they can, they are making a small pocket where this pocket where something can fit, if this is the pocket something can fit or it can be a groove.

You can see it is very simple it can be a groove, where something can fit, this is the groove and this is the antigen it can fit. Same way this is again a crystal structure with that antigen and yellow mark region is the surface of the antibody where antigens interacting it may be an extended surface like this, you can see it is extended surface. So, a big area of the antibody is actually interacting with antigen or it may be protruding surface.

So, this is a real antibody. So, you see this it is just protruding. So, something if this is the outs I mean the hyper variable region or the antigen interacting region is like that the original antigen should be like this, so that it can fit see the antigen is like that we need something like this to fit here. So, these proto sinatics in fact, real antibody against HIV surface molecule, Human Immunodeficiency Virus surface molecules so we can see very nice protrusion here.

So protruding surface also possible. So, not only the groove or pocket, it may have protrusion which I mean sometimes this may be the antibody pocket antigen can fit or sometimes this may

be the antigen antibody surface antigen can fit antigen may be like this or empty and maybe like this, it is just the replica, so it fit either this way or this way.

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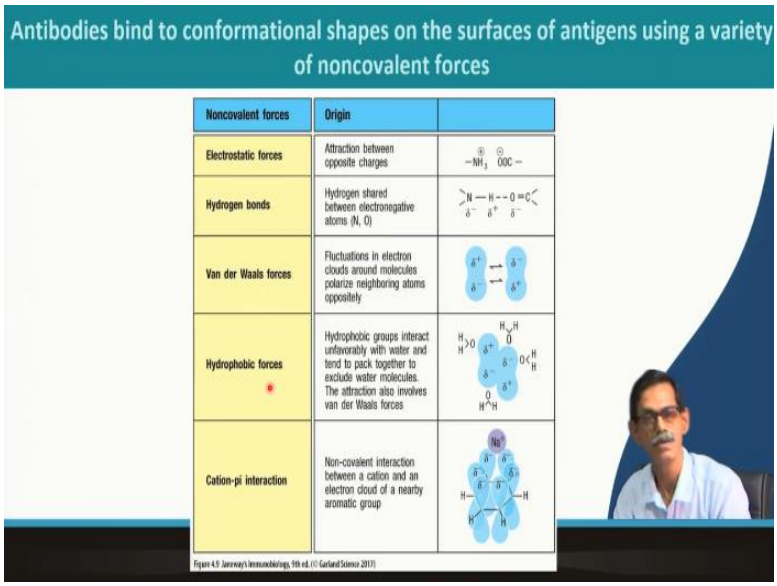

And now, what we will see actually this antigen antibody interaction is most of the time because most of the antigen what we are going to discuss like T cell response happen are mostly protein antigen covered by maybe an antigen antibody can be generated again carbohydrate can be nucleic acid but most of the time or majority of the antigen that our immune system phase is broken. So, antigen antibody interaction is just like other protein-protein interaction.

Any 2 protein interact are most interaction or mostly protein-protein interaction are mostly noncovalent in nature. So, all possible noncovalent force or forces that require to have a protein-protein interaction or their input antibody antigen interaction because antigen is also a protein, antibody is also a protein. So, any this is a noncovalent interaction and these non covalent interactions are Electrostatic force. It may be hydrogen bond between 2 antigen and antibody it may be a Van der Waals force.

That Van der Waals forces may be the responsible these all what I am talking about low energy non covalent interaction, it may be Hydrophobic force in the middle column, you can see that I mean just get an idea like what this Hydrophobic force means and what are the definition for but those were interested to know little more, you can go and check in any protein chemistry book or

molecular biology book, signal transaction book or let it is available you are welcome to see and know detail about it and Cation pi interaction.

So, all 5 noncovalent force or interaction may be present in antigen antibody interaction, but do not think that every interaction of antigen and antibody are having all 5, no, it may be all 5 it may be any 4 of them, any 3 of them and 2 of them. It is a combination which 3, which 4 it all depends what kind of surface property of antigen and what is the surface property of the antibody. So, any of these force or all of these forces or in combination of any of these force may be present in antigen and antibody interactions and it is in noncovalent interaction.

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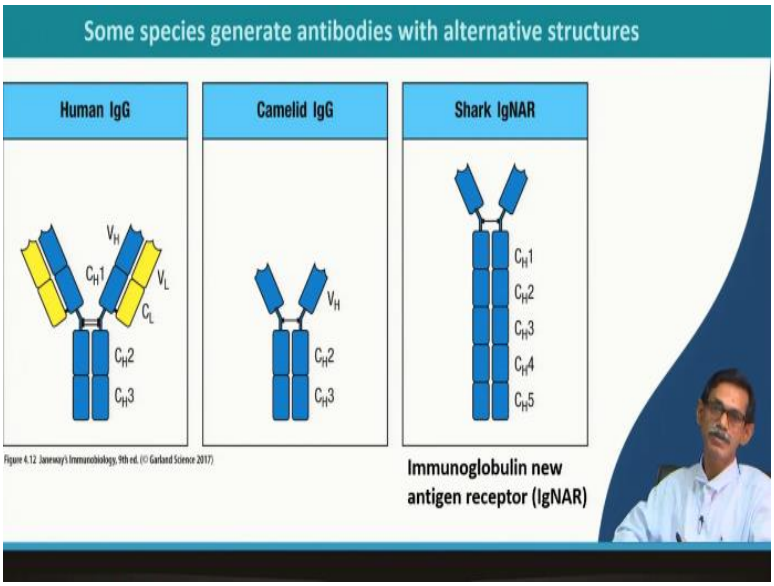

So, whatever we have discussed, so far the structure like FR1, FR2, FR3, HV1, HV2, HV3 this will see why it is important and hope you understand. So, that is mostly we discuss about the antibody of human or mouse, which we see here in this figure, which has 2 heavy chains, 2 light chains, 2 variable domains in each arm, one constant light chain domain constant heavy chain domain C H 2, C H 3 but there are few antibodies which already are seeing it is that Camelid IgG it is sometimes it is called single chain antibody.

It is present in camel you see there is no light chain, it is very clear another thing there is no C H 1 also. So only variable region and C H 2 and C H 3 is there. So these but again this is a combination of 2, it is also have a divalent. So only one variable region of and heavy chain are

doing the job or serving the purpose of antibody antigen interaction. Same way if you see the Shark one of the early antibody like molecule.

If you see the evolution, Shark has so many constant domains, C H 1, C H 2, C H 3, C H 4, C H 5 they also do not have any light chain. Both of them has hinge but they are hyper variable region or sorry the variable region is only one no light chine contribution and the Shark antibodies also known as the new antigen receptor that Immunoglobulin normally antibody also say Immunoglobulin, Immunoglobulin is represented by I and g, Ig is a small abbreviation for Immunoglobulin.

So, Shark antibody also called IgNAR which is written here Immunoglobulin new antigen receptor. So, hope you understand the basic structure of antibody molecule or Immunoglobulin molecule. So, if you understand, then my life and as well as your life will be really easy for next slide.

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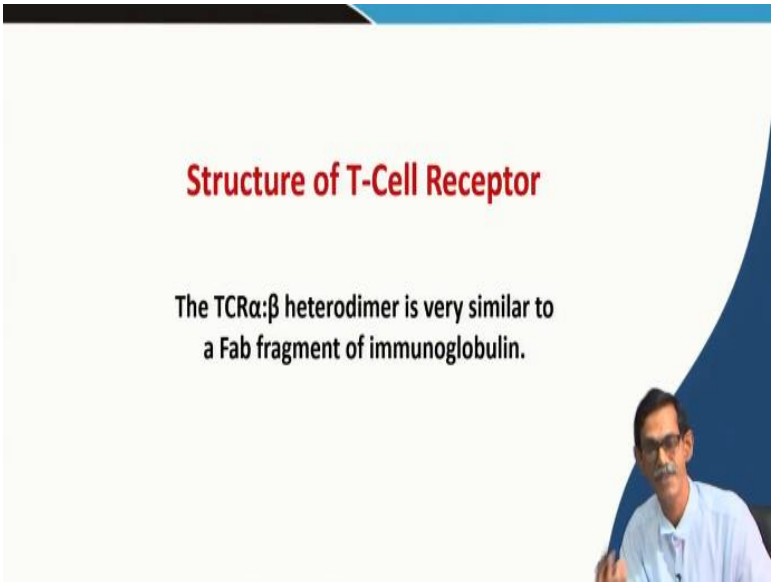

So, next part we are going to discuss is structure of T cell receptor. T cell receptor is basically the alpha beta heterodimer which is very similar to Fab fragment. So, if you see how it is similar to Fab fragment of immunoglobulin, you can understand.

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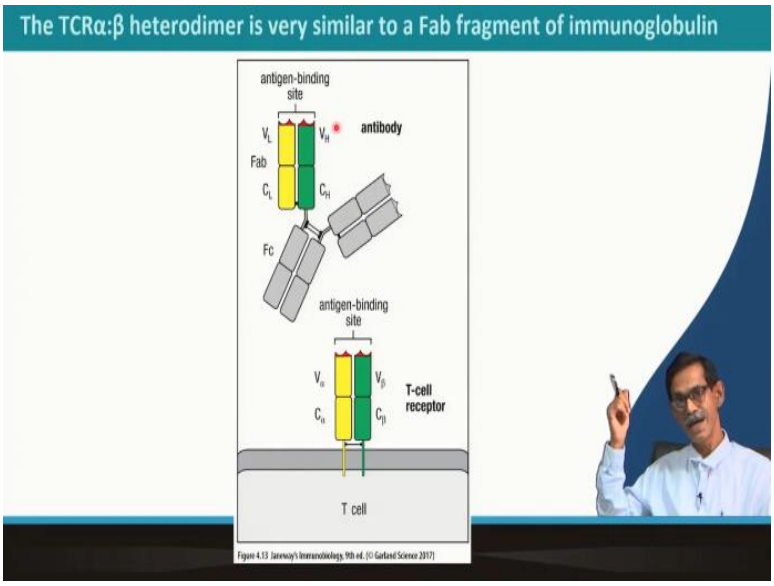

So, you know this is the Fab, so this is the Fab region, we already discussed which contain only V L and V H, C L and C H one, if you see the structure if you see the color code of yellow and green see the TCR or T cell receptor is very similar. It has one chain with 2 domain and other chain with 2 domain. Similarly, one yellow one green if you compare there is pretty much identical, but definitely there are difference, this is 2 polypeptide one is called alpha chain and other is called beta chain.

In this 2 domain upper part is variable alpha and in this case it is variable beta and then constant alpha constant beta there is no other part and each chain are integrated or just transmembrane domain are implanted them in the cell membrane. So, they cannot be free like antibody molecule it is but T cell receptor like thing, where there are 2 things. So, alpha chain and beta chain of T cell receptor, one transmembrane domain each and they are linked to the disulfide bond.

And if you see the variable region alpha and variable region beta, they are very similar to Fab fragment and if you see the antigen binding site is also same. So, I do not have to tell everything or repeat everything whatever I told during the discussion of antibody, variable region.

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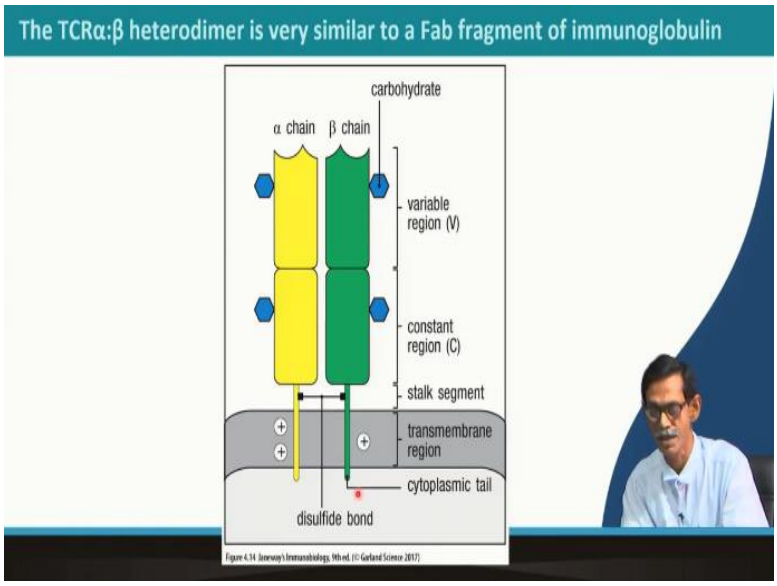

It is the same molecule slightly bigger over here also the carbohydrate or the glycosylation is there. There is a variable region constant region. So, there is a stalk segment it is not like hinge because he do not have the flexibility like this. And the transmembrane domain, this is a cytoplasmic bond a tail, which is responsible for giving the signal or transducing the signal from when it an interact with that antigen MHC complex.

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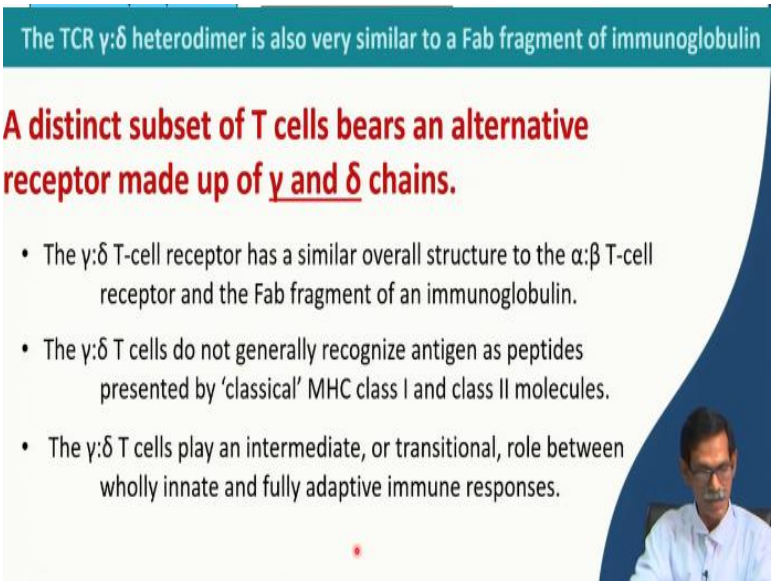

So, I assume that you understand that part, the immunoglobulin structure part and just correlate the similar thing, they are structured they also have a framework region 1234. They also have hyper variable region 1, hyper variable region 2, hyper variable region 3 or CDR1, CDR2, CDR3 exactly same but they are from antibody gene and TCR are 2 different set of genes alpha and

beta subunits are completely different gene, which is not no way similar same to the antibody gene.

But their property they are structure, they are beta sheets and loop all these structures very much similar. It now, we have just now we tell the alpha beta T cell receptor okay. So, T cell not only have alpha beta T cell receptor, there are another kind of T cell receptor which accidentally actually discovered people are looking I mean the scientists are looking for gene for alpha subunit.

Now alpha chain gene and protein and when while they are looking for alpha different alpha subunits of T cell receptor, the gamma receptor was discovered and eventually the delta also came, but it is the gamma delta T cell receptor has a similar overall structure with alpha beta T cell receptor and it is also similar to the Fab fragment of an immunoglobulin, gamma delta T cell receptor, definitely it is not exactly the same as in their function, they do not generally recognize antigen as peptide presented by classical MHC I or MHC II.

So, alpha beta T cell receptor recognizes antigen presented by MHC I and MHC II but gamma delta receptor is not. It is assumed that gamma delta T cells play an intermediate or transitional role between wholly innate or fully adaptive immune response. It is I mean, research on our delta receptor is not as much as alpha beta not as much information is available for gamma delta T receptor.

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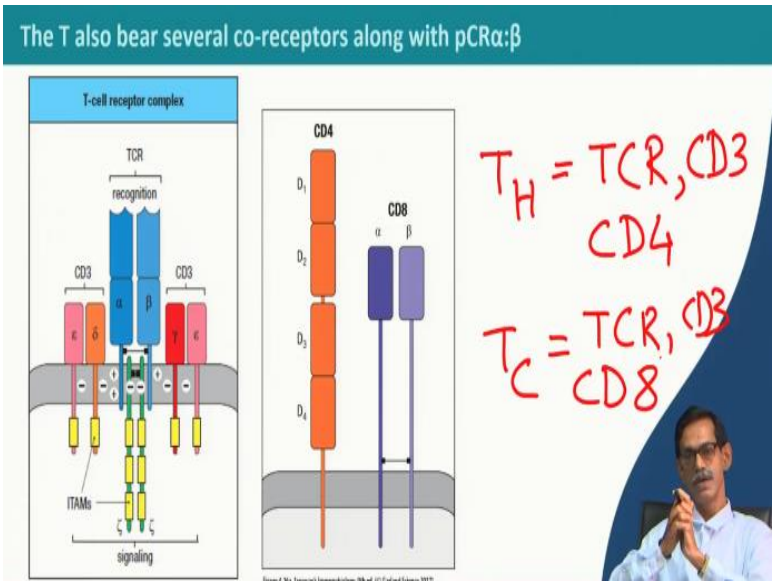

Besides these TCR gamma delta or alpha beta T cell receptor, there are few core receptors also present in T cell few I already mentioned like CD4 and CD8 here in this picture both CD4 or CD8 as shown in one but, already you know that CD4 present in T helper cells and CD8 presenting cytotoxic T cells I hope I have been as I just give a parser you will tell. So, CD4 present in T helper cells and CD8 present in cytotoxic T cells.

This is unique I mean this is not both are not present in the mature T cells. Along with the TCR or T cell receptor, there is another kind of co-receptor present which is CD3. So what are they I mean total number of receptor major or important receptor for T cell CD3, TCR, CD4 or CD8 So, now, if I say so T helper will have what definitely they will have a TCR, they will have CD3 and what they will have? They will have CD4.

Then cytotoxic T cell, but they will have they will have TCR, CD3 and they will have CD8. So, these are the total T cell receptor but CD4, CD8 there is no variety or there is no question of direct antigen binding. So, they are responsible for interaction with MHC. CD3 is responsible for signal transduction. But if I somehow can identify how many cells have CD3, you will find all T cells are having CD3 for their signal transduction.

So, this is a general marker, it said is general marker but it is the PCR is only T cell receptor which is responsible for antigen binding. So, this is overall or in general structure of anti T cell

receptor and B cell receptor or antibody molecule. So, now, we will see how this antigen variability developed in our next class. So for the timing, thank you all. Thank you very much.