Immunology
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Lecture - 13
Generation of Diversity (GOD) of Lymphocyte Antigen Receptors

So, welcome to lecture number 13. Today we are going to discuss the generation of diversity of lymphocyte antigen receptor. So, in last class we were discussing like different antibody different antigens. So, their hyper variable region is different because they need to interact with different antigen molecule or 2 specific different epitopes. But, the thing is, if you consider I already told during a basic concept discussion of basic concept of immunology.

While we are discussing that part that the number of genes predicted genes it is not exactly known the number of predicted genes in human say 25000, 30000, 35000 depending on the different algorithms but if we consider the number of antibody present in our body say at any time point it is 10 to the power 8 different variety, so if each antibody is protein and each protein should have at least one gene.

So, then we should have 10 to the power of 8 different genes but we do not have that, so how these diversity is developed so that was a big question, though it was solved a long time back by Prof. Susumu Tonegawa in MIT. What is the basis how it is; now we know but before that I mean when people was not known; what is the origin of this variety origin of this gene. What we used to?

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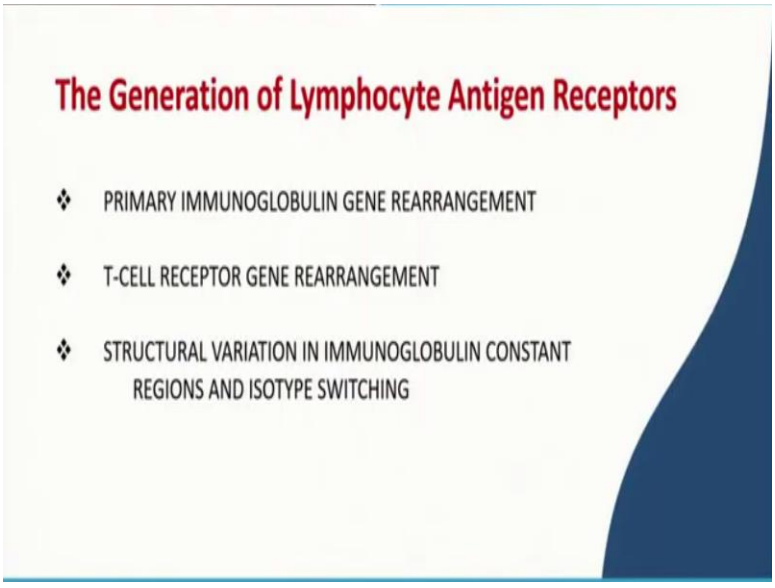

How people know this how so much variation in the hyper variable region develop or generate in antibody as well as T-cell receptor. So in this in the incoming few lectures, we are going to discuss about generation of lymphocyte antigen receptors I will mostly focus on the primary immunoglobulin gene rearrangement and their variation and which is almost equally applicable for T-cell receptor gene rearrangement also but definitely if there is anything special for T-cell receptor will discuss.

And in the last part of it, we will see the structural variation in immunoglobulin constant gene which we already know little, and how this constant region is changing and how this different immunoglobulin isotypes are formed so we will see isotype switching as a last part of it.

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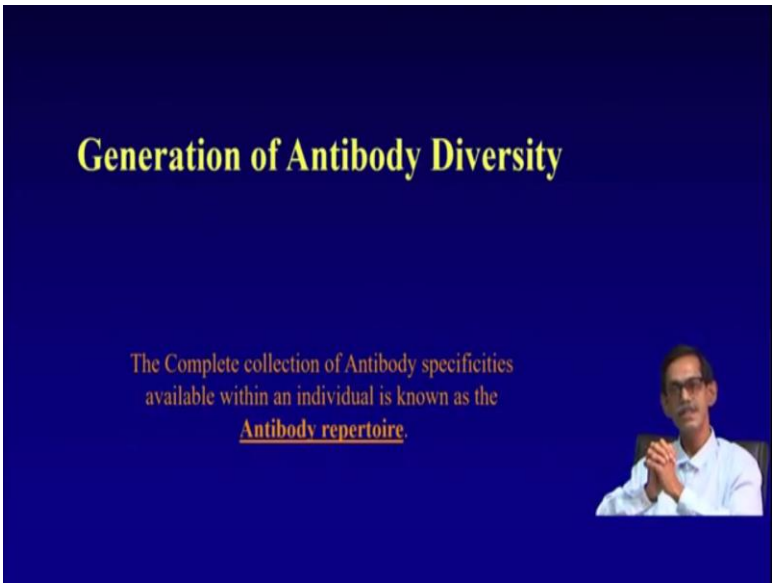

So I will start today with immunoglobulin gene rearrangement or generation of antibody diversity in one of my basic concept class I told you to go and check what is the meaning of repertoire. So, now I am giving the complete collection of antibody specificities available within an individual is known as antibody repertoire. So which time it is changing because B cell continuously develops.

So, their receptor modification and all this thing when you study the B cell development you will see it is a continuous process, so the variety of antibody is also changing their amount is changing so that is why antibody repertoire is not fixed at particular time today what I have the variety of antibody in my immune system it may be different after a month or so, this is antibody repertoire.

So, the complete collection of antibodies specificites available within an individual is known as antibody repertoire but, before the genetic engineering or recombinant technology is known or the different techniques were discovered people used to guess like what could be the origin of this diversity.

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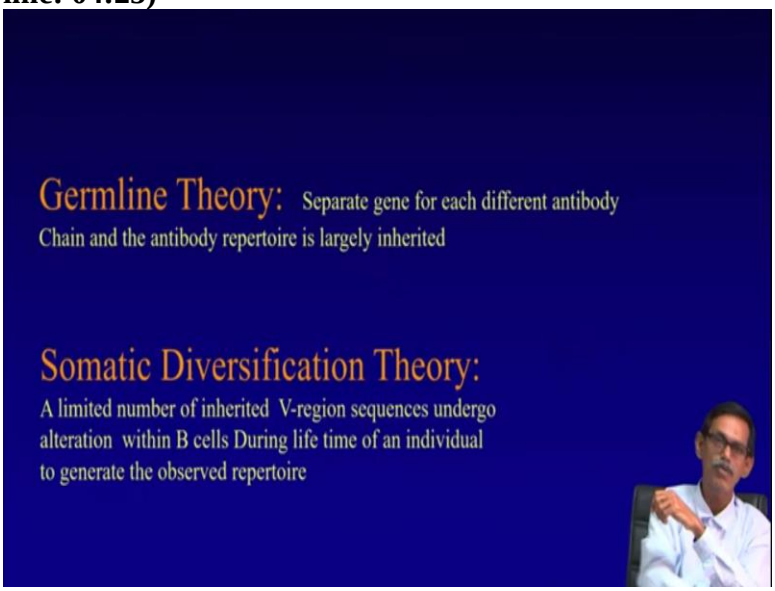

How is to come so different type of receptor is generated. So there are 2 theory are proposed one is germline theory and there is somatic diversification theory. In germline theory it has said that separate gene for each different antibody chain and the antibody repertoire is largely inherited. The germline theory is saying that separate genes are there for each antibody chain. An antibody repertoire is largely inherited that time the number of gene in human was not known.

So, the prediction was not irrational but which is not true we know because we do not have that many genes and somatic diversification theory is sake is saying limited number of inherited variable region sequence undergo alteration within B cell during lifetime of an individual to generate the observed repertoire. So, if I ask you which one is true one is saying each one have gene each antibody has individual gene or separate gene.

And it is largely inherited, another theory is saying that the V-region sequence undergo alteration during lifetime and this V-region is inherited. So let me tell you the answer the answer is both are partially correct neither one is completely true it is definite that each antibody is produced by different gene but, how this gene originate? It is not saying and

another one and it is also saying it is inherited this is also partially correct part of this gene is inherited. So now we will discuss how this diversity is originated.

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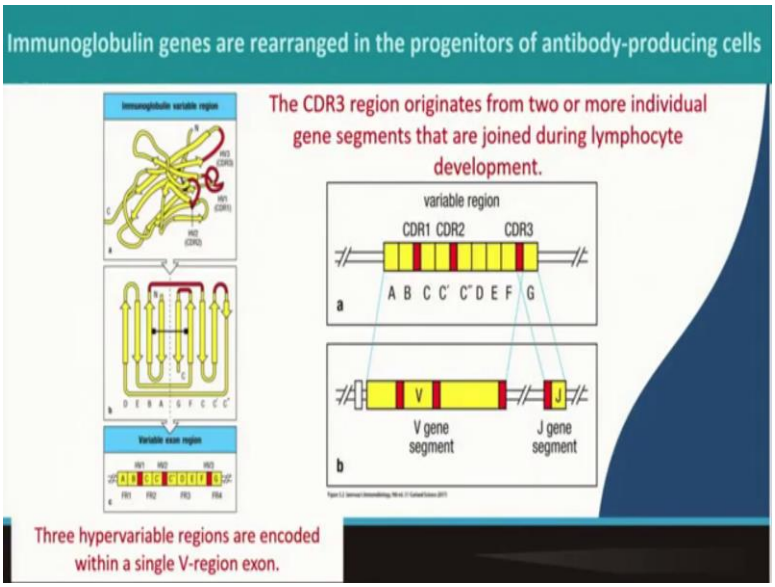

So primary immunoglobulin gene rearrangement I will discuss now. So this picture you have already seen so these parties I am coming again and again, you see so the red part is the most important region of antigen interaction this is also known picture, only difference if you see is the leveling of this region D, E, B, A, G, F, C, C Prime and C double prime because why this is actually these letters are indicating the different beta strand in this figure.

So, these beta strands is designated as D then E then B just to understand which between which beta strands this hyper variable region is here. So, if you see that B and C there is one then G and F there is another and C prime and C double prime there is another and now this is this same picture is little simple simplified form in here so it is A B then hyper variable one and then C.

So between B and C the hyper variable region between B here and C, if you see this is which one this hyper variable region is HV1, so C and C prime. So this is HV 2 and F and G what is between these 2 F and G this one is hyper variable region 3. So this is a schematic presentation of HV1, HV2 and HV 3 our main idea to see I mean this lecture will be just prove how this variation comes on how this hyper variable region are different.

How come so many variable sequence are present in this total repertoire of antibody. Let us understand first the antibody part and then or the B cell receptor part then we will see how

this is applicable for T cell receptor part. So this figure if you see this once again here we are showing so this figure if you go here and just what is the difference here difference is HV1 is replaced by CDR1 it is all same, so now if you see CDR1 and CDR2 are in this region so in the gene, I will come slowly before that I think we should go here.

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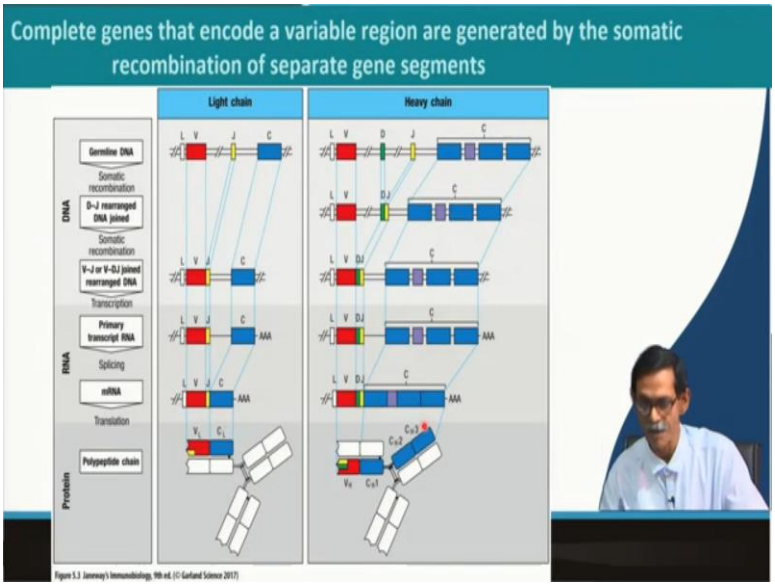

I will come back to this slide again. So what is found that in light chain the variable region is composed of 2 different segments one is V another is J and light chain has only one constant domain so there is one constant domain. So this is the DNA structure, this is the germline DNA or gene I mean before the B cell matured. So, what happened during maturation of B cell formation there is a recombination happening.

In that recombination this B and J joined together this is also at the DNA level in this figure wherever 2 lines are there this is DNA and single line is representing the RNA. So these V and J recombine and then make a continuous structure V J together and which is again there are some space and constant region is there. So, light chain actually the light chain variable region is present in chromosome or in germline level as 2 segments. One is V little bigger and where is J.

So at germline level they are separated but, by recombination they come together and make the complete variable region and here L stands for the leader sequence or the signal peptide in this figure L stands for the leader or the signal peptide. So, the V and J together makes variable region of light chain. So I am going back to the previous slide so now if you see this

B and J they are separated, they together make the variable region and if you see that both CDR1 and CDR2 they are already present in variable V segment.

But CDR3 is actually the contributed both by V segment as well as J segment and these during this recombination this part is very important. And we will see with time that means V and C, V and J will join it they made CDR3 and the CDR 3 originates from 2 or more individual gene segments in case of light chain it is 2 segments. Some part contributed by V and some part contributed by J they become together make the CDR3 same way if you see in heavy chain the variable region then there is a D segment D stands for diversity.

And J segment, so first what happened D and J joined together make D-J and this is also a DNA level and then another recombination happened between D-J and V they together make V-DJ the complete variable region of the heavy chain and this D-J together and in fact V-DJ all 3 segments together is making the CDR 3. So here the CDR3 originates in light chain by 2 components and in heavy chain by 3 different segments of DNA.

So, the chances of variability in CDR3 are maximum. So if you see the light chain now I am going back and forth so, please excuse me so from this DNA say for light chain recombination happened now it is a DNA and it is the rearranged. So V-J in light chain and V-DJ in heavy chain rearranged then transcription happened in transcription we will not see that J was just far in the genome.

So, it is together then by splicing they have become together this is one mRNA the precursor RNA with the polypeptide then splicing happened, so the variable region and constant region comes together. So variable region and constant region comes together and which gives a protein product like this and if you see this region the antigen binding region you see the yellow part which is a J region.

Which is also a part contributing in the CDR3 are very much important because it is present in the antigen binding site. Same way if you see in the heavy chain the D-J comes together make DJ and then DJ and V together make V-DJ then transcription happen. After transcription the C region in this case of heavy chain you know the C region or the constant domin as this is if you consider this is IGG there are C H 1, C H 2 and C H 3 and this violet color is a hinge region.

So this constant region is composed of C H 1 hinge and C H 2, C H 3 all these are together so, they come as the single precursor RNA then splicing happened where V-DJ is already joined by recombination, so V-DJ and constant domain come together in the mRNA and mRNA gives the product of the heavy chain which has variable region which is combined V-DJ plus C H 1, C H 2 and C H 3 the heavy chain.

But if you see carefully that VDJ all 3 are taking part in the antigen binding site, so even they are very small in comparison to the V region J in case of light chain and D-J in case of heavy chain are very very important because, that makes the antibody different with respect to antigen recognition. Now we will see how this thing happened. So this V-DJ recombined, so how this is going to help in the diversity.

Because these, this is the basis like what is the construction of heavy chain and light chain gene in chromosome but this is not saying anything about the diversity or the so much variability in receptor of antibody B cell receptor and T cell receptor.

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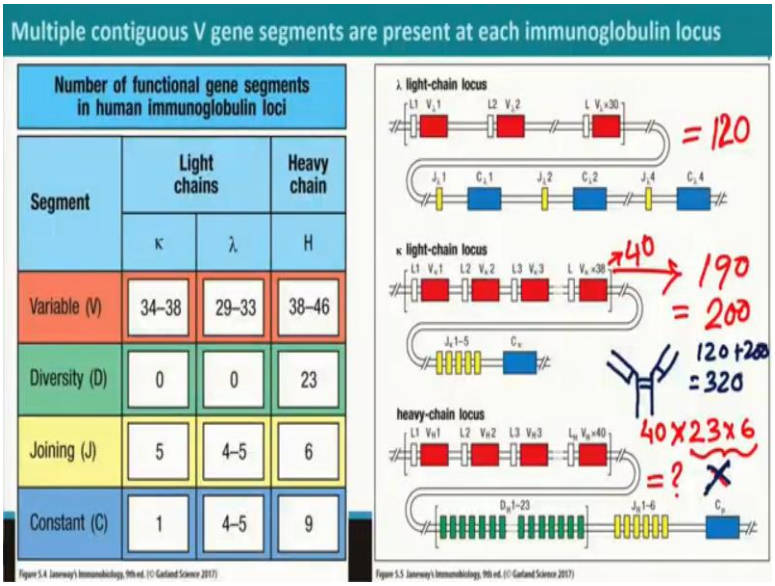

So here is the magic. It was discovered that in our chromosome we have multiple copy of variable region, multiple copy of diversity region, multiple copy of joining region which actually makes the antibody more specific or different specificity against different antigen, which I do not remember exactly but, I will repeat again repeat here if I already told you there are 2 type of light chain present.

So one antibody is composed of one type of heavy chain and one type of light chain, so there are 2 type of light chain genes are there one is called kappa and one is called lambda. So one set both kappa and lambda has V and J segment separately, so kappa has some V segment and J segment, lambda has some V segment and J segment. So what was discovered actually? Initially, when this diversity was discovered at genetic level or molecular level it was shown that in any individual we have variable region for light chain.

Say for example, kappa light chain we have 34 to 38 numbers of V region, for kappa chain joining region we have 5 and for lambda, we have lambda light chain we have 29 to 33 variable region 4 to 5 joining region. So, total if you consider the light chain it may be but in one antibody in any particular antibody the light chain should be either kappa type or lambda type it is not that in one hand it is kappa in another part is lambda, it is either lambda type or kappa type. So it is not that all are present.

In heavy chain at the same time a variable region we have 38 to 46 in number we have a diversity region of 23 or the D segment 23 and joining we have 6. And what does it mean or what is that if you see in the chromosome level or the if it may be a cartoon or the map of the genetic it is like this, in light chain this is a lambda light chain locus in chromosome, in lambda light chain locus it is, say there are all how many I said? I said 34 to 38 variable region.

So, sorry 29 to 33 variable region in case of lambda, in lambda chain we have V lambda 1, V lambda 2, V lambda 3, 4, 5, 6 and up to 30. So 1 to 30 and then there is a space, then there is one joining region, constant region, joining region, constant region how many we have, we have 4 to 5 joining region for lambda, so similarly we have say for example, this case we have 4, so we have one, J 1 constant 1, J 2 constant 2, J 3 constant 3, J 4 constant 4.

So if these, all this variable region are in series then alternate J 1 and constant region, in case of kappa the arrangement is slightly different. In case of kappa it is all this 34 to 38 variable region again in tandem or inverted y that I will come, what is this inverted tandem so they are present one after another then there is a space then all 5 J region is here then only one constant domain. So this is the chromosomal arrangement of kappa light chain. The heavy chain we have 3 domain one is V, D and third one is J.

So, how many variable domain say for example if I consider that in average there are 40 variable domain of heavy chain. We have 40 variable region then is space then all 23 D region is one after another and then 6 joining region and then this C mu, mu stands for IGM all these you the constant portion if you remember IGM, IGD, IGG it is normally the gene is name like IGM is mu, IGD is Delta, IGG is gamma like that IGE is epsilon, IGA is alpha.

So, same way C mu is IGM. I hope you understand so these are the numbers and this is the orientation clear, so lambda is this. So now so before going to do that, so now if you think that variable region say I am considering only say kappa I have for example I have 30 variable region and 5 joining region if there is an opportunity or a chance like any of the variable region can join any of the joining region.

And finally, make the complete variable region of light chain because this is a segment of variable region of light chain. So, variable segment of kappa is 30 and the joining region is 5, if it is so anyone of this variable region can join any one of this so there are say this is I am talking kappa, so there are 38 total and there are 5 total joining so what will be the number possible. It is very simple mathematics how many different kind of segments variable segment we can have, the mathematics is very simple 38 times 5.

If randomly any variable region can join with any joining region the total number of variability will be 38 into 5, same way if we consider for the lambda chain here there are 30 variable region and there are 4 joining region. So, what is the possible variation again simple mathematics 30 times 4 is equal to 120. So now there are total number of variation possible here is 30 times 4 is 120, then here 30 say 44 roughly calculation see if this is equal to 40 and we have 5 what will be the total? It will be 200.

Or to be too specific it is 190. So this is the possibility 120 possible lambda variable region 190 to 200 possible kappa light region, same way if you calculate, how many different kind of heavy chain is possible what we have to do we have to just multiply this variable segment into because any D can join with any J so that will give actually 23 into 6, so this is the combination of I mean possible combination of D and J and then that combination can join any one of these 40.

So this will be total number is 40 into 23 into 6, so you can easily calculate I am not spending time for calculations. So, now suppose this is X and so if I consider now, so what is I am telling you the number again, light chain, 120 lambda light chain 120 possible variation, kappa light chain 200 possible variation and heavy chain variation were normally any D can join with any J and any DJ combination can join with any V segments the possibility is X. So that gives some variation.

So, we do not have that many genes but after recombination different segments can produce variety of variable region of the gene segments and you know that constant region does not have any role in the antigen specificity. So they do not have many we have very few either one is 5 it may be lambda or kappa heavy chain how many are there? Heavy chain only 5 difference are there A, D, E, A, and G. So different there are few subtype that we will discuss later if when time comes.

So now what is happening in antibody molecule what is there in antibody, so let us see in antibody molecule, what is there antibody is the combination of one heavy chain and one light chain right which is linked with a disulfide bond same thing is repeated here. One heavy chain one light chain and they are also linked, so in any antibody there should be how many like how many light chain in any antibody there is one light chain and one heavy chain.

And finally they are dimer heavy chain, light chain combination is dimer. So, now if you go back to the calculation what we see 120 anyone of this 120 can make antibody molecule anyone of this X and any one of this 200 can make antibody molecule final antibody molecule with any of this heavy chain, so what will be the possible number any one of 120 and anyone of 200. So total is say see this one 120 + 200 = 320.

So, 320 is the possible light chain and we have X number of possible heavy chain if this is also random if this is random like anyone of this 320 and any of this X can recombine what is the possible number of antibody molecule very simple mathematics again you just have to multiply 320 times X that gives you the variety of different antibody even that I mean it is surprising I mean so, few number of segments just by recombination and random recombination can give so many variety of antibody.

But, if you calculate the number it is still very very low with respect to the number of antibody we are having at any time point. So next time we will see or in the next lecture we will see how that number we can reach. Today we will finish here and in the next class we will see how that 10 to the power 8 or 10 to the power 10 variety or 10 to the power 13 variety of T-cell receptor we can have these will not explain you that much but even all I mean even if it is not taking it that much number or that many number.

But it is also very good like small segments and random recombination can increase the final product in a good and much more in number than it is present we do not have to suppose just 320 if I see 320 how many pieces we know we do not have to have the 320 gene. So, if you just consider a light chain gene we do not have to have 320 gene very few segments by random recombination can give us 320 possible gene. So, in next class we will see how the diversities increase, what are the other factors of diversity of the nucleotide that immunoglobulin receptor. Thank you for today.

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