Essentials in Immunology

Prof. Anjali A. Karande

Department of Biochemistry

Indian Institute of Science, Bangalore

Lecture No. # 10

Generation of Antibody Diversity

Today's lecturer is going to be on the generation of antibody diversity.

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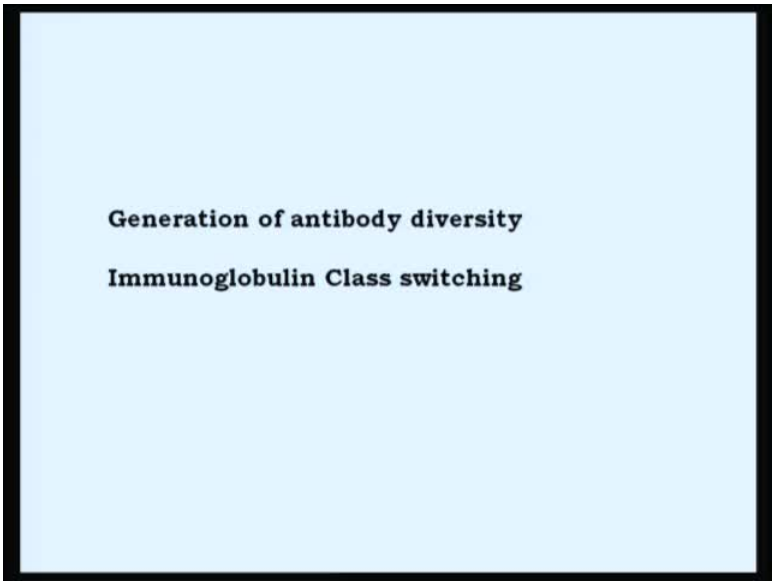

As well as, there will be introduction to immunoglobulin class switching.

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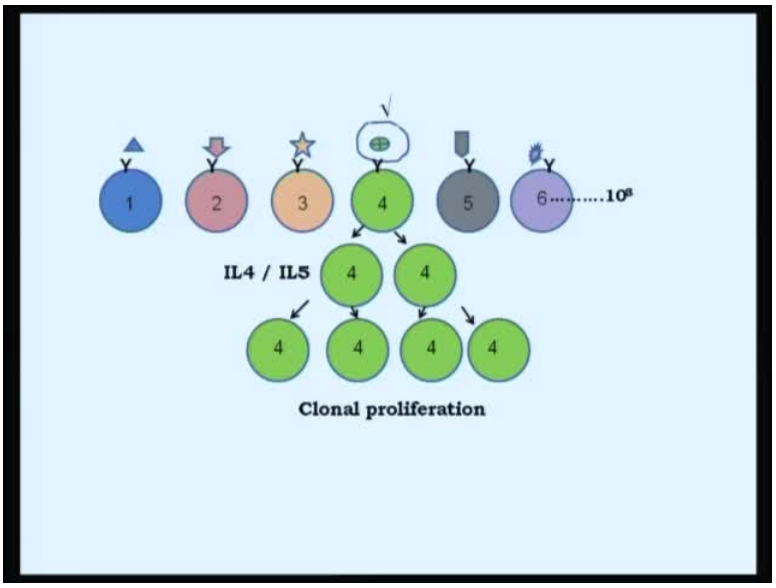

If you remember from the last class, we have already discussed how every different B cell, which is naive has not encountered antigen, has already on its cell surface specific receptor; three-fourths of which is identical in all the cells; only one-fourth is hyper variable; and, it is this hyper variable region, which is at the amino terminus, which is responsible for specific interaction with antigenic determinants on the different antigens. We are capable of mounting an immune response, which would make antibodies of at least 100 million difference specificities. Now, this cartoon shows different B cells and the recognition of a specific antigen by clone number 4 would lead to activation of this cell, proliferation in the presence of into (( )) 4 and 5 secured by T cells. And then, ultimately, differentiation to plasma cells.

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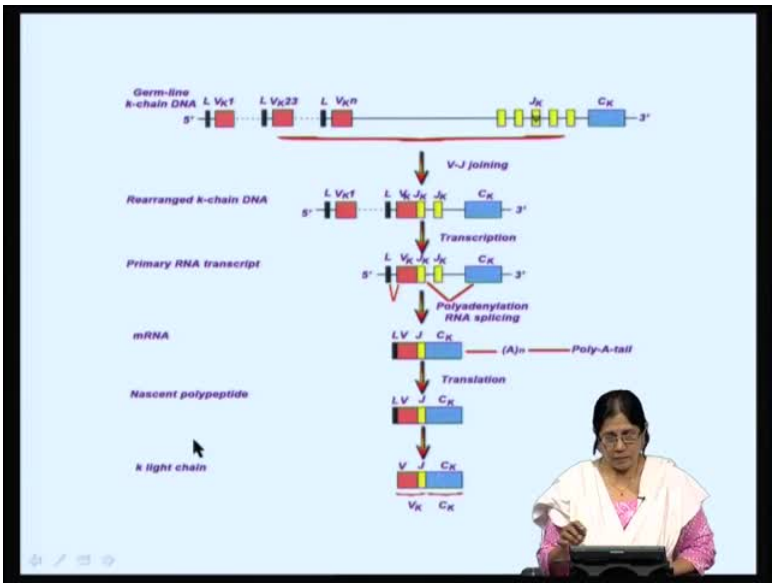

Just to recapitulate to your memory, the hyper variable region responsible for antigen binding on the heavy and light chains constitutes any one of the several V gene segments, which come in close proximity; in fact, joined with any one of the J gene segments in the case of the light chain gene.

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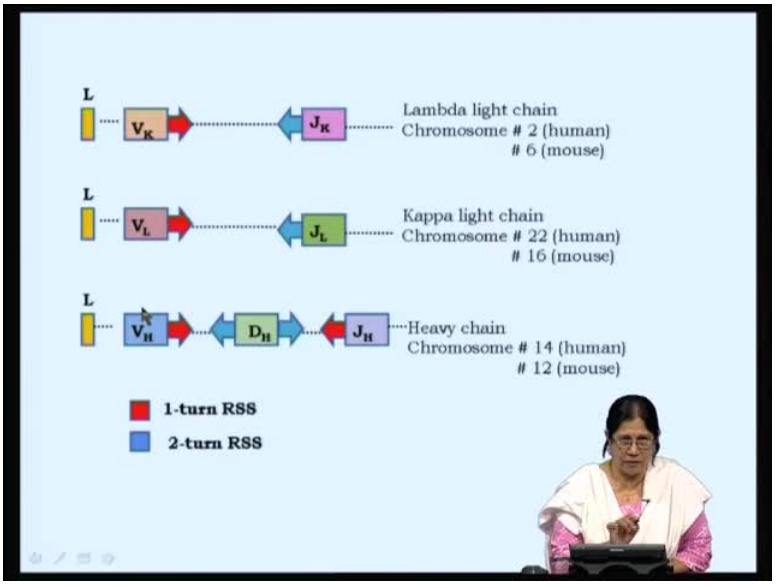

And, in the case of the heavy chain gene, it is V, D and J; addition of one more gene segment in the heavy chain. And, as I told you before, the heavy chain gene product, which is the heavy chain of immunoglobulin, gives much more regions for binding to the

antigen. How it is that only one of the several J segments present and one of the several V gene segments present combined? Now, this is because of the presence of special recognition signal sequences, which I mean, as I mentioned earlier, these are conserved through evolution. So, you have two sets of these recognition signal sequences and it is a rule that the combination or joining would take place only between the two different RSS. One, which is 1-turn RSS; this 1 turn corresponds to 1 turn of DNA helix; or, 2-turn RSS. Now, this is what would be required in case of the light chain – lambda light chain assembly as well as kappa light chain assembly.

Now, since there are three segments on the heavy chain, the two RSS rules would need to be modified. And, this has been modified in the following way. The same RSS signal in this case, which has been depicted, 2-turn RSS are present on 5 prime and 3 prime side of every D region segment. And, the corresponding, that is, 1-turn RSS are present on the 3 prime side of every V gene segment and the 5 prime side of every J gene segment. Joining between D and J occurs first. And, once this combination has taken place, one of the several V gene segments are recruited.

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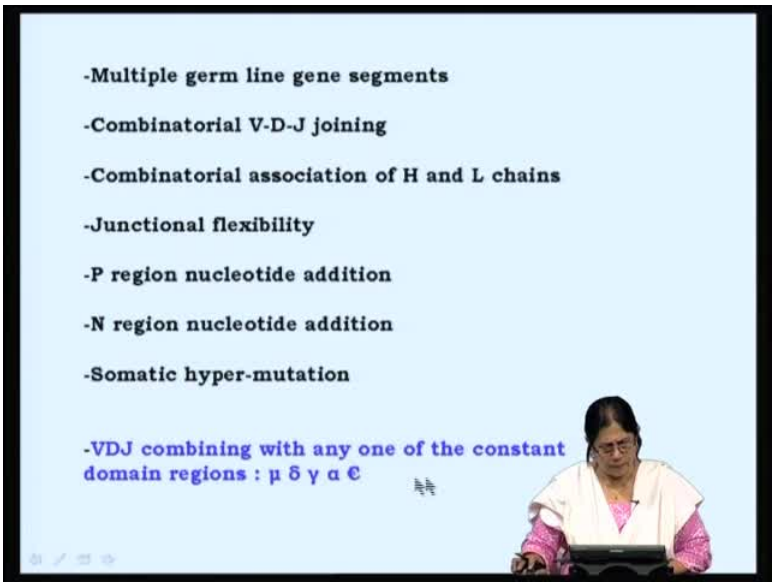

Now, the question comes – how is this variability of 100 different molecules to be synthesized; 100 different molecules, which has specificity to recognize 100 million different molecules. How are these generated? All the information lies in the genome,

but T and B cells; both of them undergo recombination with respect to their receptors. And, this is how the generation of diversity is brought about.

Now, there are 7 plus 1 different mechanisms that go to providing this process for generating tremendous diversity. First, one is multiple germ line gene segments. Second, combinatorial V-D-J joining in heavy chain; and, V and J joining in light chain; combinatorial association of recombined heavy and light chain; junctional flexibility; P region nucleotide addition; N region nucleotide addition; somatic hyper-mutation. And, last but not the least, which does not contribute to the recombination with respect to variability; yet, one can say, this (Refer Slide Time: 05:32) also provides diversity, because once the VDJ recombination has taken place, then the recombined region can combine with any one of the constant domain region in the heavy chain increasing the diversity. That means the same recombined V-D-J can associate M and D simultaneously that happens on the navies cell as well as any one of the other constant domain gene.

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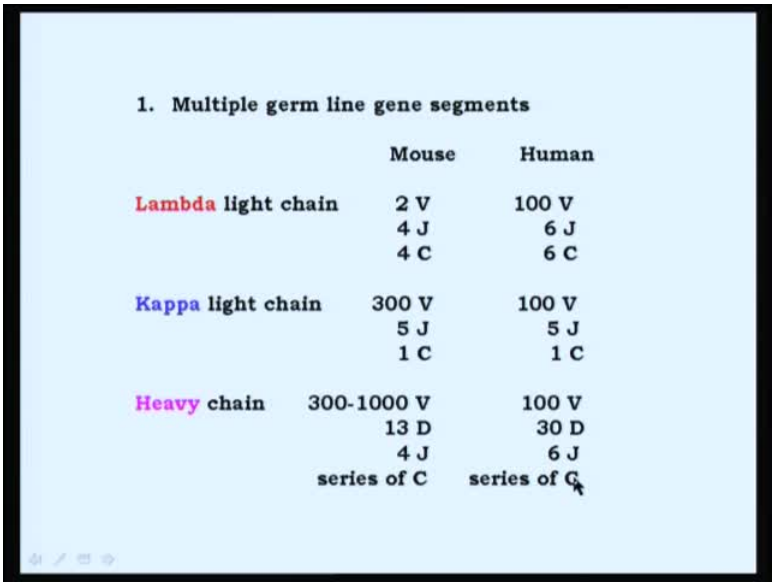

Now, let us go to the first mechanism, that is, multiple germ line gene segments. I have on the slide, the estimated V gene segments corresponding to the lambda light chain in mouse, human, the kappa and the heavy chain. I would like to take the simplest one first, lambda light chain. You can see, in the mouse, there are only two variable gene segments corresponding to the lambda light chain in mouse, in human. However, this has evolved to have 100 different V gene segments. Now, when I say different V gene segments,

please remember, we are referring to the sequence, the nucleotide sequence, which is of course, then corresponding to the amino acids. Now, why in the mouse, there are only 2 V gene segments? This would mean that the light chain corresponding to lambda is reconstructed. It is important to remember here and I have mentioned this earlier that 95 percent of the immunoglobulins in case of mouse are associated with kappa light chain and not lambda light chain gene segment. Now, let us come to the J gene. In human, there are 6 J gene segments on the lambda light chain, 4 in mouse. And, now, this is something I would like you to see that though there are 6 constant domain genes corresponding to the lambda light chain in human, and 4 constant domain genes in mouse. All these are identical.

Now, kappa light chain because remember again, the light chain would have only one constant domain. A kappa light chain – there are 300 different variable gene segments in mouse; 100 in human; 5 J in both and 1 constant domain. Heavy chain – interestingly, in the mouse, there are 300 to 1000 variable gene segments; that is really a very large number. In case of human, it is 100 V gene segments. But, the human heavy chain makes up by having more diversity gene segments; there are 30 in all. Mouse has 30, 6 J and 4 J in human and mouse respectively, and of course, series of C, that is, constant domain gene segments; each one corresponding to the class of immunoglobulin. So, if you have that many different V gene segments, and I have told you, joining of V-J or V-D-J in heavy chain is totally a random process, and therefore, one can see that this random joining is what gives rise to diversity.

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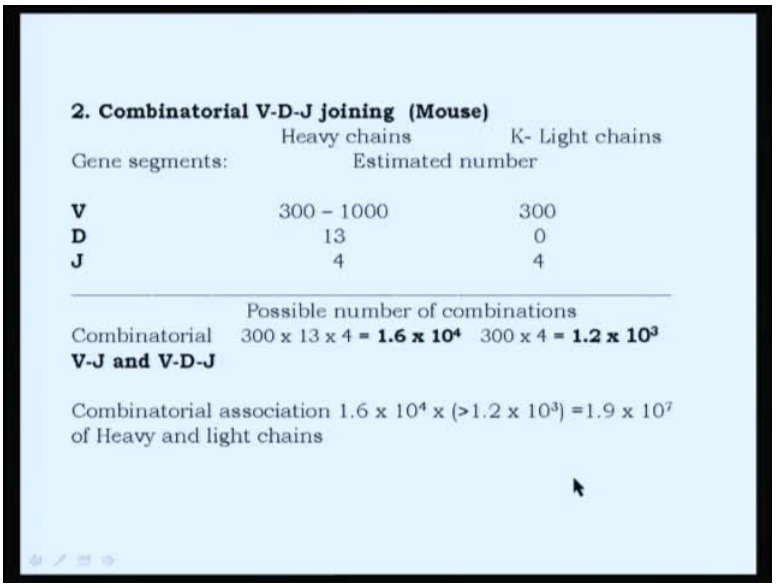

And, if you do a mathematical calculation, then if you look at just the mouse combinatorial V-D-J joining, heavy chain and in the case of light chain, V-J, then the possible number of combination would happen in the heavy chain; 300 into 13 into 4, which would give to 1.6 into 10 to the power of 4. Now, this along with the light chain, which is 300 and 4, because of absence of D segment, would give rise to 1.2 into 10 to the power of 3 combination between these two combinatorial associations. Immediately then increases the permutation to an extent, where you can have 1.9 into 10 to the power of 7; 1.9 times, I meant, into 10 million of heavy and light chain.

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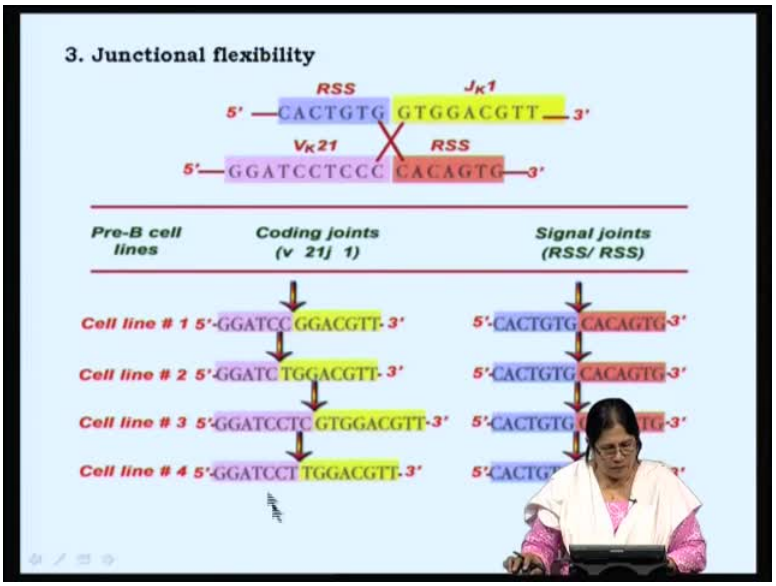

So, let us go back and just think that we have now very large number of V-D-J and combination between any one. Now, each B cell would have the capacity to join any one. So, randomly, you would have any one of J, D and then V being recruited. And then, again light chain, which is combined separately; the heavy chain combined separately; and, these two coming together after the translation when folding takes place in the cell, combination of this again would give for the diversity. Therefore, we have covered now the first two; that is, several of the gene segments, which have the capacity to recombine at the DNA level followed by combinatorial association between the heavy and the light chain.

Let us come to the third, which is junctional flexibility. What is junctional flexibility? Now, since we are talking about two segments, which join together… Now, if you remember, in the last class, the mechanism of RAG 1 and 2 cutting the single strand of DNA specifically at the RSS; the RSS, which are, if you remember, hepatoma and the AT-rich nanoma, they come very close in proximity, and this is done by the RAG 1 and 2, which recognize these signal sequences. They bind and recognize and they bring them together.

Now, cutting of the single strand DNA occurs by RAG 1 and 2 precisely at the hepatoma. Therefore, though you get signal joints, that would mean, when the hepatoma, nanoma, the RSS are deleted, if one sequences these deleted products, one can see that the joining between the hepatoma, nanoma, the signal sequences are very precise; it is identical as seen in pre-B cell lines, which are going on to becoming B cells, where recombination is taking place. This is experimental evidence that shows that signal joints are very precise. But, when the coding joints join that it would mean 1 D with 1 J or 1 V with 1 J or V with D J. The coding joints always are imprecise. And, we will come to how this happens in two minutes. You to see this, (Refer Slide Time: 12:37) the coding joints, which of four different cell lines, which are derived from one pre-B cell line; this pre-B cell line was now activated to start the recombination process in to (( )) 7 at as in V (( )) where you have the bone marrow providing into (( )) 7. Now, the pre-B cells go on to recombining the heavy and light chain. And, when coding joints were sequenced, it was seen, the several 1, 2, 3, 4 different cell lines have different sequences.

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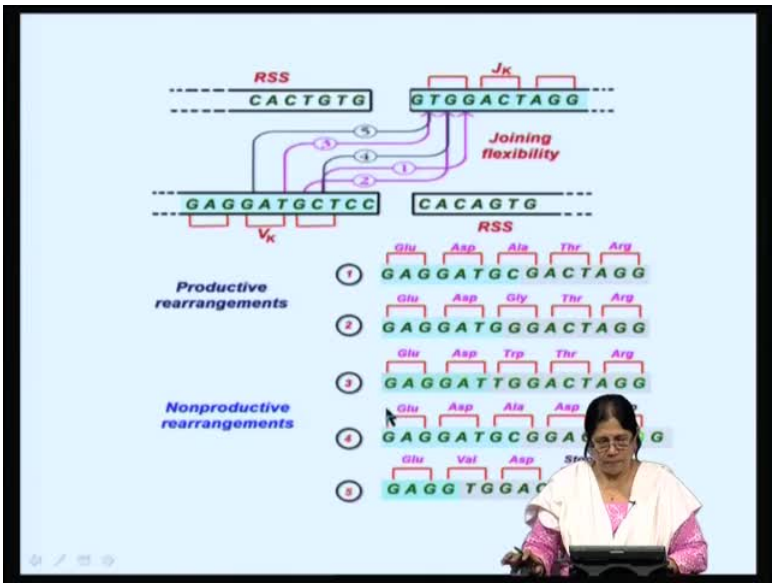

This imprecise or difference in joining in the coding joints can of course, be deleterious. And, as seen in the last experiment, (Refer Slide Time: 13:34) the coding joints joining together can also change the sequence, the reading frame in such a manner that you may have the introduction of stop codons. And, this is what has happened in the 5s and 9s that were then looked at; other five, where two of them, the recombination failed, because of the change in the reading frame, stop codons were introduced. However, productive rearrangement happened in 3.

Why it is important to know this is, because remember, when we talked about the development or the ontogeny of these cells, in the antigen-independent phase, there are large number of B cells, which undergo apoptosis, because of lack of precise joining. One of the reasons is introduction of stop codon in the reading frame when coding joints were joined together changing the reading frame.

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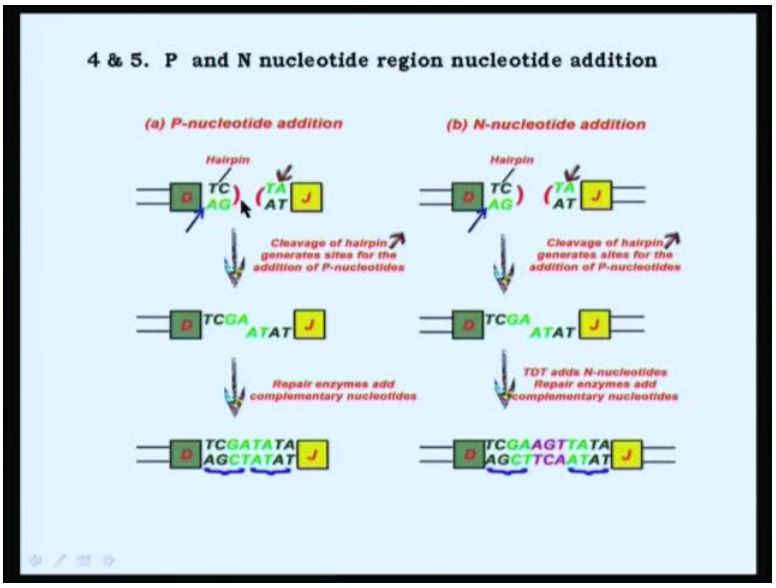

Next, would be, that is, 4 and 5. 4 – the fourth mechanism in introducing diversity would be addition of P-nucleotides; and, N-nucleotides would be the fifth one. Let us look at the addition of P-nucleotide. I have a diagram in the next slide, but let us first go to the theory part. In this particular example, one of the D is joining with one of the J and by the transesterification reaction, RAG 1 and 2 have made a palindrome sequence. You have now in the coding sequence of the D segment, TC; and, in the lacking strand, AG.

Now, remember artemis, the endonuclease, which is specific with respect to recognizing hairpin structures. Now, this protein, this enzyme binds to the hairpin structure and can cleave anywhere, because cleavage is not specific with respect to the nucleotide sequence. Now, let us see, in case artemis cleaves at the hairpin junction between the D gene segment and this hairpin structure. Now, if the cutting is in the lagging strand, closer to the lagging strand, then you would get TC; look at the coding strand first; the coding strand would have the TC, which was originally there plus would get GA from the lagging strand.

Similarly, if you look at J gene segment, you have now cleavage occurring at the joint after TA, which would mean now that the lagging strand would get here; AT from the coding strand. GA has the different color. GA and AT are not present in the sequence of the coding or the lagging strand. They are new nucleotides with respect to that particular strand. When joining takes place, you would have end filling before the ligase activity.

And, you can see now, four nucleotides in the coding strands, which are different from those that were present earlier. Therefore, now, these are unknown as P-nucleotide addition. This of course, as you can imagine, would generate diversity. But, this would also if the reading frame now adds a stop codon midway, would also be deleterious. However, this is one of mechanisms of diversity.

Let us look at this unique system of addition of N-nucleotides. When the hairpin structure has already been cut by the artemis as I already mentioned and described in the P-nucleotide addition, there is expression of a specific enzyme, which again specific to lymphocytes. Terminal deoxyribosyl transferase – you might remember from my first slide in my first lecture that TDT is the marker for B cells and T cells. Terminal deoxyribosyl transferase – immediately, you would realize that this enzyme has the capacity to add new nucleotides. So, at least with respect to P-nucleotides, the information was available not necessarily in that particular strand, but in the opposite strand. However, in the case of N-nucleotide addition, in additions of nucleotides, which are the catalyzed by the TDT and which are not present at all, are there any preferences with respect to the addition of any nucleotides? It has been seen that there is no preferences as such, but if you look at the number of nucleotides, which are present in this particular D-J joining, then, there is a preference for G-nucleotides to be added. So, now we have two more mechanisms: P-nucleotide addition and N-nucleotide addition, which would contribute to this diversity. Now, all these are going to change the frame, are going to change the amino acid sequence; the information of which was a variable on the coding strand earlier.

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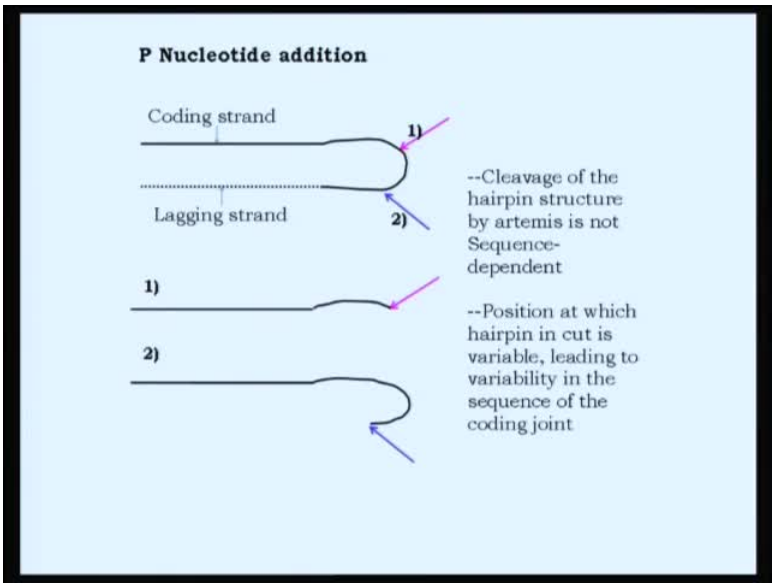

To look closely at the P-nucleotide addition and to make it a little bit clearer, I have a diagram here the coding strand and the lagging strand, which is in dotted line. Now, it is after all the coding strand that matters. And, let us look at the hairpin structure. Now, there are two instances here. The artemis is cutting the hairpin structure at two different regions. Now, in one, if the cleavage occurs closer to the coding strand, then you would have a much smaller nucleotide sequence from the coding strand, which is present, and modern half would go automatically to the lagging strand, which I have not shown over here. However, in case of two, if the cleavage occurs on the hairpin closer to the lagging strand, then you have a much larger fragment, which would encompass quite a few nucleotides from the lagging strand. And, this would be new to the segment over here, which could be let us say D. Cleavage of the hairpin by artemis is not sequence–dependent. Again, I would like to remind you, position at which the hairpin is cut is variable leading to variability in the sequence of the coding joint.

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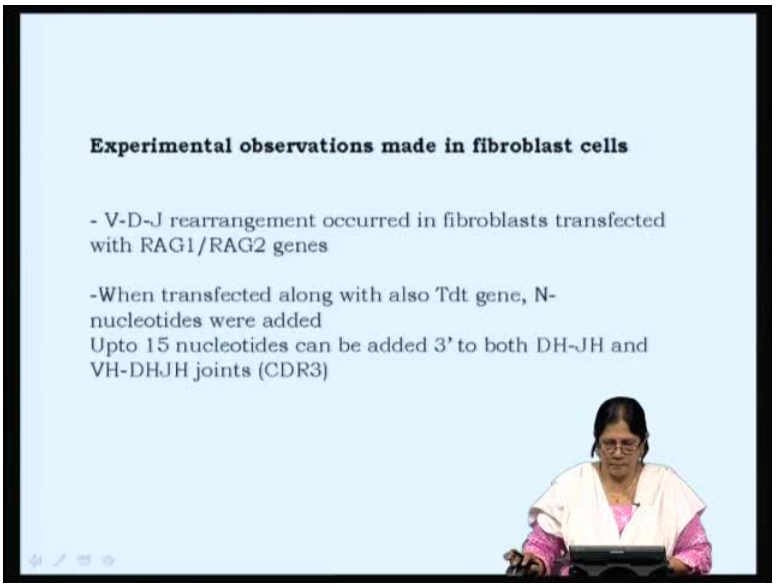

Let us look at the Tdt gene expression. When immunologists sequenced these V-D-J recombined genes, they found that there were a few nucleotides in the D-J as well as V-D; they could not count for those on the lagging of the coding strand. And then, they realized that there was this enzyme Tdt, which was adding new nucleotides. So, after that, large number of sequences were obtained and it was very clearly seen that in fact, yes; there is nucleotide addition in the heavy chain gene, N-nucleotide addition. I would like to also tell you that N-nucleotide addition happens mostly on the heavy chain, because the expression of Tdt, terminal deoxyribosyl transferase enzyme is decreased when recombination of the light chain gene is happening.

There is a very nice (( )) experiment that was carried out to show; I have told you in a few lectures ago that people have been able to induce recombination invent in fibroblast cells. Recombination of the immunoglobulin gene or rearrangement would not happen in any other cell except T and B cell. B cells in case of the immunoglobulin gene and T cell with respect to the TCR, T cell receptor; T cell receptor also has heavy and light chain, etcetera. The structure might be different, but in any case, both of these undergo reorganization.

Now, one can induce reorganization of these genes in fibroblasts by expressing a plasmate, which codes for RAG 1 and 2. So V-D-J recombination was induced in fibroblast cells and only when these fibroblasts were also transfected with the Tdt gene,

N-nucleotide addition was seen to take place. So, definitely again, the relevance of Tdt, the enzyme was very well demonstrated. How many nucleotides can be added to the cut ends? And, as many as 15 nucleotides can be added to 3 prime of both DH-JH as well as VH-DHJH joints. And, this constitutes CDR3. This is very important to remember that the CDR3 region is where N nucleotides are added. So, highest diversity would be there.

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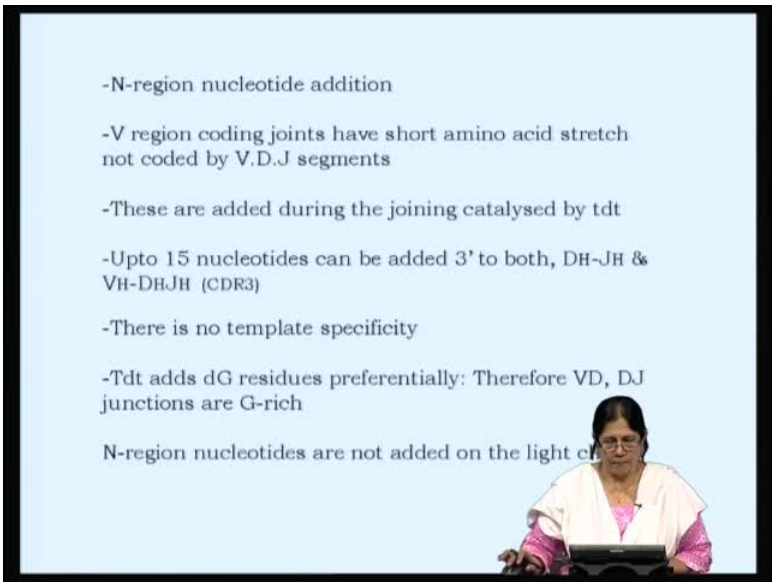

N-region nucleotide addition – just like to say a few more things with respect N; there is no template specificity. And, Tdt adds, like I said a little while ago, dG residues preferentially. Therefore, it has been also observed that VD, DJ junctions are G-rich. Again, like to mention that N-region nucleotides are not added on the light chain, simply because heavy chain assembly takes place first; Tdt expression happens at that time and the level of this enzyme starts going down when the light chain assembly is taking place.

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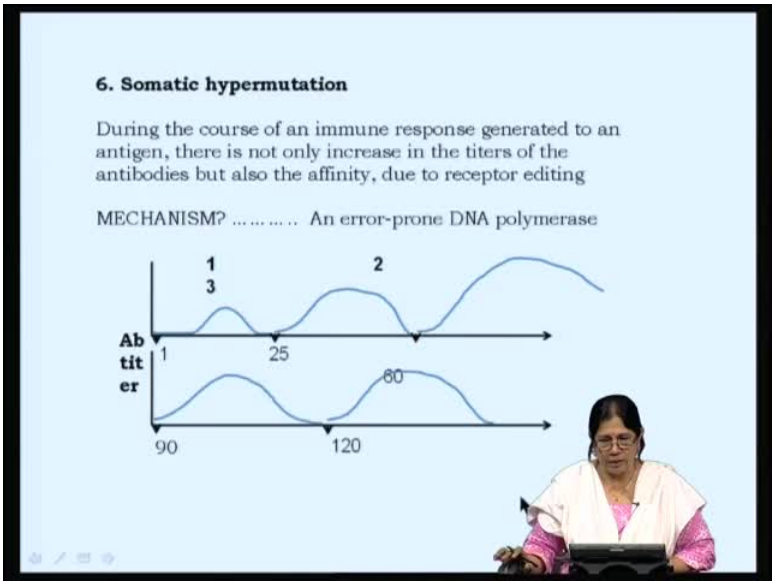

During the course of immune response generate to an antigen, there is not only increase in the titers of antibodies, but also the affinity. This was looked at a little bit closely. How does this happen? Now, remember this figure. This is experiment that was conducted in a rabbit injected with one particular antigen on day 1. Blood was collected from the rabbit every other day or every third day, and then, the titer of the antibodies generated in circulation to the antigen was determined or quantitated; so, the y-axis shows that; the x-axis shows the days over which the blood was collected. After the first response, when the titers were seen to come down, the same antigen was injected again, and also, at a third time. Now, there has been slight change; this should be here (Refer Slide Time: 26:58) 60 and this 3 should be here. That would show primary immune response, secondary immune response and tertiary immune response.

This is what I told you already that you would have a much smaller amplitude after the first, which is absolutely expected and most of these antibodies will be of IgM isotype.

You have then, memory cells, which will be generated here (Refer Slide Time: 27:28). And, when the animal sees the antigen again, there is no lag phase. Immediately, circulating antibodies start appearing and the amplitude of the response is higher. Similarly, after the third injection of the same antigen, again there is no lag phase and you would see the amplitude, which is much higher. Now, when people started to look at the affinity of the antibodies – this was only the respect to the titer, it was interesting to observe that the affinities of the antibodies increased; and, this was every time. In the third immune response, the affinity would probably increase even a 100 fold or a 1000 fold.

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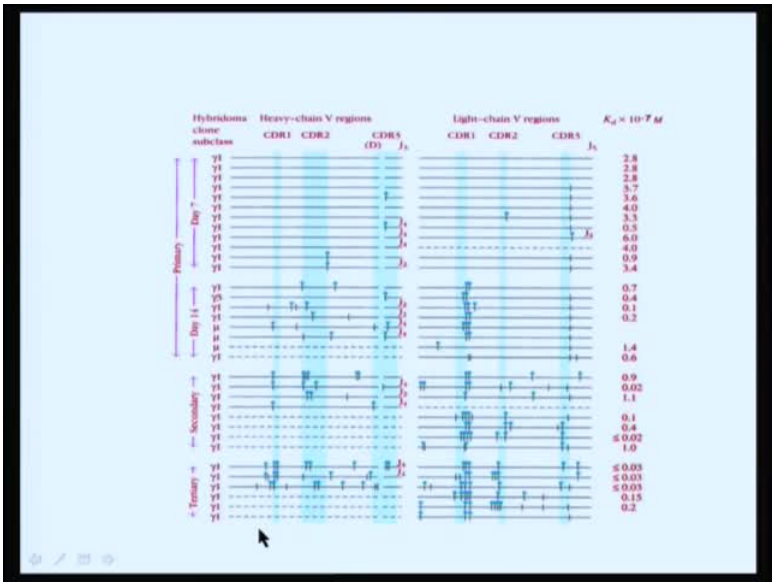

People started to look at the receptors on memory cells versus those on the naive cells. We know that the naive cell to a particular antigen already has a predetermine sequence of the hyper variable region. The mutations were found in the memory cells after the secondary and tertiary response. So, there were quite a few experiments that were carried out. One of which I would like to mention over here, because this gave a direct evidence that though the variable region once formed, that is, V-D-J, does not alter except for a few mutation, which give the recombination event a better advantage; how, let us see.

There were experiments that were carried out where a hapten – why did these people take hapten? Hapten is a small molecule; it has an antigenic determinant; it could be either a peptide or it could also be a small molecule, such as dinitrophenol. Now, such a

small molecule when needs to be conjugated to a carrier protein for it to become an immunogen; in this particular experiment, scientists have taken phenyl oxyzolone, which is a very good B cell epitope. You get a good response to this very small molecule, phenyl oxyzolone; molecular size is very small. It is conjugated and then to a carrier protein, injected into mice. And now, hybridoma was established from the spleen of these mice on day 7, day 14, and then, subsequently. They got several cell lines, several hybridoma. I have not introduced hybridoma, but I would like to just mention, hybridoma are those cell lines, which are generated by fusing a B cell with a myeloma cell, so that the B cell becomes immortal, the antibody producing B cell. Getting a cell line is always easy, because several experiments can be done including looking at the gene sequence. Now, these hybridoma are monoclonal, and therefore, all the cells that are generated in one particular culture would be monoclonal. So, every sequence would be identical in this particular cell.

Now, there are large number of cell lines that were sequenced with respect to the immunoglobin heavy as well as the light chain. And, as can be seen with the hybridoma that were generated on day 7, neither the heavy nor the light chain differ too much with respect to the immunoglobulin sequence. I would like to say that these small dots and lines represent mutations in CDR 1, CDR 2 and CDR3. I have not discussed these three regions, but sufficer to say that both heavy and light chains have three hyper variable regions, even in the variable region. And, as a name suggest hyper variable, you have tremendous variability in these three regions. And, it is through these regions that antigen binding takes place. So, you can see almost no mutations seen in the heavy and light chain in the first 7 days after immunization. Remember, now, the mice were immunized on day 1; 7 days later, hybridoma were established.

Interestingly, when hybridoma were established from spleens of mice, that immunized 14 days prior, which would mean now that this would be the secondary immune response. You can see very clearly that the light chain has accomplished or accumulated large number of mutations. Now, each one of these, for example, cell line number 1 has two; the second cell line has two; three mutations in 3, and so on. This was quite also interesting. Now, this could be only with respect to phenyl oxyzole, but on day 14, it is a CDR 1 of light chain seems to have accumulated. In fact, if you look overall, in case of the light chain, CDR 1 seems to have maximum number of mutations.

Now, the mutations were really quite random in case of the heavy chain in the CDR 1, 2 as well as 3 regions. In the heavy chain, CDR 3 is coded for by part of the day D segment and J. In case of the light chain, CDR 3 is only J. You can see the secondary immune response and you can see the mutations have increased; you can see by the number of dots. You can see that the mutations are tremendous when you come to the final tertiary. Now, what is secondary and tertiary is day 7 and 14; the animal has been injected here. Animal has been injected once more before the secondary immune response and once more after the tertiary. You can see very clearly that these mutations are accumulated in memory cells in response to the antigen.

Now, look at the right side panel; I am sure that is difficult to read, but I just I tell you what does this show. Now, the antibodies that were synthesized, secreted by the hybridoma clones, but then, assessed for their K d or the affinity to bind the antigen. And, what can we seen, let me read this out; K d into 10 to the power of minus 7 moles. So, this becomes 100 nano molar. So, first the affinity of the antibodies is quite low, does not change much from one cell line to another in hybridoma that were established on day 7. Immediately, when hybridoma were established from mice on day 14, you can see immediately an increase in the affinity 10 fold. And this became, with this increased further in the secondary as well as the tertiary, by a 100 fold. This was direct relevance.

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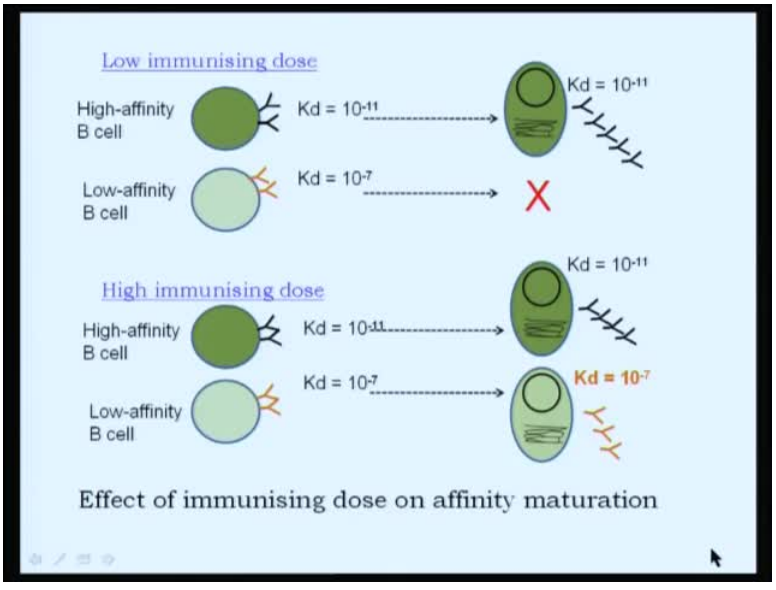

And, this experiment has been reproduced several times to show that during the generation of an immune response, we have increase in the affinity and this affinity can only happen by receptor editing. Considered by the sequence, if it remains the same, there cannot be increase in the affinity. For the affinity to be increased there has to be a sequence change. And, the sequence change happens because of the presence of an error prone polymerize. And, this error prone polymerize gets activated in memory cells. Therefore, this will of course, then the receptor editing as well as increase in affinity can only happen in case of those B cells, which are T cell dependent. Remember, memory cell generation itself is a T cell dependent phenomenon. So, affinity maturation T cell dependence. And then, may be if I have time today, I will talk on isotype switching. All these happen in T cell dependent B cells.

I would like to also tell you a relevant experiment with respect to affinity of antibodies. Several years ago, when people did not know much with regard to these hemoglobin genes and the affinity, it was believed that if you injected milligram amounts of antibodies, you would get a very robust immune response. But, it is not. In fact, I would also like to say, when one is doing experiments with let us say, mice and rabbits, rabbit would be let us say, even 1 kg and a mouse is a paltry 15 grams. The amount of antigen that is injected does not go by the body weight. There is no pharmacological dose. Immunological doses will still be in microgram amount. Typically, if you are injecting 20 micro grams of immunogen in mice, in the rabbit, you would inject not more than 10 fold, 200 micro grams; but, not corresponding to the weight of the animal.

There are two instances given over here; (Refer Slide Time: 38:00) low immunizing dose versus high immunizing dose of the very antigen that I was talking about. And, let us say, the animals are rabbits. If the animal is injected with low immunizing dose, what would happen? Because the dose is very low, only high affinity receptors of those B cells would recognize the antigen. Let say, this B cell has an affinity of binding to the antigen to the order of 10 to the power minus 11. So, it becomes absolutely to fem to molar range. So, very low immunizing dose; let us say, it would be something like 10 micrograms in rabbit. You would get an immune response generated, but very few B cells would get activated in response, because only the high affinity ones would. Only these would then get activated, proliferate, differentiate and become plasma cells. Maybe in certain instances, you may not detect the presence of antibodies in circulation, because

the numbers may be very low. At the low affinity receptor bearing B cell, would not come into the picture at all, because they would not mind. So, there is no question of activation, proliferation and differentiation.

Now, if a high immunizing dose is given, then both B cells, those bearing high affinity receptors as well as low affinity receptors, would get activated differentiate, and you would get a mixture of antibodies, which would have both high affinity as well as low affinity. So, effect of immunizing dose on affinity maturation is important. And, even if one starts with high dose of antigen immunization one, subsequently, the dosage can be decreased, so that one would get mostly high affinity binding antibodies.

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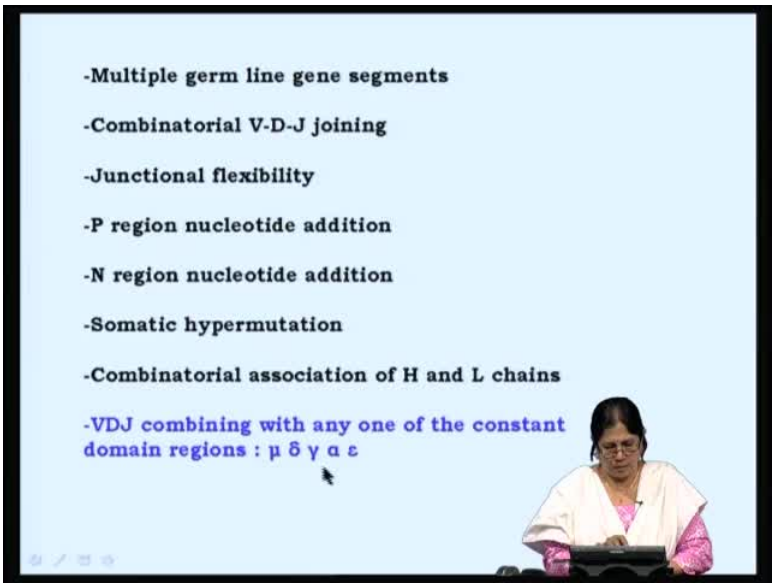

Now, let us go over this whole mechanism of the establishment of diversity in the immunoglobulin gene. It is because multiple germ line gene segments; combinatorial V-D-J joining; combinatorial association of heavy and light chain; junctional flexibility; addition of P region nucleotide; addition of N region nucleotide; and, somatic hypermutation. Now, I would like to mention finally, that we do have the V-D-J, which is already combined; maybe it has undergone mutation, affinity maturation. This does combine with always the mu constant domain, and simultaneously, with the delta constant domain. But, those B cells, which are T cell dependent, can undergo class switching, and in them, the recombined V-D-J can associate with also any one of the other five classes of immunoglobulin: the gamma, alpha, eta.

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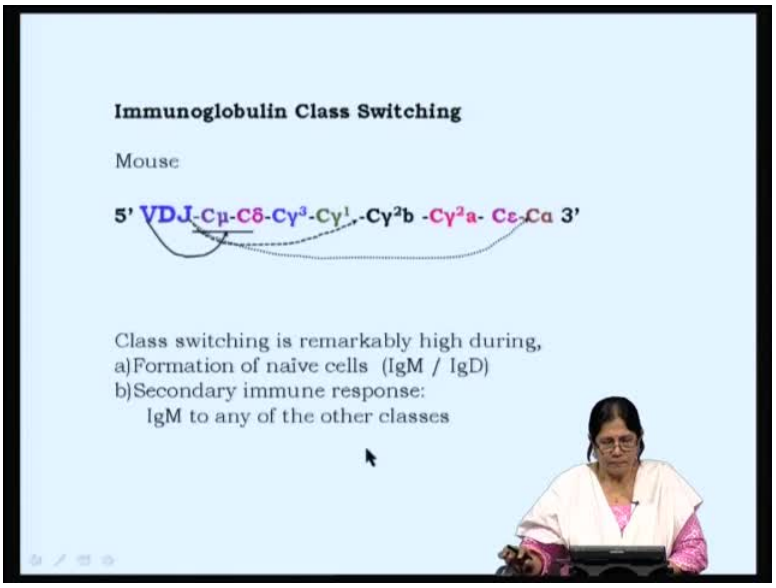

Since I have time, I will introduce to you immunoglobulin class switching. Class switching – I have not introduced again already the five different classes of immunoglobulin, but I would have mentioned when I was talking about the genes that you have in the constant domain; you have gene segments that code for constant domain of IgM, that is, mu, delta for D. Then, there are four gene segments for the immunoglobulin gene: constant domain G 3, constant domain G 1, constant domain G 2 b and constant domain G 2 a. Now, this is mouse, because in human, it is the simpler; it is 1, 2, 3, 4. So, human also have 4. Then, follows; in the immunoglobulin gene organization, you have constant domain eta, that is, IgE, and then, constant domain alpha.

Immunoglobulin class switching is maximum and very high during the naive cell generation. Remember, in the ontogeny of B cell, IgM is the first receptor that gets suffice expressed. Now, by alternate splicing, IgD is also simultaneously expressed. So, during this, (Refer Slide Time: 43:00) though this is not class switching totally from one to the other, there is alternate switching. And, this is, like I said again, by alternate mRNA splicing, that you have information of IgM and IgD of naive cells. Now, class switching is remarkably high in the first instance and remarkably high during the secondary immune response when IgM switches to any one of the classes immunoglobulin. Classes again would only be referring to the constant domain genes.

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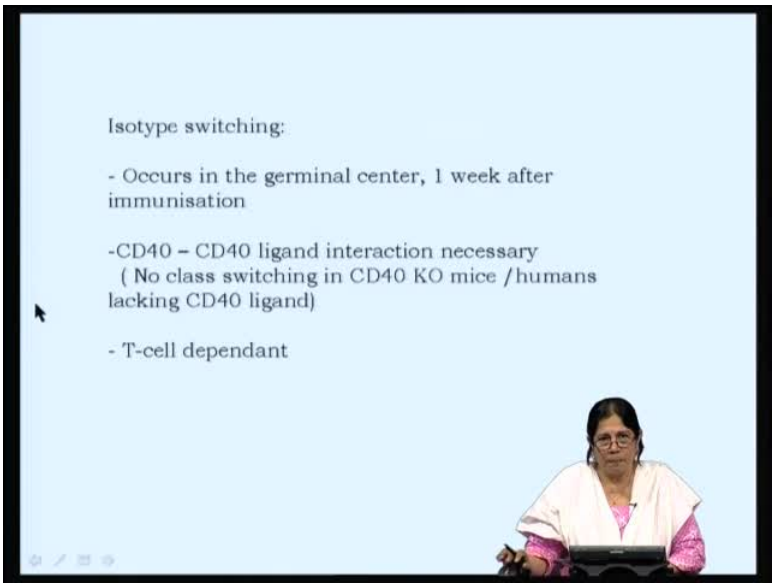

Isotype switching occurs in germinal center, 1 week after immunization. You have already been familiarized with the secondary lymphoid organs and you already know what germinal centers are. And, this is where you have memory cells; you have T, B and macrophages cells. And importantly, memory cells of B cells, which 1 week after the primary immunization, if the antigens still persist, then there is activation of memory cells and there is isotype switching. So, the memory cells, which would be IgM… One thing, which I missed telling you all earlier that though we have IgM and IgD, both the immunoglobulins on the naive cell, IgD is almost never expressed; and, the only time once this IgD is (( )) in myeloma cell (( )). So, there is no IgD in circulation of very low amounts. It is always IgM, IgG, IgE, IgA; mostly, IgM and IgG and IgA.

Now, 1 week after the primary immunization, isotype switching takes place and this isotype switching is absolutely requires CD40 receptor, CD40 ligand interaction. Again, I would like you to remember that CD40 receptor is present on B cells constitutively, but require the presence of CD40 ligand, which is made by the T cell for certain processes of activation. It is this interaction which now tells the cells to produce the cytokine receptors and also gives the progression signal to the B cells for proliferation. If there is no CD40, CD40 ligand interaction, no class switching takes place; also, no memory cell generation. This has been shown by conditions in human, who lacks CD40 ligand; there T cells cannot make CD40 ligand, and therefore, there is no CD40 receptor ligand

interaction. Therefore, no memory cell generation, no class switching and no affinity maturation, because this process is totally T cell dependent.

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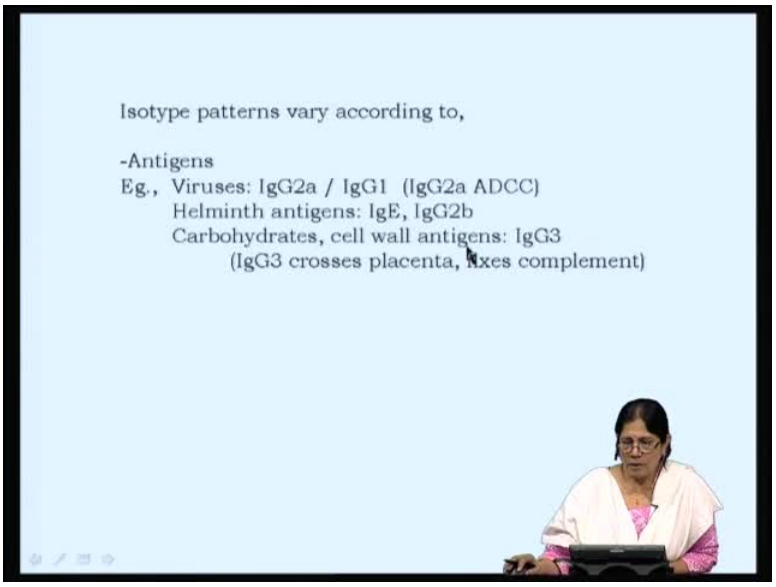

Isotype – the same variable, again and again I would like to mention this that it is already combined V-D-J, which now can associate or disassociate from the IgM and associate with any one of the isotypes. Forget the IgD, because in fact, there is no switch region between these two. Isotype pattern varies according to antigens. Now, I have another slide, which goes a little bit more detail for, but suffice here to say that, for example, why do I say isotype patterns vary according to antigen? Antigens such as viruses usually induce IgG2a type of immune response; why, because of the fact that IgG2a can bind to a specific receptor on the T cells, which allow now antibody dependent cellular cytotoxicity.

Helminth antigens – they induce IgE type of an immune response or IgG2b. I will be dealing with this a little later. Carbohydrates on the other hand or cell wall antigens typically induce IgG3 type of an immune response, because IgG3 10 crosses placenta; IgG3 can fix complement better, and the other isotypes. Now, of course, one can look at it from the point of view that the (( )) element response to bacteria can be conferred to the fetus. And also, because IgG3 can fix complement, these bacteria or pathogens can very well eradicated even (( ))

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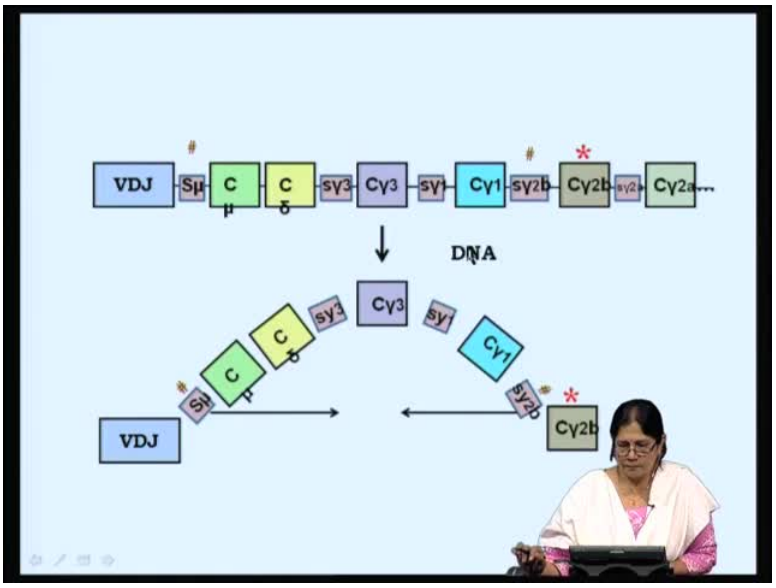

Just introduced to you this recombination event that takes place during class switching; this also of course, again happens at the level of DNA. So, let us look at recombination. Let us first look at the mechanism, how does this happen. Now, very clearly you can see, VDJ has already recombined and fine waiting over here. You have now, on the 5 prime side of the constant domain gene segments, which are in different colors switch region… There is no switch region, please note here, between the constant domains of mu and delta. Therefore, you can have RNA splicing and alternate expression on the naive cell.

Now, switch region, mu, needs to combine with any one of the switch regions for the isotype switching. How does this happen? Let us say, in this particular case, now you remember, the organization of the constant domain is exactly the way I have mentioned earlier. And, this does not change. So, you have now this cell (Refer Slide Time: 49:23) that is going to undergo class switching once to switch from mu, that is, IgM to IgG2b. Now, this happens by the joining coming together of the switch region genes come in cross proximity, and then, you have the deletion of all the sequences, which were in between the m and the IgG2b.

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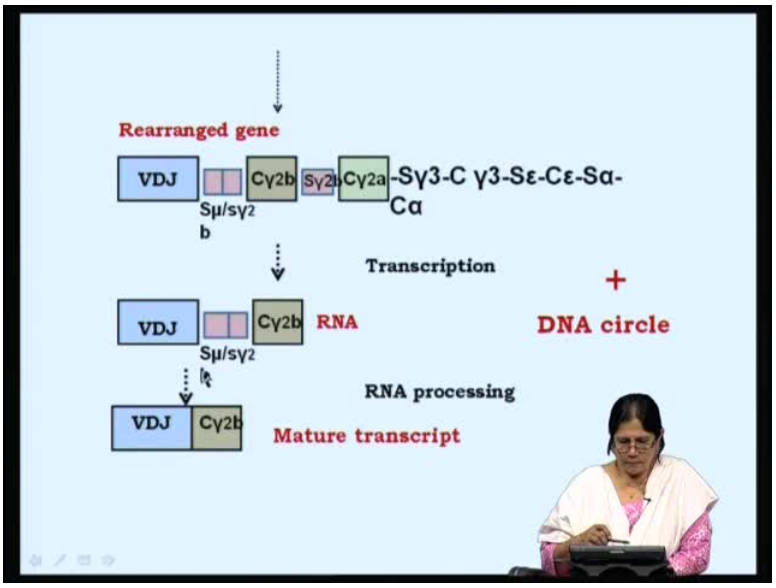

This recombination between the switch region S mu, that is, for the mu or the IgM and gamma2b. Now, during transcription, again you will have RNA and then you have the matured transcript.

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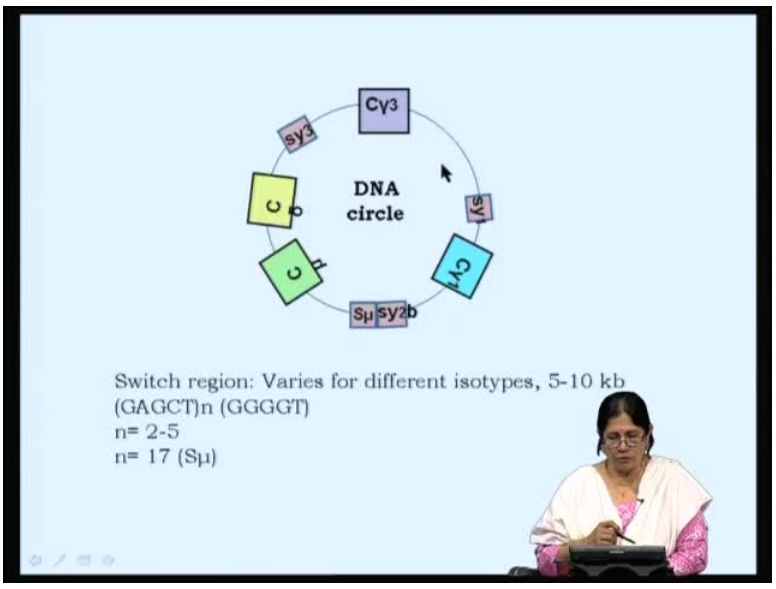

Along with this, you will of course, have this interesting structure. This is the path that has been thrown out of the DNA sequence. You have, just like in the other strand that is retained, you have a part of the switch mu and a part of this switch gamma 2b, which have joint, and this is part, which has been deleted. And, you can see the constant

domain mu, because now that cell can no more make IgM; neither IgD, IgG3 nor IgG1, because all these are in between the switch regions.

All these (Refer Slide Time: 51:10) are between the switch regions mu as well as switch region gamma 2b. So, you can just visualize very well that you have the switch regions that come together; the recognized come together. Then, you have cleavage. So, you have a part of the switch region now, which is still in this sequence (Refer Slide Time: 51:34) and a part which is here (Refer Slide Time: 51:37). Now, the same thing would happen if the switching has to take place between mu to let us say, IgE or mu to IgA.

Now, also look at something interesting that if the cell has already undergone switching (Refer Slide Time: 52:01) from mu to IgG1, then this memory cell can further switch from IgG1 to let us say, immunoglobulin IgG2a; or, what is not shown here is immunoglobulin alpha. But, of course, since this part would be deleted, the cell can no longer go back to making these isotypes. So, once again, there is cleavage (Refer Slide Time: 52:34) and throwing off or deleting an entire sequence. In this case, it would be the constant domain genes.

Now, I would like to stop here. And, in my next class, I would like to talk about what brings about this recombination, is it known? What are the different proteins, which bind to this? What induces? How much is known with respect to class switching? What can be these factors that govern? Obviously, one can imagine and these factors would be somehow associated with the kind of the antigens that go in, because it is antigens finally, which determine which kind of immune response with respect to the isotype. I already given example of viruses, which induce IgG2a type in case of mice and IgG2 in case of human; whereas, helminthes, what switch IgM to immunoglobulin E. Obviously, then, the immune system has been involved in such a manner that the immune response should help to eradicate that particular foreign object in the body. Therefore, there should be generation of some transcription factors; there should be generation of interns, some proteins by the antigens themselves; the immune response that is seen in these antigens, which would culminate in the production of those kind of immunoglobulins, which would be highly effective.

So, next class, we will be looking at the mechanism of this class switching further, and also, looking at the immunoglobulin gene regulation; what actually regulates all these processes; are there any genes, which are above RAG 1 and 2 and what these are.

Thank you.