Essentials in Immunology

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Lecture No. # 12

Structures and Functions of Immunoglobulin's

Today's topic is Structures and Functions of Immunoglobulin. You have been studying for over several lectures that B cells are the only cells that make immunoglobulins as cell surface receptors; and, the same cells start secreting antibodies, once they are differentiated to plasma cells. Now, it was already known that there is a soluble component in the blood, which can counteract very effectively with the immunogen or pathogen as it was known earlier. And, this was known already in beginning of the twentieth century.

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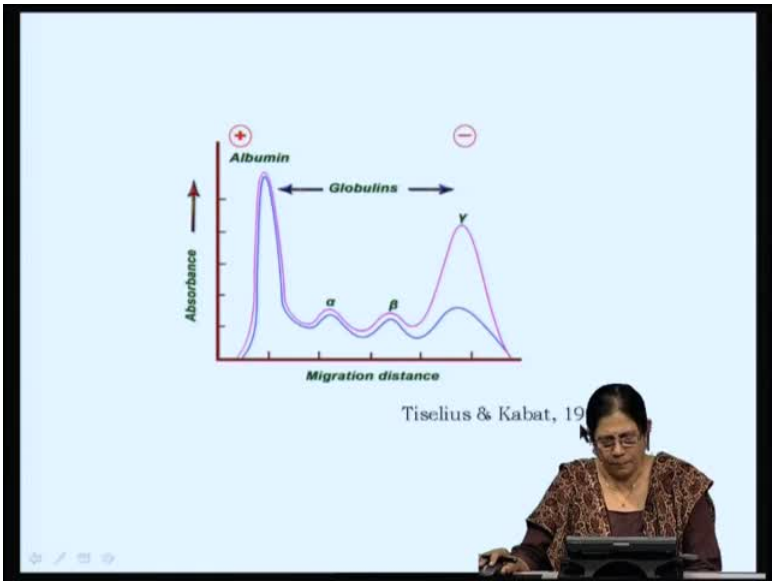

People since then, were trying to look at or identify what is this molecule or what are these molecules that participate in the humoral immune response. It was around 1939 that Tiselius and Kabat were the first report that this component antibody is present in the gamma globulin fraction of the serum. Technology was primitive those days. And, the only method by which scientist could identify which were the fractions present in the

serum was electrophoresis, simple electrophoresis, where proteins could be separated based on the net charge. Now, Tiselius and Kabat did a very simple experiment and it took them several months, perhaps several years to do. They have made antiserum to ovalbumin. When they electrophoresis the antiserum as is known for even just serum from any individual, there are essentially two sets of proteins that one can identify: albumin and globulins. Now, globulins also could be separated as can be seen on this graph – into alpha, beta and gamma.

Now, when they have taken the same aliquot of antiserum from rabbit raised against ovalbumin, that is, egg albumin to which now, they added or a certain amount of T antigen ovalbumin. They already knew that, on addition of antigen to antiserum, always or most likely, resulted in precipitation; precipitates that could be centrifuged down to a pellet. So, they did that. Now, they have taken two aliquots like I said: one, where they have not added the antigen; and, the other one, where they have added the antigen. After what they thought was appropriate antigen-antibody interaction, they centrifuged down the particulate material, which was nothing but the immune precipitate. And now, they have run electrophoresis both the samples on their native gels, where proteins were separated as albumins, and globulins: alpha, beta and gamma.

Now, what could be seen very clearly that in the aliquot, which showed precipitate on adding the antigen, there was a decrease only in the gamma globulin fraction. And, it was quite clear; you can see the decrease. Therefore, it was easy for them to then conclude that the immune response that one can generate by way of secreting antibodies in the blood resided in the gamma globulin fraction, and therefore, the name immunoglobulin came about. So, it was Tiselius and Kabat, who showed for the first time that antibodies were gamma globulin fraction. Since then, people started to do more search on how to find out the structure of this gamma globulin fraction or the antibody that we know now.

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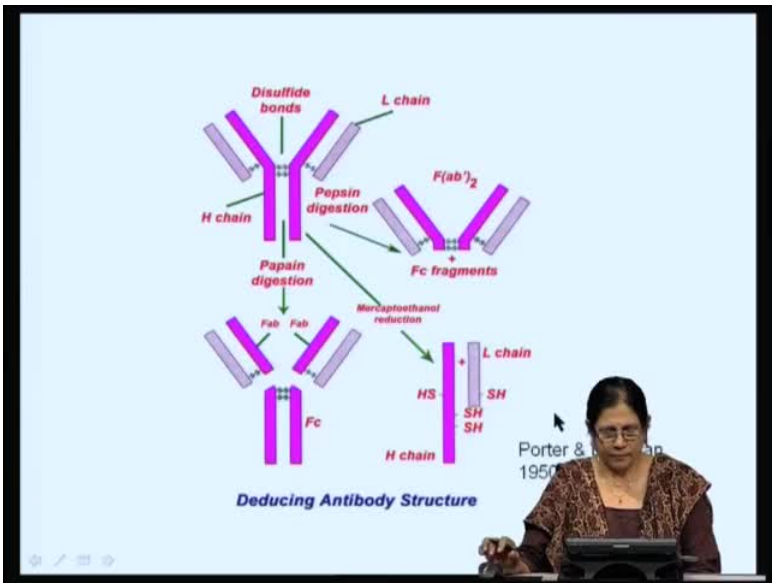

Now, there were large number of scientists, who were looking at the structure. And now, we know the structure has two heavy chains, which are held together by disulfide bond, which inturn hold the light chain. But, it took many years for Porter and Edelman to come to this, just because the technology then was not as advanced as it is today. And, they always needed to work with milligram amounts of the molecule anyway.

This picture here shows the results of two of the major experiments. One, where they have taken the antibody molecule, immunoglobulin; and now, reduced the disulfide bonds with mercaptoethanol. Always, this resulted in identification of two bands; they called heavy and light. There are always two bands. But, when they looked at the size of the two, they realized immediately that the structure should be much large. Now, the size of the heavy chain is only around 55 kilo Dalton and the light chain is around 25. And, they knew that the whole protein molecule was around 150000. Therefore, they could do simple calculation and surmise that the immunoglobulin molecule is made up of two heavy chains and two light chains.

Now, in addition to these experiments, they also were digesting the proteins with different enzymes and they were able to come to a good conclusion with respect to the structure by digesting the molecule with pepsin or papain. Interestingly, pepsin digestion attacks the site on the immunoglobulin molecule, which is after the disulfide bonds, which holds the heavy chain together. Papain on the other hand, cleaves the molecule at

a site, which is above the disulfide bonds. Therefore, the pattern of the fragments that they obtained was quite distinct. What they obtained with pepsin digestion had a fragment, which could bind to molecules of antigen; whereas, with the digestion with papain, they got a fragment, which could bind only one; it was monovalent; only one antigen molecule. Upon papain digestion and pepsin digestion, they were able to get fragments, which they called FC. And, we know that this is the constant domain of immunoglobulin. Now, putting all these observations together, they came to the conclusion that the antibody binds two antigens from the amino terminus and that antibody molecule is at least bivalent. But, this was quite remarkable considering the time at which they were working in trying to elucidate the structure of immunoglobulin.

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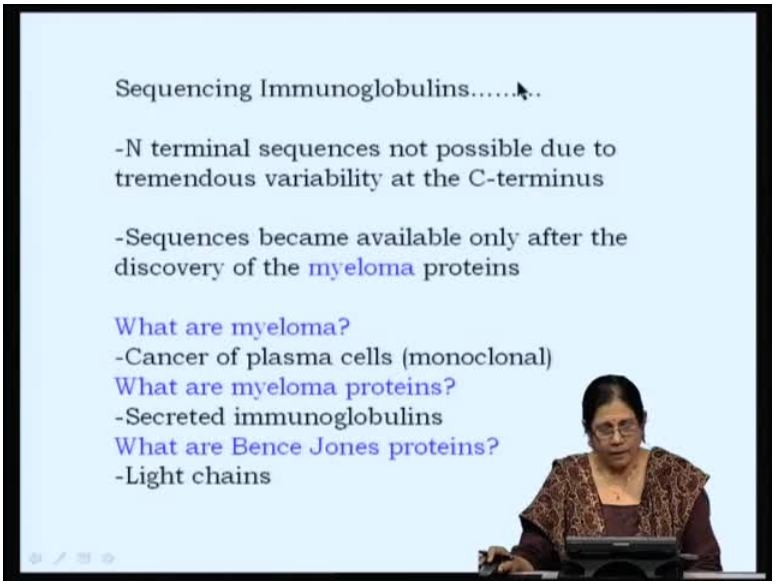

Now, already in the 1930s, sequencing of protein, the methodology at least, came to be established. So, scientists started to sequence immunoglobulins. How were the immunoglobulins purified? By the method of either sorting out with ammonium sulphate or by electrophoresis as I have shown in the first slide; but, this was always from the immunoglobulins from serum. Now, they could not come to any conclusion with respect to the sequence. Now, it is very easy for us to see why, because there is tremendous variability at the N terminus, and therefore, the N terminal sequencing was possible due to tremendous variability at the N terminus. So, how did they then get the sequence of immunoglobulins? Now, you know that in the database, we have large number of sequences of various immunoglobulins. So, it was after several years that scientists

found, in fact, immunoglobulins could be sequence, because of the discovery of myeloma proteins.

Now, what are myeloma proteins? Myeloma proteins are products of cancer of plasma cells. Myeloma proteins are also monoclonal; why, because cancer is usually monoclonal; almost any cancer one can think about whether it is epithelial or let us say from hematopoietic lineage; cancer is usually monoclonal. If a plasma cell gets transformed – plasma cell remember, the cell that secrets antibodies, becomes immortal now or becomes cancerous, then this cell and the progeny of this cell would make antibodies of only one class, only one type. And, those proteins were known as myeloma proteins. At that time when myeloma proteins were discovered, it was not known what these molecules are; they came to be known later. So, what are myeloma proteins? They are secreted immunoglobulins from the cancer of plasma cells.

Now, at this junction, those not really relevant, I would also like to say that people were also able to identify Bence Jones proteins, which were not the whole molecule of immunoglobulin, but light chains. Now, myeloma patients secrete in their circulation not only myeloma that is the whole molecule immunoglobulin, but also some of them express large amounts of the light chains. And, those were called Bence Jones proteins. Finally, anyway they realized, is a whole molecule myeloma or Bence Jones like chains alone.

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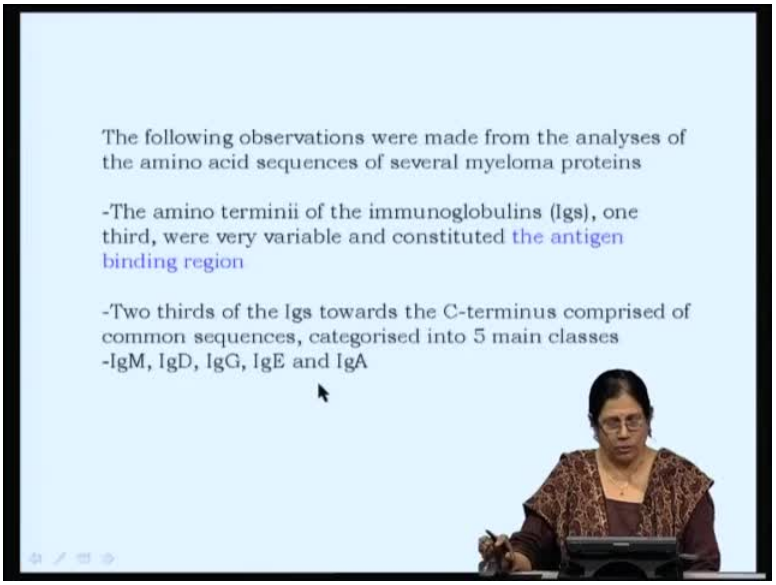

Now, soon after that came to be known, almost every myeloma protein that could be identified was sequenced. The amino acid sequences then were analyzed and the following observations were made that the amino terminii of the immunoglobulins, that is, one third, were very variable. So, when they looked at all the sequences of the myeloma protein that they had now a bank of, they found that the amino terminii always were very variable. And therefore, it was logical to conclude that this region would constitute the antigen binding region. Two thirds of the immunoglobulin sequence towards the C-terminus comprised of common sequences. When they looked at those common sequences, they could see that these myeloma proteins then could be categorized into five main classes. And, we know them as IgM, IgD, IgG, IgE and IgA.

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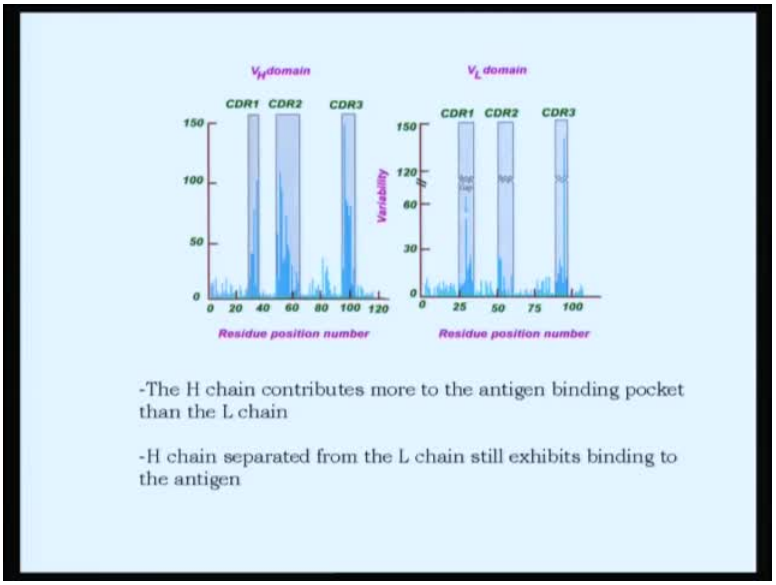

Now, once the sequences came to be known on closer look of the variable domains… There are now two graphs here before me: one shows the variable domain of the heavy chain; and the other one, variable domain of the light chain. Now, when people analyze these variable sequences closely and they try to see how variable these variable regions are, they did a very simple analysis and which is shown here.

Now, the variable domain, first of all, they could see was around 110 amino acids from any and every myeloma protein; both heavy as well as the light; about approximately 110. Now, they made these graphs by putting on the x-axis the amino acid number, and then, they tried to look at how many times how different is that particular amino acid at

that particular residue, that number. So, y-axis shows the variability. The peaks that you can see here would mean, in this particular case, where it is maximum, let us say, in the 94th position or 95th position, the amino acid varied almost more than a 100 percent; which would mean that this would be the most variable region and residue, and definitely, this would contribute tremendously to the antigen binding.

Now, another observation – in fact, you can also make looking at this, that both in the heavy and the light chain variable region, there is a hyper variable or there are hyper variable regions. Look at the residues from around 35 to 38. Similarly, let us just say, 50 to about 55; and similarly, around 90 to 94. Now, these varied tremendously all the myeloma. Now, these three regions are flanked by more or less constant domain. There is a region here, which is more variable than the others. So, they coined the terms that these were the CDR1, 2 and 3; CDR stands for complementarity determining regions, because by then, they already knew that these were the regions by which antibodies of B cell receptors bound their cognate antigen. Now, flanking these are the framework regions, which remain more or less constant. Now, in fact, today, it is possible to design primers corresponding to the framework region and amplify the hypervariable domain, because one cannot determine or design primers to get only the hypervariable domains.

Again now, let us look at the heavy and the light variable domains and you can see immediately, that in both cases, the CDR3 is most variable. And overall, the heavy chain domain is much more variable than the light chain domain. The CDR2, for example, of the light, contributes minimal to the antigen binding pocket. So, the H chain contributes more to the antigen binding pocket than the L chain. Now, people have done various experiments even at the protein level trying to see when you separate the heavy and the light chains, whether they are still capable of binding the antigen. And, almost with about 95 percent cases, when H (heavy) chain is separated from the light chain, a simple reduction of the disulfide bond, the heavy chain still can bind the antigen; whereas, light chain almost cannot. I would like to mention here that the heavy chain separated from the light chain still exhibits binding to the antigen, but the affinity with which the heavy chain still binds could be 100 and sometime may be a 1000 fold lower.

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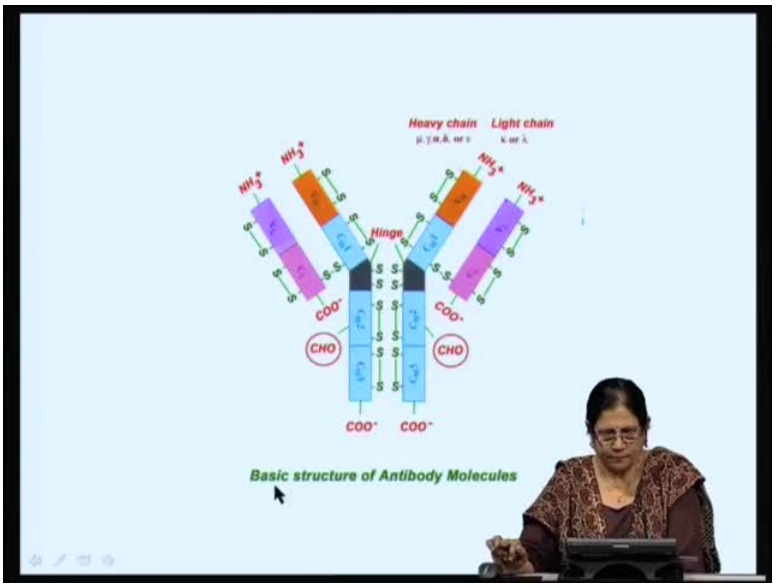

Now, here after all the information that was gathered with respect to the amino acid sequence after reduction of the heavy and light chain, and the bindability, and all the data, now, could be put together to come to the final structure of the monomeric immunoglobulin. Why I say monomeric, is because there are classes of immunoglobulin that show oligomeric structure. This is a basic structure of an antibody molecule; or, you can say immunoglobulin molecule. What does this structure? We know very well now that every immunoglobulin is made up of two heavy chains, which are held together by disulfide bonds. Now, the heavy chains themselves are again held to or hold the light chain through disulfide bond. Both heavy chains of an immunoglobulin molecule are identical; so, also the light chains. An antibody molecule is at least bivalent. Now, in comparison to those, which have more than one monomer, where the valency can go as high as 10; and, we will see that in a while.

The heavy chains could be either mu gamma, alpha, delta or eta. And, in the antibody molecule, the class is known by the heavy chain; so, M, G and so on. The light chain can either be kappa or lambda. It is important to know that apart from the heavy chain being identical to each other, even the kappa would be identical not only with respect to the variable domain, but also with respect to the constant domain. Remember, in my last lecture, I did mention that always, the recombination of the kappa light chain allele 1 starts first and if that fails, the second allele takes over. If the recombination of the second kappa allele also is disaster, then the lambda allele 1 starts the recombination

process. Therefore, we know that an antibody molecule always has only one type of heavy chain, one type of light chain. I should have mentioned something about the constant domains here, which I forgot and let me go back to the basic structure again; that the variable domain and the constant domains are almost similar with respect to the number of amino acids. The heavy chain after the variable domain has three constant domains in three out of the five classes of immunoglobulin that we just talked about. The constant domain of the light chain is only one. Immunoglobulins are glycoproteins; they are glycosylated. The amino acid that get glycosylated are on the constant domain 2 of the immunoglobulin molecule. Both the heavy chains are glycosylated.

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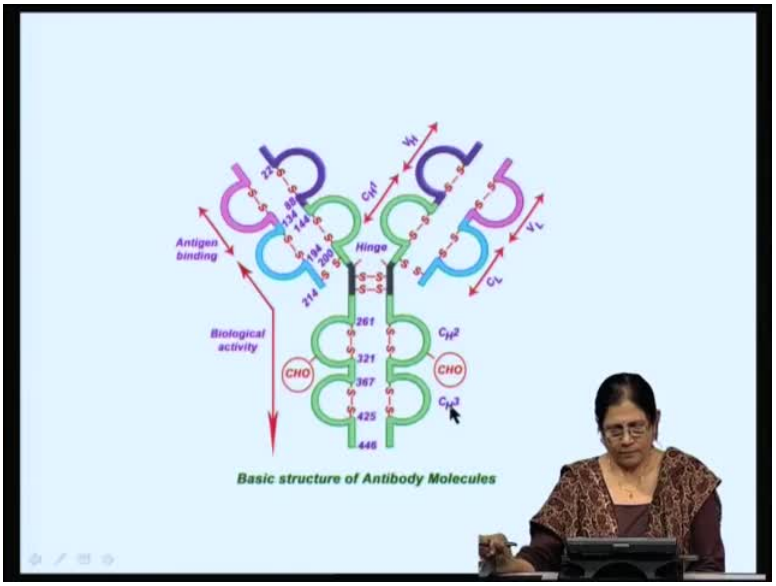

This is a structure, which shows the fold of the molecules. It is the same immunoglobulin molecules, but this elaborates the function of the intra disulfide bonds. And, let us look at this a little bit closely. You can see very clearly from here that the heavy chain has 1 2 3 and 4 folds. These are called immunoglobulin folds. In fact, there is a super family, where immunoglobulin is the prototype molecule; and this family, I can give you a few examples, the alpha 1, beta 1, the co receptor for the immunoglobulin, the B cell receptor or the CD4 or the CD8 or the TCR receptor and the co-receptor. All of these belong to the immunoglobulin super family. And, all of them would have perhaps different sequences at these different regions and the structure may be very different. But, all of them are characterized by the presence of these immunoglobulin folds. These immunoglobulin folds are held together or stabilized by intra disulfide bonds. So, you

can see the complexity of the molecule here. Now, why is it necessary that there should be these immunoglobulin folds, is because… Now, where do you find these immunoglobulin molecules either as cell surface receptors or in circulation as the antibodies? Now, there is a lot of… Even these cells are built in the blood plasma or in body fluids; all of which contain large number of proteases. To enable these molecules to survive the proteases, nature has allowed the evolution of such molecules, which are very stable to proteases.

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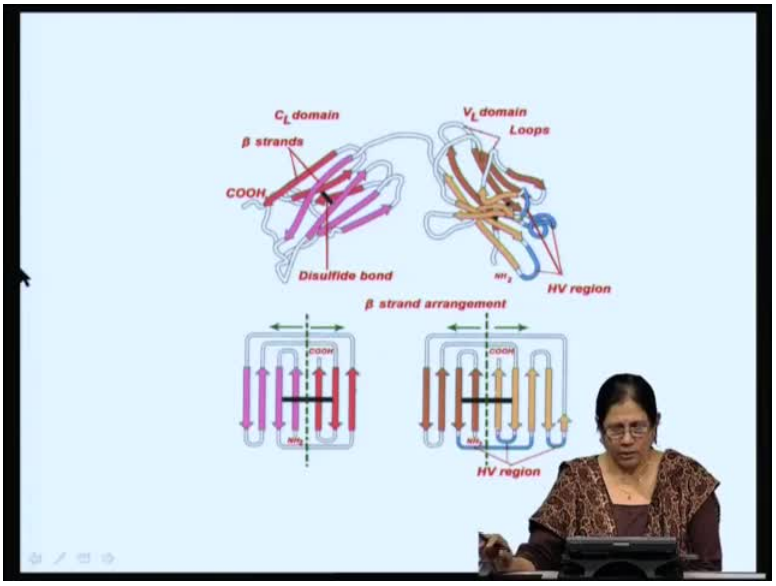

Now again, one fourth of the molecule is the variable region and the rest of the molecule is more or less constant. And, this definitely then should have common functions biological activity. Let us look at that. This is one more structure, which is based on crystal structures that were obtained for all the myeloma proteins and it was seen… Now, this is very characteristic and I would like to spend a little time on this. Now, the immunoglobulin fold – each one of this is a fold and this is the structure of the light chain; you can see constant light and variable light.

Now, very clear here are the presence of these antiparallel beta pleats. These are held together as a barrel. And, if one can think in terms of roof of the barrel and the flow, one can see that the roof and the flow are again held together by the presence of that disulfide bond, which I had pointed out in the previous picture. Now, these antiparallel beta sheets are joined together by these loops: (Refer Slide Time: 26:01) beta bends or loops. And,

as one can imagine that it is the loops, which constitute the antigen binding pocket. This is the variable domain and these are the antigen binding pockets. What is very interesting to see, that the immunoglobulin molecule does not have any helices; total absence of helices.

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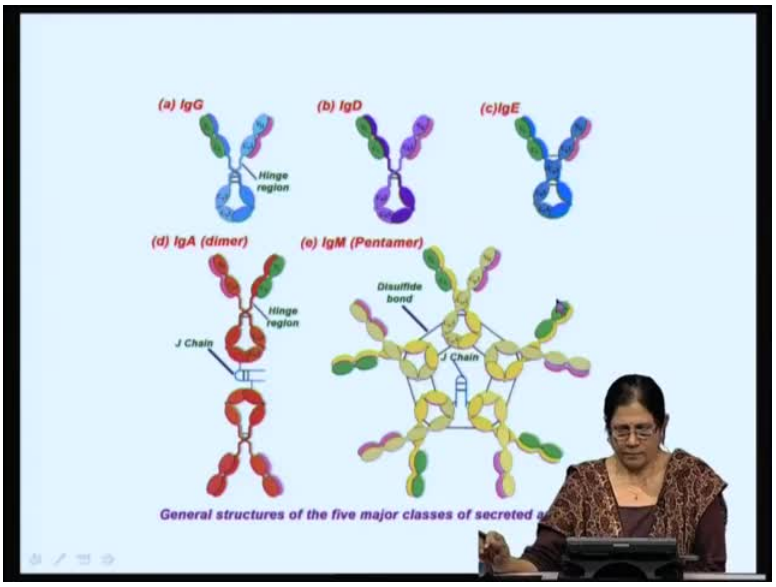

There are five classes of immunoglobulins or antibodies. Are these similar; are they identical? Obviously, not; so, where are they different from each other? Before we come to the molecules level, let us just look at the domain organization. IgG, IgD and the monomer of IgA are very similar; they have the heavy chain, which has variable one domain and then constant three domains; you can see. However, the remaining two classes: IgE and IgM; both have four domains. And, what you might see very clearly here, they do not have the hinge region, which the other immunoglobulins have; there is lack of the hinge region. In fact, the hinge region in IgE and IgM has been modified to yet another constant domain. Therefore, now, IgE monomer and IgM monomer are longer; the heavy chain is longer than the heavy chains found in the other three.

Is there any similarity between IgA and IgM? Yes, both of them can oligomerize. And, the oligomerization – you can see two molecules of IgA forming a dimer; IgA can also find form a trimer. The dimer and the pentamer seen in the IgM are held together by the J chain, which is a 15 kilo Dalton, 16-rich molecule, which is also synthesized by the B

cells. You can see the J chain here and the J chain here (Refer Slide Time: 28:48). So, you have similarities between these two molecules because of the presence of J chain.

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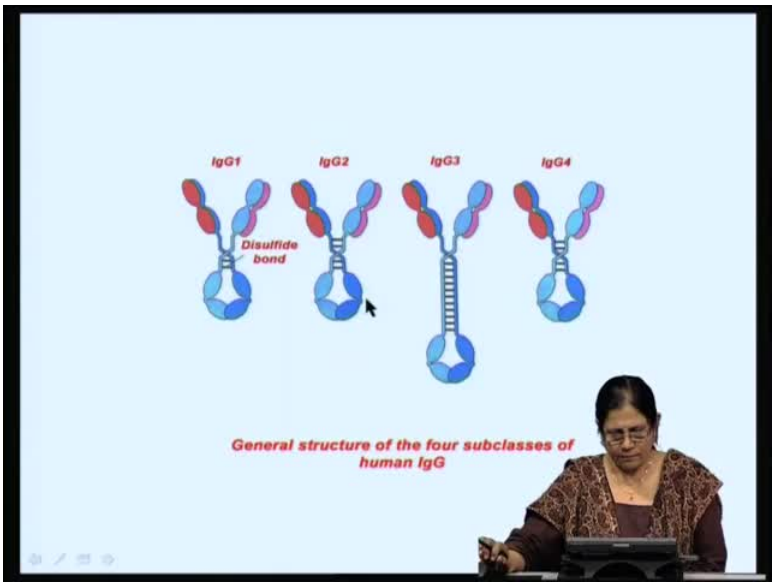

Let us see if there are any other similarities. Now, let me come to the subclasses of IgG. I have already discussed this with you earlier that IgG is present in the serum as four distinct subclasses: IgG1, 2, 3 and 4; in human, IgG1, 2a, 2b, 3. And, these are also present on memory cells as the antigen receptor. Remember that there are constant domain regions in the immunoglobulin gene itself. And, these not in the sequence, but these are found soon after the gene for IgD. So, you have IgM, IgD and then you have the IgGs, the different domains of the four types of IgG: IgG1, 2, 3 and 4.

Now, if you look at these four, immediately you can see, IgG3 is different, because it has a very long hinge region. Hinge region is that region, which joins the constant domain 1 with the constant domain 2. Now, there can be as many as 13 disulfide bonds in the extended hinge region of IgG3. IgG2 and 4 are similar in their hinge region disulfide bonds. All of them again would be similar with respect to the domains. They have only three constant domains and the heavy chain. Why do these subclasses differ from each other? The difference lies mostly in the amino acid sequence, which is in the hinge region and a little bit in the FC region. Therefore, one can have reagents or antibodies, which can recognize IgG1, but not the others and so on. So, one can generate isotype-specific antibodies. These are isotypes of IgG.

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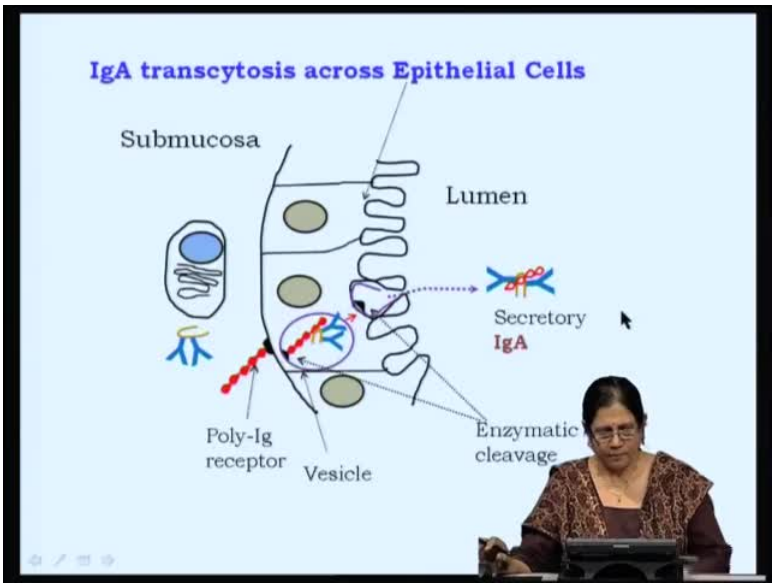

Something very interesting about the IgA molecule; I will be coming to that a little later, but IgA molecules are present mostly the dimeric form, is present mostly in the body fluids. Therefore, the molecule has to transcytose travel across the epithelial barrier to go on the other side. How is this achieved? Now, the transport of the IgA across epithelial cells was discovered a little later; if I remember correctly, it was sometime in the early 90s. Now, how does this happen, is what I will tell you that now you have the plasma cell, which synthesizes IgA. IgA dimerises by the J chain. Now, this allows accessibility to a region on the FC portion of the IgA, one of the monomers, to recognize or bind to a receptor, which is also a member of the immunoglobulin super family, which is known as poly-Ig receptor, which is present on the basolateral surface of the epithelial cells. So, this is IgA synthesize (Refer Slide Time: 32:41).

The dimer now binds to the poly-immunoglobulin receptor triggering receptor mediated endocytosis of the entire complex in the vesicle now; this is a vesicle, (Refer Slide Time: 32:55) where there is cleavage of the immunoglobulin receptor. This is the site, where cleavage takes place. And now, the lose end can wrap itself around the entire molecule finding a binding site on the other monomer of the dimer. Now, this complex through receptor mediated exocytosis is thrown out. And, you have now, this molecule of the dimer of IgA, which has the J chain and the secretory component now. The same poly immunoglobulin receptor on wrapping itself around the dimer is known as secretory IgA. What does the secretory component do? As the receptor helps the transcytosis and after it

is secreted, you have the wrapping of this secretory chain, which is around quite large, is about 7000 Dalton protein. And, this can further add to the stability of the dimer stabilizing the protein against proteolytic degradation.

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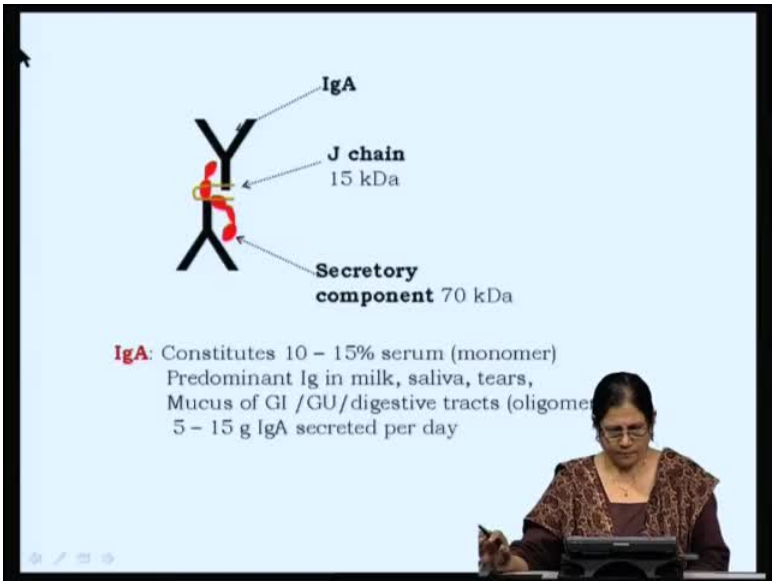

Like you to look more closely at the immunoglobulin that is secreted; the IgA that is secreted. You have the dimer formed by J chain and then the secretory component. Now, this is something quite interesting though we know that in the serum is the IgG, which constitutes major part of the immunoglobulin. But, it is the IgA, which is secreted at the rate of something like 5 to 15 grams in humans; but, most of it is secreted or transcytosed across epithelial barriers into body fluids. Now, IgA constitutes only 10 to 15 percent of the serum. And, in the serum or blood, the protein is present as a monomer; as an oligomer either dimer or timer. IgA is present in milk, saliva, tears, mucus of the gastrointestinal tract as well as urogenital tract as well as digestive tract. Therefore, this in fact, protects the body surfaces from pathogens.

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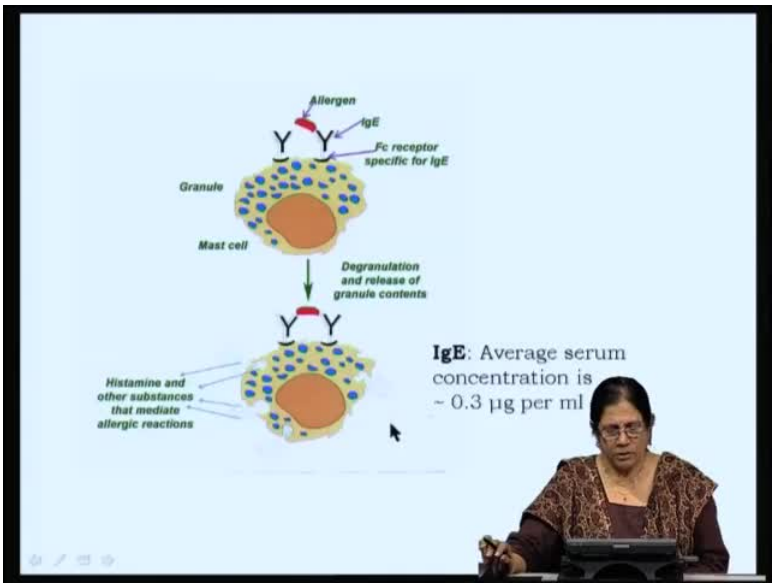

Let us talk now about IgE. When we think about IgE, we always think in terms of the hypersensitivity reaction that IgE mediates. Now, that is a separate lecture. But let us look at IgE, which is secreted into the blood. Average serum concentration of IgE is only 0.3 micro grams per ml, because as soon as IgE, as soon as a memory cell now, upon activation by the antigen, undergoes class switching. And, if it is an allergen and the class switching is to IgE, IgE synthesizes immediately captured by specific high affinity receptors present on mast cells; and, this is the picture of the mast cell, basophils as well as eosinophils (Refer Slide Time: 36:28).

And, IgE may not have a very long half-life in circulation. But, present as bound to the FC receptors, IgE molecules can be stable for weeks. Cross linking of such molecules by the allergen will induce cross linking of the FC receptors, which are present on mast cells. And, this leads to degranulation and release of the preformed granules, stored granules components of which are histamine and several mediators, which we will again deal with later. So, this is what IgE does, You might remember that both IgE and IgM have four constant domains.

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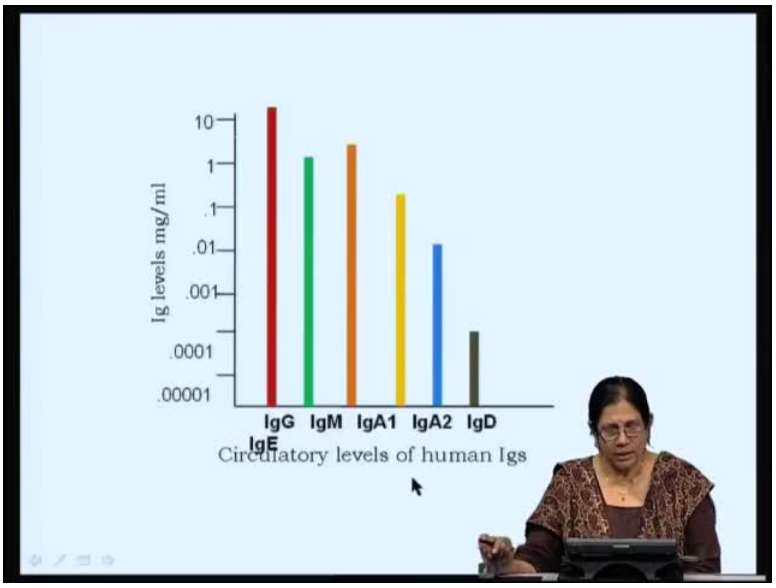

Now, this is a graph just to tell you that the concentrations, the circulatory levels of human Igs. Now, there has been a little bit of a shift of the writing here. The first one is IgG; the x-axis is all the immunoglobulins. The first one is IgG; the green is IgM. This is IgA 1, 2 – the monomer, the dimer; this is IgD; and, this is IgE. Now, the y axis says immunoglobulin levels milligram per ml. But, I would like you to look at the numbers; we start with the highest number, which is 10 mg per ml, which goes down to 1, 0.1, 0.01. So, this is increments of 10 fold. So, you can see the concentration of IgE, which is this as being not more than 3 micrograms per ml; whereas, IgG in circulation is about 13 milligrams per ml. So, IgG is maximum in the serum.

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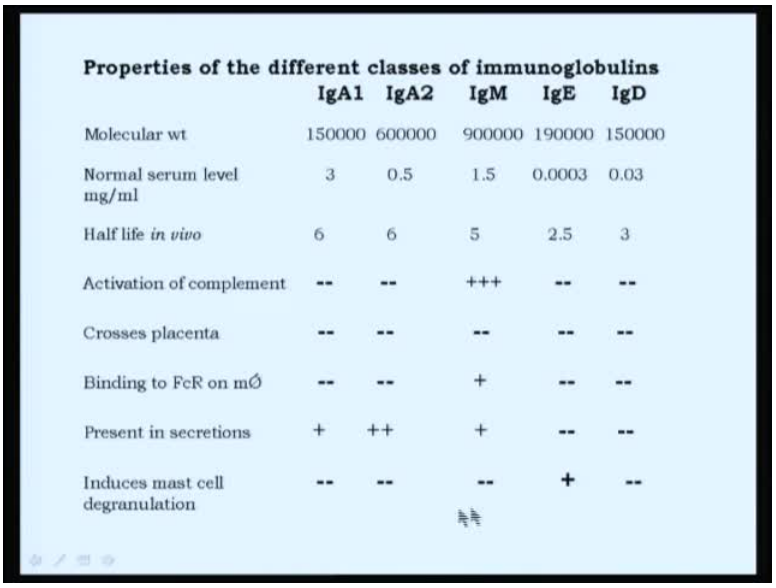

What are the properties of the different classes of immunoglobulin? I will just point out a few. Now, this is a table that talks about the molecular weight and the other properties of the subclasses of immunoglobulin G, IgG1, 2, 3 and 4. And, you can see that all of them are around the same molecular size. I should have in fact said around 150000. The normal serum levels are IgG1 is maximum 9, 3, 1 and IgG4 is very low – 0.5 milligram per ml. The half life of IgG in circulation is 23 days. This says days; I missed that. Half life in vivo days – 23 except for IgG3; now, you can immediately link this to the presence of that very long hinge region, which allows proteolytic degradation. So, IgG3 has a stability of only 8 days. Can all of them activate complement? That is going to be my next lecture. IgG3 of the classes can, but you will see that IgM does that much better. But, you can see IgG1 and 3 do; IgG3 does that better; and, IgG4 does not activate the complement. Do all of them cross placenta to confer immunity to the fetus – IgG scan, but IgG2 has minimal ability to cross the placenta.

IgG1 and 3 can bind to the FC receptors on macrophages. This allows antibody-dependent – the antibody recruiting the cells of the innate immune response. You may have studied thus in some of your earlier classes. None of them are present in body secretions; none of them can induce mast cell degranulation, which IgE can. Now, properties of different classes of immunoglobulins other than IgG, that is, IgA1, IgA2; this is a monomer; this would be a dimer, (Refer Slide Time: 41:01) trimer; IgM, E and D. You can see that IgM being a pentamer along with the J chain, has a molecular size,

which is around 900000; IgE because of an extra constant domain has a molecular size larger than IgA1 and IgD, which is 190000. IgA1 is present in 3 milligrams per ml; IgM half of that, 1.5; and, IgD as I have told you several time, that IgD is mostly present as cells of this receptor and almost no antibodies in circulation, which represent the IgD class. Half life in vivo – all of them have much shorter half life than IgG, which I have told you is 23 days. These are only maximum 6 days and IgE is only about 2.5 days in circulation. But, remember on the mast cells, it is few weeks.

Activation of complement – only IgM can activate complement from this group; IgG can as I have told you while referring to the earlier slide. None of them can cross the placenta; only IgG can. Only IgM can bind to the FC receptors of the macrophages or monocytes. IgA and M can be present in body fluids; definitely, the dimer form of IgA2. And, except IgE, none of them can induce mast cell granulation.

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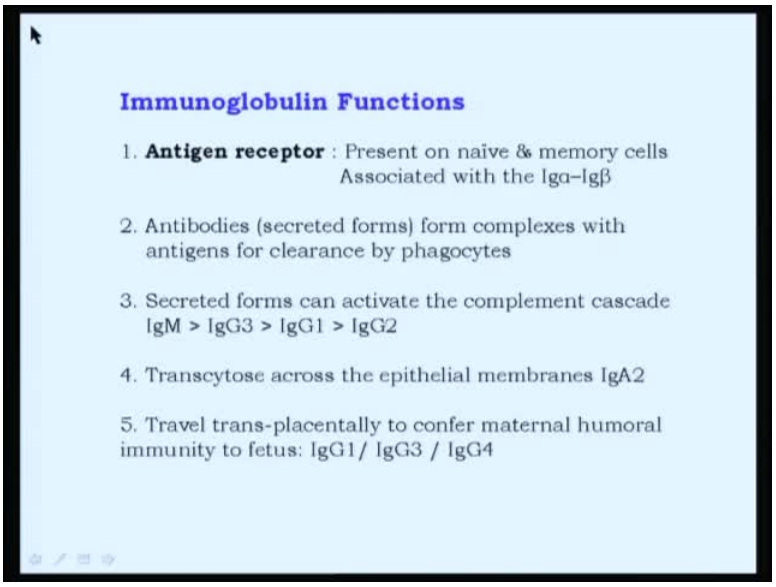

And, we have talked about the structure of the immunoglobulins what are their functions. Since we have been discussing so much with respect to antigen binding and specificity, obviously, that would be the first on the list. And, let us begin with the molecule as it is made as the cells of its receptor. Now, immunoglobulins are antigen receptors. They are present on naive and memory cells; and, they are associated with the immunoglobulin alpha-immunoglobulin beta for signaling. This signaling can happen only because the antigen receptor can bind very specifically to the cognate epitope on any antigen. In the

plasma cells, secrete antibodies; the secreted forms of immunoglobulin can form complexes with antigens. And, these are very nicely cleared by phagocytes.

The secreted forms can activate complement cascade. And, like I said, my next lecture is going to be that. And, the complement activation is a very important part of the immune system. Again, one part of that complement cascade recruits proteins, which otherwise would have been proteins of the innate immune system, which is now recruited to the acquired immune system. So, IgM can; then, IgG3, IgG1 and 2; these can these molecules can activate the complement cascade. IgA2 can confer immunity in the body fluid by transcytosis itself across the epithelial membranes. IgG1, 3 and 4 can travel trans-placentally to confer maternal human immunity to the fetus.

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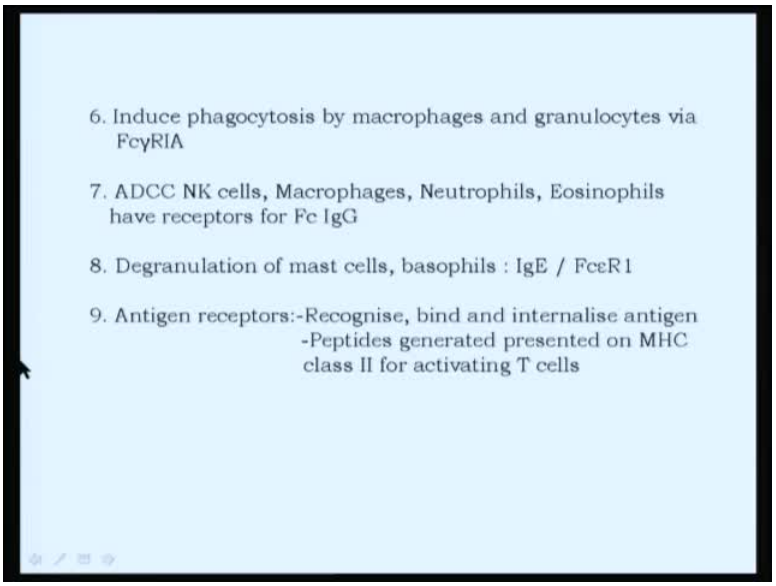

The FC receptors – FC gamma RIA, which are present on phagocytes or macrophages, now, can bind to antibodies, which have inturn bound to pathogens. Now, once such a complex is bound to the FC gamma RIA, this activates the macrophages to internalize the pathogen. Therefore, antibodies here by binding FC gamma RIA, induce phagocytosis macrophages and granulocytes. Antibodies can all also by similar method, bind to NK cells. And, now NK cells here in case of the phagocytes, it is phagocytosis, which is enhanced in case of NK cells. Proxicity is increased by ADCC, which is antibody dependent cellular cytotoxicity, because these cells have FC receptors for IgG.

And, again by a similar mechanism, can now bring the pathogen closer to the cells; the linking would be through IgG.

I have already told you that IgE brings about degranulation of mast cells, basophils and eosinophils; thereby, again contributing to the immune system or the humoral immunity. Now, what I talked about with respect to antigen receptors a little while ago is that antigen receptors can recognize bind and internalize antigen. What I talked about only was recognition. But, remember that binding of the antigen triggers the cells to internalize the antigen, where peptides are generated in the cell in the lysosomal compartment. And, these peptides can be presented on MHC class II for activating T cells. Thereby, antigen receptors are now allowing B cells to become antigen presenting cells.

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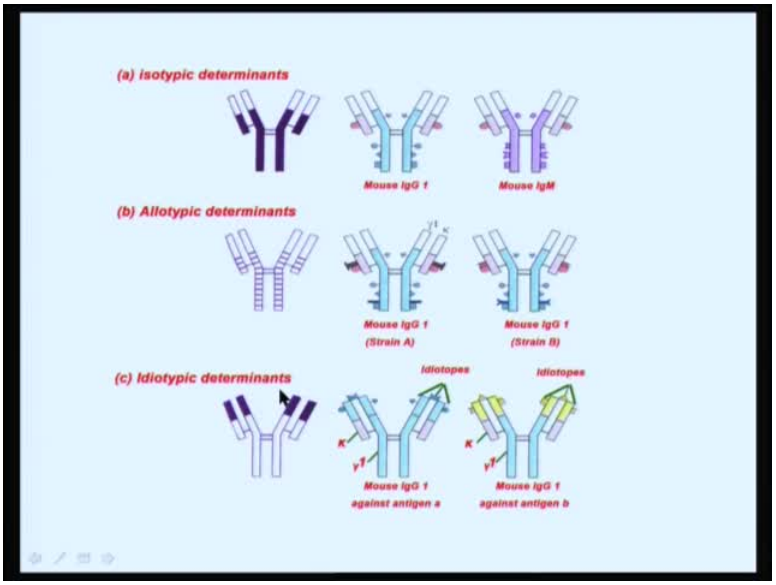

Can immunoglobulins themselves be antigens? Yes, immunoglobulins are glycoproteins. They are very complex structures as you have seen so far. And therefore, they themselves can evoke an immune response by way of dictating B cells to make antibodies to them. So, what are the regions on the immunoglobulin molecules, which can be antigenic? Let us come to the isotypic determinants. Now, if one takes let us say, IgG from human and injects into mouse, antibodies will be made to the isotypic determinants, which are the determinants, which are present on mostly the constant domain of the immunoglobulin. Now, here the example was human immunoglobulin.

Therefore, those antigenic determinants that are present on the constant domain on the human immunoglobulin… So, antibodies would be made by one species to the immunoglobulins of another and though would be called isotypic determinants. And, you all may have used the secondary antibodies; what we call secondary antibodies as reagents in a LISA, western blotting, where we use secondary antibodies are nothing but… Let us say if you are using the primary antibodies mouse, then you would be using rapid antibodies to mouse immunoglobulins. And therefore, those rapid antibodies would be binding to such determinants.

What are allotypic determinants then? Allotypic determinants are those that have arisen during evolution. Now, though we say that the constant domain is exactly the same in all IgGs, let us say, all IgG1 from the human species would be identical yet, during evolution, there is every chance that there could be one amino acid mutation. Now, if those mutations 1 or 2 lie in antigenic determinants, that determinant now would become antigenic, which would be different from the isotypic determinants. Now, there are these allotypic determinants that have been identified. And, let me elaborate little further. When would you see generation of an immune response to allotypic determinants? In blood transfusions; it is very apparent that normally, we should not be making antibodies even if we receive antibodies from other members of our species. But, in blood donation, people found that there was a reaction or antibodies were made by the recipient or the receiver from the donors' immunoglobulins. And, on closer look, these allotypic determinants were identified.

What are idiotypic determinants? Idiotypic determinants are those which are present on the variable region. Now, as is shown here, the idiotypic determinants would be those that would bind to that region or an immune response that is generated to that variable region. And in fact, which I have not mentioned earlier that these idiotypic antibodies, which can be generated, can also regulate the immune response; not can too regulate the immune response. Idiotypic antibodies can be shown very easily in an experiment; in fact, you can think of doing that experiment; a rabbit, which is injected with one antigen, you will see no idiotypic antibodies made in the first two exposure to the same antibody. Now, let us say, the rabbit have received ovalbumin; there will be antibodies to ovalbumin. In the first generation, the primary dose, the secondary immune response also will give you mostly antibodies to ovalbumin. But, let us say, from the third

immunization onwards, you may start getting antibodies to the antibodies to ovalbumin. And, these in fact are regulators of the immune response not allowing the immune response to (( )) And, there is a decrease in the amplitude of the immune response. So, antibodies to idiotypes can also have several uses apart from the regular immune response, which dictates the suppression to some extent of response to the primary antigen. We will come to that a little later.

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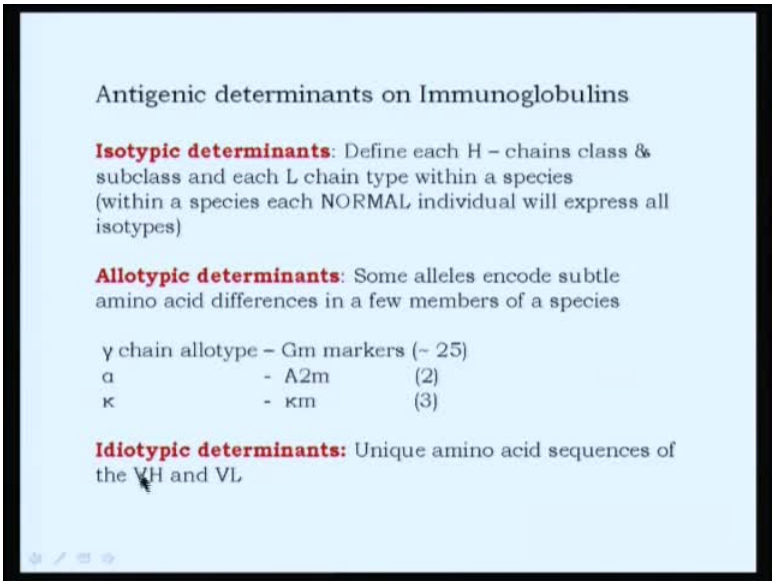

So, just to go over the antigenic determinants on immunoglobulins, we have isotypic determinants, which define each heavy chain class and subclass and each L chain type within a species; within a species, each normal individual will express all isotypes. This is important. However, allotypic determinants – some alleles encode subtle amino acid differences in a few member of a species. Now, these are the ones that have been well–known; and therefore, I have written them here. In case of human, the gamma chain allotype, there are about 25 such allotypic determinants. And also on IgA, there are two; and kappa light chain, there are 3. Idiotypic determinants – I have already said – are those determinants that are found because of the presence of unique amino acid sequences of the VH and VL.

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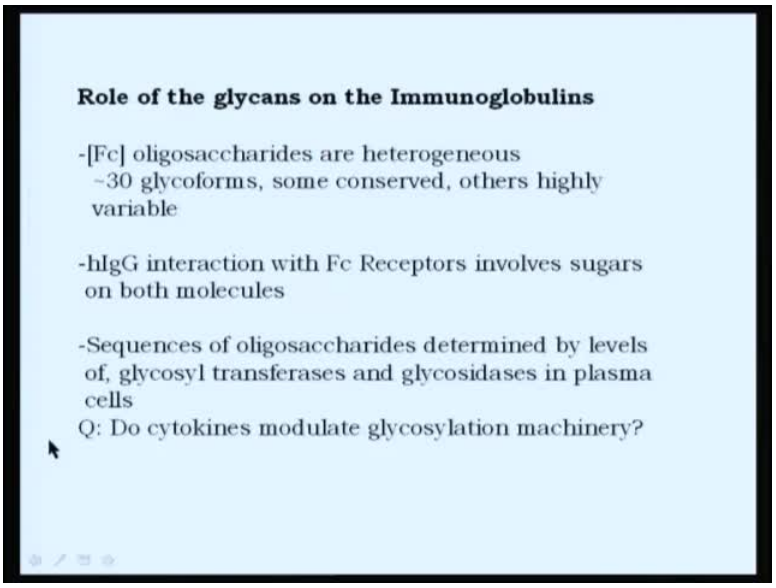

We should also think about immunoglobulin molecule as being a glycosylated protein or a glycoprotein. So, there has to be some role that glycans play. It was thought that since immunoglobulin, when it is secreted in B cells, it is secreted as heavy chain and light chain and these have to come together. And then, when they held together, disulfide bonds have to be formed. Now, these are exported outside either cells of receptors or secreted as antibodies. Therefore, the glycosylation function is only to help the protein folding. But, we know that the glycans also stabilize the molecule protected from proteolytic attack. The importance of the sugars have become known as glycobiologists have started looking at the glycans. And, I would like to just mention here that there are about 30 different glycoforms of immunoglobulins; and, some of these glycoforms are conserved; others are highly variable. This glycans change, which I have told you are present on the ch2 region of the FC region.

These molecules, the glycans are very important for the immunoglobulin to bind to the FC. I just told you about the importance of these FC receptors. And, it is the sugars, which allow this linking. Decrease in the glycan structure now, does not allow a tight binding of the immunoglobulin with the FC receptors. Also, the sequences of these oligosaccharides, the sugars that are present have been shown to inhibit an auto immune response; the auto immune response, which might result in rheumatoid arthritis.

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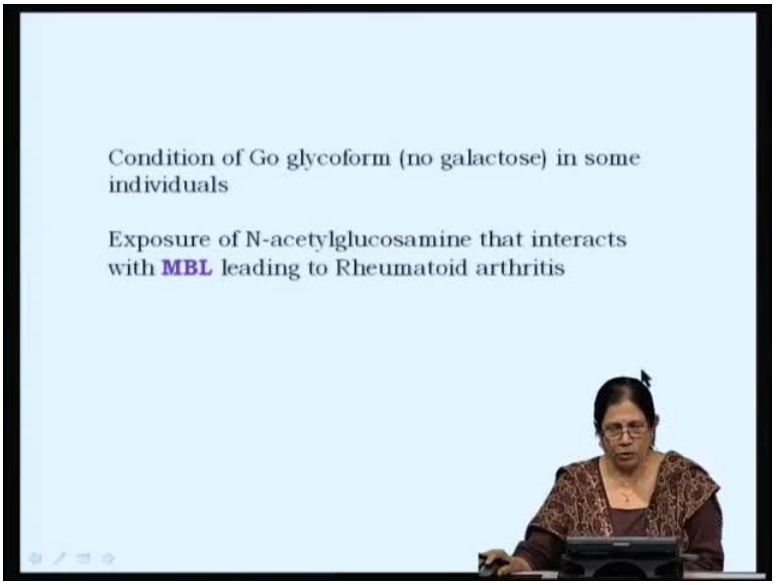

Now, let us see how that happens. There are people who have the condition of G0 glycoform. That is, they do not have those enzymes, which transfer galactose, terminal galactose. Therefore, in the absence of galactose, there is exposure of N-acetylglucosamine. Now, this allows binding to the MBL, which is mannose binding lectin. And, we will be discussing what MBL is in the next class. And, this MBL is a cascade of the complement pathway, which can lead to inflammation and one type of rheumatoid arthritis.

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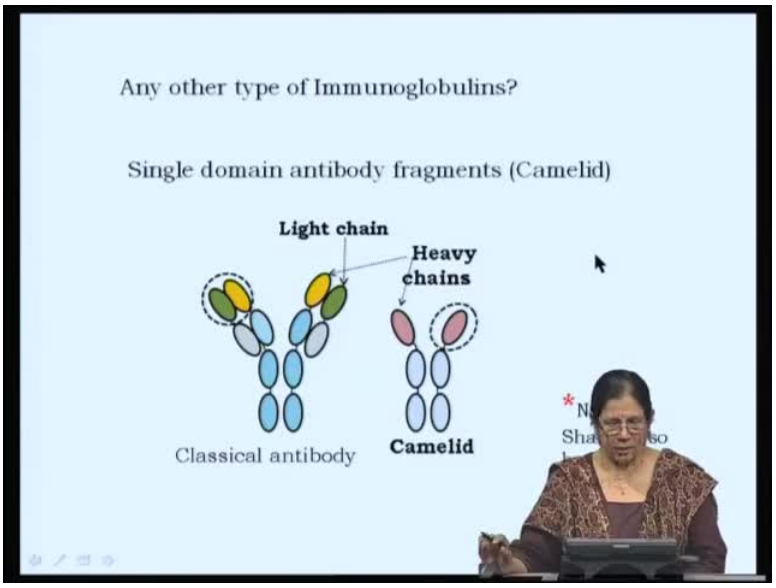

Now, what I would like to do is, stop here and then begin my next lecture with two immunoglobulins that I have not discussed with. But, they are quite important; specially, one of them, the single chain domain antibody, which is known as camelid antibodies, because this has helped molecular biologist to be able to express only the variable domain of the immunoglobulin. And, these are known as single chain variable fragment. And, nature has done this in camels, where 30 percent of the antibodies in camels are made up of only heavy chain.

So, I will stop here. And, in my next class, I will start with the introduction on the camelid antibodies as well as the IgY antibodies.

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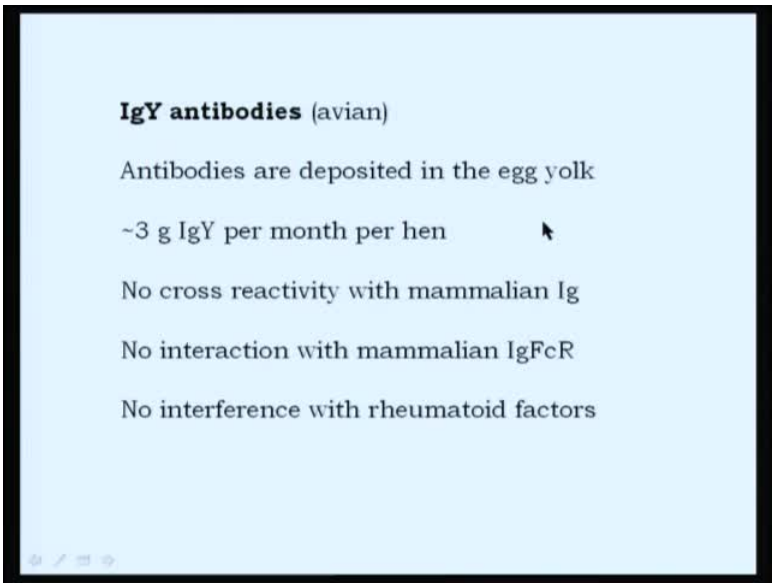

Now, why IgY antibodies, which are from the avian system? Why this is important is, because they can be used much in diagnostics. Also, immunoglobulins made by births are deposited in the egg yolk. And therefore, it is much easier to be able to purify antibodies form the egg yolk rather than from the serum, because of the presence of large amount of albumin. More importantly, 3 grams of immunoglobulin Y can be got from eggs in a single month from one hen. So, I will talk about this as well as introduce you all to the complement cascade.

Thank you.