Essentials in Immunology

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Lecture No. # 13

The Three Complement Pathways

In my last class, I had discussed the structure and functions of immunoglobulins.

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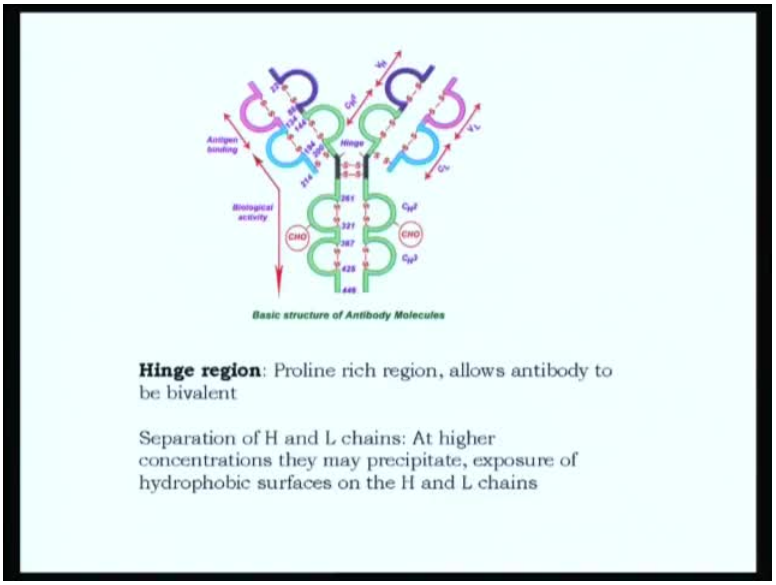

Now, today's class is going to deal with complement and activation of the complement at three different pathways. But, since one of the pathways involves antigen-antibody interaction, I thought I will deal with little bit of the structure of immunoglobulin, so that one can understand how complement can be activated through this pathway. I had also forgotten to mention a few important molecules while talking about the structure and functions of immunoglobulin. And, I will also in very brief describe those.

Now, let us first look at the structure again of immunoglobulin. This is the prototype structure as you know. And, you might remember that this is the prototype structure for IgG, also IgD and IgA. IgE and M have a modification of the region called the hinge region. And, this is what I would like to describe. In IgE and IgM, the hinge region is

modified into a domain itself. And therefore, only these classes of immunoglobulins have; apart from the three constant domains, one extra constant domain. So, the hinge region now becomes CH 2; then, there is CH 3 and CH 4. Is there anything specific with regard to hinge region? It was found that the hinge region has a large number of the amino acid proline. Why proline, because the addition or inclusion of this amino acid lends flexibility to the primary amino acid sequence or the polypeptide chain.

Now, why should there be in the hinge region, number of prolines, which give flexibility to the molecule, because we do not know that immunoglobulins are at least bivalent. Now, monomeric immunoglobulin molecule, which is made up two heavy and two light chains now, can bind two domains or antigenic determinants of an antigen; two at one time. Now, if one is occupied, the other one may experience steric hindrance by which it will not be able to make contact with another molecule of antigen or the same epitope on the same antigenic structure. Therefore, nature has it that the hinge region is very flexible and allows sufficient movement of the two fab molecules for efficient binding to antigen, so that an antibody molecule can be at least bivalent in its function. I had already mentioned to you that, if you take the heavy chain separate from the light chain; that is, it is an easy experiment; one can use DDT or any reducing agent beta mercaptoethanol to break the disulfide bonds, which hold the two heavy chains as well as the each of the heavy chain to one light chain. Now, after reduction of these bonds, you can have two heavy chains or light chains separate.

Experimentalists have tried to see whether the light chains can bind to the antigen when separated from the heavy chain. It was seen that, at higher concentrations of the immunoglobulin reduction leads to precipitation. As I have probably mentioned to you, heavy chain alone if soluble, can bind to antigen. The light chain usually does not show any binding to antigen. We do know that binding of the immunoglobulin molecule or the antibody molecule to the antigen is more dependent on the structure of the heavy chain.

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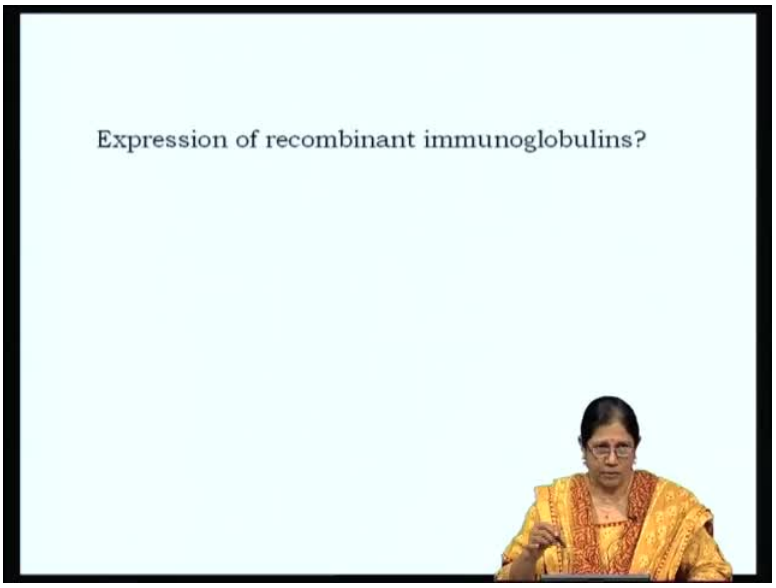

Now, like I said that heavy chain separated from light chain though it can bind to the antigen, the affinity of the binding comes down tremendously. It could be 10 fold or even a 100 fold. In any case, one needs to think in terms of the solubility of the molecules; like I said, at higher concentration, the immunoglobulin chains precipitate, and of course, heavy chain more likely than the light chain.

Now, what is the reason for that? That is because the coming together of the heavy and the light chain to form a structure, which can bind to the antigen, this interaction is through hydrophobic surfaces. So, when these two chains are separated, the hydrophobic patches are exposed and, this will bring about aggregation of the self molecule; that is, two heavy chains would come together and this would bring about precipitation of the molecule. Therefore, expression of immunoglobulins in recombinant systems, specially prokaryotic system is rather difficult.

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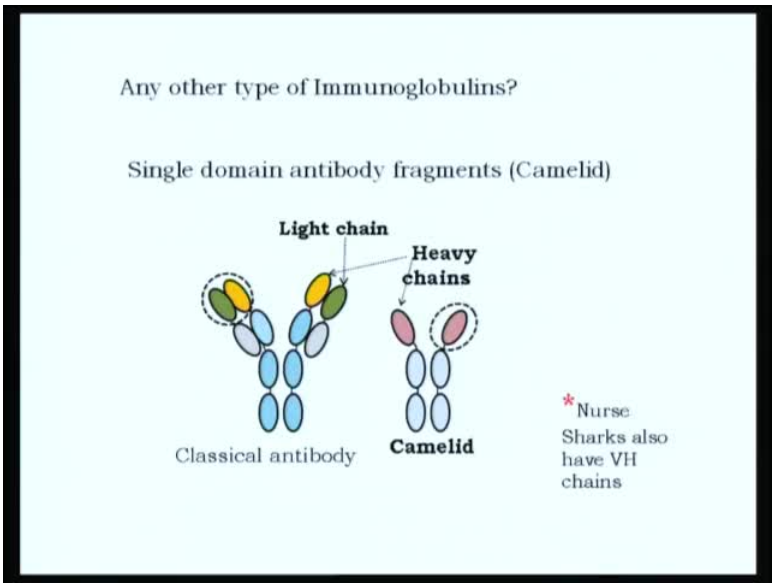

Now, why did I say all this is because sometimes in the late 90s, there were reports on a type of antibody, which is specifically found amongst mammals found in camel group of animals like dromedary and camel. Now, interestingly, it was found that 33 percent of the immunoglobulins in camels are made up of two heavy chains only. Just to recapitulate your memory that it is two heavy chains and two light chains, which comprise the classical antibody; and, the N-termini of both these chains makes the antigen binding pocket. In case of camelid antibodies, which again I would like to stress, is only around 30 to 33 percent of the total immunoglobulins found on the camel. But, they are much smaller and they are made up of just two heavy chains, which are of course, identical. Just to tell you, we do not deal with the low vertebrates. And, whatever immunology we discussed is that described in mouse and human. But, I would like to tell you that sharks also have similar molecules. Of course, the repertoar is much restricted in sharks. The camelid antibody is made up of two heavy chains.

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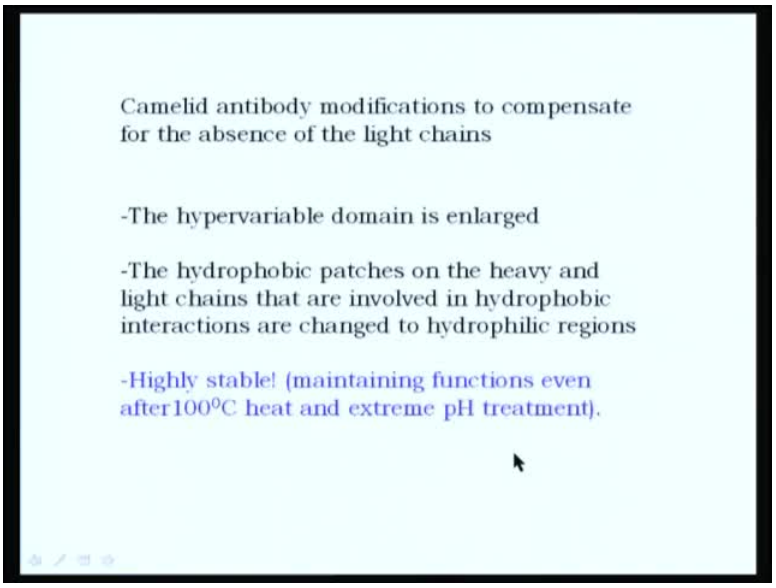

Now, this would mean that there have to be some kind of modifications in the camelid antibody, so that it could do away with the light chain, because all along, we have been learning that both heavy and light chains come together and they form the antigen binding site. And therefore, how does the heavy chain in camelid antibodies manage without the light chains? Because like I said, the repertoar appears to be the same in the camelid antibodies compared to the classical antibodies.

Now, I am not going to deal with the entire paper that was described by two scientists, who are from Belgium, but I thought I will just tell you what could be the main two modifications that the camelid antibody chains needed to undergo, so that they now becomes soluble protein and are effective. To compensate for the light chain, the hypervariable domain of the heavy chain is enlarged by 7 to 8 amino acids. Importantly, the hydrophobic patches on the heavy and light chains that are involved in hydrophobic interactions with each other are changed to hydrophilic regions. So, hydrophilic amino acids would not bring about this cascade of precipitation as one sees when the classical antibody is reduced to separate the light and heavy chains.

Interestingly, this camelid antibody is very stable and it maintains function even at 100 degree centigrade and also extreme pH treatment. Now, looking at the structure of camelid antibodies, looking at the sequences, has actually provided immunologist to start thinking in terms of modifying the classical antibody at the gene level, so that one could

probably get antibodies as recombinant proteins and just the heavy chains, which would be much simpler than if we were to clone and express both heavy and light chain genes into a prokaryotic system. Therefore, camelid antibodies have provided enough of interesting ideas to biotechnologists.

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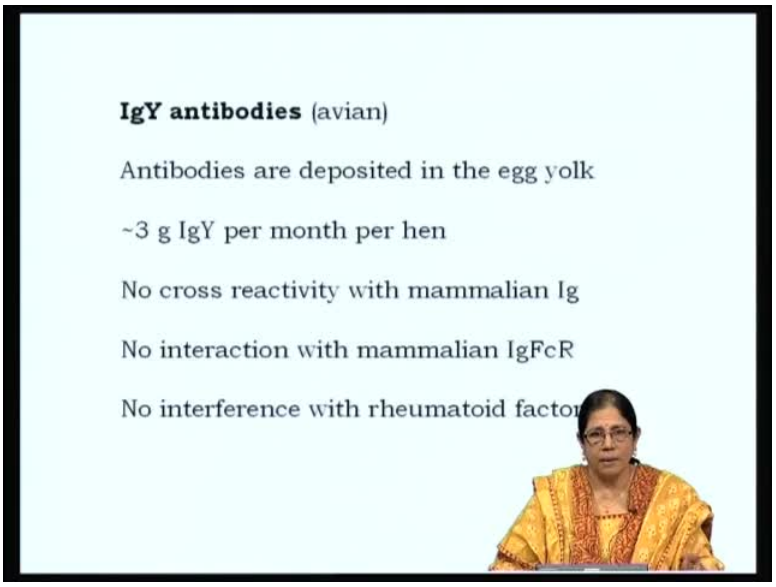

There is another set of antibodies, which I thought I should introduce to you, because in the last 20 years or so, IgY antibodies from the avian system are being utilized in diagnostics. Why IgY antibodies? IgY is an antibody that is found in the avian system and chicken or hens are now recruited for generation of this kind of an antibody. Why is it interesting? Because in hen, it has been seen that circulatory proteins are deposited in the egg yolk. Therefore, in immunoglobulins, when hen is immunized or in the natural condition, is exposed to pathogens, the antibodies that are generated are automatically deposited in the egg yolk. The immunoglobulins are taken across those site membranes and deposited. Therefore, now, the egg becomes a store house of these IgY antibodies.

Interestingly, one can get 3 grams of immunoglobulin Y per month per hen, so that becomes also an extremely cheap way of getting large amounts of antibodies. Immunizing hen is not any more difficult than immunizing rabbits. Also, most importantly, apart from the commercial aspect, no cross reactivity is seen between IgY and mammalian immunoglobulin. Therefore, there is no interaction with mammalian FC receptor. Therefore, they will not be any interference specially when IgY antibodies are

used in diagnostics. So, apart from camelid antibodies, IgY antibodies also have a very interesting commercial aspect.

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Now, we come to the complement system. Complement activation is an important or an integral part of the humoral immune response. And, it is integration between the acquired immune response and the innate immune response.

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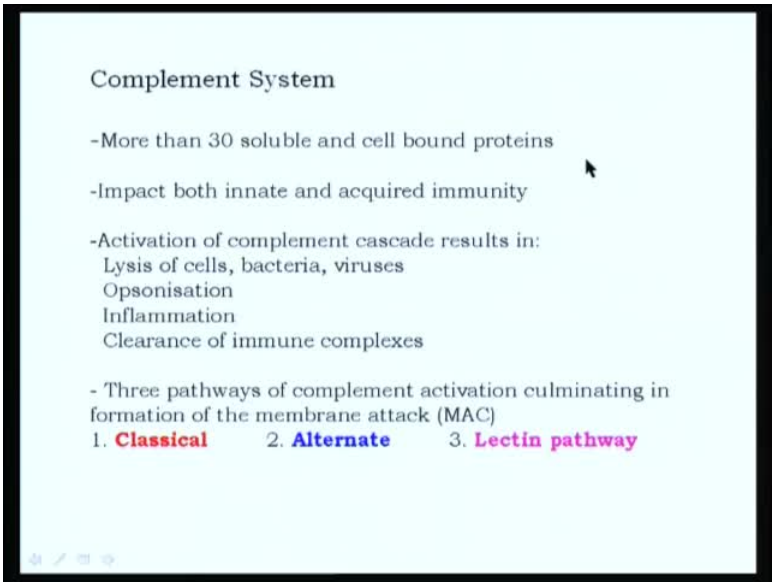

The complement system comprises a very large number of soluble and cell bound proteins. There are more than 30 soluble and cell bound proteins in this system. And, like

I said, these impact both innate and acquired immunity. What is the complement cascade tool? Amongst all the functions of the immunoglobulins, you might recall that immunoglobulins or antibodies bind to their cognate antigenic epitopes on antigens in a very specific manner. Antigen-antibody interaction activates the compliment cascade and will be dealing with mechanism part. But, what does this activation of the complement system whether it is classical, alternate or lectin, what is the ultimate result of the activation? It is the pathways forming membrane at a complex, which now induces lyses of cells, bacteria, viruses; also, causes opsonisation; inflammation; importantly, clearance of immune complexes. So, we will be dealing with all these when we look at the activation of the complement cascade. Complement cascade or activation culminating in the formation of the membrane attack complex can be antigen and antibody interaction dependent as happens in the classical pathway or the alternate pathway and the lectin pathway. Now, the alternate pathway and lectin pathway are both systems of the innate immune response.

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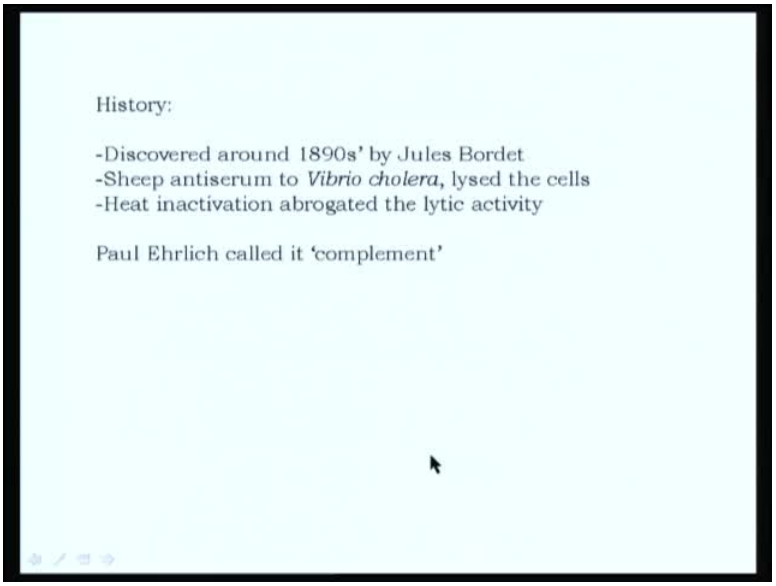

Always, good to know a little bit of the history; when were these complement components discovered? The complement system itself was first described in very brief around 1890s by a scientist Jules Bordet. Now, what was his observation on which he described the complement? He had made sheep antiserum to the bacteria vibrio cholera and found that under certain conditions, when the vibrio cholera or the bacteria were incubated with this antiserum, there was lyses of cells. He found also that this was…

When this antiserum collected was rather fresh or rather recent. Therefore, now, he chose to heat inactivate the serum and found that heating the antiserum at 58 degrees, did not abrogate the reactivity or agglutinating activity of the antiserum to the bacteria. But, it abrogated the lytic activity. Therefore, he knew then that there was a component in the serum apart from antibodies, which is able to bring about lyses of cells. It was couple of years later that several observations were made by the scientist Paul Ehrlic and he called this complement; complement, because it complements the activity of the serum.

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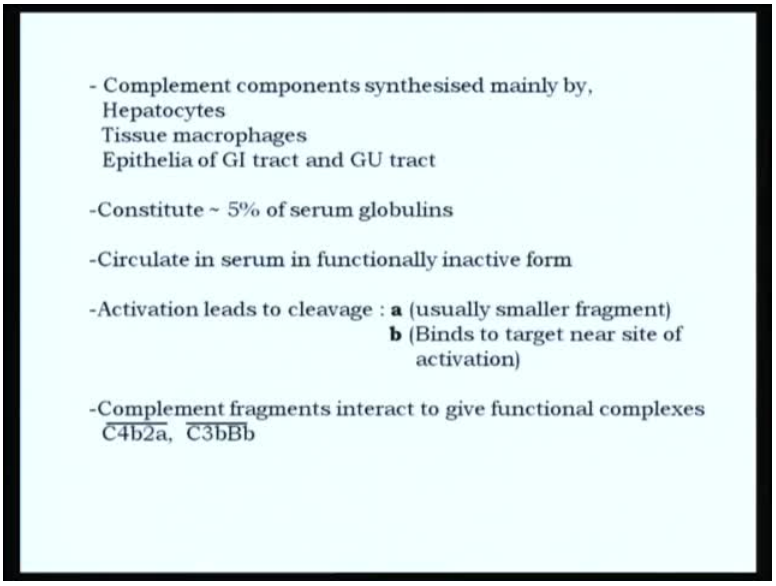

Now, even before we come to the complement components, let us see, where are these complement components synthesized; mainly, by the hepatocytes; also by the tissue macrophages; and, by the epithelia of the gastrointestinal tract and the genito urinary tract. Complement components are quite large in number, because 5 percent of serum globulins are constituted by complement components. All the complement components circulate in the serum in a functionary inactive form. You can call them psammogeton. Activation of the complement leads to cleavage. And, the cleavage can be a – smaller fragment and b – larger fragments. Of course, there are some differences in the size of the fragment and which one now becomes the active complex of the complement cascade; and, I will be dealing with that in detail and in a sequential fashion. Complement fragments interact with each other to give functional complexes.

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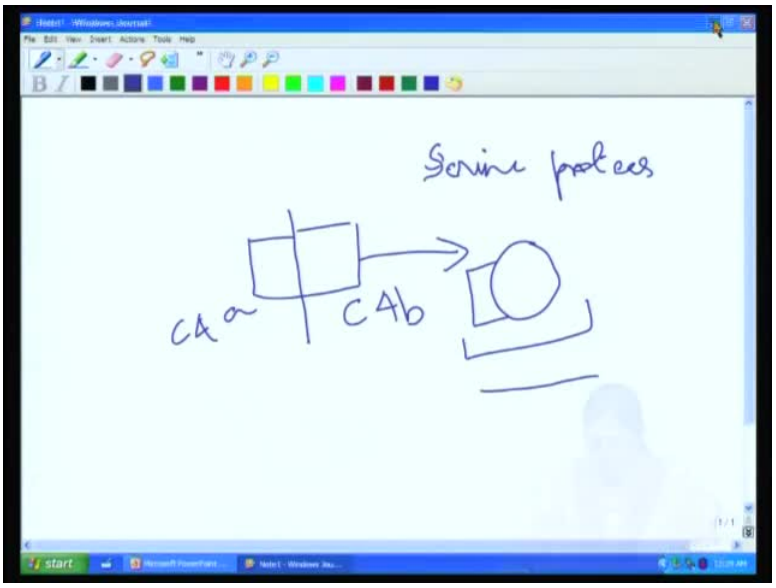

Now, I would just like to tell you all about all complement components. There is a common strategy. So, you have now, let us say, this is one of the components; after this gets activated and all these are usually serine proteases, if they are enzymes. Now, when the compliment component gets activated; and, I will tell you how the activation happens; then, there is usually a cleavage. Let us say this one is C4. Now, the larger fragment, which would be C4b now and the smaller fragment C4a. And, the larger fragment gets associated with a component, which is already present at the site of the activation. So, you have now C4b, which would come and bind here. The binding of these two would bring about a complex, which may have an enzyme activity. Now, whenever a complex has an enzyme activity, such a complex is denoted by a line above the components to say that this one is the enzyme.

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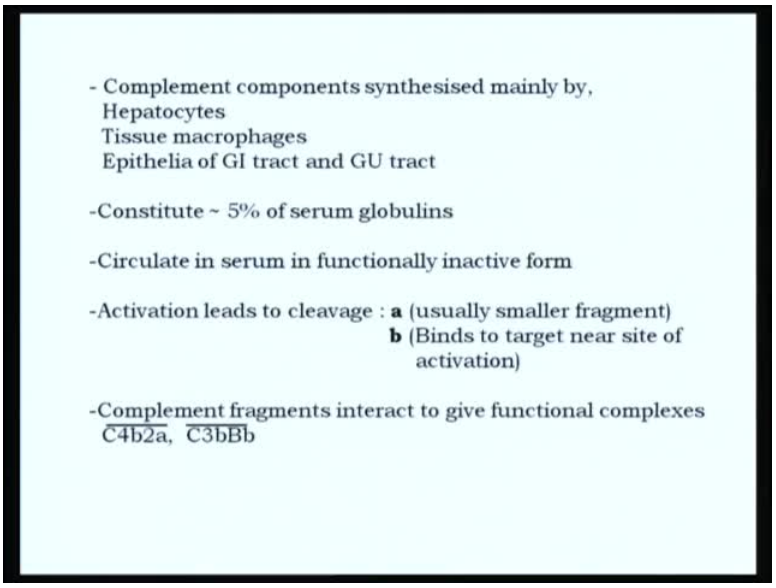

So, we will come back now to the activation of the complement cascade. So, the example that I have given here; complement fragments interact to give functional complexes, for example, C4b2A or C3bBb. As such on their own, they are not enzymes when they come together; they have specificity with respect to a particular substrate.

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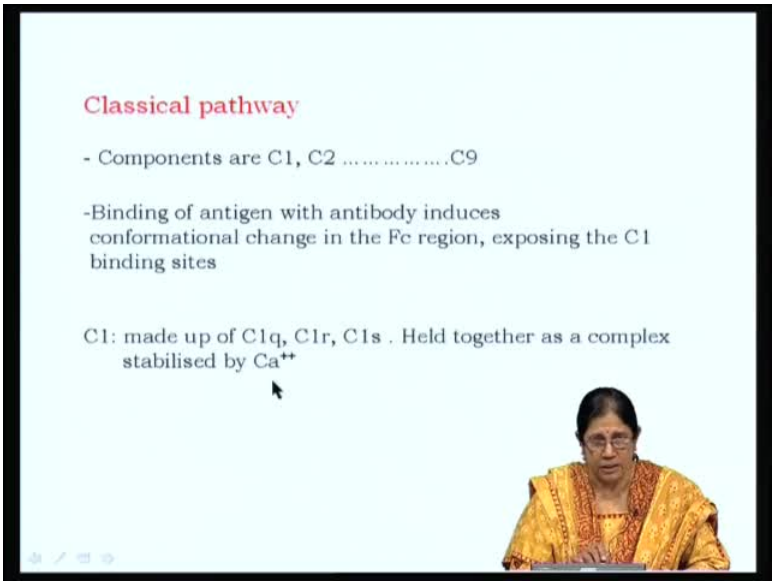

Let us first come to the first pathway, which is activated by antigen-antibody interaction. The classical pathway has components, which comprise of C1 to C9 different components. Because the classical pathway is activated by an antigen-antibody

interaction, we need to see how this actually brings about activation of the molecules. Now, binding of antigen with antibody induces a conformational change in the FC region of immunoglobulin. And, just to recall, there are two classes of immunoglobulins that can fix. And now, activate the complement components are IgG and only three subclasses of IgG and IgM. In these two, there is upon antigen binding, exposure of the C1 binding sites on the FC region. Now the C1; what is C1? The C1 itself, which is the first component of the classical pathway is itself a complex. It is made up of q, r and s. And, these three are held together as a complex, which is stabilized by calcium. So, without calcium, there is no activation of the complement cascade.

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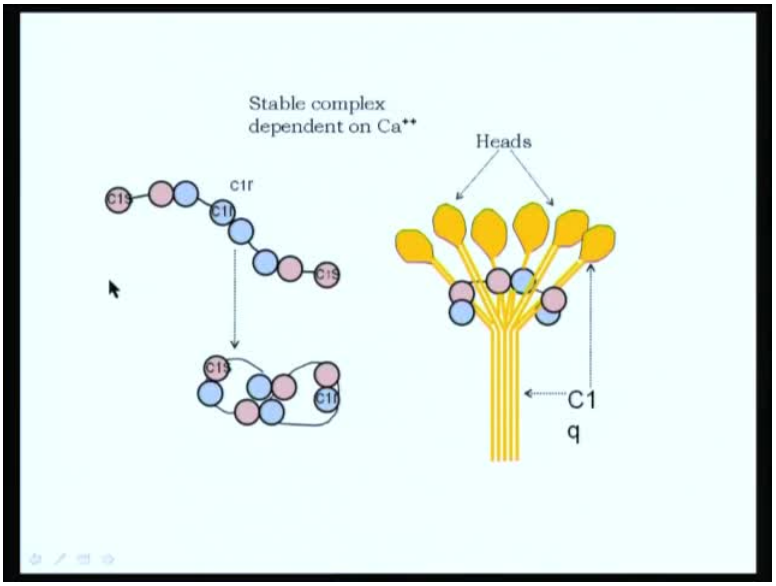

Let us look at first the C1 q. This is the molecule C1q. As you can see, this is a flowery structure, which is made up of actually, which does not show here in the diagram, but is made up of 18 polypeptide chains, which now are held together with one part, which is like petal and three of such polypeptide chains come together to give one such head. There are 6 heads.

On the other hand, the C1s and C1r are complex together very closely. And in the serum, they are usually present as an s-shaped complex. But, the same can bind to the C1 q in a calcium-dependent manner. And, when bound to C1 q, they have a structure, which is 8 shaped. In the serum of course, this complex is not active.

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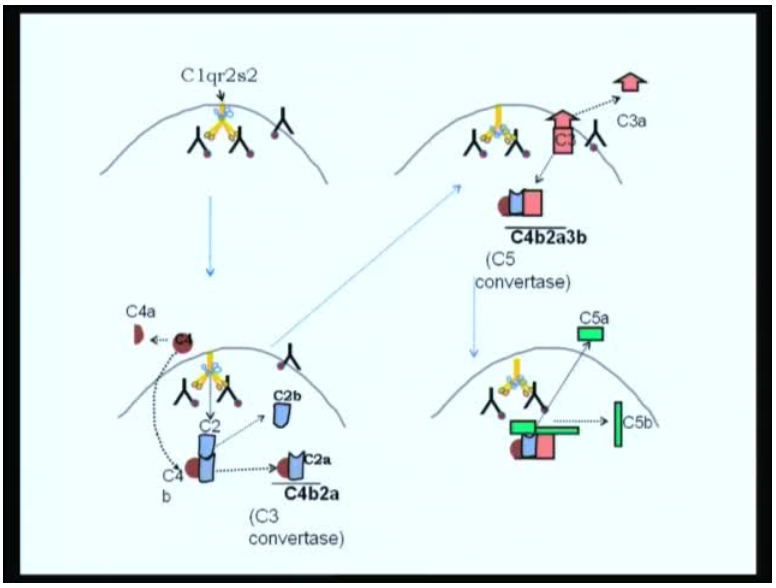

Let us look at what happens when the complex is recruited to the site, where an antigen-antibody interaction is taking place. Now, the C1 component, which is made up of three molecules is recruited to the site, where an antigen, which is a component of a bacterial cell. Now, this is the bacterial cell; half a cell. And, you can see that there are specific antibodies, which are these y-shaped structures, which are binding to specific antigens present on the bacterial cell surface. Though I have put the antigen in the same color, it need not mean that all the antibodies here should be identical. There could be two different antibodies recognizing two different antigens on the bacterial cell surface. Now, the C1 q binds to an exposed region present on the FC region of the immunoglobulin molecule after it is bound to the cognate antigen. So, it is very important to know that antigen-antibody interaction brings about a conformational change not only in the antigen binding site, but also the FC region, which is the constant domain of the immunoglobulin molecule.

There should be at least two heads of one C1 q molecule such that it can bring about activation of the r2 s2; both of which are serine proteases. It is also important here to make you remember that IgM is a better activator of the complement cascade. If you remember the table that I showed in the last lecture, IgM is much more efficient in activating the complement cascade as compared to any one of the subclasses of IgG. The reason being that IgM is the pentameric structure and since two heads of one C1q molecule is required to activate the complement cascade, IgM, which has a pentameric

structure is more likely or to be able to involve or engage to heads of the same C1 q at one particular position. Therefore, IgM is much more efficient.

Now, what happens here? Here of course, I have only IgG and let us go on with that. So, at least, two immunoglobulin molecules should be in close proximity so that two heads of the C1 q molecule get engaged. The binding of the C1q to this exposed site on the FC region now brings about a conformation change or induces a conformational change in the C1 molecule. The C1 molecule, upon its conformation alteration, induces a conformation alteration in the molecule that is closely associated with it; that is, r2. The r2 now, activates s2 by chopping off or cleaving a part of the s2. The s2 is an enzyme and has two substrates: one is C4 and the other one is C2. So, remember all this is happening on the surface of the bacterial cell. And though the various activities that I have shown in pictorially, all this is in fact happening at the site of the antigen-antibody interaction and the C1 complex, which is binding to it. So, everything happens at this (Refer Slide Time: 26:59) position. But, to make it clear and to think in terms of the sequence of events that are taking place, we draw or depict the antigens far away from that of the antigen-antibody and the C1 q binding to antibody molecule. So, C1 q binds to the FC region of the immunoglobulin bringing about a conformational alteration, which is now passed on to the r2 activating it, which inturn activates the serine protease, s2.

The cleavage of s2 to its active form also now exposes the binding site for the second molecule in this sequence, which is C4. Now, though the complement components of the classical pathway are made up of C1 to C9, the sequence in which they are recruited may not necessarily be so; and, this is the pathway after C1. The next complement component to get activated is C4. C4 is recruited to s2, which cleaves now; which remember by now is activated. So, it cleaves C4 to C4a and C4b.

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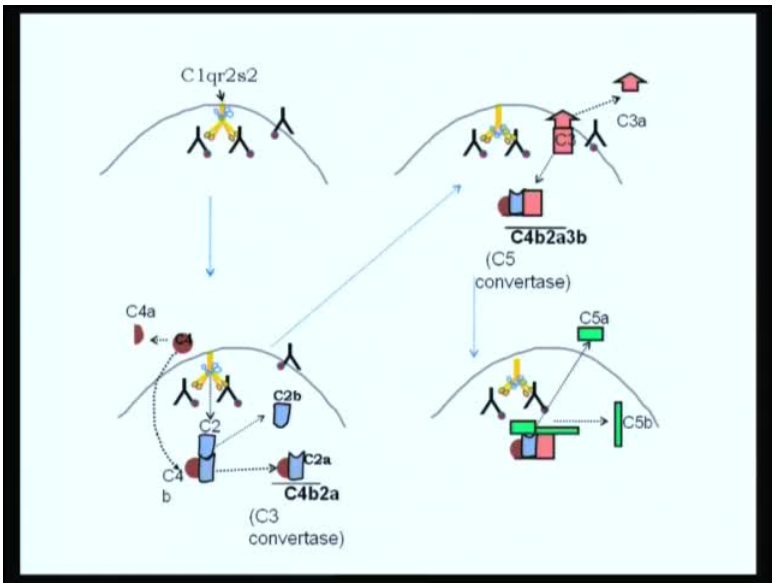

C4b is the larger fragment and gets associated with s2. C4a on the hand, is the smaller component and diffuses away. Now, I will come back to what C4a does. But, let us come back to the C4, which is now bound or in close proximity to s2. Now, s2 recruits yet another component, which is C2. C2 is cleaved by s2 again. I told you s2 has two substrates: C4 and C2. Now, C2 gets cleaved to two fragments again: C2b and C2a. C2 is the only molecule now in this particular category of molecules, where the C2b or the larger fragment diffuses away; whereas, the smaller fragments C2a now complexes with C4b. And, this complex, which is stabilized by a calcium again is an enzyme, which is C3 convertase. And, you can see that the complexes now, because it is functionally active is denoted by a line, which is across the C4b2a name.

Now, C3 convertase as the name suggests, recruits C3, which is yet another protein in the serum again in its inactive form. C3 binds to C4b2a. The C3 convertase gets cleaved to a larger fragment C3b and a smaller fragment C3a. The C3a diffuses away and C3b binds to C2. This now; (Refer Slide Time: 30:13) binding of C3b to this C3 convertase now changes the specificity from being a C3 convertase to a C5 convertase. So, C4b2a3b – actually, it is C4b C2a C3b; but, since C is common, it is written as C4b – one component; 2a – another component; and, 3b. The C5 convertase as the name suggests has a binding site for the next component, which is C5. C5 convertase now cleaves C5. Now, just remember every time, there is some activation. Like in this case, there would always be a conformation alteration like, for example, in this case, binding of C3 to the

earlier components; now, changes or makes accessible a binding site for the next component, C5. C5 is therefore, recruited, because there is now a binding site on the complex. And, this is cleaved from C5 to C5a and C5b.

C5a again diffuses off. Now, at this juncture, (Refer Slide Time: 31:49) I would like to tell you, C4a C3a C5a – all these three are anaphylatoxin. And, we will be dealing with this in detail in the hypersensitivity context. But, these are molecules, which induce information and inflammatory response through mast cells, basophils as well as eosinophils. There are specific receptors that bind to these components. So, they have used C2b on the other hand is a question mark. What does C2 b do is still a question mark. Now, let us get back to the complex. We have now C5b, which is formed by the enzymatic cleavage of the whole C5 molecule. What happens next?

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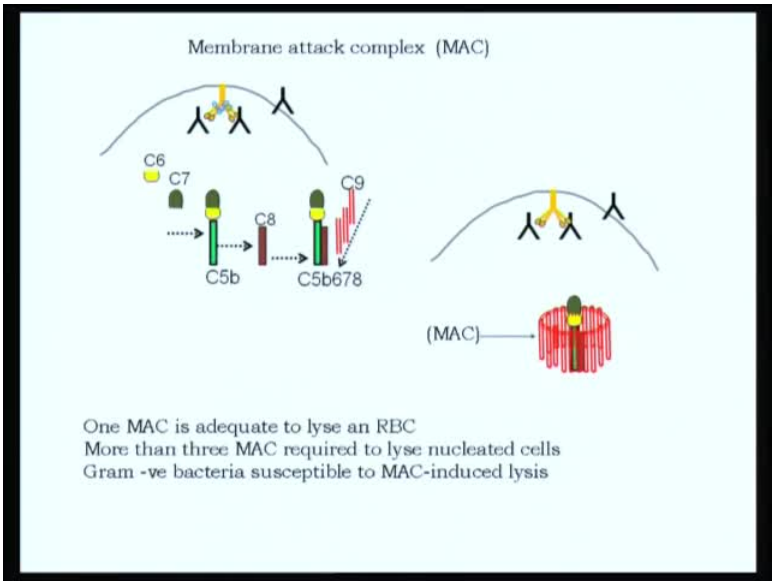

Now, C5b has a tendency to bind to a surface membrane, but it is not very stable. And, it is not stable and starts to degrade unless the next component, which is shown in yellow here; C6 binds to it. The moment C6 binds, C5b, which is held loosely on the membrane gets stabilized. The binding of C6 to C5b brings about a conformation alteration in the molecule C6, so that a binding site for the next components C7 gets exposed. This complex now can recruit the next component, which is again in sequence C8.

Once C8 molecule is recruited to the already existing C5b, C6, C7, which now… This is the complex here (Refer Slide Time: 33:56). You can see that there are four components;

each one in different colors. Now, this is an important step, because this component now undergoes a change from hydrophilic to a hydrophobic status. Why is this required? Now, what I mean by this is that in the serum, all proteins need to be soluble. When they come now onto a membrane and they need to be anchored on the membrane, they need to have hydrophobic surfaces or regions exposed. So, this entire complex now when 678 are bound to C5b, undergo a hydrophilic to hydrophobic transition, so that now, this complex can get into or anchor in the lipid bilayer. The moment that happens, the next complement component, C9 molecules are recruited to the site. C9 on its own is present in soluble form. But, when the binding site for C9 is exposed on the C5b6, 7 and 8, there is polymerization of the C9 and there are large number of C9 molecules, which are recruited. And, you can see, this (Refer Slide Time: 35:30) forms a structure, which is known as membrane attack complex.

A polymerization of C9 and the hydrophobic activity of the C5b678 bring about a pore forming complex, which is called membrane attack complex or MAC in short. Now, what does MAC do? As the name suggests, it lysis cells. How will you do that? It actually makes a pore in the membrane. One MAC is adequate to lyse an red blood cell. However, in case of lysing nucleated cells, more than three MACs are required. Gram negative bacteria are susceptible to MAC-induced lysis; whereas, gram positive bacteria, which are peptidoglycan layers, are resistant.

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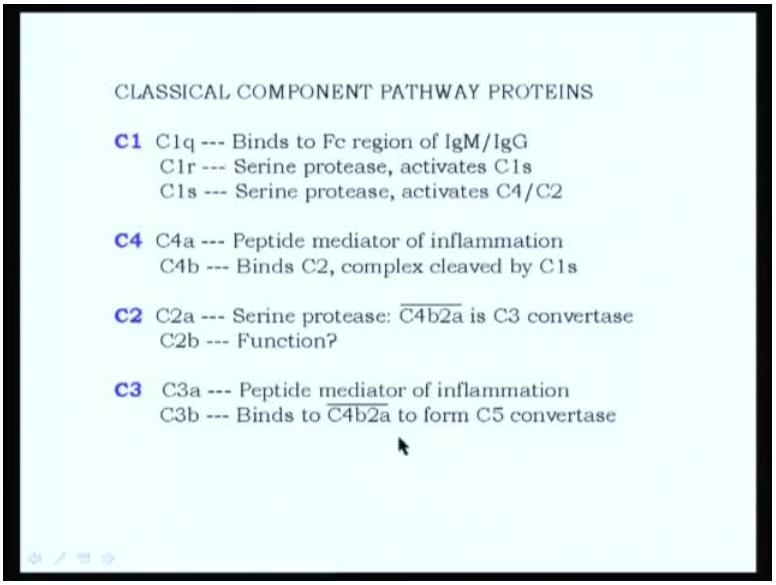

I have already talked about these complement components. Let us just go over them in brief. So, the classical component pathway proteins are made up of first those the initial components, which are all enzymes except of course, C1, but the C1q complex is. C1, which is made up of C1q, C1r, C1s; C1 starts or initiates for binding to the FC region of IgM or IgG upon getting activated in such a way, that is, by conformational change, it induces the serine protease C1r to activate C1s, which is also a serine protease. Now, what are serine proteases? Those proteases, which have serine in the active site; so, all of these are more or less same in terms of classification of enzymes.

Now, C1s activates C4 and C2. So, substrate for C1s is C4, C2. C4, which is made up of C4a and b; C4a is the peptide mediator of inflammation; C4b binds to C2 and the complex is then cleaved by C1s. Now, C2a is a serine protease and C2b – the function is unknown; and, both these are a part of the C2. When C2a binds to C4b, it forms now an active C3 convertase. C3, which is one of the most important molecules of the complement components again is made up of two C3a and C3b or rather C3 gets cleaved to these components. Some of them have more than one sub unit. But, we will not go into that. Now, C3 gets cleaved to C3a by the C3 convertase, which is the peptide mediator of inflammation. The C3b on the other hand, binds to the C3 convertase to form now the C5 convertase.

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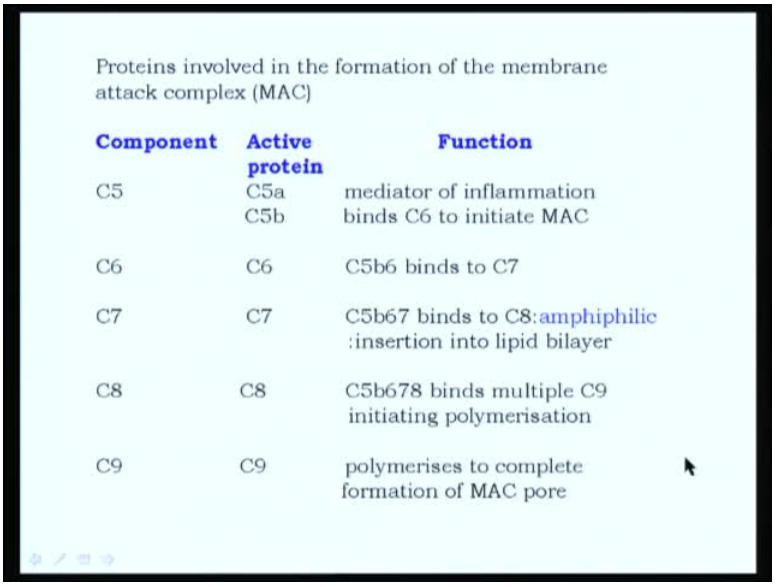

Proteins involved in the formation of membrane attack complex are C5, C6, C7, C8 and C9. The active forms are C5b. And, the others only after they bind to the components, which are activated before, which is C5b. Now, it is at this stage, C5b67 that there is a transition of the soluble component to become now amphiphilic, which will have hydrophobic surfaces inserting into the lipid bilayer. C9 of course, polymerizes to complete the formation of the MAC pore. Now, there are some reports that say that in the absence of C9 in some cases, just the complex form between C5, C6, C7 and C8 is enough in fact to kill a cell.

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Now, let us come to the alternate pathway of complement activation. Like I told you before, the alternate component is a part of the innate immune system. So is the classical component, except that the classical component or the cascade is initiated only upon antigen-antibody interaction. Let us look at now, what is the pathway of the alternate cascade.

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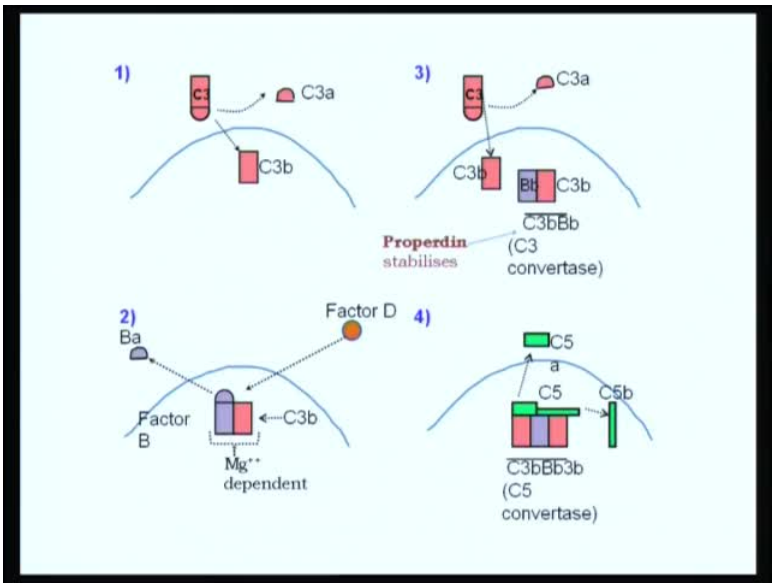

Let us start off with the first component in this, which is C3. Now, the molecule C3, which is present at the serum in an inactive form, has a (( )) thioester bond. And therefore, there is auto conversion to C3a and C3b, but this is very slow process. But, since C3 in the serum and since there is this autocatalysis, there is always C3b, C3a formation continuously in the blood. And, if there are bacteria in the blood, this cleaved C3b binds to the bacterial cell surface.

Now, does C3b bind to even mammalian cells? Yes, in fact, C3b can bind to all surfaces, all cells, but on mammalian cells, since the surface is sialic acid-rich, this molecule gets degraded very fast. Bacterial surfaces on the other hand, have very few sialic acids; almost none. And therefore, C3b binds a little bit more stably. Now, C3b bound to the surface, recruits a factor B. C3b bound to the cell surface, has a binding site for another serum factor called factor B. And, this binding is magnesium ion dependent. If this complex is not now bound to factor D, in fact, this complex is also not very stable. But, when factor D is recruited to this site of the complex factor B, C3b, factor D, which is the serine protease, cleaves factor B to factor Ba and Bb. And, this is where we come from here (Refer Slide Time: 42:29). So, you have now C3b, which is bound to cleaved Bb.

Now, factor D has its substrate, is factor B. Once B is cleaved to Ba and Bb, which is bound now with C3b, this becomes a convertase (Refer Slide Time: 42:55). Now, this

needs stability. And, unless there is another serum protein called properdin, which binds to this complex and makes it stable, this is highly unstable complex. After stabilization of properdin, the complex C3bBb, which is a C3 convertase, now, recruits C3. Now, C3 is recruited to this site (Refer Slide Time: 43:27) and the C3 convertase converts C3 to C3a and C3b. And now, C3b is recruited to this site, which becomes a C5 convertase. So, you have C3bBb, which is C3 convertase; and, you have C3bBb and C3b. Now, C5 convertase has two molecules of C3b, one molecule of Bb. As the name suggests again, C5 convertase recruits C5, because there is a binding site on this complex, where C5 binds. And, the C5 convertase cleaves the molecule to C5a and C5b.

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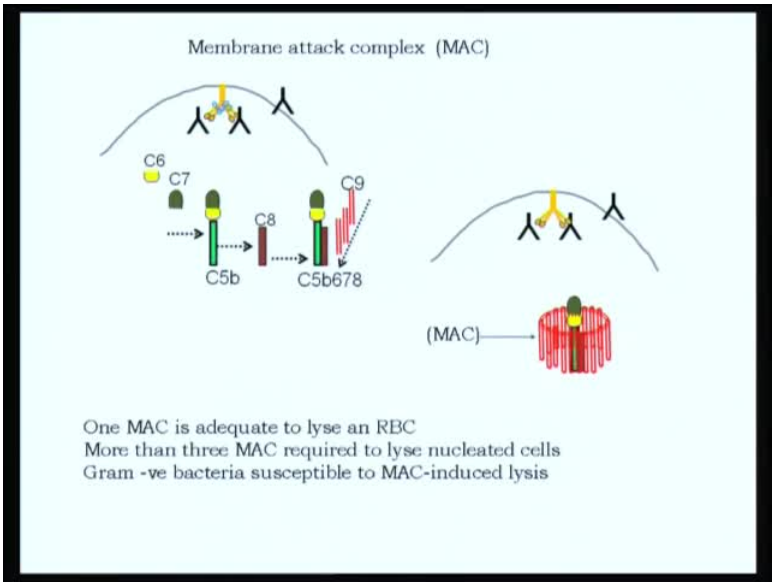

Now, the rest of the sequence of events is identical here on between the classical component and the alternate component, because you have formation of C5b, which recruits C6 first. And, the binding of C6 to C5b brings about a binding site or makes accessible a binding site for C7. This undergoes along with C8, a transition to a hydrophilic or amphiphilic structure, which can insert itself into the lipid bilayer; there on, recruiting C9, because there is exposure for on this complex for this polymer of C9 and you have the formation of a pore forming complex called MAC.

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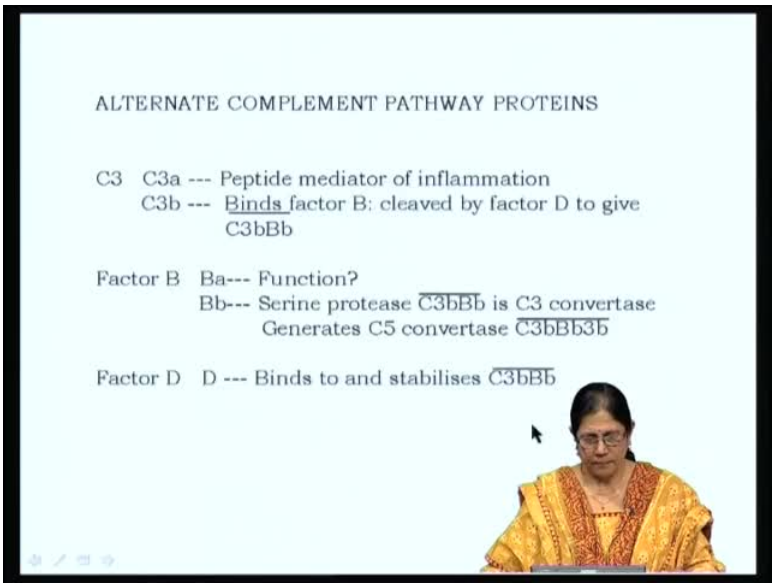

What are the alternate complement pathway proteins? They are C3 mainly, because the complement cascade actually starts with the auto conversion of C3 to C3a and C3b. Now, remember that there is a fast way of getting C3 converted to C3a and b and that would be by the enzyme C3 convertase. But, in the serum, a slow reaction is always taking place, where C3a and C3b are formed; C3b binds factor B, which is now cleaved by factor D to give C3bBb. The C3a as in while discussed earlier, is a peptide mediator of inflammation. Factor B, which is also a serum protein, gets cleaved by the factor D to Ba and Bb. The Bb complexes with C3b to make the C3 convertase and then recruits another C3 molecule, so that there is generation of a C5 convertase. Factor D binds to and stabilizes C3bBb.

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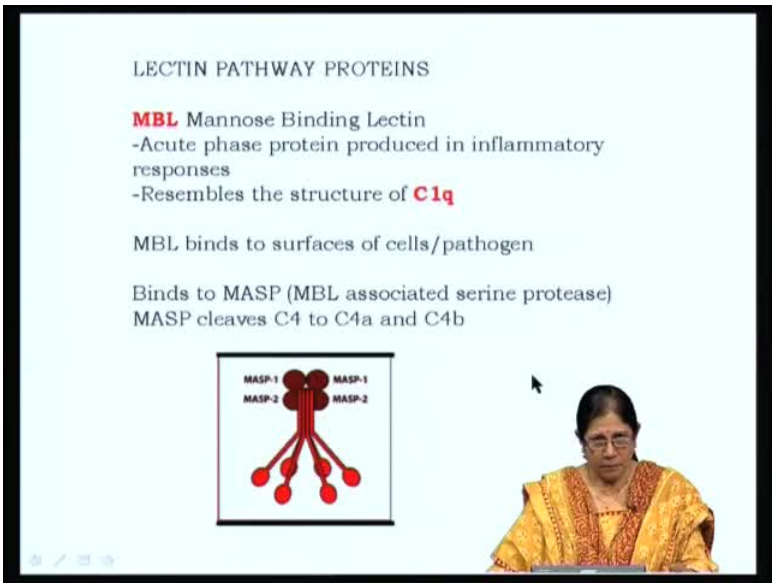

Let us come to the third pathway, which is also a part of the innate system in the true cells, because its initiation or activation also is through the innate pathway by no antigen-antibody interaction, needs to happen. This is a lectin pathway. Lectins are proteins that bind to sugars. Each one has different specificity. Now, MBL or mannose binding lectin has specificity for mannose as the name suggest. And, this protein is an acute phase protein. It is made or it is synthesized and secreted when there is an extensive inflammatory response or inflammation.

MBL – short for mannose binding lectin resembles the structure of C1q. It is not identical, but resembles. Now, MBL, once it is made or synthesized, binds to surfaces of cells and pathogen. And, when it is bound in a fashion, which is similar. Now, all these heads bind to mannose present on bacterial surfaces and you might remember again that bacterial surfaces have more mannose exposed on their cell surface than mammalian cells. The MBL is associated with serine proteases and there are two of such sub units called MASP 1 and 2, which form a tetrameric structure bound to the MBL. Now, MASPs is the short form for MBL associated serine proteases. Now, you have a bacterial surface, where the MBL is bound. This binding activates MASP 1 and 2, the serine proteases 1 and 2. And, this recruits C4 to the site, cleaves C4 to C4a and C4b. And, the rest of the cascade is identical to what one sees in the classical pathway.

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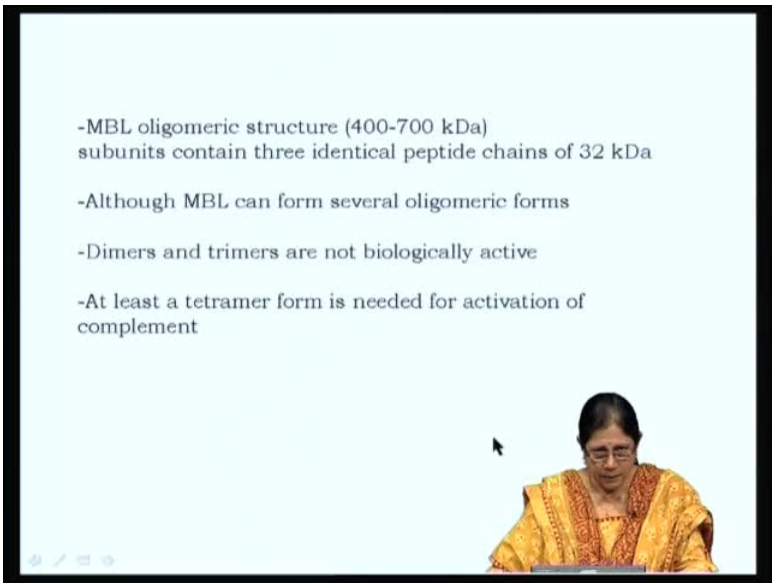

Little bit with regard to the MBL or the mannose binding lectin; it has an oligomeric structure, which could be 400 to 700 kilo Dalton. Sub units contain three identical peptide chains of 32 kilo Dalton to make that flowery structure. But, it is known that MBL can form several oligomeric forms: dimers, trimers, tetramers and so on. But, it is the tetramer, which is the minimum oligomer, which can be activated for this; or, other can activate the complement cascade. Dimers and trimers have been seen not to be biologically active.

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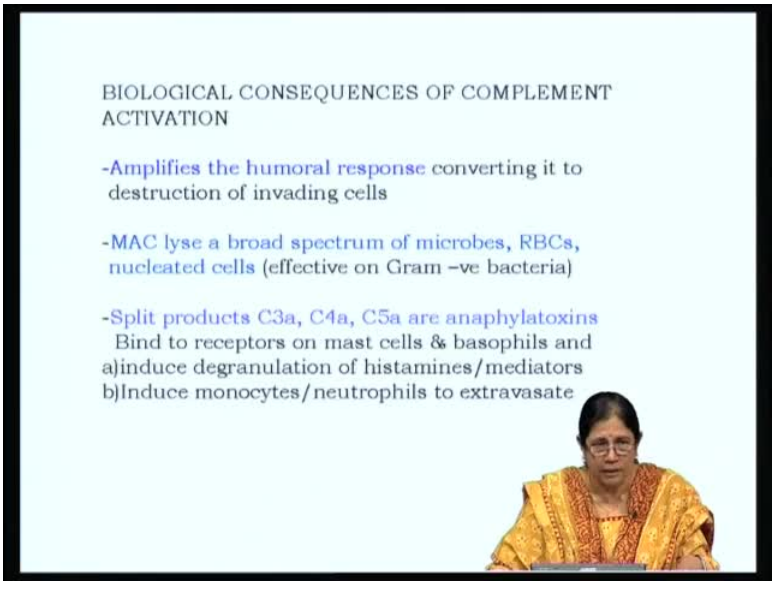

What are the consequences of complement activation? One can imagine immediately that since the classical pathway is dependent on the antigen-antibody interaction and since there are enzymes involved, there could be an amplification of the humoral response. So, the classical complement components or cascade amplifies the humoral response converting it to destruction of invading cells. Antigen and antibody binding can bring about inactivation of the protein by way of complex formation or immune complex formation, which can be thrown out of the body by phagocytosis. However, does not antibodies do not kill themselves. So, here is the strategy by which antibodies can recruit the complement and trigger the cascades, so that membrane attack complexes are formed. And, they can kill broad spectrum of microbes, red blood cells and nucleated cells; we should not forget. And, though we are not going to talk too much in detail with respect to this split product that are formed, C3a, C4a, C5a – these are called anaphylatoxins. They bind to receptors on mast cells and basophils; and, induce degranulation of the preform granules of these cells, which have histamines and mediators of inflammation. The split products also induce monocytes and neutrophils to extravasate.

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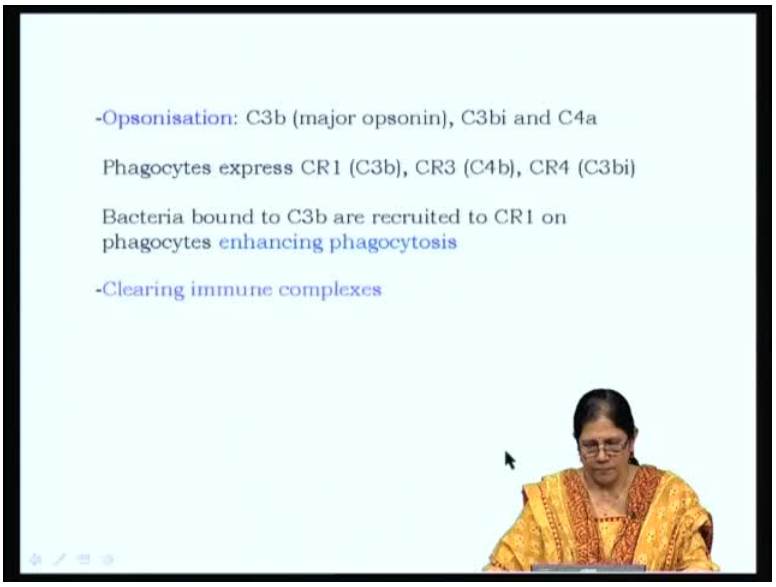

Complement components can also opsonize, that is, bacteria surfaces since they bind to C3b. And, quite stably then, the opsonized bacteria, which would mean bacteria that have bound to C3b, can now be recruited two phagocytes, which express complement component receptors CR1, CR3, CR4, which are the receptors for each of the

complement components. Now, bacteria bound to C3b are recruited to CR1 for example, to the phagocytes. And, this enhances phagocytosis; thereby, mediating clearing of immune complexes.

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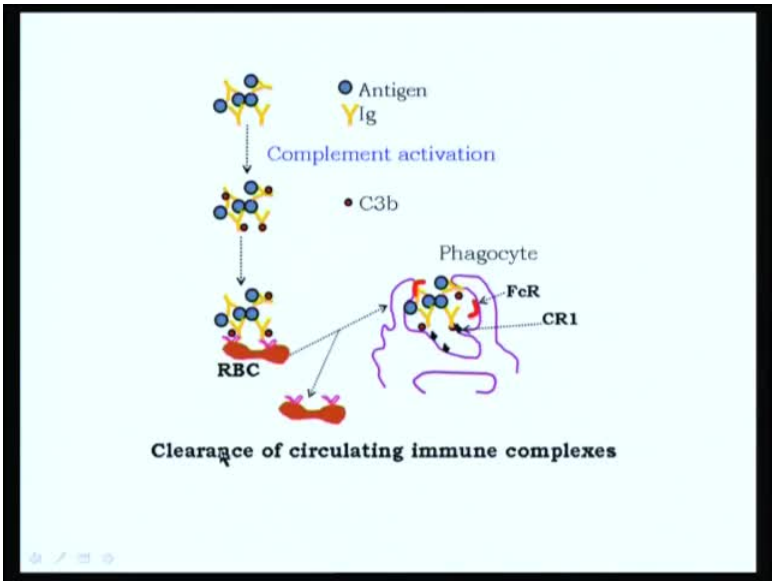

In fact, this complement cascade, which brings about clearance of the immune complexes is something that is an important function, because in diseases, where this cannot take place, would lead to hypersensitive reactions. And, we will come to that in a later lecture.

Clearance of the circulating immune complexes – how does that happen? You have the complex formation, antigen-antibody interaction. This now brings about activation of the complex such… What is not depicted here is the formation of the C1q binding here and then C4b2a and then formation of C3b. So, we come directly to C3b, which would be attached to this site and the C3b is here in red. The C3b has receptors on red blood cells as well as phagocytes. The red blood cells, CR1 – because you can imagine that the red blood cells are large in number and in circulation. And therefore, the complex can load onto the CR1 receptor on the red blood cells. And, this RBC with its cargo now donates this complex to the phagocytes, because the CR1 phagocytes have many more CR1s and have good affinity; and, phagocytes can also phagocytose this complex. And thereby, this is in fact cleared from the system. RBCs then go to the spleen for their final destruction.

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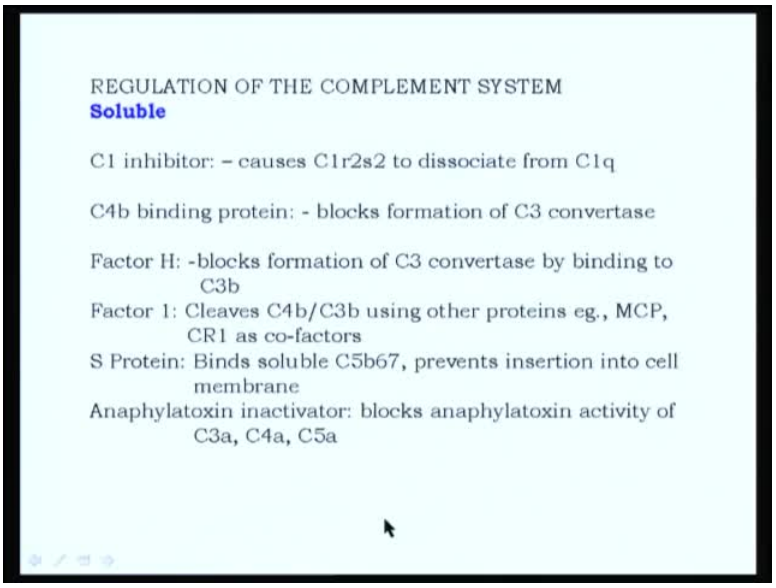

Is there any regulation of the complement system? There should be, because after all, complement cascade involves enzymes. And, once these enzymes are triggered, there is a possibility that the entire cascade can bring about hypersensitive reactions. There has to be a regulation of the complement system therefore, so that by standards are not hurt or normally tissues are not hurt.

There are both soluble as well as membrane bound proteins, which regulate the complement system. C1 inhibitor – all these components are present in serum, because they are soluble. C1 inhibitor that causes dissociation of the r2s2 from C1q; that means the complex is constantly broken. So, the cascade cannot. C4b binding protein, which blocks the formation of C3, binds to the surface of C4b, which should bind to C3. And therefore, now, the enzyme is not allowed to act. Factor H blocks formation of C3 convertase by binding to C3b. Factor 1 – it cleaves C4b/C3b. S protein binds soluble – you remember the C5b67, which should insert into the cell membrane, but does not because of the presence of S protein. And then, also, anaphylatoxin inactivator, which blocks anaphylatoxin activity.

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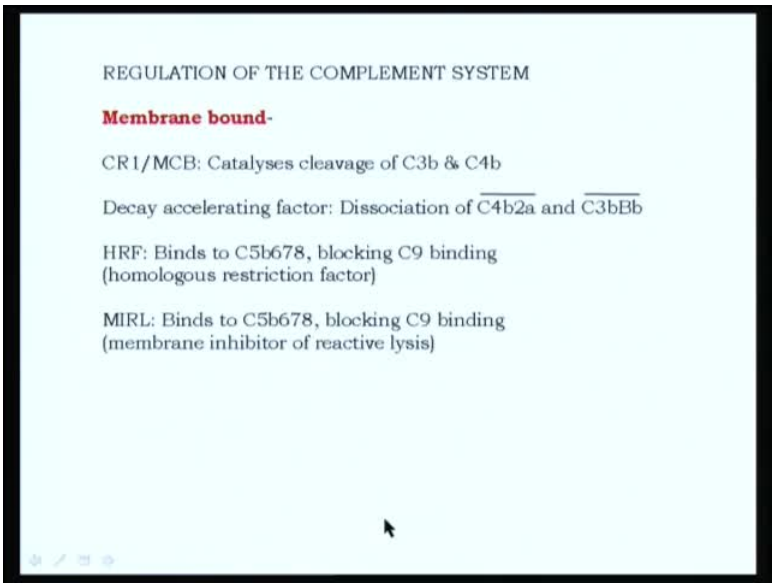

Membrane bound – there are molecules CR1/MCB, decay accelerating factor, HRF and MIRL. All these block. Now, the last two block C9 polymerization and CR1 catalyzes cleavage of C3b, C4b. And, decay accelerating factor dissociates the C4b2a or the C3 convertase.

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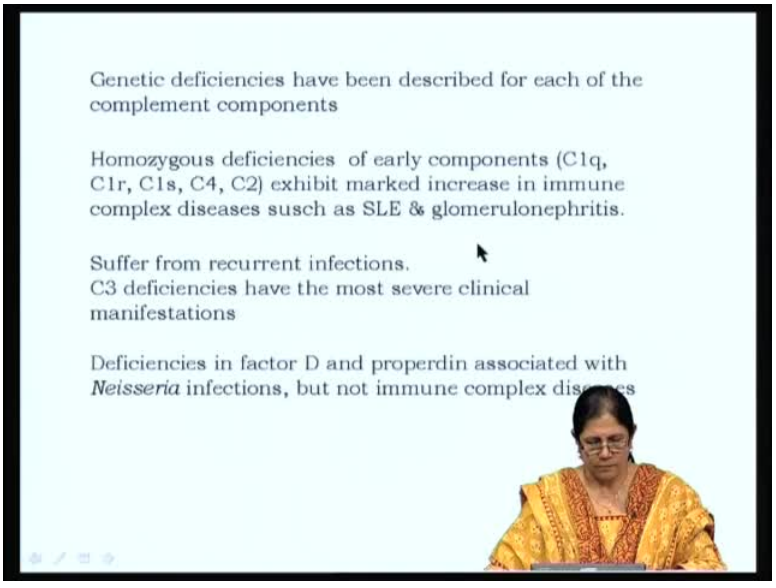

There are genetic deficiencies, which have been described for each of the complement components. And, homozygous deficiencies of early components exhibit marked

increase in immune complex diseases such as SLE and glomerulonephritis. These patients also suffer from recurrent infections.

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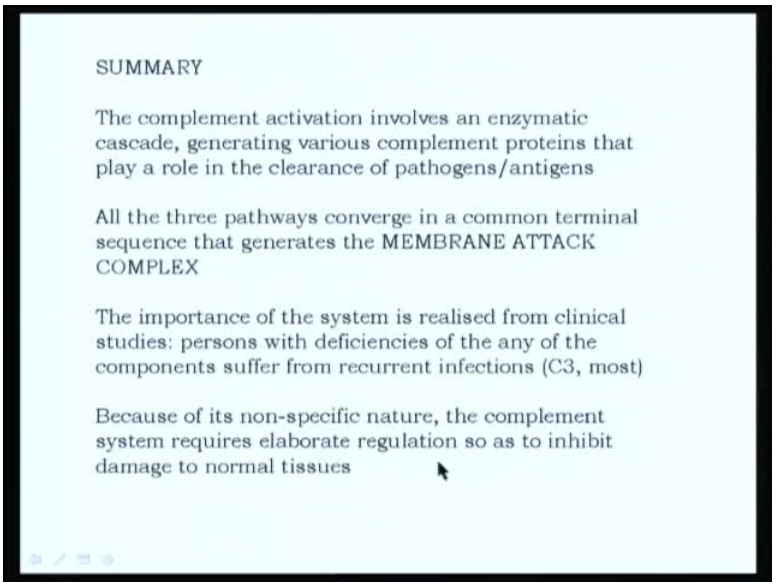

Now, to summarize the complement system – the complement activation involves an enzymatic cascade, generating various complement proteins that play a role in the clearance of pathogens and antigens. There are three pathways: the classical, the alternate and the lectin pathway. All of them have different ways by which they get activated, but converge in a common terminal sequence that generates the membrane attack complex.

The importance of the system is realized though it is a part of the innate immune system. The importance is realized from clinical studies: persons with deficiencies of any of the components suffer from recurrent infections. And, has been found, those with C3 deficiency are most prone. Because of its nonspecific nature, the complement system requires elaborate regulation so as to inhibit damage to normal tissues. And, all the inhibitors that are talked about in the last two slide, are there to limit to an extent, but not to abrogate the complement cascade.

Thank you.