Immunology
Prof. Agneyo Ganguly
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture No -44
Complement Biological Consequences (Contd)

So welcome to the immunology lectures and in this lecture we are going to discuss mainly about the regulation of the complement system.

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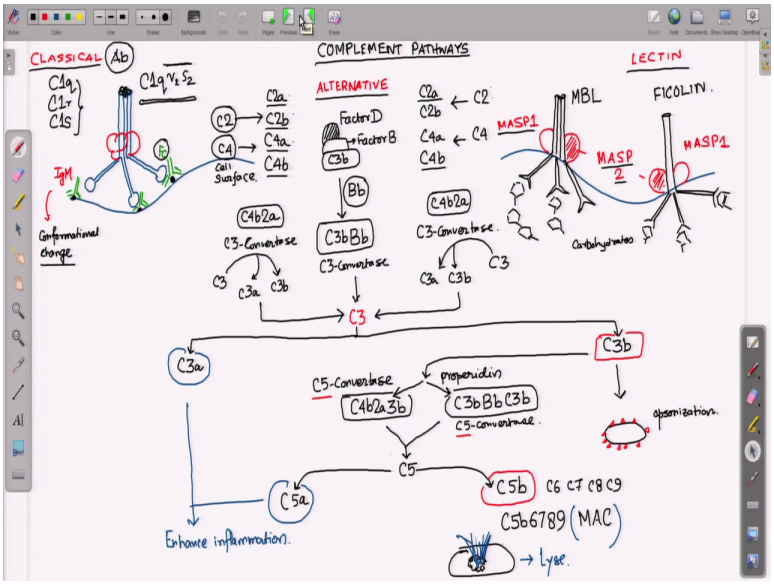

So in our previous classes about the complement system we had discussed about the complement activation pathway. So how the complement is being activated how the complement pathway is being activated and in this complement activation pathway we have learned that at least three different pathways are active the classical alternative and the lectin pathways. And if we see that the major part of this complement activation pathway or the central part of this complement activation pathways break down of the C3.

So I told in my last lecture as well that this complement proteins they are always present in our system but they are normally not doing any harm to us they are not killing ourself cells or our own cells or healthy cells they are not acting on the healthy cells or they are not killing our own cells. They are mostly active on the pathogens or the foreign cells. So they can recognize the

foreign cells and the complement protein or the complement system as we told previously as well that it is the effector part of the humoral immunity.

So one of the major effector pathways of the humoral immunity that is in conjugation with antibodies it can lead to the complement activation leading to formation of the membrane attack complex or it can lead to opsonization of the pathogens. So but the complement system does not work on our own cells it does not do any harm to us although the proteins are they are so why they do not get activated why, the question is why and how?

So of course there is some tight regulation of this complement system and there is a class of proteins a group of proteins rather which are known to function as regulators of complement. So they work as the regulator's of the complement and they are known as the regulators of complement activation or the RCA proteins. And these proteins are encoded on a single location in the human chromosome number one and they are produced from there and they work on the this complement pathway at different, different stages of the complement pathway.

Now if we quickly look into the complement activation pathway what we have seen in the last lectures what are the major significant steps in the complement activation pathway. One of the significant step is activation of the C1 complex that is C1 forming C1 q r 2 s 2 and then it gets activated and Cleaves the C2 and the C4 and once this C2 and C4 are cleaved then only they can form the corresponding clip product like the 4 P the 2 way these are formed and then only this 4b 2 we can associate so one of the important steps in the complement activation pathway is the initial cleavage of the C2 and the C4by the C1 and for that you need the activation of the C1.

So one of the major steps where this complement regulators can act or they can inhibit. So these regulators you have to remember that these regulators are kind of inhibitors that mean they inhibit certain steps of the complement activation pathway or regulate certain steps in the complement activation pathway. And how do they do that and which steps do they really act on so which are the major steps one as I told is the C1 activation and the subsequent cleavage of C2 and C4 in to 2a 2b and 4a 4b.

The second major step is formation of the C3 convertases you see the C3 convertases the C3 convertase is there are two major C3 convertases that are formed one is the 4b 2a and the second one is a 3b Bb. So these are the two major C3 convertase that are formed. So the second stage of regulation is the formation of the C3 convertase that is do not allow the C3 convertase to form does not allow the C3 convertase to be formed.

A third stage of regulation would be if the C3 convertase is formed let us say the C3 convertase see the the regulation at the initial stage is somehow did not work and the C3 convertase is formed by association of 4b and 2a or 3b and Bb. So a third point of regulation is dissociation of this complex that is destruction or decaying of the C3 convertase. So that the C3 convertase cannot work on the C3 and lead to subsequent cleavage of C3 into 3a and 3b another point of regulation is one of the major points of regulation is the regulation of formation of the membrane attack complex that is this part.

The last part so here so another point of regulation is do not allow although you can have all these C3 convertase C5 convertase but do not tell how the formation of the membrane attack complex. So that the membrane attack complex is not formed and a very tight control at the 4 you can understand a very tight control is required at this level of formation of the membrane attack complex because sometimes not all not all the times usually as I told that the complement proteins they assemble and activate each other on the surface on the cell surface surface of the pathogen.

But sometimes these complexes can also occur not on the cell surfaces. So they can just occur outside the cell and these complexes if they form and they can form a C5b67 complex as we described. So if that kind of a complex is already formed then that complete complex is competent enough to go and get inserted into a membrane and cause a hole or a pole or a channel in the membrane leading to the lysis of an innocent cell that means a cell which is not destined to lies which does not is not required to lies that kind of an innocent cell can be also lized and that is know also known as innocent bystander lysis.

So to avoid innocent bystander lysis you can also have regulations or checkpoints at the level of formation of membrane attack complex. So we see at least there are 1 2 3 here 3 and here for so at least 4 different areas or 4 different parts of the complement pathway where you can have checkpoints or regulations.

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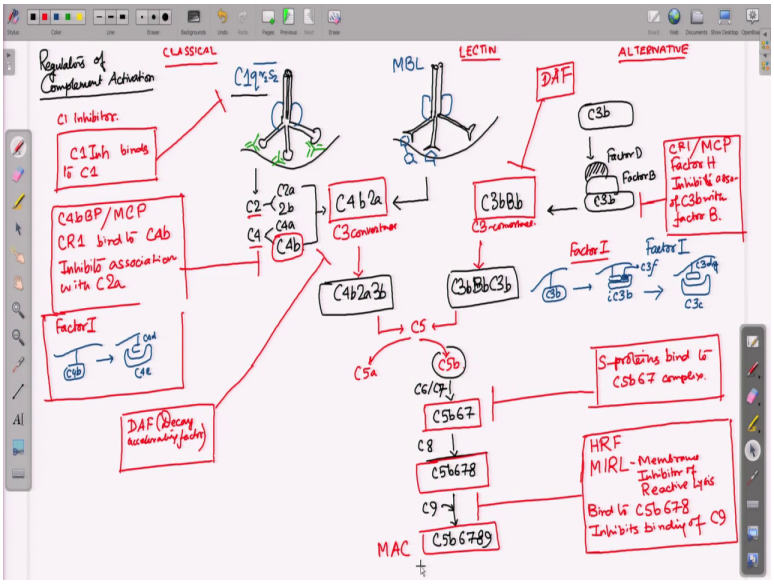

Now let us have a closer look into these regulatory pathways how the complement system is actually regulated. So we start from the very beginning in the classical pathway where we have this C1q r2 s2 and there is so this as I told or describe them as regulators of complement activation. So who are they and how they were. So one of the very initial stages where these RCA proteins can work is on the C1 compliment plodding.

And one of the important RCA is the C1 Inh or the C1 inhibitor. Now the C1 inhibitor it points to the C1 and inhibits the activation of the C1. So what is the C 1 or the C1 q r 2 s 2 known to do it is known to cleave the C2 and the C4 and produce C2a 2b and from C4 C 4a and C 4b and then this 4b and to a they associate to form C4b 2a which is also the same from the lectin pathway. So the first step of inhibition is the C 1 Inh or the C1 inhibitor also known as a C1 inhibitor which basically inhibits the activation of the C1 and inhibits this step of cleavage of C2 and C4 into C2a 2b or 4a 4b.

And thereby this can inhibit the subsequent steps of formation of C4b 2a as well. So this as we know is known as C3 convertase. So this is one of the initial checkpoints where you can actually inhibit the formation of a C3 convertase. Now what is the C3 convertase in the alternative pathway we know it is the C3b Bb how does it come from it comes from? The C3b here is the C3b and this C3b can associate C3b which can associate with factor B and factor D.

And lead to the formation of C3b Bb ok. So this is another C3 convertase. Now as I told apart from inhibition at the level of C1 that is at the beginning there is also regulation at the stage of formation of the C3 quantities. So there are a regulator which does not allow the formation of the C3 convertase and what are they. So one of such regulators is C4b binding protein as the name suggests it can bind to the C4b.

So one of them is C4b binding protein or there is also MCP the membrane cofactor protein or you have the complement receptor one or the Cr1 they all can bind to the C4b and does not allow it so it inhibits its association inhibits its association with C2a. So now this C4b which was supposed to associate with C2a to form C4b 2a cannot associate with the C2 way anymore and thus these proteins C4 4b Bb or MCP or Cr1 they can inhibit the association of C4b with C2a so it inhibits this particular process.

Similar to this what we have seen in case of the classical pathway is similar to this in the alternative pathway as well there are similar inhibitors which inhibits the step of association of the C3b. So for example the Cr1 or the MCP these are the common factors this is a common regulators along with that there is another factor H this also inhibits association of C3b with factor B. So the C3b now cannot associate with factor B and if it cannot associate with factor B then it cannot form the C3b Bb.

So this cleavage is does not occur that means the factor B cannot be cleaved into effect of Bb and this C3b B C3 convertase is not formed. So these are the two major these are the two major steps of regulation where the C3 convertases are not formed. Apart from so in this same stage of regulation when this C4b binding protein or the MCP or the Cr1 they can bind to the C4b they also activates another factor known as the factor I.

The factor I is interesting what does it do so this factor I it basically it cleaves the factor 4 so this is for example C4b and this C4b is further cleaved into the 4 C4c and the C4d. So C4b which was the membrane bound form it can now get cleaved into the C 4c and the C4d still remains bound to the membrane while the C4c is the soluble part which lives or goes away after the cleavage it goes away thus it leaves the C4b inactive.

So the factor I is a factor which is primarily activated after binding of C 4b Bb or the MCP or the Cr1 to the 4b and leading to cleavage of the C4b. So the C4b is then cleaved into 4d and 4c where the 4d is the bound part 4c is the soluble part. And this soluble part goes away so leaving out that the factor factor B is quite inactive and it cannot further form the C3 convertase. Similar to similar to this in case of C3 as well this C3b here can also be further cleaved by the factor I.

So the factor I here clips the C3b so the C3b is also membrane bound form we have seen previously it can get bound to the membrane. So C3b which is initially in the membrane bound form now it gets cleaved into; so this is the C3f and this is the iC3b so the iC3b is the in soluble fraction so it still remains bound to the membrane and the C3f is a small fraction there is the soluble part that gets cleaved and goes out.

So the C3b is broken down into the iC3b and the C3f. Now this iIC3b can be further broken down and it can be broken down into a soluble and an insoluble fragment. So we have the C3c and the C3 dg. Now this dg part remains bound to the membrane while the C3c is the soluble part. So now this C3b by the factor I it can be cleaved by this factor I, similar to what we have seen in case of the C4b this C3b can also get cleaved by the factor I in to iC3b and the C3f which the iC3b can further be cleaved into C3 dg and the C3c.

And this cleavage leads to inactivation of the C3b. So the C3b as we have learned from our previous lectures that the C3b is one of the central mediators of the complement activation pathway it is one of the central molecules in the complement activation pathway. Now this C3b is completely destroyed or degraded and once the C3b is completely degraded or destroyed it

cannot further associate with the factor B or the factor D and form and lead to the formation of the C3Bb.

Now this C3Bb or the 4b 2a either of these 2Cbe converted they converge at the formation of two different C5 convertase. So the forms the C5 convertase is the C4b 2a forms C4b 2a 3b and this form C3b Bb C3b okay. So these are the two C5 convertase. So now another checkpoint in this whole activation pathway is decaying or dissociating the C3 convertase is by itself and that is done usually by a factor known as the decay accelerating factor or the DAF decay accelerating factor.

And this decay accelerating factor also works on the DAF can also work on the C3 the C3b Bb convertase is that is the convertase the C3 convertase from the alternative pathway. So this DAF the decay accelerating factor is active on the C3 convertase so they work on the C3 convertases and they can quickly or very fast they can dissociate the to a from the 4b or the Bb from the 3b. So dissociation of this 3b from the Bb ensures that the C3 convertase is not active anymore.

Similarly from dissociation of 2a from the 4b ensures there is no further C3 convertase available to convert C3 or to decay or destroy cleave C3. So this is another point of regulation where we have the decay accelerating factor or the DAF. So now once we have kind of seen the checkpoints starting from the C1 to the C4 C2 C3 and mainly the C1 one of the checkpoints is the C1 this initial part.

And the second checkpoint is in the formation of the C3 convertase and the third checkpoint is the stability of the C3 convertase that means the C3 convertase is a decay. Now once the C3 convertase if they skip these checkpoints and there is formation of a C5 convertase. So what do they do they cleave the C5 and the C5 is clipped into 5a and 5b C5a and C5b. So it is cleaved into these two molecules or the two fragments and C5b associates with C6 C7 to form the C5b67 complex.

Now this C5b67 is a complex which is already competent to go and lead to lysis of the cells C5b67 can already because the 7 the C7 the outer part of the C7 it it undergoes are structural

transition and leads to exposure of the hydrophobic patches. So it can very easily go into the membrane and can lead to lysis of the cells. Now if the complexes are 5b6 7 complexes are formed on the surface of the cell there is no problem or the target cell there is no problem.

But if it is not formed on the surface of the target cell then what happens if it is, it can trouble can travel to the next cell and can kill an innocent self. So there can be no sin bystander lysis. So the next checkpoint of the point of regulation is how to stop this kind of bystander lysis. So there are complement regulators or complement proteins regulator proteins which can bind to this. So, which can bind to this C5 b67 complex and inhibit its insertion into the cell membrane?

So one of them is for example the soluble serum protein or the S protein that can bind to C5 b67 complex and inhibit its insertion into the membrane. Now if even if it skips this checkpoint you still have the C8 binding to it and forming C5b678. So C5b678 and finally you have C9 or policy nine binding leading to C5 b6489 which is actually the membrane attack complex or the MAC. So this is the MAC, so now there is one final more checkpoint where there are two major proteins one is known as the homologous restriction factor or the HRF another is MIRL also it's also known as the membrane inhibitor of reactive lysis.

So membrane inhibitor of reactive lysis, so now this MIRL or the HRF they can bind to this complex the C5 b67 complex bind to the C5 b678 complex and inhibits binding of the C9. So this inhibits the binding of the C9 so this MIRL or the HRF the homologous restriction factor or the MIRL that is the membrane inhibitor of the reactive lysis this both of these two proteins they binds to C5 b678 complex and inhibits thereby inhibits the binding of the policy 9 and formation of the membrane attack complex.

So there is no formation of the membrane attack complex. So these are mostly the different stages where these regulators of complement activations or the RCA proteins they act upon. So quickly I go through or revise this whole thing. So that the there are the different checkpoints. The first checkpoint is here at the level of C1 it inhibits the activation of the C1 and cleavage of the C2 and the C4 downstream. The second checkpoint usually is in the classical pathway it is the binding to the C4b there is a cleave product of C4.

So C4b binding protein or the MCP of the Cr1 the binds to C4b and inhibits its association with the C2a so that this C3 convertase that is C4b 2a is not formed. Similarly this is also part of the second checkpoint of the second regulation that this CVAR Cr1 or the MCP the membrane cofactor protein or the factor H they bind to C3b and inhibits the association of C3b with factor B so that C3b cannot interact or cannot associate with the factor B and the finally inhibition of C3b B complex.

Now apart from doing this apart from doing this both of them they initiate a third process that is they activate this factor I activate the factor I. So here and here as well they activates the factor I which can cleave the C4b that is the cleaved product is the one of the main mediators or one of the main components of the C3 convertase. So factor I can cleave C4b into C4c and C4d so that there is no further C4b available for the formation of 4b 2a.

Similarly in the alternative pathway the factor I can cleave C3b into C3f and iC3b, iC3b is further cleaved into C3c and C3 dg and so by that the factor I can cleave or completely destroy this C3b and inhibits or does not allow the formation of this C3b Bb complex but what if this C3b Bb complex has already been formed or the C4b 2a complex has already been formed that has been taken care of this fourth step or the 4th checkpoint which is the decay acceleration factor here and here in both the cases.

The decay acceleration factor usually is responsible for quick decay of or dissociation of 4b from 2a or 3b from Bb. So once this C3 convertase is like 4b 2a or 3b Bb is formed this decay acceleration factor can dissociate the 4b from 2a or the 3b from Bb and thereby inhibiting the C3 convertase tape. So it does not allow the C3 convertase to be formed. Further to this we have two more steps in the process of the membrane attack complex formation.

One step involves immediately after formation of 5b so immediately after formation of the 5b we have 5b67 complex and this complex is a very important complex in a way that this 5b 67 can get inserted into the cells and can lead to innocent bystander lysis. So, S protein one of the

soluble serum proteins, so the S protein it can bind to this 5b67 complex and thereby inhibiting it inhibiting this complex to get inserted and how does it do that?

So the after this S protein leads to a hydrophilic transition so usually this C7 is hydrophobic and that that is what favors it in its insertion into the membrane. So now it is this binding of this s protein leads to a hydrophilic transition so that it cannot get accommodated in the hydrophobic environment of the membrane. So it does not get inserted. So this is another checkpoint number 5 and finally in the last step you have the checkpoint where these two proteins that is the homologous restriction factor the HRF or the MIRL that is a membrane inhibitor of reactive lysis these two factors they can bind to the C5b 678, 5b 6 7 8 is the penultimate step of memory and at a complex.

Now these two proteins they can buy they are expressed by the expressed on the surface of the host cells and they can bind to C5b 678 and thereby inhibiting or restricting the binding of the C 9 to this 5b 678 and if C9 cannot bind to 5b 678 it would not form this policy 9 and the membrane attack complex. So the membrane attack complex will not be formed and this is the sixth of the final regulatory steps by the complement regulators or the regulators of complement activation.

So in today's lecture we have learned briefly about regulations that are there in the complement activation pathway and how these regulators of complement activation they regulate the different steps in the complement activation pathway and thereby does not allow the complement proteins to just get activated and destroy our own cells or work on the self cells. So that is all for today's lecture and we have mostly completed or finished with the complement proteins.

And I hope you have understood the complement proteins by far have understood the complement proteins. So we will finish the complement chapter here and you can go through any standard textbook what which has been referred to in our website. You can consult any of the text books and read them and I hope things get clearer to you and thank you very much.