Host-Pathogen Interaction (Immunology)
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Module No # 09
Lecture No # 45
Innate Immunity – Complement Introduction

Hi so we are in the middle of this course and this course was started with historical aspect of immunology and then we learned about the immune organ then we have learned various immune cells. And then we have learned about the cytokine molecules which basically coordinate the cells and various component of immunity. And after that we have discussed about there is a pattern recognition receptor we, have completed this pattern recognition receptor we learned in quite a deep length.

And today I will start one more component of immunity or more precisely innate immunity that is complements. So complement is kind of a junction between innate and adaptive immunity. This complement activation do need the support from adaptive immunity in terms of antibody and you know that antibody is, produced by the b cells and b cells are adaptive immune cells.

So complement is also a component of innate immunity it is a humoeral component it is a soluble component which is playing a very important role against microbial infection. Today I will begin or in this session I will begin the compliment and before beginning I would like to take you again in historical part how it was, discovered?

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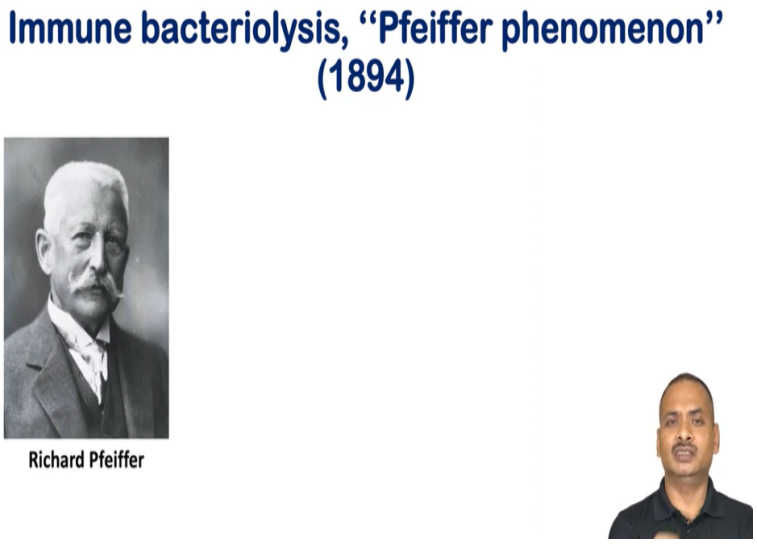

So if you remember my previous or initial sessions where I have discussed about the history. So if you remember then you probably remember the name Pfeiffer or Pfeiffer phenomena who; which was discovered by Richard Pfeiffer around 1894. So this work basically started the initial observation was started with his work and later on this work was taken up by Jules Bordet. And he established that there is some factor which is playing very important role in Bacteriolysis.

So let me explain the Pfeiffer s work so Pfeiffer basically working on a guinea pig and he was using Vibrio Cholerae as model bacteria for his experiment. So what he has done? He has performed one simple experiment where he challenged the guinea pig which is immunized with a, Vibrio Cholerae. And he noticed that in this guinea pig the when he exposed this guinea pig with Vibrio Cholerae which is already immunized with her vibrio cholera.

Or you can consider that a guinea pig recovered from the vibrio Cholerae~~corollary~~ infection so then in that animal he injected in peritoneal cavity he injected vibrio and then he noticed that this bacteria is very quickly disappeared, or it will be very quickly realized. So this observation and he along with this experiment he performed another experiment he also performed this experiment using non-immunized animal.

And when he injected please listen carefully when he injected the non-immunized guinea pig with the serum which is taken from the immunized guinea pig and the bacteria the bacteriolysis was happened. So, when he performed the similar experiment in vitro condition he was not able to show the bacteriolysis consistently. And finally what he concluded out of all these experiment that there are some phagocytic cells which is present in peritoneal cavity and those cells basically cause the bacteriolysis.

So this work was till this point where this Pfeiffer has completed this work and concluded his conclusion was it is some peritoneal cavity phagocytic cells which occurs the bacteriolysis.

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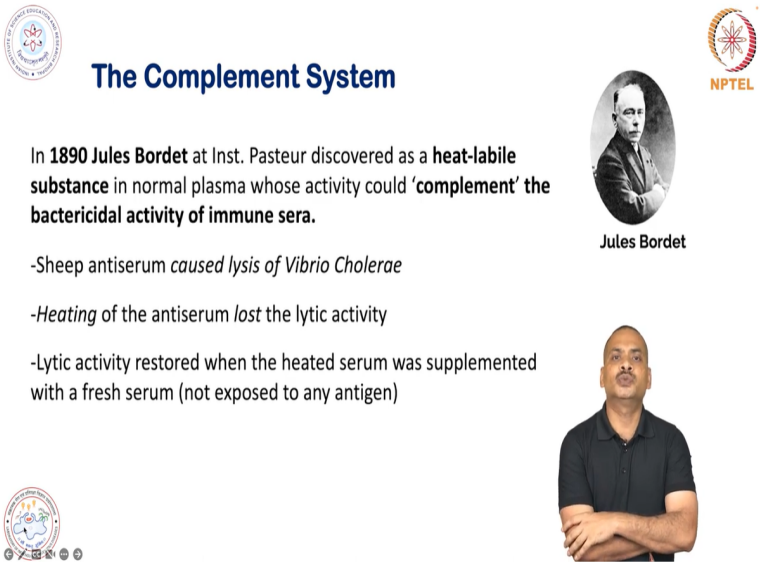

Then what happened in around 1895 Jules Bordet basically initiated his experiment with his observation Pfeiffer's observation and he was basically working in the lab of Elie Metchnikoff you remember the Metchnikoff who discovered the phagocytosis so he was working in his laboratory and his, project is to investigate this Pfeiffer phenomena in extensively. And he performed a series of experiment using vibrio and guinea pig using anti serum and serum.

And he finally concluded that there is a some factor in the serum and this factor is partially play an important role in bacteriolysis. And if this serum is not kept properly or if it is not fresh if it is a old or if the serum is exposed to the temperature fluctuation particularly if the serum temperature increase more than 55 degree then this loses its bacteriolysis capacity. So this was the beginning of the concept of complement he very firmly showed that.

In fresh serum besides antibody or besides a specific molecule there is a molecule which is needed for the bacteriolysis and this molecule is, irrespective to the specificity. It means if the serum is old which is received from the immunized animal then it will not show the lysis activity. But if it is ~~replenish~~replenished with fresh serum from non-immunized guinea pig then that complement activity will be restored or the activity of the serum will be restored irrespective of any other thing.

So this was a great experiment and great, observation and that result to the discovery of complement. So this work was a done by Julie Bordet ~~it~~ beside this he has performed various work during his lifetime. And he also discovered the aessay which we call it as a complement fixation test or aessay which is used for the identification of a specific antibody or specific antigen when I say antigen it means a particular microbial, pathogen.

So in addition to these 2 this very key discovery he also discovered the causative agent of whooping cough. Probably you are aware that whooping cough is caused by Bordetella pertussis and so he is the discoverer of this Bordetella pertussis which cause the whooping cough. For all this work he received the Nobel prize in 1919 so probably you might be aware that 1919 or 1918 was a, the pandemic time the influenza there was a pandemic all over the world so at that time he received this Nobel prize.

Anyway let us come back to the compliment so basically he was working in a Metchnikoff laboratory at Institute pasture. And he after his experiment he his observation was that there is a some factor in the serum which is heat labile factor and this heat labile factor is, responsible for the bacteriolysis. And he also suggested that these complement proteins or these factors are present in Zymogen form.

So this Zymogen form is inactive form like a pro you know that there are several enzymes which are present in inactive form and we call it as a Zymogen form. A pro form and this pro form get activated when there is a there is some cleavage of this, protein. So this was the observation he also showed this phenomena in sheep and the way I have explained he have performed this experiment in using sheep anti-serum.

And he showed this if you heat the serum at particular temperature that is 55 degree centigrade then serum will lose its lytic activity. And lytic activity can be restored when this heated serum is mixed with fresh serum which, is irrespective of a pre-immunized or immunized.

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Components of Complement

- **Soluble proteins and glycoproteins**
  - Synthesized mainly by liver hepatocytes and other cell types
  - 5% of serum globulins
    - Circulate as inactive proenzymes (Zymogens) – proteolytic cleavage removes inhibitory fragment and exposes active site

So this was his all work and finally this heat sensitive component is he established that heat sensitive component is important for bacteriolysis. And this ~~agumented~~augmented activity of antibody is also observed by Paul Ehrlich you know that he is very important in giving the side chain theory. So and he since; he observed that this antibody, activities ~~agumented~~augmented by these factor he gave the name complement so the complement word is given by him.

Now let us look at the component of complement so complement are basically a group of protein which is present in Zymogens form in inactive form and this is basically a protein or glycoprotein and synthesized mainly in the liver and other cell types. And 5% of serum globulin is a, is the complement circulated in inactive form in Zymogen form and there will be a cascade of signaling or proteolytic cleavage that makes it inactive to active form.

Basically, it clears the inhibitory fragment and exposes its active site and in that way this complement get activated.

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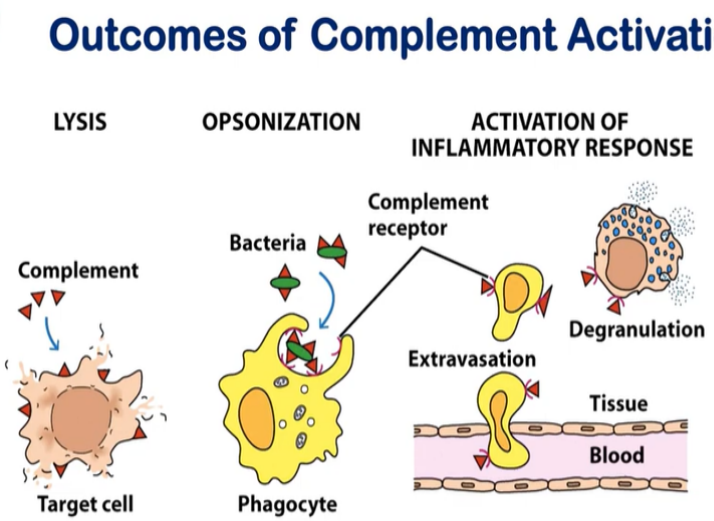

The outcome of complements are 3 major outcomes one is I think told you several times in previous, session the first outcome is or one of the outcome is the lysei~~cense~~ of target cell. So when complement gets activated this will lies the target cell another is opsonization. So opsonization is basically the target microbe will be coa~~quo~~ted by some group of molecules and once it is coated then phagocytic cells will readily phagocytose these microbes.

So we call it as a opsonization or you can, call it as a there will be a more quick phagocytosis. The third outcome of the complement activation is to induce inflammation so some of the

complement the some of the complement part if it is cleaved from the parental complement molecule then that will act as a inflammatory substance it induces inflammation. And you know that inflammation is a very well I have discussed, inflammation in quite big length in previous session so this complement can also cause the inflammation.

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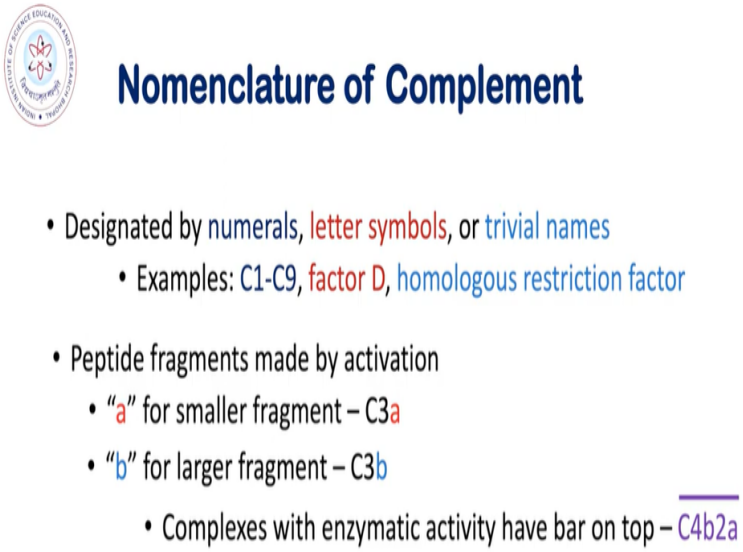

Now I will talk about the nomenclature of complement it has some set of naming system. So all complements are basically designated by C so most of complements which was earlier discovered they were they were given the name as a C~~A~~ and there will be a numbers and there is also some complement name is also as a as a trivial name. For example, factor D and factor B something like that.

So this is first step in complement and generally when this complement get cleaved or proteolytically activated by previous complement then this breaks into 2 fragments generally these fragments are one larger component and another is a smaller, component. So the smaller component in general designated by a. So for example there is a complement C3. And if C3 is activated then it will make a 2 fragment C3a which is a smaller fragment and there will be a C3b which is a larger fragment.

And this naming is present in this whole complement system you will see a shortly when you will see the pathway activation pathway of, complement. The b part is always the larger fragment in general again it is not always the case in general the b part is the larger fragment and a part is the smaller fragment.

So there is a larger fragment which we designate as a C3b and this larger fragment is a basically in various complement you will see there is a larger fragment and they are designated as a b. For example, if C3 will, cleaved then it will make a smaller component that is C3a and the larger component C3b. And these complement upon cleavage and binding with other complement they gain the enzymatic activity. And this enzymatic activity we basically denote by a bar as you can see that here there is a C4b2a.

So this C4b2a protein complex is basically interacted with C4b and 2 a complement C4b, and complement C2a, this makes a complex and when this complex is formed then this will gain the enzymatic activity. And when the when the complex gain the enzymatic activity; this is denoted by bar.

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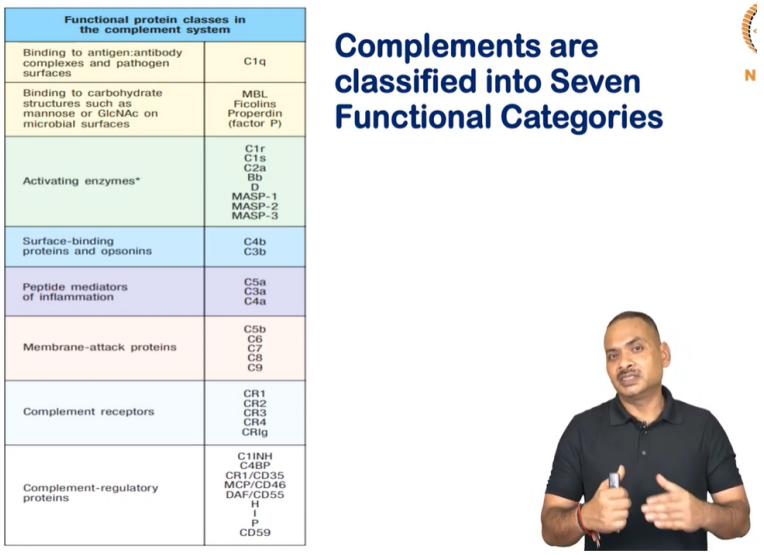

So now I will talk about the complement basically these complements are classified into 7 functional categories here you can see there are various functional category. The first category, is basically initiator the complement which initiate the complement fixation pathway. Here you can see that there will be a C1q is a initiator and later on you will see that this C1q is an initiator of one particular pathway which we call it as a classical pathway.

The complement molecules which activate another pathway we are also categorize these proteins in as an, initiator for example MBL, Ficolins, Proderdin factor P. So these are all these molecules are basically initiator which will access activate or initiate various complement pathway. There are 3 complement pathway which you will see soon after this

slide. Another is a activating enzyme so there are complement which basically activate the enzymatic activity like C1r, C1s, C2a, Cb, B b, factor D, d MASP 1, MASP 2, MASP 3.

So these are basically activate the enzymes they trigger the enzymes another is a surface binding protein and opsoninance I have explained you what is opsonization? So all those molecule which is involved in opsonization we call it as opsonaince. So these surface binding protein and opsoninobstinence are C4b and C3b. So if microbe is, coated by C4b or C3b then it will be readilyRidley Phagocytose so this is the 1 category.

Another is peptide mediator of inflammation I told you the complement breaks into 2 components and one of the component basically induces inflammation. So those component we call it as a peptide which basically induces inflammation that is C5a, C3a and C4a. The outcome of complement activation I told you the lysis one of the outcome is lysis. So this lysisicense is basically mediated by one protein complex which we call it as a membrane attack complex.

All those complement which is involved in this making this complex we call it as a membrane attack proteins so all these molecules like C5b, C6, C7, C8 and C9 basically they make a constituent of this membrane attack complex. Basically, it makes a kind of pore or whole in the target cell this membrane attack proteins makes a pore or whole another is a complement receptor.

So these complements for example see c4 b and c3b they after coating there will be a receptor on phagocytic cells. So those receptors are categorized as a complement receptor like CR1, CR2, CR3, CR4 and CRIg so these are the, complement receptors. There are regulator protein in the this whole complement pathway we call it as a complement regulatory protein that is C1 i-n-h. C4-BP and CR1 or it is alternative name is CD 35 MCP or CD 46, DAF or CD 55 factor H, factor I, factor P and CD-59.

So these are the various regulatory protein and I will discuss these some of these protein when I will talk about complements and disease.

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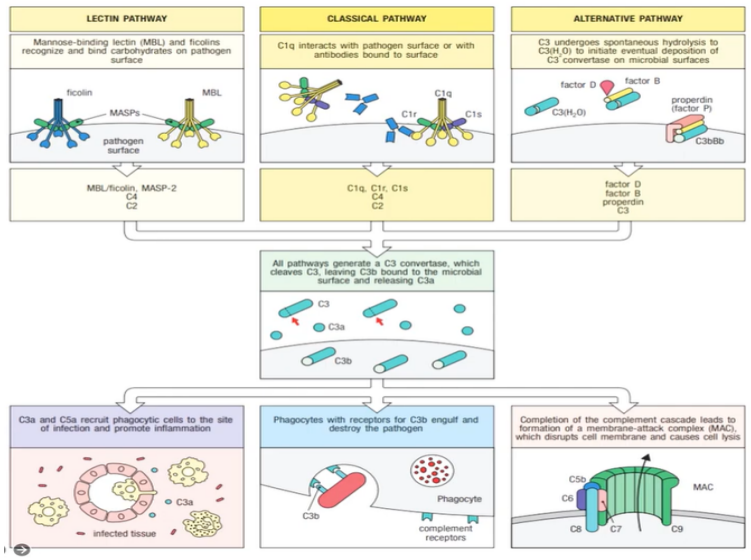

Now this complement pathway are basically there are 3 major pathway or three pathways not major they all these 3 pathways are existing first is alternative pathway. And this alternative pathway is basically activated spontaneously when there is a microbial infection. So in this pathway basically there is a complement C3 which basically spontaneously hydrolyze and initiate, the ~~depositation~~deposition of this C3 converters on microbial surface.

So what is basically happening this when there is a microbial infection there are some factor over the microbes which is distinguishing feature between our own cell and microbial cells. I will discuss when I will take up the alternative pathway but just you remember here that there are some distinguishing features which, basically allow the initiation of hydrolysis of C3 is a complement 3.

So this initial hydrolysis basically caused the deposition ~~depart agitation~~ of C3b over the target cell and then this will interact with another factor and then that will acquire the enzymatic activity. So alternative pathway is spontaneously triggered when; there is a microbial infection. Another is the classical pathway and, this classical pathway is basically initiated as you have seen in previous slide there will be a molecule known as C1q.

This C1q molecule will interact with antigen antibody complex then this complement initiated. So classical pathway is triggered by antigen antibody complex and eventually towards next step this will basically make a C3 convertase~~r~~. And then the subsequent pathway, will be triggered another pathway is lectin pathway this lec~~a~~tin pathway I have told you there are l~~a~~ectin proteins.

So what is lactin is basically protein molecule which is binding with the sugar molecule. So this lectin pathway is triggered by manos binding lectin MBL I have told you in previous session when I was discussing the humoeral component MBL. So MBL can also activate the, complement pathway and this we call it as a lectin pathway. So this will be triggered by various molecules like MBL, Ficolins and then this will activated.

And then it will initiate the complement pathway please remember the aim of all these 3 complement pathway is to make C3 convertases. Please remember again I am telling the aim of all these pathway alternative classical or this lectin, pathway is to make the C3 convertase is and what is C3 converters? It will cleave the C3 are this clear C3 will generate C3a and C3b so this C3a will be a kind of anaphylotoxin or it will induce the inflammation and C3b will be deposited over the target cells.

And once it will be deposited then various the most important thing which will happen is the formation of membrane attack, complex which you will see in subsequent slide. So here you can see that all pathway basically generates C3 convertasers which cleave the C3 and leaving C3b over the over the target cell surface. And this C3b will be bound to the microbial surface and release C3a so C3a will be causing a inflammation and C3b will be will be present on the microbial surface.

And once it will be present over, the microbial surface it will be readily phagocytose and there will be a various things will happen for example here the C-3a and C-5a recruit phagocytic cells. So it is inflammatory molecule please remember this is inflammatory molecule so this inflammation will attract the phagocytic cell and this will once it will be attracted then they will clear the microbial pathogen.

And C3b as I have explained you see 3b if the microbial pathogen is coated with C3b then it will be readily phagocytose we call it as the opsonization. Opsonization is a process of this coating of this target microbial pathogen by C3b and after that this will be phagocytose. The third outcome here you can see which I have shown you in previous slide that will be the formation of membrane attack, complex and this membrane attack complex will make a pore in the target cells.

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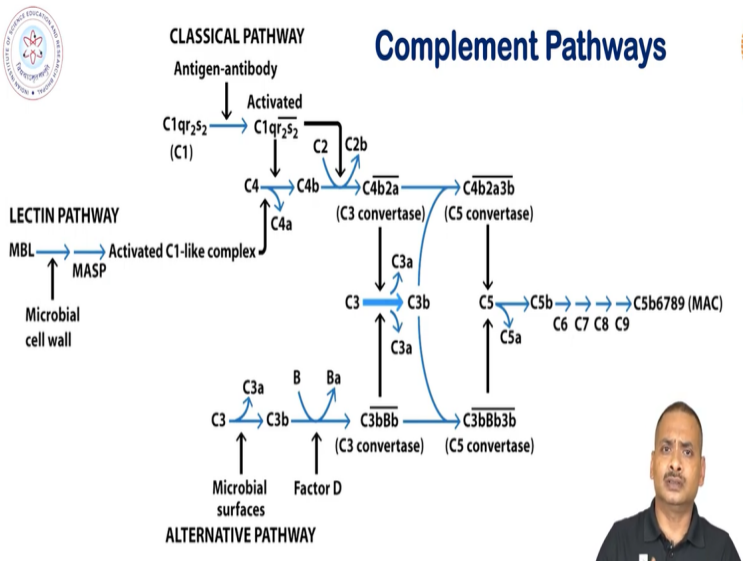

Here this is a little more complex and less interactive here you can see that there are alternative pathway and this alternative pathway is basically initiated. If you remember the previous slide this basically spontaneously there will be hydrolysis of C3 and then this spontaneous hydrolysis will make a C3b. And, then this will interact with the factor there will be a factor b and this factor b will again cleave and then that will make B-a.

And there will be a small is the factor b will once it will be clear it will make a factor B a which will be a kind of anaphylotoxin. And inducing the inflammation the bigger component that is factor b means B and b that will make that will, interact with C-3b and this C3b will be the C3 convertaseers. In case of alternative pathway in case of classical pathway there will be a C1qr2 s-2 this will interact with antigen antibody and then this will basically make a, C3 convertaseers after cleavage of a C4 complement and C2 complement.

And eventually that result to the formation of C4b2a ~~to a~~ this will have a c three convert phase activity. As you can see in this slide there is a bar both classical and lectin~~atin~~ pathway will generate this kind of C3 converters. So I will discuss all this pathway in more detail when I will take up individual pathway. So with this I am stopping here and next session we will discuss about the particular pathway thank you.