`Host-Pathogen Interaction (Immunology)
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Module No # 09
Lecture No # 47
Complement – Alternative and Lectin Pathway

Hi in previous session we have discussed about the complements and we have discussed in great detail about classical pathway. And this classical pathway is basically initiated through antigen antibody complex. If you remember the Fc portion of antibody interact with C1q and then that initiate the classical pathway. So in this session we will move on to the complement and in this session we will discuss about alternative and lectin complement pathway.

So if you remember that these 2 pathways are basically antibody independent and since it is the antibody independent the soume of initiator is also act as antimicrobial protein and they are enhanced during the infection and then that trigger the particular complement pathway.
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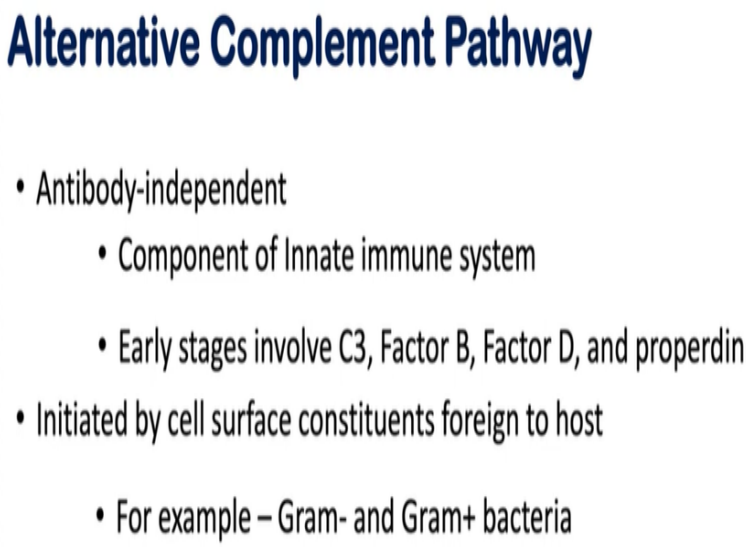

So let us begin with alternative complement pathway and here you can see that this, alternative complement pathways antibody independent. And component of it is a one of key component of innate immune system it is you can consider it as a humoeral component of innate immune system. And this is initiated or there is a involvement of C3 one of the key molecule which is a which is playing important role in this complement is C3 another is factor B, D and properdintey.

So we will see how, these molecules are involved in activation of alternative complement pathway. There are several factors which initiate this alternative complement pathway and most of these factors are basically foreign to the host and for example there is a gram-positive bacteria and gram-negative bacteria. I will show you in more detail what are the factors from gram positive and gram negative bacteria that, can initiate the alternative complement pathway.

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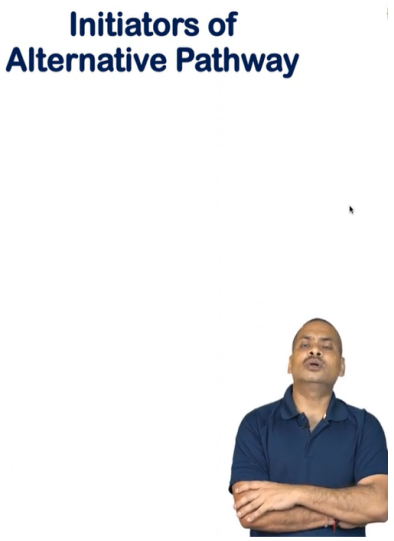
TABLE 7-1 Initiators of the alternative pathway of complement activation

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)
NONPATHOGENS
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)

SOURCE: Adapted from M. K. Pangburn, 1986, in *Immunobiology of the Complement System*, G. Ross, ed., Academic Press, Orlando.



Here you can see that there is pathogen or particles of microbial origin and these molecules basically initiate the alternative pathway. Here you can see that many strain of gram negative bacteria you know that these bacteria poses lipopolysaccharide LPS. And these components can activate the complement pathway Teichoic acid from, gram-positive bacteria which is present in bacterial cell wall they can also trigger the alternative pathway.

In addition, besides bacteria fungus can also trigger the complement alternative complement pathway and one of the key component of fungal cell wall is beta glucan which is also known as Zymosan. So this beta glucan or Zymosan can also initiate the alternative pathway some, virus and virus infected cells can also trigger the alternative complement path. Interestingly the tumor cells can also trigger the complement pathway and some parasites can also trigger the complement pathway.

Here now you can see that this complement can be triggered by all kinds of microbial pathogen that is gram positive bacteria, gram-negative bacteria, viruses and parasites. In addition modify, itself better to say that modify itself can also trigger the complement

pathway. For example this the normal cell which is turned to the cancerous cell they can also trigger the alternative pathway.

In addition bigger parasites can also trigger the alternative pathway there are some non-pathogen factors are also there which is which can also trigger the alternative pathway. Here you can see that, there are some immunoglobulin like IgG, IgA and IgE in complexes they can they can trigger the alternative pathway. So immunoglobulin G complex in complex form this which is originated from rabbit and guinea pigs they can also trigger the complement pathway.

It is very interesting to learn that the cobra venom can also trigger the alternative pathway and heterologous source the RBCs or, red blood cells it is then this can also trigger the complement pathway. Interestingly some anionic polymer which is like a dextraon sulfate and pure carbohydrate like agarose or inulin they can also trigger the alternative pathway.

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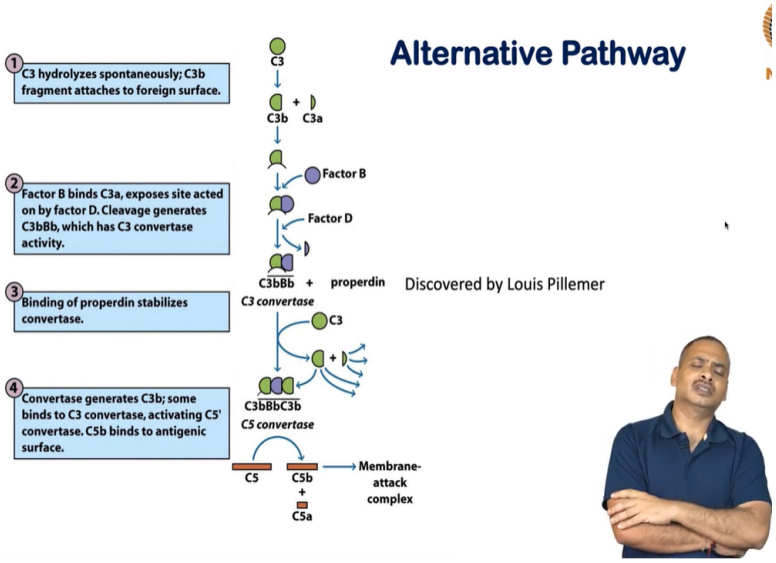

Now you look at how this alternative pathway is operated we will basically look at the initial part because later part is same as classical pathway. So first there will be a, hydrolysis of C3 which is spontaneous. And then this hydrolysis result to the formation of C3b which will attaches or attach to the foreign surface. Foreign surface mean the bacteria or parasite, viruses so and so forth as you have seen in previous slide.

And once this C3b is generated then there is a factor B this factor BD binds C3a and exposes a site acted on the factor D. So here you can, see that this factor B will be basically binding and with C3b and then this factor B will be cleaved by factor D it if factor D has a property which

will trigger the cleavage of factor B. And then there will be a generation of a factor B means B is b that will be the larger fragment of factor B and there will be a generation of factor B a.

And once this C3b, bind with factor B, b then that will acquire the enzymatic activity and that enzymatic activity is C3 convert phase activity here you can see. And all these things basically stabilized by a very important molecule known as properdin. So properdin is basically this stabilizes the C3 convertersase which is consists of C3b, B is b so here there will be a larger, fragment of C3 and larger fragment of factor B.

So this makes a complex and this complex is acting as a C3 convertase and this complex is basically stabilized by properdinanine it binds with this C3 converter is basically bind with this property and it basically stabilizes this complex. So this property was discovered by Louis Pillemer and basically there is a little interesting point with him, that he was basically initially he was a biochemist and he turned to the immunologist.

So he basically purified since he is a biochemist he purified a tetanus as well as diphtheria toxin. And he basically his work resulted to the generation or development of DPT vaccine which is a very important vaccine if you see the vaccination of small kids. So he discovered that as well as he, discovered the properdin name so initially when he discovered properdinty it was not accepted by the scientific community.

But later on after few years he again repeated the experiment and then he established that this properdinty is playing very important role in alternative pathway. So once this C3 convertase is generated then of course there will be a lot of generation of C3b and there are, 2 fate of C3b one fate will be it will be deposited to the target microbe or microbial cell. And then it will be readily phagocytosed it will be opsonizedxidized and it will be readily phagocytosed by the phagocytic cells.

Another fate will be this will generate the C5 convertersase convertis here you can see that the C3b, B, b which is a Cc 3 convertase convertis now interacting with C3b, one more unit of C3b and that acquires C5 convertase activity. And this C5 convertaseconverter is activities needed for the generation or for making the membrane attack complex there are several molecules which are involved in formation of membrane attack complex. So this I will not discuss I have discussed in classical pathway so it is not needed.

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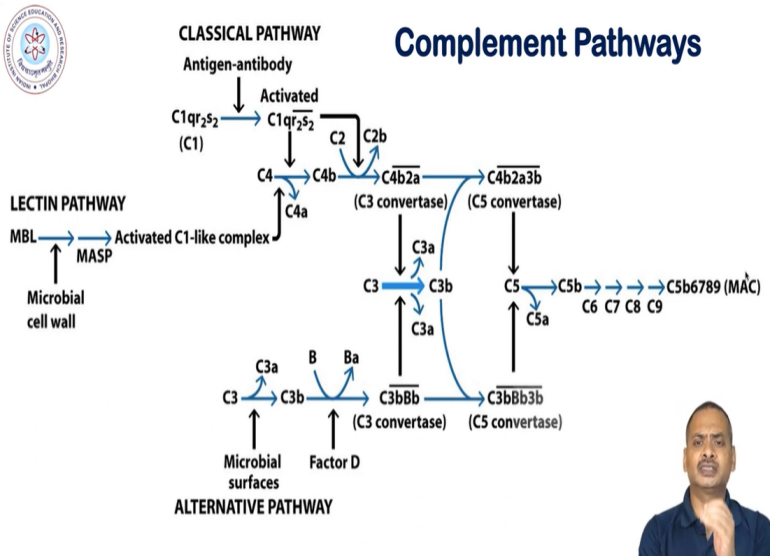

Here you can see that this is another plan or, schematic here you can see that initially C-3 is break down into C3a and C3b. C3a will be anaphylotoxin and this C-3b will be interacting with a factor B larger fragment that is C3b, B, b which has a C-3 convertase~~converters~~ and this will be mediated by a factor D. And the C3 converters is stabilized by properdin~~ty~~ and then there will be a generation of C5 convertase~~converters~~.

Here you can see that C5, convertase~~converters~~ is C3b, C3b, B, b C3 b so this C 5 convertase ~~convert~~ is so here you can notice that C3 convertase in case of classical pathway is composed of different protein complex. In case of classical pathway it is consists of C-4-b and 2a and the C-5 convertase~~convertage~~ is also different this is C-4-b-2-a-3-b. So this is a one of major difference please, remember that think the C-3 convertase~~converter~~ is in case of classical pathway is different and alternative pathway is different.

Once the C 5 convertase~~converters~~ is generated there will be a series of proteolytic cascade will be there and eventually there will be a formation of membrane attack complex. And this also involves the formation of a membrane attack complex also involves C 6, C 7, C 8 and C 9.

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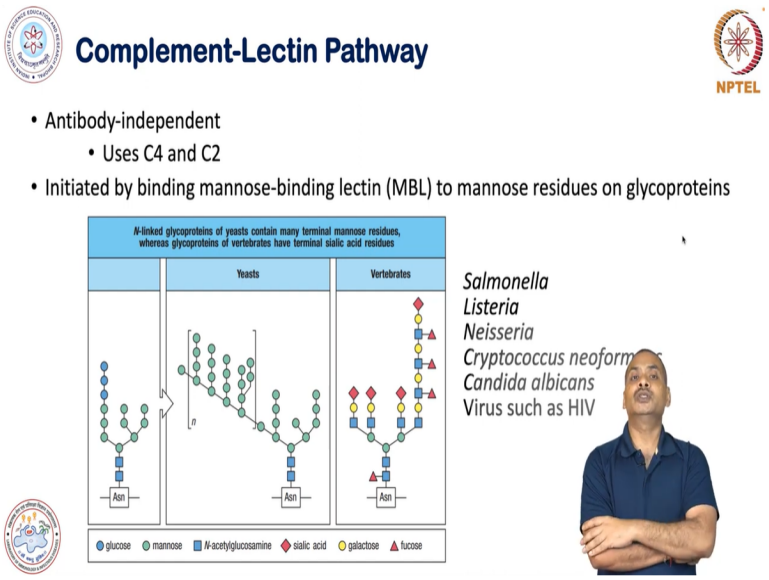

Now I will take, you to the lectin pathway and this lectin pathway are basically again antibody independent and it uses C4 and C2. And it is basically initiated by binding with mannose binding lectin which we call it as a MBL. Mannose binding lectin to mannose residue which is present on glycoprotein of microbial pathogen so you know that these proteins are there are the presences of protein over the microbes.

And these protein are having some sugar residues and in case of microbe the terminal residue is mostly or mainly the mannose. So this MBL can bind with these mannoseers and that can trigger the lectin pathway. Here you can see for your convenience I have a very good schematic here you can see that yeast has a lot of mannose and MBL can bind with the mannose. But on, another hand in case of vertebrate proteins they are generally terminated with in general terminated with sCialic acid.

And this scialic acid basically this is a kind of distinguishing feature now I will tell that this lecaetin pathway can be triggered by Salmonella can be triggered by Listeria, Neisseria, Cryptococcus neoformens. So this is a Cryptococcus neoformens is a pathogenic, fungus candida albican is also pathogenic fungus and this can be the lectin pathway can be also triggered by viruses such as HIV.

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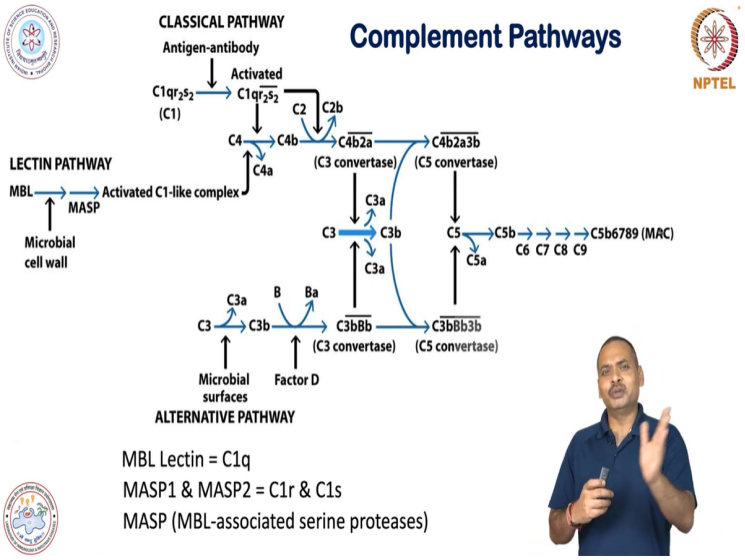

So here; you can see the lectin pathway and this lectin pathway is a basically initiated by MBL mannose binding lectin. So MBL if you if you remember the classical pathway there is a C1 subtype known as C1q. So MBL is like a C1q and there is a protein known as, MASP there are 2 kinds of MASP 1 is MASP 1 and MASP 2. So MASP 1 is like a C1r and MASP 2 is like a C1s.

So here there is a good comparison between classical and lectin pathway that what I am highlighting and MASP 1 is basically MBL associated serine protease. And this basically trigger the lectin pathway and the downstream pathway is similar or same as a classical pathway the C4, will split into C4a and C4b. And this C4a basically interact with C2b which will be split into C2 will split into C2a and C 2 b.

And this c4 ~~for~~ a 2~~to~~ b or 2a has a enzymatic activity the C3 convertase~~converter~~ is activity which will break C3 into C3a and C3b. And this C3b will also make a complex with C 3 convertase~~converters~~ which is consists of C4 ~~for~~ b and 2a and the C5 will be 3 b will be also, present in that complex and that will have a C5 convertase~~converters~~ and eventually that will make a membrane attack complex. So this is a all about the alternative pathway and lectin pathway here I have a one very nice video which you can see and this will basically clear your all this all this pathway.

Because there are a lot of complicated thing like a b and all those things so, it is little difficult to remember. So in order to understand more clearly I have a one very nice video and you can watch it.

(Video Starts: 19:52)

The complement system is an important part of the innate immune response and plays a major role in the killing and clearance of invading pathogens including bacteria, viruses, fungi and parasites. The system is composed of over 30 proteins most of which are found in, serum while the remaining proteins are membrane-bound proteins and receptors. On activation complement proteins that participate in the activation of either the classical or alternative pathways of complement.

Interact in a highly specific enzymatic cascade and generate proteolytic fragments that mediate the numerous biological functions of the complement system. In this video we will focus on the, activation and biological functions of complement. Activation of the classical pathway the classical pathway is typically activated by antigen antibody complexes. Although there are many other activators of this pathway only 2 isotypes of antibody IgM and as shown here IgG will activate the classical pathway.

While one pentameric IgM antibody will activate the classical pathway a minimum of 2 IgG, antibodies in close proximity are required. In either case antibody must be bound to antigen in order to activate the classical pathway. The classical pathway is activated when C1 the first protein in the pathway binds to the FC portion of the antibody. C1 is a large macromolecule composed of a C1q molecule and 2 molecules each of C1r and C1s.

It is the globular head portion of the C1q that binds to, the fc binding site on the antibody this binding causes a conformational change in C1q in subsequent autocatalytic conversion of C 1r to an active serine protease. Activated C1r cleaves C1s to an active serine protease activation of C1 by binding antibody is also termed complement fixation. The next step in the activation of the classical pathway is the cleavage of C4 by the activated C1 molecule.

C4, is cleaved by the activated C1s molecule in C1 into 2 proteolytic fragments a small peptide called c4a and a larger fragment C4b. C 4b attaches covalently to the antigen antibody complex and has a binding site for C2 the next protein cleaved in the classical pathway. C2 is also cleaved into 2 fragments and the resulting C2a fragment binds to C4b this bimolecular complex of C4b2a is a C3 convertase, of the classical pathway and serves to cleave C3 through the enzymatic activity in the C2a portion of the complex.

Classical pathway C3 convert days the C molecule; is cleaved by the C3 convertase into C3a and C3b. The C3a fragment has potent biological activities which will be discussed shortly the

C3b fragment can covalently attach to the C3 convertase resulting in a trimolecular complex, consisting of C4b 2a 3b.

This multimolecular complex is called the C5 convertase of the classical pathway and is specific for cleaving C5. Classical pathway C5 convertase the C5 molecule is cleaved by the C5 convertase into C5a and C5b both of which mediate important host defense functions of a complement system. Both C3a and C5a are potent chemo attractants C3a attracts mast cells to sites of, complement activation and binding of C3a and C5a to these cells as well as basophils induces degranulation and release of histamine and other vasoactive amines.

C5a is a chemo attractant for macrophages and neutrophils and binding of C5a to these cells primes them for mediating their host defense functions. The terminal complement pathway or formation of the membrane attack complex one of the best, known host defense functions of the complement system is its ability to lysei~~e~~ as many bacteria envelope viruses and nucleated cells.

Lysis~~Slices~~ of cells by the complement system are mediated by a large macromolecular structure called the membrane attack complex or mac. The mac is formed through what is called the terminal complement pathway which starts with the generation of C5b. C-5-b associates with C6 C7 and, C8 forming a large multi-molecular complex that associates with and begins to disrupt the cell membrane.

Subsequently C9 binds to C8 and this is followed by the binding of many additional C9 molecules leading to the formation of a large pore in the cell membrane. The pore in the membrane is large enough to allow water ions and small molecules to enter the cell leading to lysis. Activation of the alternative pathway of complement is activated by LPS and other lipopolysaccharides found on the surface of invading pathogens.

The alternative pathway is always active at a very low level this activation is due to the spontaneous hydrolysis of an internal file ester bond in C3. Hydrolyzed C3 called C3 water has a binding site for factor b another protein in the alternative, pathway. Factor b binds to C3 water and becomes a substrate for cleavage by factor d denoted here as fd factor d cleaves factor b into 2 fragments ba and bb.

The enzymatically active bb fragment remains associated with C3 water and this bimolecular complex is the alternative pathway C3 initiation convertase alternative pathway C3 initiation

convertaseconvert days. This initiation convert case cleaves C3 into, 2 fragments C3a and C3b the newly generated C3b can covalently attach to nearby surfaces just as C4b does in the classical pathway.

In the absence of an appropriate activator for the alternative pathway complement regulatory proteins block further activation of the pathway. However; C3b on interacting with an activating surface such as a bacterial cell will bind factor b which will be cleaved by, factor d resulting in the formation of the alternative pathway. C3 convertase of C3b bb alternative pathway C3 convertase the C3 convertase of the alternative pathway leaves C3 to C3a and C3b.

Some of the C3b generated by this cleavage does not interact with the activating surface and remains soluble some of the C3b generated will covalently interact with the activating surface and start the, formation of additional alternative pathway convertases. Thus rapidly amplifying activation of complement through this pathway the surface of an activator such as a bacterial cell can rapidly be covered with many thousands of C3b molecules and a few minutes as a result of this amplifying effect.

And as with the classical pathway some of the C3b generated will interact with the C3 convertase itself, resulting in the formation of a trimolecular complex of 2 C3b molecules and bb. This multi-molecular complex is called the C5 convertase of the alternative pathway and is specific for cleaving C5. The C3a and C5a fragments generated by activation of the alternative pathway mediate the same functions of chemoattracting and activating granulocytes and phagocytic cells.

C3a attracts mast cells to, sites of complement activation and binding of C3a and C5a to these cells as well as basophils induces degranulation and release of histamine and other vasoactive amines. C5a is a chemoattractant for macrophages and neutrophils and binding of C5a to these cells primes them for mediating their host defense functions. Activation of the alternative pathway results in the formation of the membrane attack, complex leading to the lysis of the invading pathogen.

LysisSlices of cells by the complement system are mediated by a large macromolecular structure called the membrane attack complex or mac. The mac is formed through what is called the terminal complement pathway which starts with a generation of C5b. C5-b

associates with C6 C7 and C8 forming a large multi-molecular complex that associates with and begins to, disrupt the cell membrane.

Subsequently C9 binds to C8 and this is followed by the binding of many additional C9 molecules leading to the formation of a large pore in the cell membrane. The pore in the membrane is large enough to allow water ions and small molecules to enter the cell leading to lysis.

(Video Ends: 30:53)

So you have seen this is a very nice video which was a presenting in a very systematic manner, you can understand there are so many a, and b and all those things so it is little complicated initially to understand and to remember. But you have to understand and try to remember at least the key steps of the complement that is what are what kind of C3 converters are present in case of alternative pathway, classical pathway or lectin pathway and what is the composition of C5 convertase~~converters~~ in, these pathways.

So I will stop this session over here and in next session I will discuss about the regulation of this complement pathway because you can understand if it is dysregulated then that will result to the various level of complication. And I will also discuss about some diseases associated with complement deficiency so see you in next session thank you.