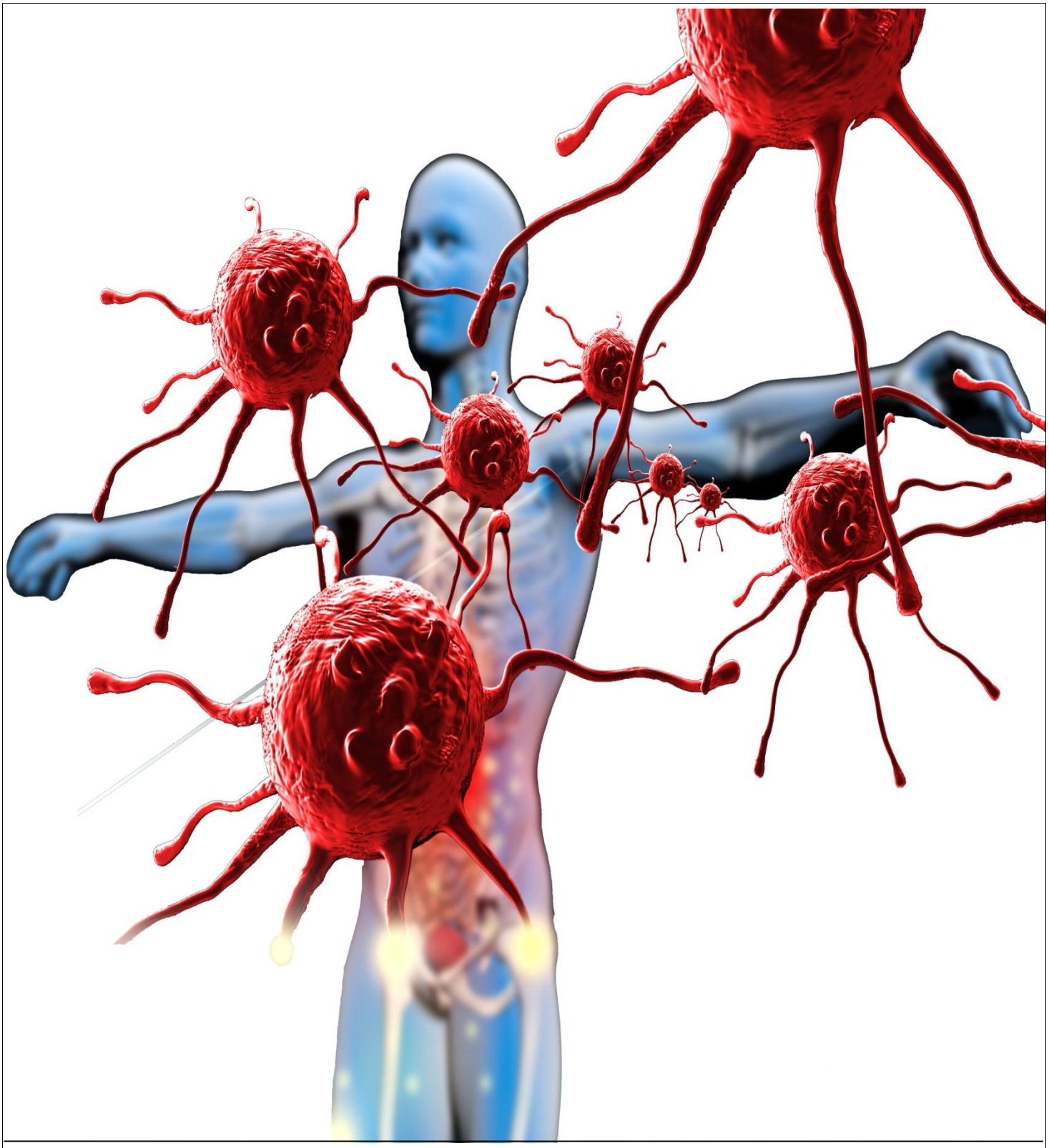

IMMUNOLOGY

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6

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Immunity lecture #6

Complement system

#Functions of complement system:

1-opsonin.

2-lysis.

3- Production of inflammation .

4-chemotaxis.

5-clearance of immune complexes .

6- Prevention of precipitation of immune complexes (when immune complexes are formed they will precipitate--> can be solubilized using complement system) .

Now, we will talk about complement system in more details:

Complement system is a collection of proteins, 30-40 or more, that are actually activated in sequence and release products that are responsible for the action of the complement system.

Of course, there are 3 ways by which complement system can be activated:

1- **Classical pathway**: it's called classical; because it was the first to be discovered .

2- **Alternative pathway.**

3- **Mannan lectin pathway** (mannose binding proteins pathway) .

Before we talk about these pathways, let's talk a little bit about nomenclature of the complement system. The main proteins, usually, are given the name "**components**", usually these components that can be activated, we have nine components c1-c9. All of complement proteins are usually present in their inactive form (except one protein which is **factor D**), they need to become activated in order to continue the cascade of activation from one

component to another. Most of these proteins are soluble, they can bound to cell membranes.

Other proteins are given the name "**factors**" example: factor I, B, D, H. Those proteins are not components, they are factors because they are really important .

There's another way for naming the complement system: **trivial name**, it describes the function of proteins, for example C4 binding protein, what does it mean?! It's actually protein that binds C4. Another example is complement receptor 1 this is the first complement receptor ,which is present on other cells.

During activation of the complement system, there will be breakage of some bonds ,so really it's enzymatic reaction, so **thioester esterases** are needed to break down proteins , these proteins will activate another proteins in the cascade. So in the beginning there will be an inactive protein--> will be activated by esterases--> this protein will be broken down into two pieces -->the piece that has been splited is the active one, which will do the job, if it can't perform the job, it needs to be inactivated. The half-life of these broken products is very short, it is measured in milli seconds, so the complement system is a very destructive system.

If you come across some examples:

$\overline{C3b}$: c3 means its the third complement protein, b means it's a break down product of c3 and the line on top of it indicates that this is the active form of C3b. The activity does NOT pass long, just few milli second then it gets inactivated. Once it become inactivated we call it: **i**C3b, the (**i**) stands for **i**nactive.

#Note: we don't usually use the line to indicate that this is the active form of complement system. It's understood when we just use c3b that this is the active form.

So when we break down a complement protein like c2,c3 , c4, usually we will get two pieces: one small piece will be given the suffix (a) or c3a , and another one which is the bigger one will be given the suffix (b) or c3b. So, during complement activation once you cleave c3 you will get two pieces: c3a (small) and c3b (large).

Now, this has been introduced you to nomenclature and general distribution of complement system, and let's talk about pathways of activating complement system.

First pathway is the classical pathway

It's really part of the adaptive immune system, why?! Because it's usually activated by immune complexes (antibodies + antigen) ,the site of contact between the complement c1 and cH2 constant domain in the immune globulin is not exposed but once you get an immune globulin attached to its antigen , this site of contact will be exposed on the complement protein and then you will get activation of this protein. It does make sense that antibodies on their own do NOT activate the complement system, only immune complexes (antigen + its antibody) will activate the complement system but sometimes, certain bacteria or viruses can activate the complement system.

Wiki says about initiation of classical pathway: It is triggered by antigen-bound antibody molecules. (The only antibodies capable of binding are two units of IgG or one IgM, although IgM is more effective at activating complement. IgG4 cannot bind, but the other three IgG types can.) It is the binding of Fc region to the C1 component that initiates this pathway. The classical pathway can also be triggered by binding of C1 to some types of structures on the target microbe. Also, it can be triggered by C-Reactive Protein binding to microbic polysaccharides such as phosphocholine.

The first component that will get activated in the classical pathway is : C1 its made of 3 polypeptide chains, 6 globular heads and collagen like sticks this is known as c1q, there are 4 molecules that are contained between globular heads(not sure) : c1r and c1s. So we have one c1q molecule, two c1r and two c1s.{ $C1qr^2s^2$}

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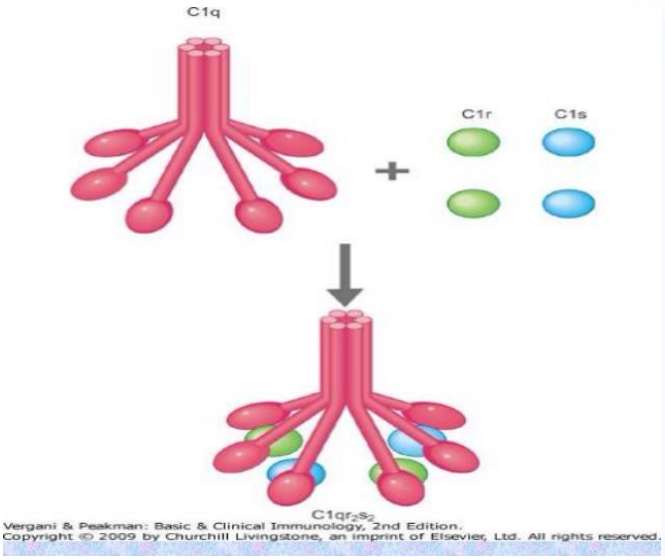

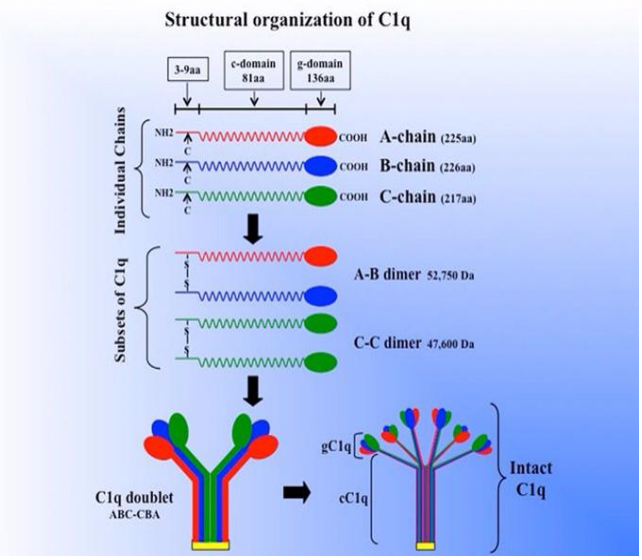

In order to activate c1, you need an immune complex (antibody+ antigen+ activated cell (sRBC)), ch2 domain of these antibodies is exposed and can bind to globular heads of c1, it's a provisor in order to have activation of c1, you need to have at least two globular heads to be engaged on the same c1 molecule. So you need to have a lot of igGs attached to the activated surface so that you can have double engagement of two globular heads and that's actually give rise to complement activation. With IgM it easier, why?! Because IgM has got many many Fcs; because it's a pentamer so it has a great opportunity to engage two adjacent globular heads on the c1 molecule. So we conclude that IgM is a more potent activator of the classical pathway than IgG.

Once this happened, you will find some of conformational change in the c1 molecule, and this conformational change exposes the active site of c1r which will act as serene protease and it will act on c1s, c1s become active and acts as a serene protease.

In brief: conformational change of c1 --> expose the active site of c1r--> c1r activates c1s--> c1s gets active.

So what happens now?! (See the picture below as you are reading the following text) C1 complex (c1+ immune complex) --> conformational change of c1--> activates c1r--> activates c1s--> any c4 molecule passing by in the environment will bind --> once c4 binds--> c1s will act on it cleaving it into two pieces: c4b (big) and c4a(small) --> c4a will go into the circulation but c4b stays attached to the activated immune complex --> c4b sitting their become attracted to c2(second component of the complement) so it binds the c2, once the c2 is bound it will be cleaved by c1s(enzymatic activity, c4b doesn't

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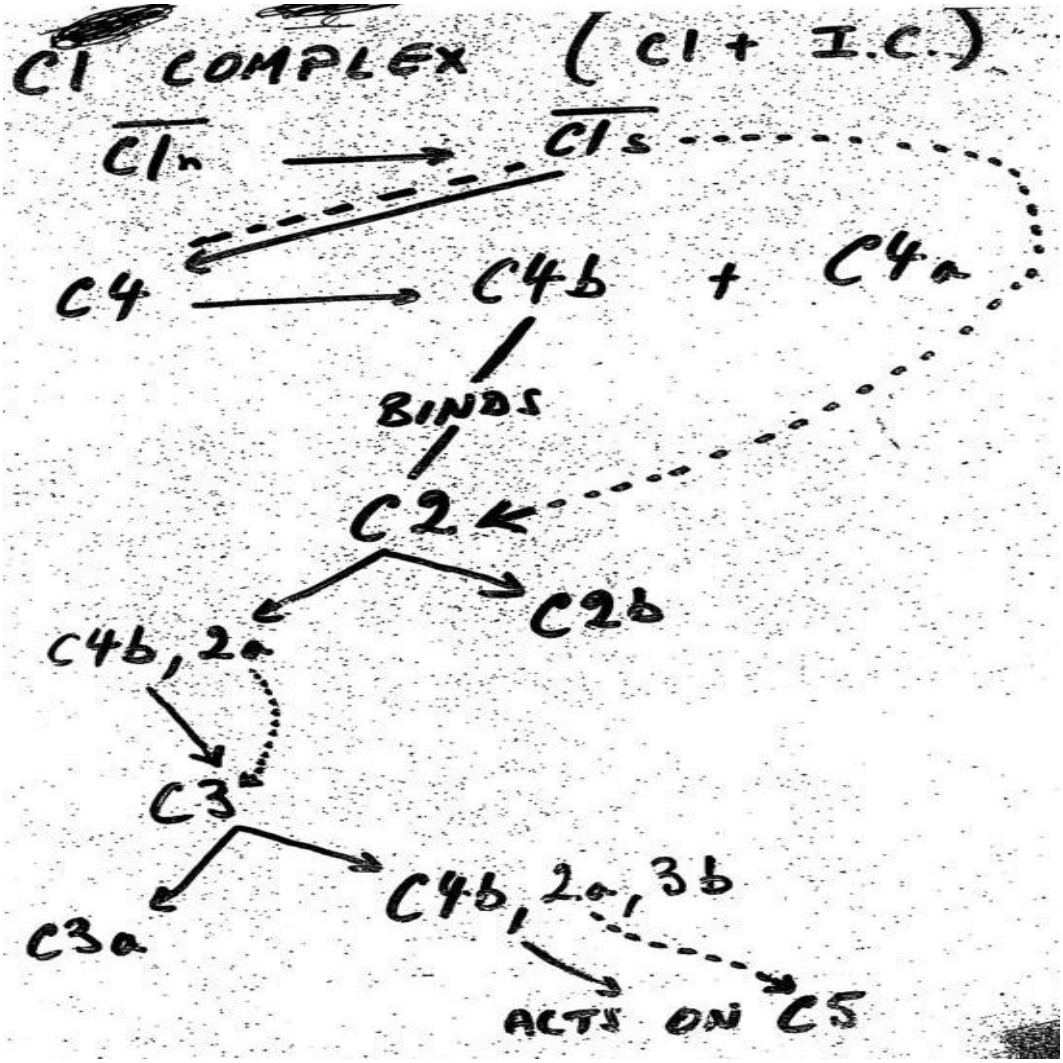
have an enzymatic activity) into two pieces : c2a (small) will be attached to c4b , and another piece which is c2b released into the environment--> now we end up with [c4b,2a] on the activated surface --> **[c4b,2a]** which is called **c3 convertase of the classical pathway**, will get attracted to c3 once c3 is bound, the enzymatic activity is done by c2a --> cleaving c3 into two pieces: c3b (big), c3a(smaller). c3a will go to the solution in the environment ,while c3b is bound with the rest of complex--> now we end up with**[c4b,2a,3b]** which is called **c5 convertase of the classical pathway**, on the activated surface --> attracted to c5, the caltalytic activity is driven by c2a. c5 will be cleaved into two pieces: c5b (big) and c5a (small).

#Simple note: when we say c4b,2a --> it means c4b and c2a .

Why does cleavage of c4 occur before c2 or why the sequence is not c1 then c2 then c3 then c4 ? Because they discovered the first component and give it the name C1 and then discovered the 3rd component and give it the name c2 and after the discovery of C3 they discovered the 2nd component and give it the name C4 (so the numbers after the {C} is based on the sequence of discovery)



C3b(mainly) or c4b can attach to the activated surface which can be a bacterial cell or red blood cell or immune complex... Whatever! These c3b or c4b have receptors present on many cells known as complement receptor 1 present on macrophages as well as neutrophils--> so what happens then? Anything covered by C3b or c4b will be attached to macrophages by these receptors .

Remember when we talked about antibodies that will attach to the bacteria, these antibodies will bind receptors found on macrophages and neutrophils--> so they work as opsonins . It's the same here! Anything(bacteria) that is covered by c3b will bind complement receptor 1 found on macrophages(mainly) and neutrophils--> so c3b work as an opsonin --> macrophages will phagocytize the pathogen.

This is the first function on complement system which *is* **Opsonin** .

Also, red blood cells have CR1(complement receptor 1) on their surfaces, if you have immune complexes in the blood stream, they will come in contact with RBCs, the immune complex will activate the complement system --> deposit c3b on the immune complexes and they will attach to the RBCs, so really you can notice that RBCs are like small magnets that are floating in the blood, while immune complexes are like Iron filings, all these immune complexes will attach to RBCs through the interaction between CR1 and c3b which is present in the immune complexes--> RBCs will go to the spleen --> splenic macrophages will interact with these RBCs--> CR1 on the macrophages has more affinity toward c3b and immune complexes than CR1 found on RBCs so CR1 found on macrophages will attach to immune complexes and take them out from RBCs. So we conclude that RBCs are weak magnets while splenic macrophages are strong magnets that will attract immune complexes and will destroy these immune complexes. So RBCs have two functions: transport of O2 and CO2, and function in clearance of immune complexes through CR1 receptors present on their surfaces.

This is the function #5 (check the first page) of complement system which is clearance of immune complexes.

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Now let's talk about ***the second pathway of activating complement system: The Alternative pathway***

The major complement system protein c3 is very important

Most proteins of the complement system are synthesized in the liver, others are synthesized in the adipose tissue and macrophages also can produce some of these proteins like c3 and c4.

C3 has a biggest concentration in the serum and what happens actually to c3? You have continuous activation and break down of c3 component.

(see the picture below as you are reading the following text)

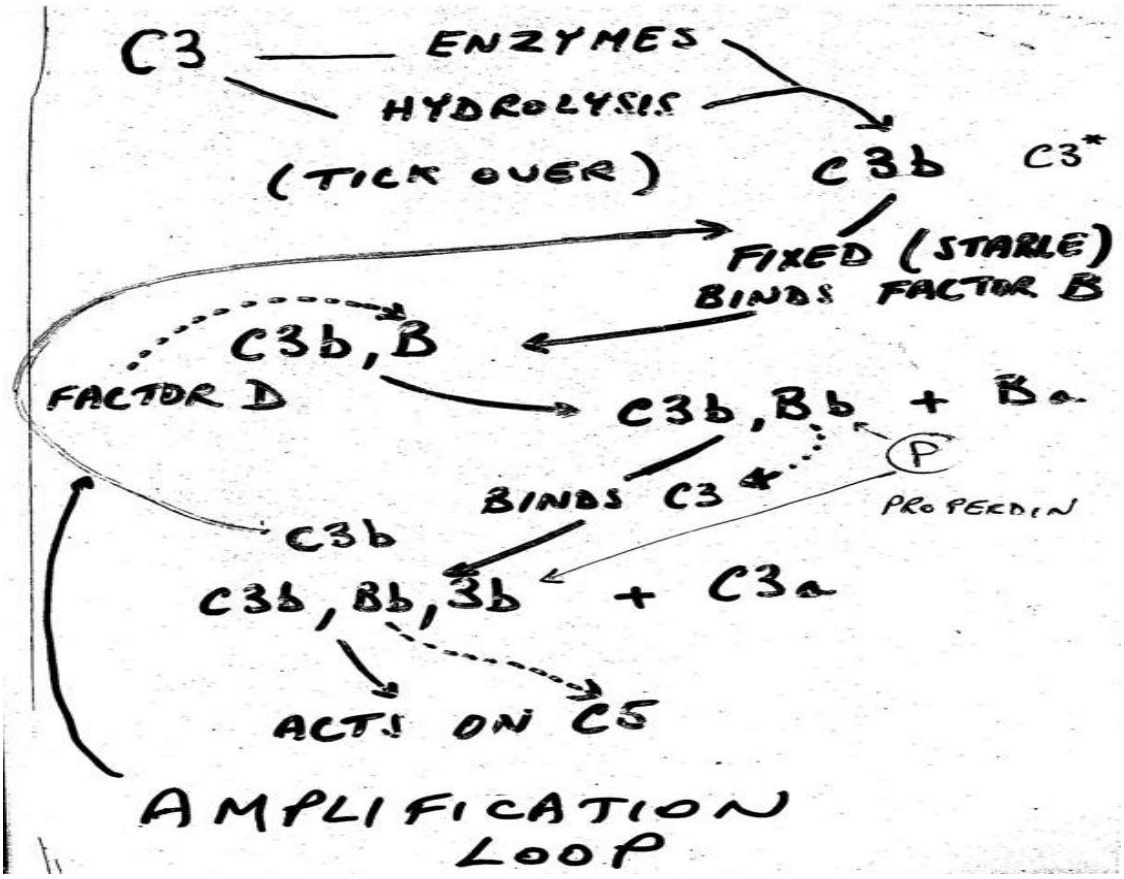
Some scientists said that c3 is hydrolyzed by water to become active c3 for a temporary short period, others said that some enzymes catalyze the activation of c3 --> whatever its, whether c3 undergoes hydrolysis or under the effect of enzyme, the net result of continuous activation of small amount of c3 enable us to have continuous activated c3 --> c3b or c3* will result (continuously produced but at the same time it is destroyed very quickly) --> c3b will activate other proteins in the activation cascade, this is called **tick over mechanism**(means that the alternative pathway is all the time active) --> if c3b meets the foreign surface (bacteria, virus, dead tissue), it will stick to it and once it sticks to it , it becomes protected against destruction by regulatory proteins of the complement system, so it really gives a chance for continuous activation of the complement system, and that's why its nonspecific, there's no immune complex start the activation as in the classical pathway, here in the alternative, activation occur by sticking to any foreign surface in general, whether bacteria or virus or whatever it is! And this is nonspecific, that's why the alternative pathway belongs to natural (innate, nonspecific) immunity. Indeed, the alternative pathway is more ancient, and the more advanced or more recent is the classical pathway --> lets go back! C3b is sticking to a foreign surface--> it will bind factor B(which is another protein of complement system , we call it (B) probably because it belongs to the beginning of the alternative pathway) --> c3b + factor B are on the surface --> another protein swimming in the serum in its active form which is **factor D** (probably it's the only complement protein that is found in its active form) --> factor D binds [c3b + factor B] --> factor D cleaves c3b producing two pieces: Bb (large) and Ba (small). Ba will go to the circulation, while Bb remains bound to c3b--> we will end up with **[c3b, Bb]** we call this complex **c3 convertase of**

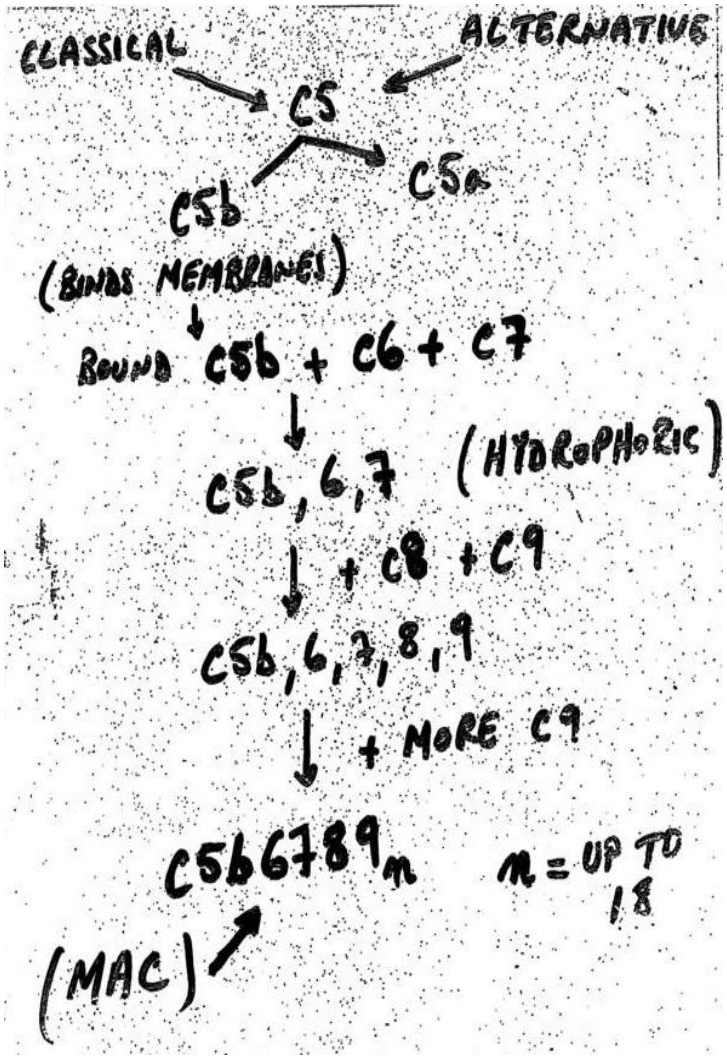
the alternative pathway, on the activated surface--> bind c3, Bb becomes enzymatically active, cleave c3 into two pieces: c3b (big) and c3a (small). C3a will go to the solution, c3b remains attached--> now we end up with **[c3b, Bb, 3b]** we call this complex **c5 convertase of the alternative pathway** --> cleave c5 into two pieces: c5b (big) and c5a (small).

There is a protein called **properdin**(it's a positive regulator because it can bind to different phases of the alternative pathway and prolong their half-lives so gives more chance for activation ,it is an exception, because all of the regulator proteins of the complement system are negative (destructive))

All of them, actually, are enzymatic activities, means that each c5 convertase will convert 50-60-80 molecules of c5 not only one, you know that enzymes can work on different substrates and break down. Same for c3 convertase, it's an enzyme that will activate many many c3 molecules.

When you produce more c3b by c3 convertase, these c3b molecules ,on their own, can sit on the activated surface and produce the cycle all over again-> c3b bind factor b ....drive the activation of alternative pathway [this is known as the **amplification loop**] the more you produce c3b, the more complement activation you will end up with.





Classical pathway and alternative pathway converge into c5, c5 is broken into two pieces: c5b (big) and c5a (small) . C5a will go into circulation while c5b will not join c5 convertase, but it has the ability to stick into plasma membrane --> c5b binds c6 (after this there is NO MORE cleavage, so c5 is the last component to be cleaved) --> c5b will bind c7 so we end up with c5b,6,7 on the activated surface, this complex become hydrophobic --> become inserted into the membrane--> bind c8 and c9, it can take more than one c9 up to 18, so we will have polymerization of c9 around this complex forming a donut or a cylinder so we will end up with hole in the plasma membrane--> causes leakage --> the cell will swell up--> burst--> **c5b,6,7,8,9n** is called **MAC** (membrane attack complex) ;n=up to 18. It attacks the cell membrane--> causes lysis

Chemotaxis: these small fragments of c5a (the most potent) , c3a which is less potent and c4a which is very very weak, these are known as **anaphylatoxins** , they act as chemotactic agents, also, they stimulate mast cell degranulation --> release histamine and also themselves have actions like histamine , they can attract Igs , activate neutrophils, affect endothelial cells and cause vasodilation just like histamine does--> we will end up with Inflammation (another function of complement system) .

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We said that immune globulin-antigen complexes will get bigger and bigger, precipitate (produce problems by blocking blood vessels), one of the functions of the classical pathway is to prevent these immune complex from precipitation. There are interactions between immune complexes (in addition to antigen-Ig interactions there are Fc-Fc interactions which actually join Igs together that will induce precipitation) so once you get activation of the classical pathway, c3b settle between fc fragments and the cell --> it will prevent accumulation together preventing their precipitation. So clearance of immune complexes is a function of the complement system more specifically the classical pathway.

If immune complexes are already precipitated due to any reason, this will stimulate solubilization of these complexes by alternative pathway, same mechanism as mentioned above.

Third pathway: Mannose binding protein [mannan lectin]

Mannose sugar is present on the surfaces of bacteria. Mannose binding protein is an **acute phase protein** produced in infections, it's nonspecifically produced in the liver , it seeks mannose sugar from bacteria and add saline , it acts like c1 . Associated with this protein, you will find proteins known as masp(masp 1,2,3) they are serene proteases, they do the work of c1s and c1r and activates the classical pathway .

So classical is very very adaptive while alternative is very innate.

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GOOD LUCK