Immunology course / 2017

Dr Heyam Awad

LECTURES 2: the innate immune system ½

Complement system

INTRODUCTION

As we agreed in the previous lecture, the immune system is composed of the innate and the specific or adaptive immune systems.

The innate immunity is very important to protect us from infections and it has three main components.

1. The complement system

2. Phagocytes: which are the neutrophils and macrophages

3. Natural killer cells.

THE COMPLEMENT SYSTEM

The complement system is composed of more than 20 proteins synthesized in the liver. They circulate in the blood in an inactive form. To perform their function these proteins must be activated by proteolytic cleavage. Each complement component is cleaved, when stimulated, by an enzyme (a convertase) that cleaves the complement components to two parts, a smaller part named a (like C3a for example) that is soluble and performs certain functions like acting as a chemotactic agent ,and a larger component termed b (C3b for example) which can be fixed to tissue. These b components are important for forming convertases needed to complete the cascade of cleavage (each activated component activates another) which results in activation of all the complement proteins needed for the innate immunity response.

Note: In humans complement system develops very early; in the first trimester (0-12 weeks) of pregnancy.

This activation of the complement cascade occurs via three pathways:

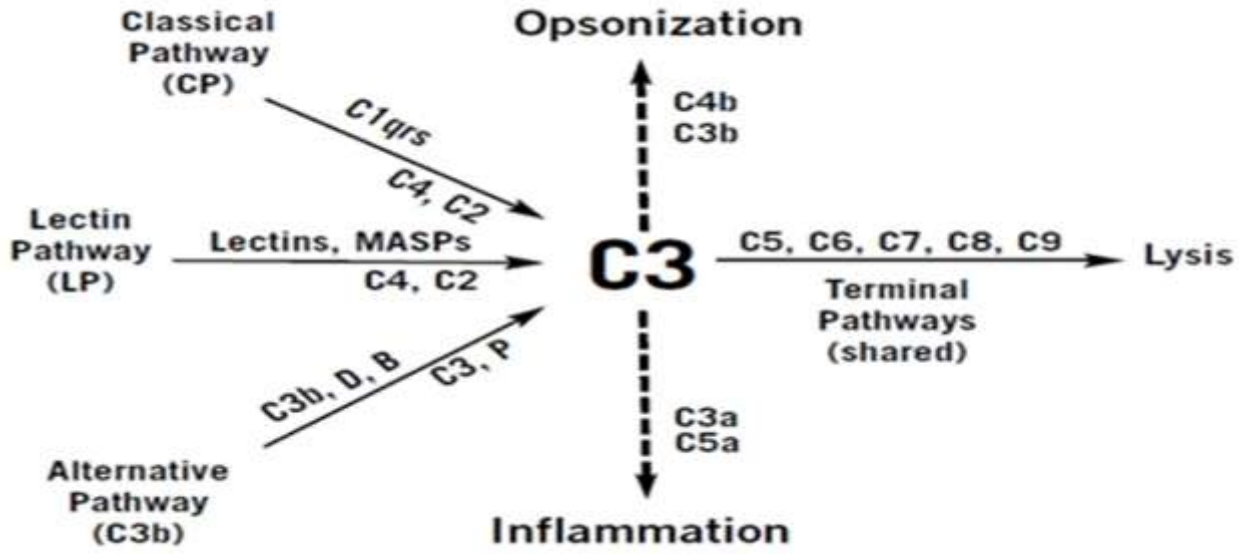
1. Classical pathway
2. Alternative pathway
3. Lectin mannose pathway

All the above three pathways cause activation **of C3 convertase** which plays a central role in complement effects. Once activated (by any of the three pathways) bacterial killing happens through three mechanisms which are:

1. Killing by opsonization: some complement components like C4b and C3b (sometimes called iC3b) coat pathogens so they are recognized by phagocytes and are targeted for killing

2. Formation of membrane attack complex (MAC) by C5- C9 and accumulation of several C9 proteins to form pores within the pathogens' membrane which results in water entry to the pathogen and death by lysis

3. Some complement components are used as inflammatory mediators (anaphylatoxins) like C3a and C5a. These kill pathogen via inflammation.

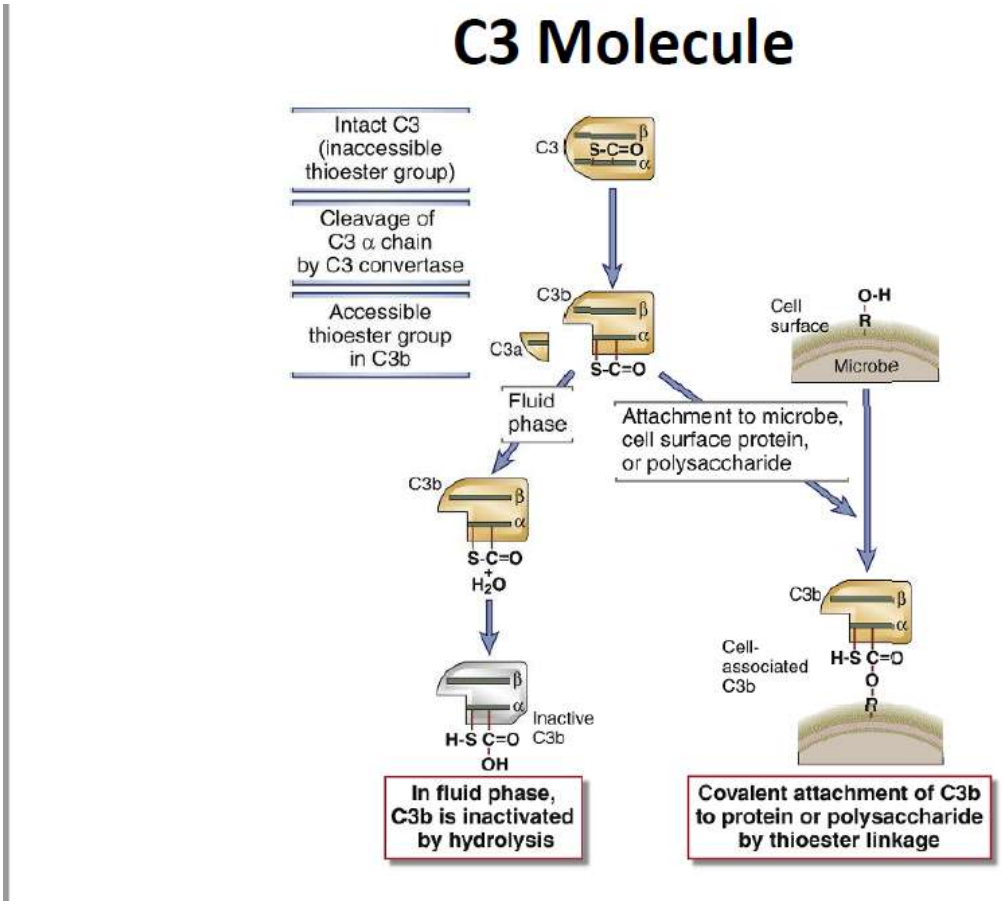


ALTERNATIVE PATHWAY:

C3 is the most abundant complement protein. It is continuously cleaved to c3b (spontaneous cleavage) even without the presence of pathogens. C3b is very reactive and can bind to amino or hydroxyl groups which are abundant on bacterial surface. If there is no bacteria and nothing to react with c3b is neutralized within 60 microseconds by binding to water.

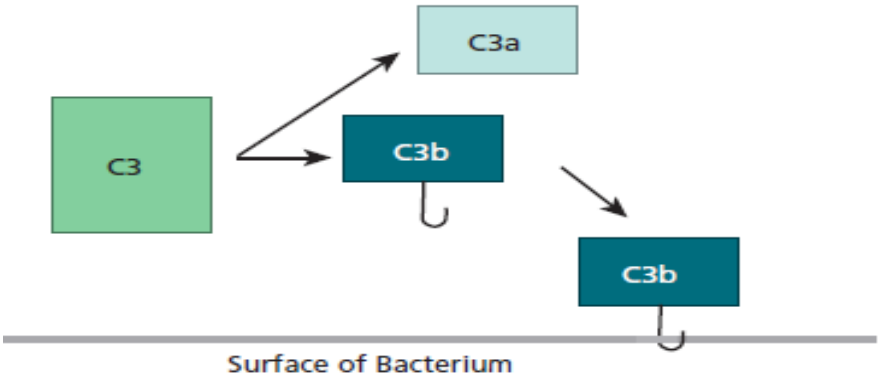
If C3b finds a hydroxyl or carboxyl group on a pathogen it is stabilized and another protein(protein B) attaches to it. Protein B is cleaved by protein D to create protein Bb Now the C3b and the Bb form **C3bBb which is the C3 convertase** of the alternative pathway.

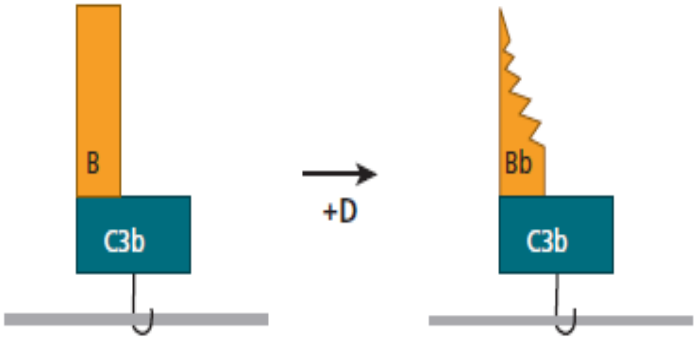
The C3 convertase cleaves more and more of C3.A chain of reaction follows, Another C3b will bind to C3bBb, making (C3bBbC3b);that can also be called C5 convertase. Then complement proteins are activated by cleavage done by the convertase enzymes and the C6- C9 form the membrane attack complex (MAC) which causes lysis of the pathogen.



Note from this figure that C3 contains inaccessible **thioester bond S-C=O**, cleavage makes the bond accessible in C3b. it is throgh this bond that attached to bacterial surface covalently

Steps of alternative pathway:





SO: C3 is continuously cleaved, why it doesn't attack our tissues:

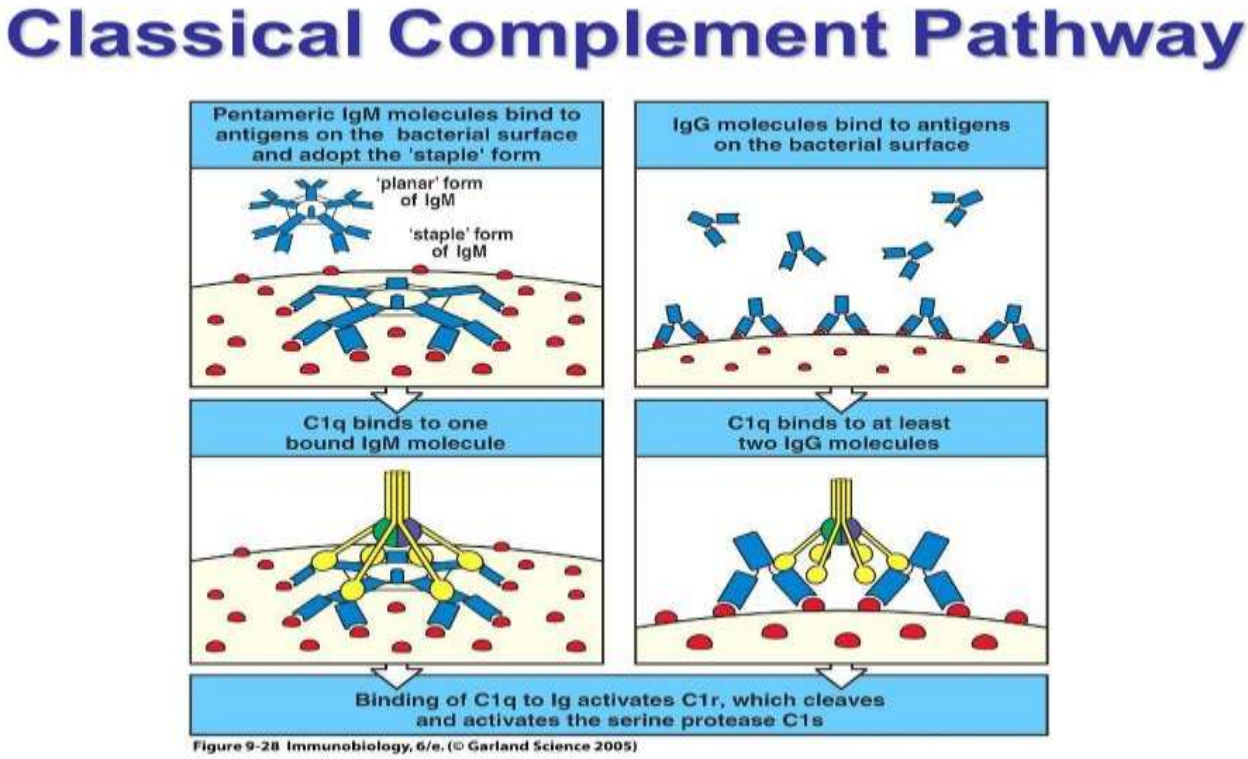
1. Because it will not be stabilized without reacting with a hydroxyl or amino group.
2. There are several proteins that inactivate the system which include

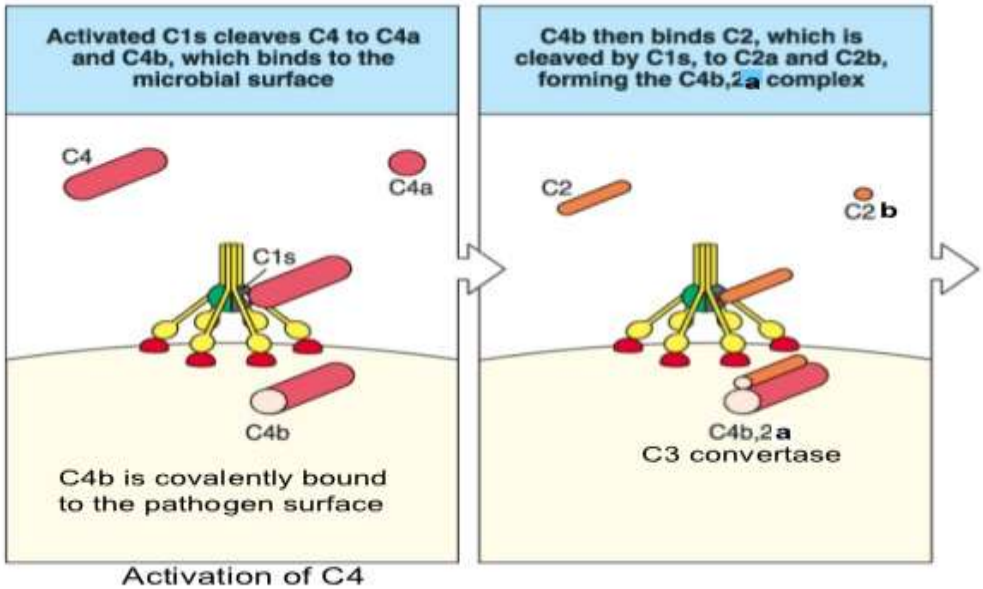
1. MCP (membrane cofactor protein) enzyme and CR1 (complement receptor one). These two which inactivates C3b.
2. DAF= decay accelerating factor can accelerate destruction of C3 convertase
3. CD59 prevents C9 to assemble and form MAC.

THE CLASSICAL PATHWAY:

The first step in this pathway is activation of the C1-complex. The C1-complex is composed of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, or C1qr2s2. The activation occurs when C1q binds to IgM or IgG complexed with antigen. Such binding leads to conformational changes in the C1q molecule, which leads to the activation of two C1r molecules. C1r is a serine protease. They then cleave C1s (another serine protease). The C1r2s2 component now splits C4and then C2, producing C4a, C4b, C2a, and C2b. C4b and C2b bind to form the classical pathway C3-convertase (C4b2b complex), which promotes cleavage of C3 into C3a and C3b. the cascade can now begin.

IMPORTANT NOTE REGARDING C3 convertase of the classical pathway: there is much confusion and disagreement in literature about the C3 convertase of the classical pathway, some people call it C4b2a, and others call it C4b2b. Our main reference for this course (Abbas et al) call it C4b2a. the dilemma came because some researchers decided to call the larger fragment of the C2 as C2a which is different from all other complement cleavage products! So the convertase is composed of the larger fragment of C3 (C3b) and the larger of C2 (C2a)! other people didn't agree with this awkward naming (why C2 is unique??? And why to use this strange exception), these people call the convertase C4b2b…. the main idea is to know that the convertase is composed **of the larger fragments of both C3 and C2** .





Note from the pic above that C2a is designated as the larger component!

Please note that only IgG and IgM can fix (stimulate) complement.

Mannose lectin pathway

Mannose binding lectin (MBL) is a protein produced from liver and is present in moderate amounts in blood and tissues. Lectin = means a protein that can bind a carbohydrate molecule.

Note that Mannose is a carbohydrate found on the surface of many pathogens

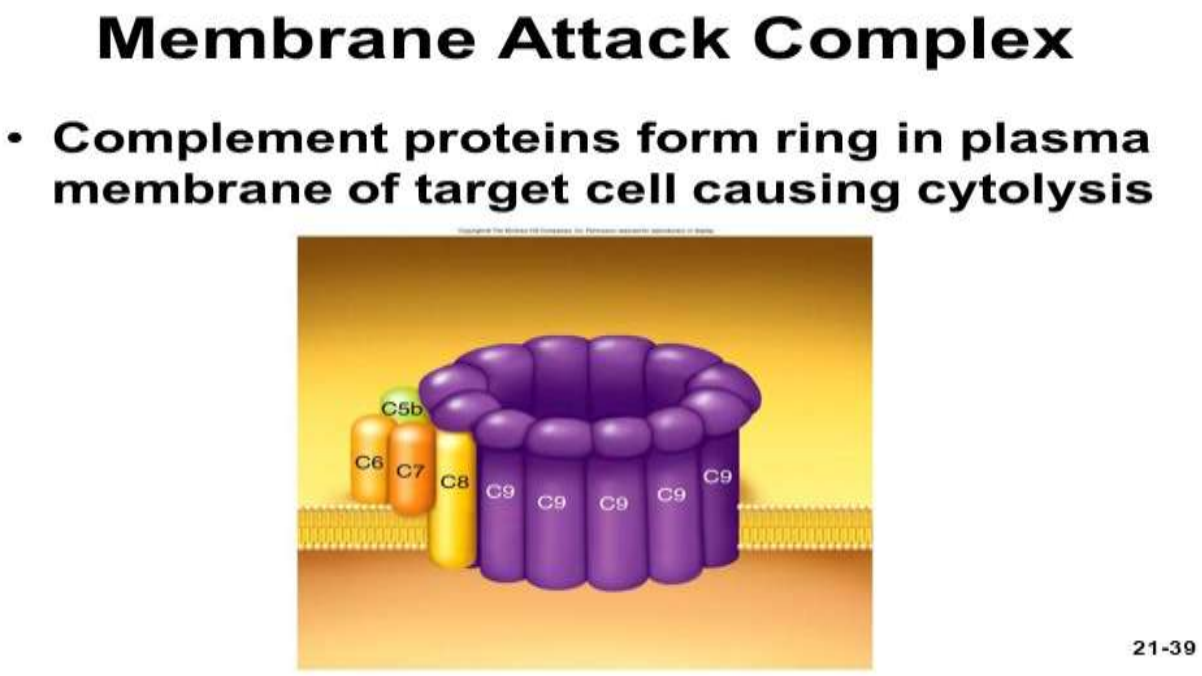
MBL binds to carbohydrates found on many pathogens but it doesn't react with carbohydrates on human tissues.

MBL circulates in the blood and it binds to another protein called MASP. When MBL recognizes a pathogen's mannose it binds to it and the MASP is activated.

Once activated MASP can directly C3 but it also cleaves C2 and C4 to form C4b2a (or C4b2b) which is a C3 convertase.

OK: again how the activated complement (b ant of the above pathways) kills pathogens:

1. by the MAC causing lysis of cells. Note: MAC is a complex of several complements, C9 is the most important, this complex attacks membranes of pathogens by creating holes within them.. This allows water to enter the cell.. Lysis happens



2. C3b acts as an opsonin (an antibody or other substance that binds to foreign microorganisms or cells, making them more susceptible to phagocytosis) .. It coats the pathogen and targets it for phagocytosis

3. C3a and C5a are anaphyltoxins that elicit an inflammatory response and act as chemotactic agents

SUMMARY:

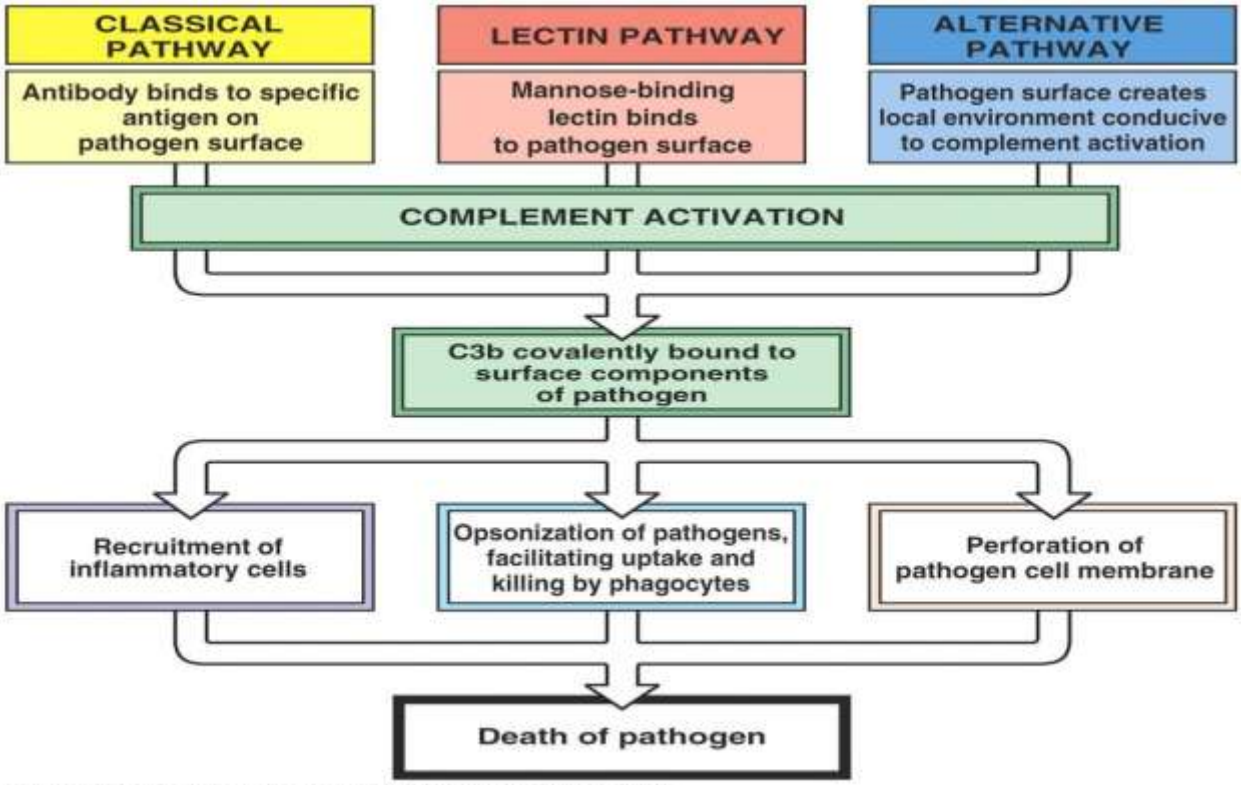


Figure 7-29 The Immune System, 2/e (© Garland Science 2005)

Case study related to the complement system: Hereditary angioedema

Hereditary angioedema is a disease caused by genetic deficiency in C1 inhibitor (C1INH), C1INH is a serine protease inhibitor (serpin). It is the sole inhibitor of C1 and is the most potent inhibitor of the classical pathway. It doesn't affect other complement activation pathways.

C1INH is a serine protease which also inhibits:

1. Clotting system

2. Kinin

3. Hageman factor (factor XII)

Note that the main product of the kinin system is **bradykinin** that causes **vasodilation and edema.**

HOW DOES C1INH work?

Patients with C1INH have C1INH inhibits C1r and C1s .It inhibits them by presenting them to a bait site: which is an arginine bond that they cleave . When they cleave this arginine bond they covalently bind to C1INH and are dissociated from C1q .Remember for the classical pathway activation you need C1qr2s2 complex. Because C1INH causes dissociation of this complex.It inhibits the classical pathway of complement activation.

Genetic deficiency of C1INH means results in **loss of inhibition of the classical pathway**. So active complement components are produced and also more bradykinin is produced. The active complement components are C4a ,C4b ,C2a, C2b. These are produced because there is no inhibition of C1. So there is more C1 complex that can cleave C2 and C4. Because C2 and C4 are continuously cleaved there is a decrease in C2 and C4.

The active components will be neutralized as long as there is no infection which means there will not be inappropriate activation of the complement system in these patients.

Note that other that C2 and C4 the rest of complement proteins are normal and not affected because the other pathways are not affected.

Because C1INH also inhibits kinin, if there is deficiency of C1INH, kinin is not inhibited which means **more bradykinin will be produced so the patients will have edema in several parts of their body**.

CLINICAL SYMPTOMS

Swelling in skin, intestine, extremities, face trunk, airway. Skin and submucosal swelling is not dangerous, it only causes temporary disfigurement

Swelling of the intestine causes pain, vomiting and watery diarrhea

Swelling of the larynx is dangerous, it can block the airway and can be life threatening

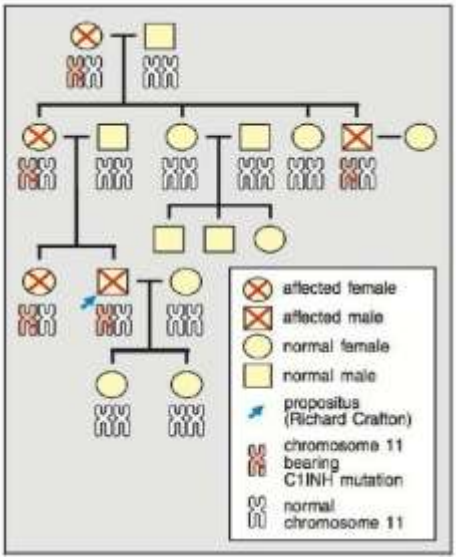
These patients do not have itching or hives.. This differentiates it from allergic angioedema (The disease is not allergic and not related to histamine)

Although there is decreased C2 and C4, patients are not at increased risk of developing infections because the other complement pathways compensate

NOTE THAT: **Bradykinin is the main mediator responsible for hereditary angioedema attacks**. This is because bradykinin causes vasodilation and increased vascular permeability by causing endothelial cell contraction.

INHERETANCE

Autosomal dominant



IMPORTANT NOTE:

Patients with the disease inherit one defective chromosome.The C1INH levels are decreased. Because one chromosome is normal you might expect the C1INH level should be half the normal ,However patients usually have less than 50% of the C1INH This can be explained by: decreased production from the normal chromosome and increased consumption of C1INH due to icreased C1 activation

TREATMENT

1. C1INH
2. Kallikrin inhibitor
3. Bradykinin receptor antagonist