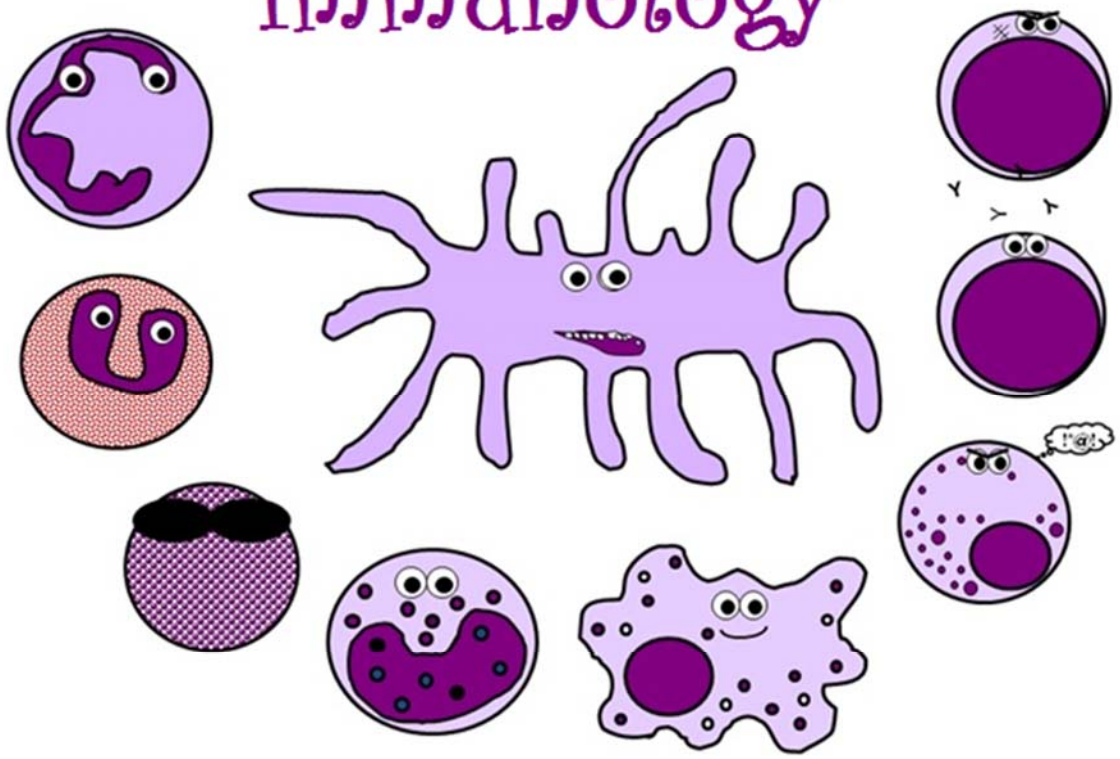
Immunology



◉Sheet ○Slides

Number: 3

Subject: Hereditary Angioedema

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- In immunology course, there would a few case-study-based lectures. These will bridge our scientific knowledge of immunological stuff with every-day clinical life in context of immunology. For these lectures, Dr. Abu Dayya told us that a few chapters are required from a book, titled "Case-Study Immunology". However, to make your task easier, we will highlight the need-to-know concepts and information in the book as well as lecture notes.

- Please, don't memorize every single sign and symptom of the disease by heart. Just study it from immunological perspective (i.e. focus on the abnormalities in the immune system that led to the sign or symptom not the sign or symptom itself).
- Last lecture, we talked about the alternative and lectin pathways of complement activation. Now, we will talk about the classical pathway and one of the, although rare, important diseases associated with it, which is Hereditary Angioedema.

Hereditary Angioedema

- C1 inhibitor is the sole inhibitor of C1.
- C1 inhibitor evaluation test is done in medical laboratories to look for certain diseases. If C1 inhibitor is low, we suspect hereditary angioedema.
- What distinguishes the classical pathway from other pathways of complement activation?
  - It needs antibodies.
  - It starts with C1.

- Normal Classical Pathway:
  Look at the figures 31.1 and 31.2.

- On the surface of a pathogen, there are antigens. These antigens are going to be bind antibodies. This forms antigen-antibody complexes.
- The classical pathway is triggered by antigen-antibody complex. That said, these antigen-antibody complexes formed on the surface of the pathogen will act as complement fixers and activators.
- Two isotypes can act as complement activators, which are IgG and IgM.

- For simplicity, let's assume that we have an IgM-antigen complex. C1q will bind to this complex, activating C1r which then activates C1s.
- C1s is a serine protease that can initiate the classical pathway. C1s acts on two complement proteins called C2 and C4, producing C4b and C2b.
- This C4b,C2b is the C3 convertase of the classical pathway. Please remember that the formation of C3 convertase is the main goal of the three different complement activation pathways.

  - In the previous lecture, we said that the complement shouldn't be activated unless there's a need for innate immune response and consequently, there are different ways to limit its activation.

  - The only inhibitor of the classical pathway is C1 inhibitor. Read about it in the text below figure 31.1

CASE 31 Hereditary Angioedema

Regulation of complement activation.

Complement is a system of plasma proteins that participates in a cascade of reactions, generating active components that allow pathogens and immune complexes to be destroyed and eliminated from the body. Complement is part of the innate immune defenses of the body and is also activated via the antibodies produced in an adaptive immune response. Complement activation is generally confined to the surface of pathogens or circulating complexes of antibody bound to antigen.

Complement is normally activated by one of three routes: the classical pathway, which is triggered by antigen:antibody complexes or antibody bound to the surface of a pathogen; the lectin pathway, which is activated by mannose-binding lectin (MBL) and the ficolins; and the alternative pathway, in which complement is activated spontaneously on the surface of some bacteria. The early part of each pathway is a series of proteolytic cleavage events leading to the generation of a convertase, a serine protease that cleaves complement component C3 and thereby initiates the effector actions of complement. The C3 convertases generated by the three pathways are different, but evolutionarily homologous, enzymes. Complement components and activation pathways, and the main effector actions of complement, are summarized in Fig. 31.1.

The principal effector molecule, and a focal point of activation for the system, is C3b, the large cleavage fragment of C3. If active C3b, or the homologous but less potent C4b, accidentally becomes bound to a host cell surface instead of a pathogen, the cell can be destroyed. This is usually prevented by the rapid hydrolysis of active C3b and C4b if they do not bind immediately to the surface where they were generated. Protection against inappropriate activation of complement is also provided by regulatory proteins.

One of these, and the most potent inhibitor of the classical pathway, is the C1 inhibitor (C1INH). This belongs to a family of serine protease inhibitors (called serpins) that together constitute 20% of all plasma proteins. In addition to being the sole known inhibitor of C1, C1INH contributes to the

This case was prepared by Raif Geha, MD, in collaboration with Arturo Borzutzky, MD.

Topics bearing on this case:
Classical pathway of complement activation
Inhibition of C1 activation
Alternative pathway of complement activation
Inflammatory effects of complement activation
Regulation of C4b

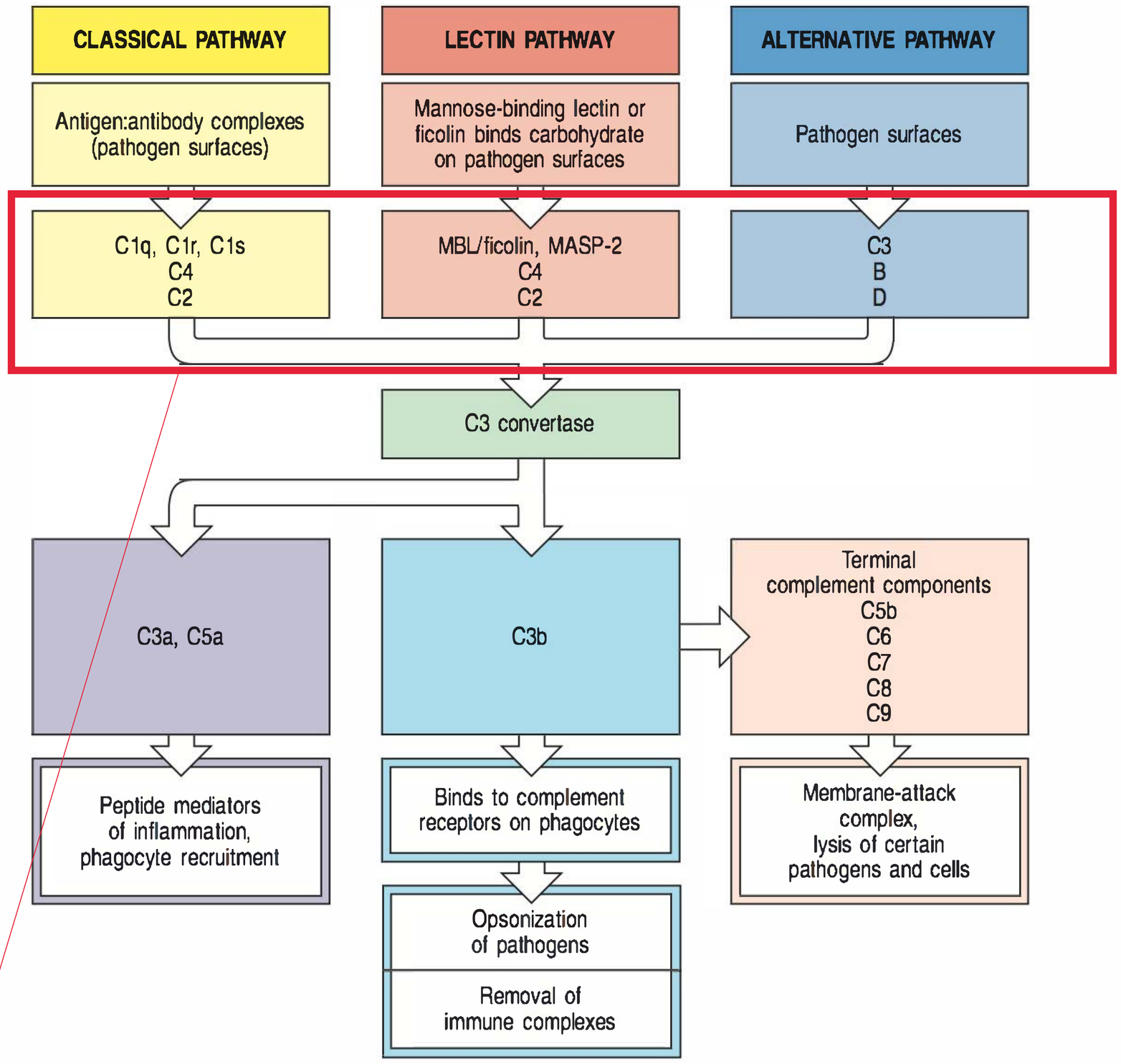


Fig. 31.1 Overview of the main components and effector actions of complement. The early events of all three pathways of complement activation involve a series of cleavage reactions that culminate in the formation of an enzymatic activity called a C3 convertase, which cleaves complement component C3 into C3b and C3a. The production of the C3 convertase is the point at which the three pathways converge and the main effector functions of complement are generated. C3b binds covalently to the bacterial cell membrane and opsonizes the bacteria, enabling phagocytes to internalize them. C3a is a peptide mediator of local inflammation. C5a and C5b are generated by the cleavage of C5b by a C5 convertase formed by C3b bound to the C3 convertase (not shown in this simplified diagram). C5a is also a powerful peptide mediator of inflammation. C5b triggers the late events in which the terminal components of complement assemble into a membrane-attack complex that can damage the membrane of certain pathogens. Although the classical complement activation pathway was first discovered as an antibody-triggered pathway, it is now known that C1q can activate this pathway by binding directly to pathogen surfaces, as well as paralleling the lectin activation pathway by binding to antibody that is itself bound to the pathogen surface. In the lectin pathway, MASP stands for mannose-binding lectin-associated serine protease.

- The main goal of the three pathways of complement activation is to produce C3 convertase.
- C3 convertases of different pathways are differenet but they are functionally the same.
- The three pathways converge at the C3 convertase. Then activation continues in the same way in all pathways.
-

regulation of serine proteases of the clotting system and of the kinin system, which is activated by injury to blood vessels and by some bacterial toxins. The main product of the kinin system is bradykinin, which causes vasodilation and increased capillary permeability.

C1INH intervenes in the first step of the complement pathway, when C1 binds to immunoglobulin molecules on the surface of a pathogen or antigen:antibody complex (Fig. 31.2). Binding of two or more of the six tulip-like heads of the C1q component of C1 is required to trigger the sequential activation of the two associated serine proteases, C1r and C1s. C1INH inhibits both of these proteases, by presenting them with a so-called bait-site, in the form of an arginine bond that they cleave. When C1r and C1s attack the bait-site they covalently bind C1INH and dissociate from C1q. By this mechanism, the C1 inhibitor limits the time during which antibody-bound C1 can cleave C4 and C2 to generate C4b2a, the classical pathway C3 convertase.

Activation of C1 also occurs spontaneously at low levels without binding to an antigen:antibody complex, and can be triggered further by plasmin, a protease of the clotting system, which is also normally inhibited by C1INH. In the absence of C1INH, active components of complement and bradykinin are produced. This is seen in hereditary angioedema (HAE), a disease caused by a genetic deficiency of C1INH.

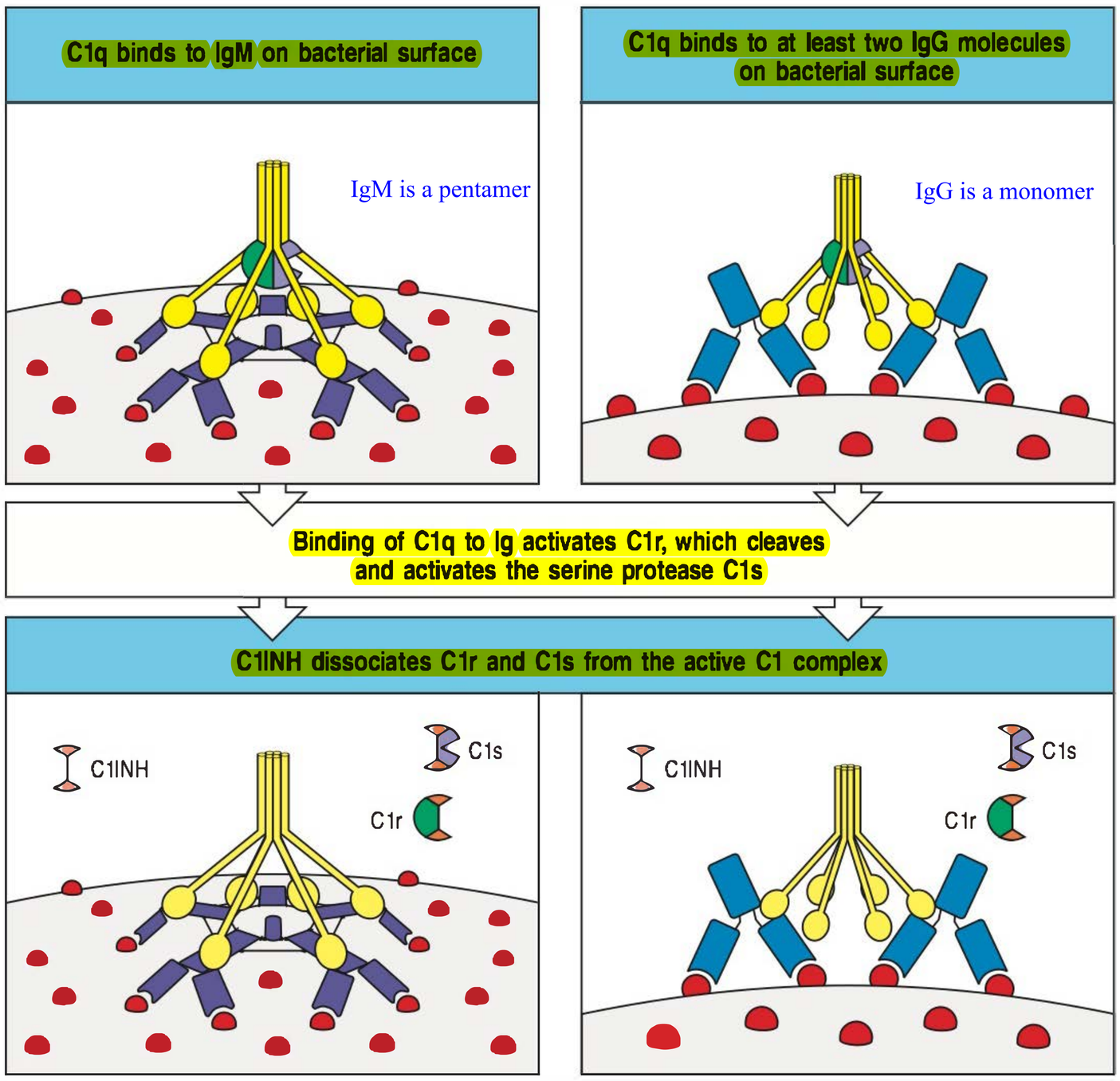


Fig. 31.2 Activation of the classical pathway of complement and intervention by C1INH. In the left panel, one molecule of IgM, bent into the 'staple' conformation by binding several identical epitopes on a pathogen surface, allows binding by the globular heads of C1q to its Fc pieces on the surface of the pathogen. In the right panel, multiple molecules of IgG bound to the surface of the pathogen allow binding by C1q to two or more Fc pieces. In both cases, binding of C1q activates the associated C1r, which becomes an active enzyme that cleaves the proenzyme C1s, a serine protease that initiates the classical complement cascade. Active C1 is inactivated by C1INH, which binds covalently to C1r and C1s, causing them to dissociate from the complex. There are in fact two C1r and two C1s molecules bound to each C1q molecule, although for simplicity this is not shown here. It takes four molecules of C1INH to inactivate all the C1r and C1s.

The two isotypes that are able to activate the complement are IgG and IgM.

- IgM is the most effective isotype at activating the complement. Why?

1- IgM is the first isotype of antibodies to recognize the antigen, so it's more logical to activate the complement at an early stage of the immune response with a great potency. It would be illogical to wait for the appearance of IgG to start activating the complement.

2- Because of its multimeric structure (IgM is a pentamer).
- Remember: IgG is a monomer.

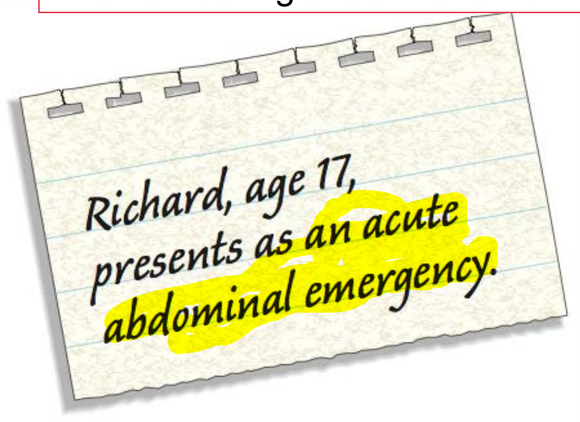
The case of Richard Crafton: a failure of communication as well as of complement regulation.

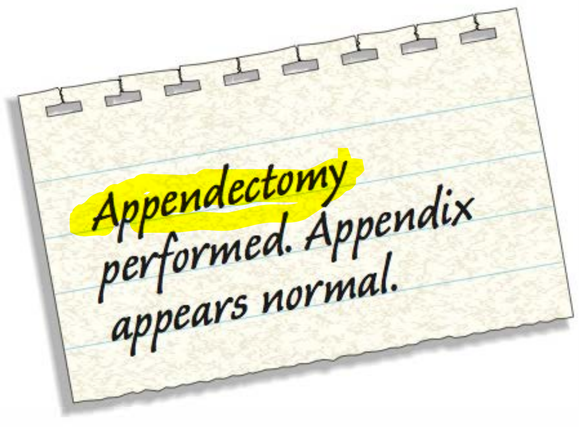
Richard Crafton was a 17-year-old high-school senior when he had an attack of severe abdominal pain at the end of a school day. The pain came as frequent sharp spasms and he began to vomit. After 3 hours, the pain became unbearable and he went to the emergency room at the local hospital.

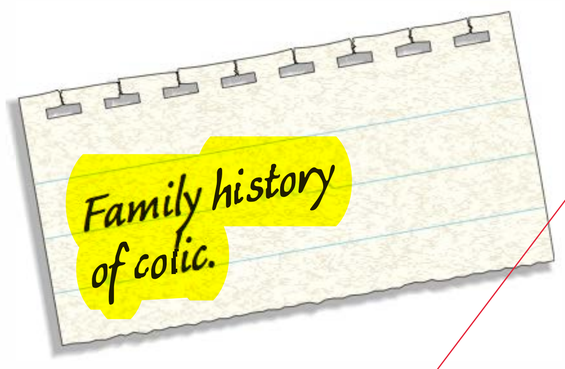
At the hospital, the intern who examined him found no abnormalities other than dry mucous membranes of the mouth, and a tender abdomen. There was no point tenderness to indicate appendicitis. Richard continued to vomit every 5 minutes and said the pain was getting worse.

A surgeon was summoned. He agreed with the intern that Richard had an acute abdominal condition but was uncertain of the diagnosis. Blood tests showed an elevated red blood cell count, indicating dehydration. The surgeon decided to proceed with exploratory abdominal surgery. A large midline incision revealed a moderately swollen and pale jejunum but no other abnormalities were noted. The surgeon removed Richard's appendix, which was normal, and Richard recovered and returned to school 5 days later.

What Richard had not mentioned to the intern or to the surgeon was that, although he had never had such severe pains as those he was experiencing when he went to the







Bad History --> Incorrect Diagnosis

In Hereditary Angioedema:

- C1 inhibitor is low
Because it's deficient in this disease

- C4 and C2 are low
When C1 inhibitor is deficient, C1
- C3

emergency room, he had had episodes of abdominal pain since he was 14 years old. No one in the emergency room asked him if he was taking any medication, or took a family history or a history of prior illness. If they had, they would have learned that Richard's mother, his maternal grandmother, and a maternal uncle, also had recurrent episodes of severe abdominal pain, as did his only sibling, a 19-year-old sister.

As a newborn, Richard was prone to severe colic. When he was 4 years old, a bump on his head led to abnormal swelling. When he was 7, a blow with a baseball bat caused his entire left forearm to swell to twice its normal size. In both cases, the swelling was not painful, nor was it red or itchy, and it disappeared after 2 days. At age 14 years, he began to complain of abdominal pain every few months, sometimes accompanied by vomiting and, more rarely, by clear, watery diarrhea.

Richard's mother had taken him at age 4 years to an immunologist, who listened to the family history and immediately suspected hereditary angioedema. The diagnosis was confirmed on measuring key complement components. C1INH levels were 16% of the normal mean and C4 levels were markedly decreased, while C3 levels were normal.

When Richard turned up for a routine visit to his immunologist a few weeks after his surgical misadventure, the immunologist, noticing Richard's large abdominal scar, asked what had happened. When Richard explained, he prescribed daily doses of Winstrol (stanozolol). This caused a marked diminution in the frequency and severity of Richard's symptoms. When Richard was 20 years old, purified C1INH became available; he has since been infused intravenously on several occasions to alleviate severe abdominal pain, and once for swelling of his uvula, pharynx, and larynx. The infusion relieved his symptoms within 25 minutes.

Richard subsequently married and had two children. The C1INH level was found to be normal in both newborns.

Hereditary angioedema.

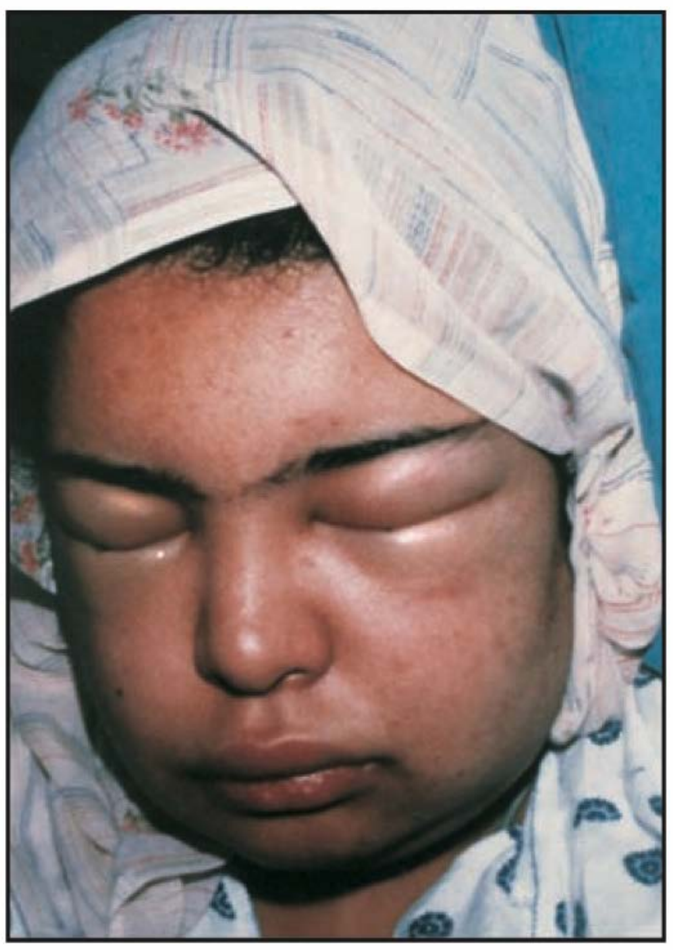


Fig. 31.3 Hereditary angioedema. Transient localized swelling that occurs in this condition often affects the face.

Individuals like Richard with a hereditary deficiency of C1INH are subject to recurrent episodes of circumscribed swelling of the skin (Fig. 31.3), intestine, and airway. Attacks of subcutaneous or mucosal swelling most commonly affect the extremities, but can also involve the face, trunk, genitals, lips, tongue, or larynx. Cutaneous attacks cause temporary disfigurement but are not dangerous. When the swelling occurs in the intestine it causes severe abdominal pain, and obstructs the intestine so that the patient vomits. When the colon is affected, watery diarrhea may occur. Swelling in the larynx is the most dangerous symptom, because the patient can rapidly choke to death. HAE attacks do not usually involve itching or hives, which is useful to differentiate this disease from allergic angioedema. However, a serpiginous, or linear and wavy, rash is sometimes seen before the onset of swelling symptoms. Such episodes may be triggered by trauma, menstrual periods, excessive exercise, exposure to extremes of temperature, mental stress, and some medications such as angiotensin-converting enzyme inhibitors and oral contraceptives.

Pathogenesis

HAE is not an allergic disease, and attacks are not mediated by histamine. HAE attacks are associated with activation of four serine proteases, which are normally inhibited by C1INH. At the top of this cascade is Factor XII, which directly or indirectly activates the other three (Fig. 31.4). Factor XII is normally activated by injury to blood vessels, and initiates the kinin cascade, activating

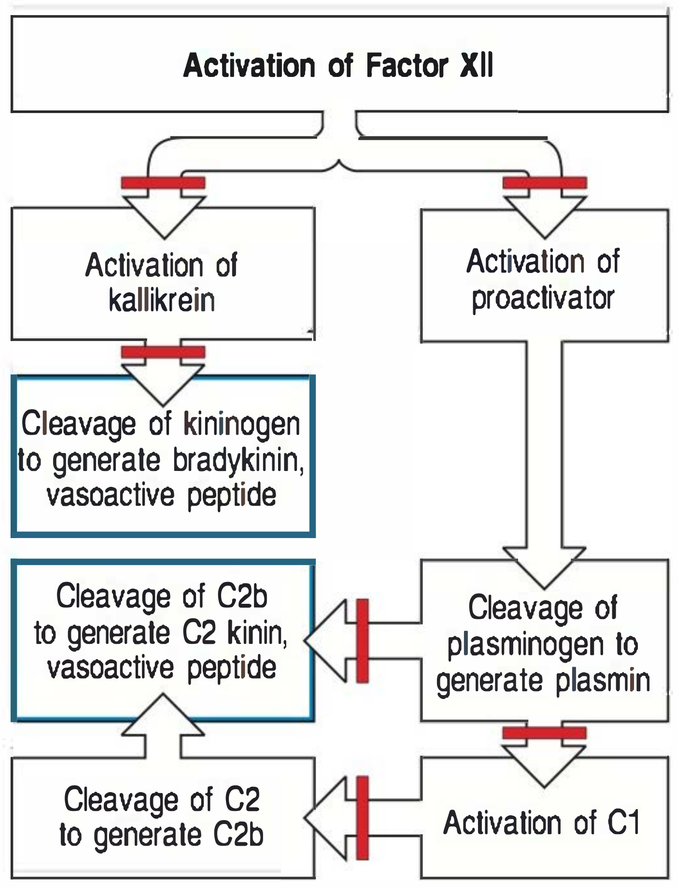


Fig. 31.4 Pathogenesis of hereditary angioedema. Activation of Factor XII leads to the activation of kallikrein, which cleaves kininogen to produce the vasoactive peptide bradykinin; it also leads to the activation of plasmin, which in turn activates C1. C1 cleaves C2, whose smaller fragment C2b is further cleaved by plasmin to generate the vasoactive peptide C2 kinin. The red bars represent inhibition by C1INH.

kallikrein, which generates the vasoactive peptide bradykinin. Factor XII also indirectly activates plasmin, which, as mentioned earlier, activates C1 itself. Plasmin also cleaves C2b to generate a vasoactive fragment called C2 kinin. In patients deficient in C1INH, the uninhibited activation of Factor XII leads to the activation of kallikrein and plasmin; kallikrein catalyzes the formation of bradykinin, and plasmin produces C2 kinin. Bradykinin is the main mediator responsible for HAE attacks by causing vasodilation and increasing the permeability of the postcapillary venules by causing contraction of endothelial cells so as to create gaps in the blood vessel wall (Fig. 31.5). This is responsible for the edema; movement of fluid from the vascular space into another body compartment, such as the gut, causes the symptoms of dehydration as the vascular volume contracts.

Treatment of HAE can focus on preventing attacks or on resolving acute episodes. Purified or recombinant C1INH is an effective therapy in both these settings. A kallikrein inhibitor and a bradykinin receptor antagonist have also been developed to target the kinin cascade and bradykinin activity.

Questions.

1 *Activation of the complement system results in the release of histamine and chemokines, which normally produce pain, heat, and itching. Why is the edema fluid in HAE free of cellular components, and why does the swelling not itch?*

2 *Richard has a markedly decreased amount of C4 in his blood. This is because it is being rapidly cleaved by activated C1. What other complement component would you expect to find decreased? Would you expect the alternative pathway components to be low, normal, or elevated? What about the terminal components?*

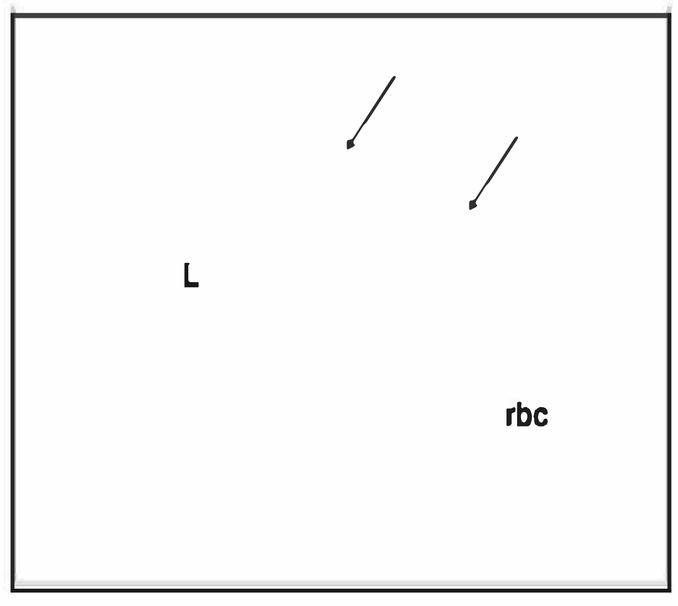


Fig. 31.5 Contraction of endothelial cells creates gaps in the blood vessel wall. A guinea pig was injected intravenously with India ink (a suspension of carbon particles). Immediately thereafter the guinea pig was injected intradermally with a small amount of activated C1s. An area of angioedema formed about the injected site, which was biopsied 10 minutes later. An electron micrograph reveals that the endothelial cells in post-capillary venules have contracted and formed gaps through which the India ink particles have leaked from the blood vessel. L is the lumen of the blood vessel; P is a polymorphonuclear leukocyte in the lumen; rbc is a red blood cell that has leaked out of the blood vessel. Micrograph courtesy of Kaethe Willms.

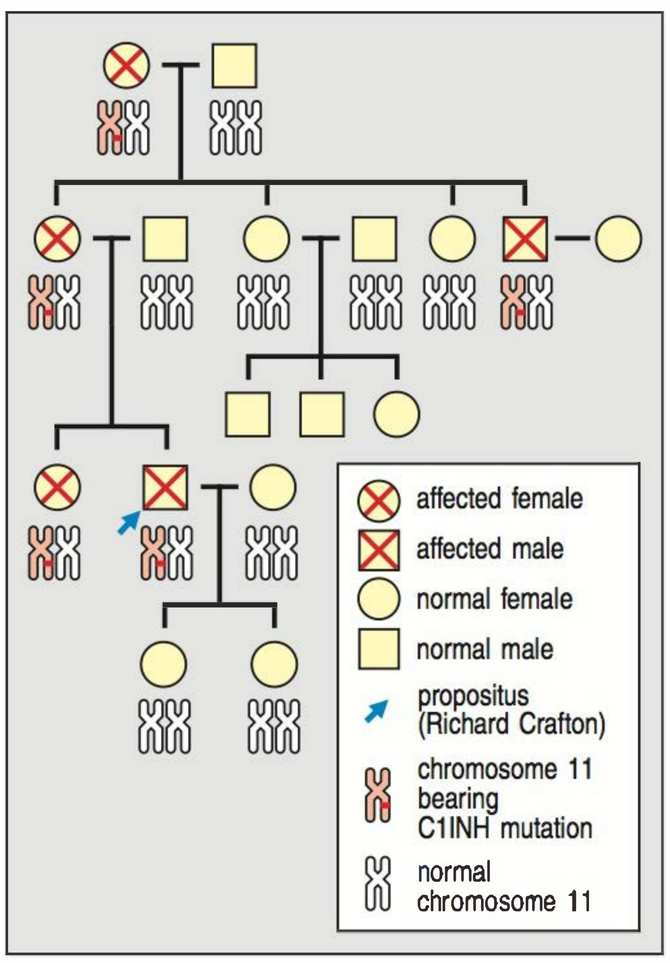


Fig. 31.6 The inheritance of hereditary angioedema in Richard's extended family.

3 Despite the complement deficiency in patients with HAE, they are not unduly susceptible to infection. Why not?

4 What is stanozolol, and why was it prescribed?

5 Emergency treatment for HAE cases is sometimes necessary because of airway obstruction. In most cases, however, a patient with obstruction of the upper airways is likely to be suffering from an anaphylactic reaction. The treatment in this case would be epinephrine. How might you decide whether to administer epinephrine or intravenous C1INH?

6 Figure 31.6 shows Richard's family tree. What is the mode of inheritance (dominant or recessive, sex-linked or not) of HAE? Can Richard's two children pass the disease onto their offspring?

- In the case of Richard Crafton, people in the emergency room didn't take the appropriate medical history, and forget to ask him about a lot of things. Hence, the surgeon performed exploratory surgery which was definitely not needed.

  Bad History → Incorrect Diagnosis → Bad Management
- So, always think of all possible diagnoses and perform the needed test to avoid performing unneeded operation or starting an unneeded treatment.

Signs and symptoms of hereditary angioedema:
1- Recurrent episodes of circumscribed skin swellings.
2- Swellings in the intestine, and the airways.

Swelling of the intestine → obstruction → Vomiting

3- **Swelling of the larynx is the most dangerous symptom.**

Pathogenesis:
Highlighted in the text below.

Questions:
1- Why is Richard's C4 low?
Because C4 is cleaved by C1.
C1 inhibitor is deficient in hereditary angioedema, so more C1 will act on C4 and consume it.

No C1 inhibitor → More activity of C1 → less C4

2- What other complement component should be low?
C2

3- Is the alternative pathway affected by C1 inhibitor deficiency?
No. Because hereditary angioedema affects complement proteins 2 and 4 which are not needed for the alternative pathway.

4- Are these patients more prone to infections?
No. Other complement activation pathways are not affected, and these would compensate.

5- In the ER, how can we distinguish between a case of C1 inhibitor deficiency or anaphylactic shock?

We administer epinephrine and then we monitor the response. If he/she improves, then it's anaphylactic shock. If not, then it would be another cause.

- We administer epinephrine because anaphylactic shock is more serious, while hereditary angioedema is not that serious and epinephrine won't cause anything even if the patient doesn't have anaphylactic shock.

6- Can Richard's children pass the disease to their offspring?

Look at figure 31.6
- Not X-linked because it affects males and females.
- Autosomal dominant/recessive?
We look at skipping generations. If it skips generations, then it's autosomal recessive. If not, then it's dominant.

In hereditary angioedema, there's no skipped generations.

Hereditary angioedema is autosomal dominant.

Richard may pass the disease to his children. If his wife is not diseases, the probability of getting the disease would be 50%.

7- Will Richard's daughter pass the disease to their children?

No. Because the disease is autosomal dominant and they are not affected.