MAIN PATHWAYS OF COMPLEMENT SYSTEM

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• The term complement refers to a set of serum proteins that cooperates with both the innate and the adaptive immune systems to eliminate blood and tissue pathogens.
• Like the components of the blood clotting system, complement proteins interact with one another in catalytic cascades.
• Various complement components bind and opsonize bacteria, rendering them susceptible to receptor-mediated phagocytosis by macrophages, which express membrane receptors for complement proteins.

• Other complement proteins elicit inflammatory responses, interface with components of the adaptive immune system, clear immune complexes from the serum, and/or eliminate apoptotic cells.
• Finally, a Membrane Attack Complex (MAC) assembled from complement proteins directly kills some pathogens by creating pores in microbial membranes.
• The famous immunologist Paul Ehrlich, working independently in Berlin, carried out similar experiments and coined the term complement, defining it as “the activity of blood serum that completes the action of antibody.”

• In the ensuing years, researchers have discovered that the action of complement is the result of interactions among a complex group of more than 30 glycoproteins.

• Most complement components are synthesized in the liver by hepatocytes, although some are also produced by other cell types, including blood monocytes, tissue macrophages, fibroblasts, and epithelial cells of the gastrointestinal and genitourinary tracts.

❖ Complement components:

- Complement components include a group of proteins/glycoproteins.
- These components are designated by numerals (C1–C9), by letter symbols (e.g. factor D), or by trivial names (e.g. homologous restriction factor).
- Designation C1, C2,…C9 denote the order in which the components were discovered, rather than their position in the activation sequence.
- In the circulation, the concentration of all complement proteins is about 3 mg/mL.

• Some complement components are found at high concentrations (e.g. C3 at about 1 mg/mL), while others (such as factor D and C2) are found in only trace amounts.

❖ Complement activation pathways:

• Most complement components are present in the serum in functionally inactive forms as proenzymes or zymogens.
• Complement activation pathways involve the activation of one component that triggers the activation of the next component in the sequence.

• Upon activation, individual components are split into fragments, designated by lower-case letters.
• The smaller of the cleaved fragments is generally designated with a lower-case a, and the large fragment with a lower-case b; for historical reasons, however, the large cleavage fragment of C2 is usually referred to as C2a and the smaller fragment C2b.
• The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and can initiate localized inflammatory responses.

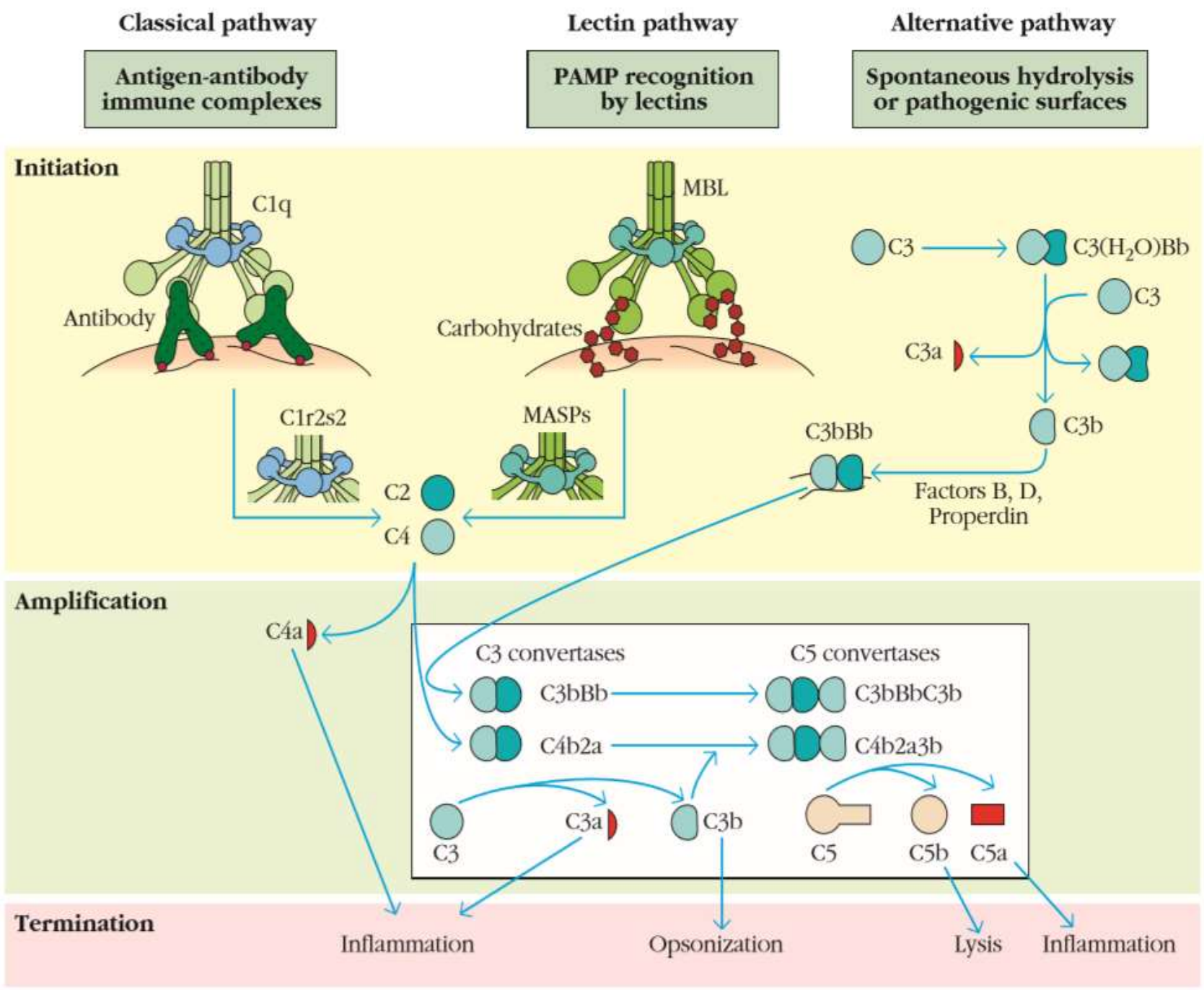
• Small complement fragments act as inflammatory mediators.
• These fragments enhance the blood supply to the area in which they are released, by binding to receptors on endothelial cells lining the small blood vessels and inducing an increase in capillary diameter.
• They also attract other cells to the site of tissue damage.
• Because such effects can be harmful in excess, these fragments are called anaphylatoxins, meaning substances that cause anaphylaxis (“against protection”).

• The complement fragments interact with one another to form functional complexes.

• Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g. $\overline{C3bBb}$).

• There are three pathways of complement activation.

• These are the classical, lectin and alternative pathways.


Classical pathway
Antigen-antibody immune complexes
Lectin pathway
PAMP recognition by lectins
Alternative pathway
Spontaneous hydrolysis or pathogenic surfaces
Initiation
C1q
Antibody
MBL
Carbohydrates
C3
C3(H2O)Bb
C3
C3a
C3b
C3bBb
Factors B, D, Properdin
C1r2s2
MASPs
C2
C4
Amplification
C4a
C3 convertases
C5 convertases
C3bBb
C3bBbC3b
C4b2a
C4b2a3b
C3
C3a
C3b
C5
C5b
C5a
Termination
Inflammation
Opsonization
Lysis
Inflammation

❑ Classical pathway:

• The classical pathway is initiated by Antibody binding.

• The classical pathway of complement activation is considered part of the adaptive immune response since it begins with the formation of antigen-antibody complexes.

• These complexes may be soluble, or they may be formed when an antibody binds to antigenic determinants, or epitopes, situated on viral, fungal, parasitic, or bacterial cell membranes.

• Soluble antibody-antigen complexes are often referred to as immune complexes, and only complexes formed by IgM or certain subclasses of IgG antibodies are capable of activating the classical complement pathway.

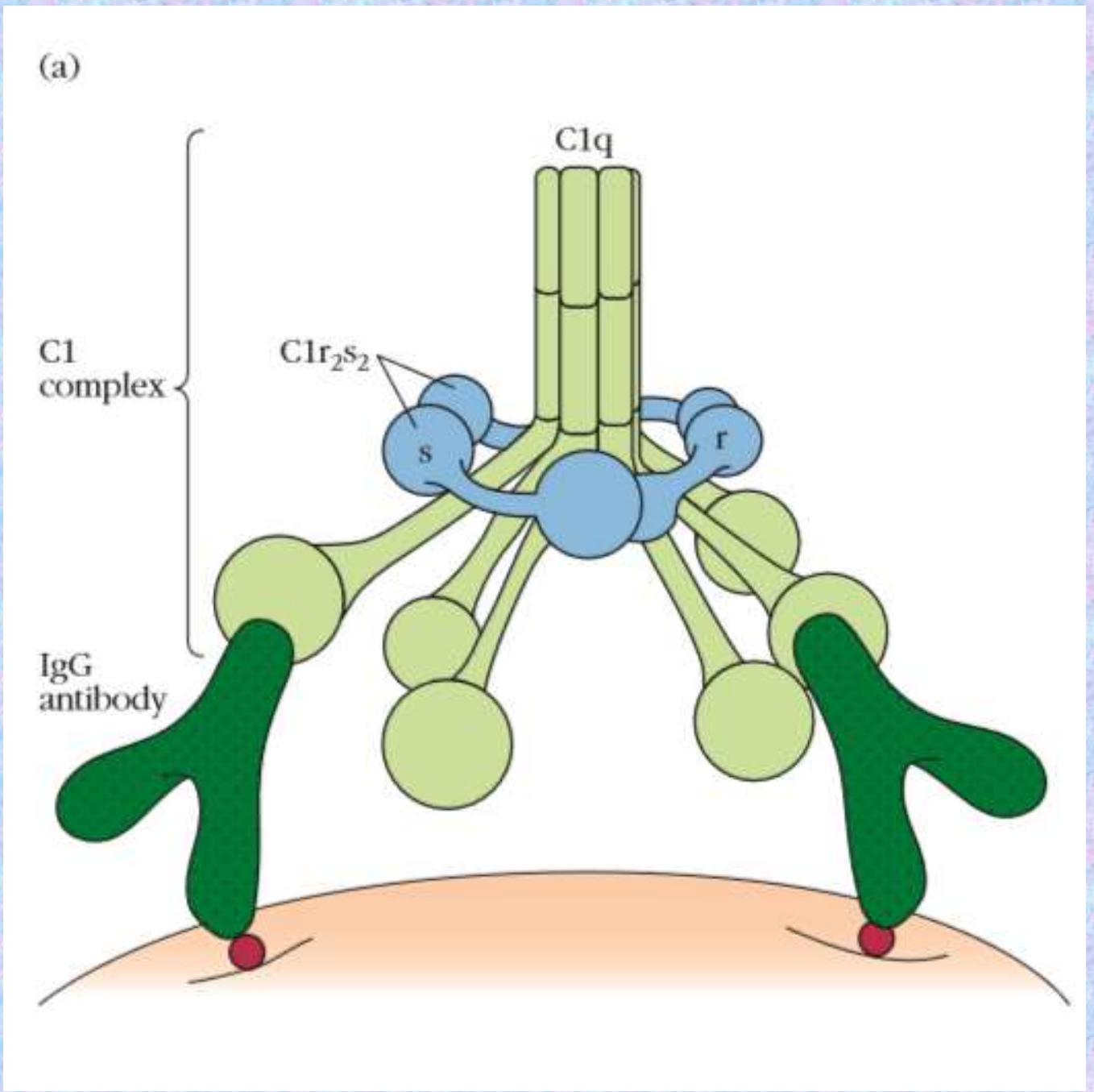
• The initial stage of activation involves the complement components C1, C2, C3, and C4, which are present in plasma as zymogens.

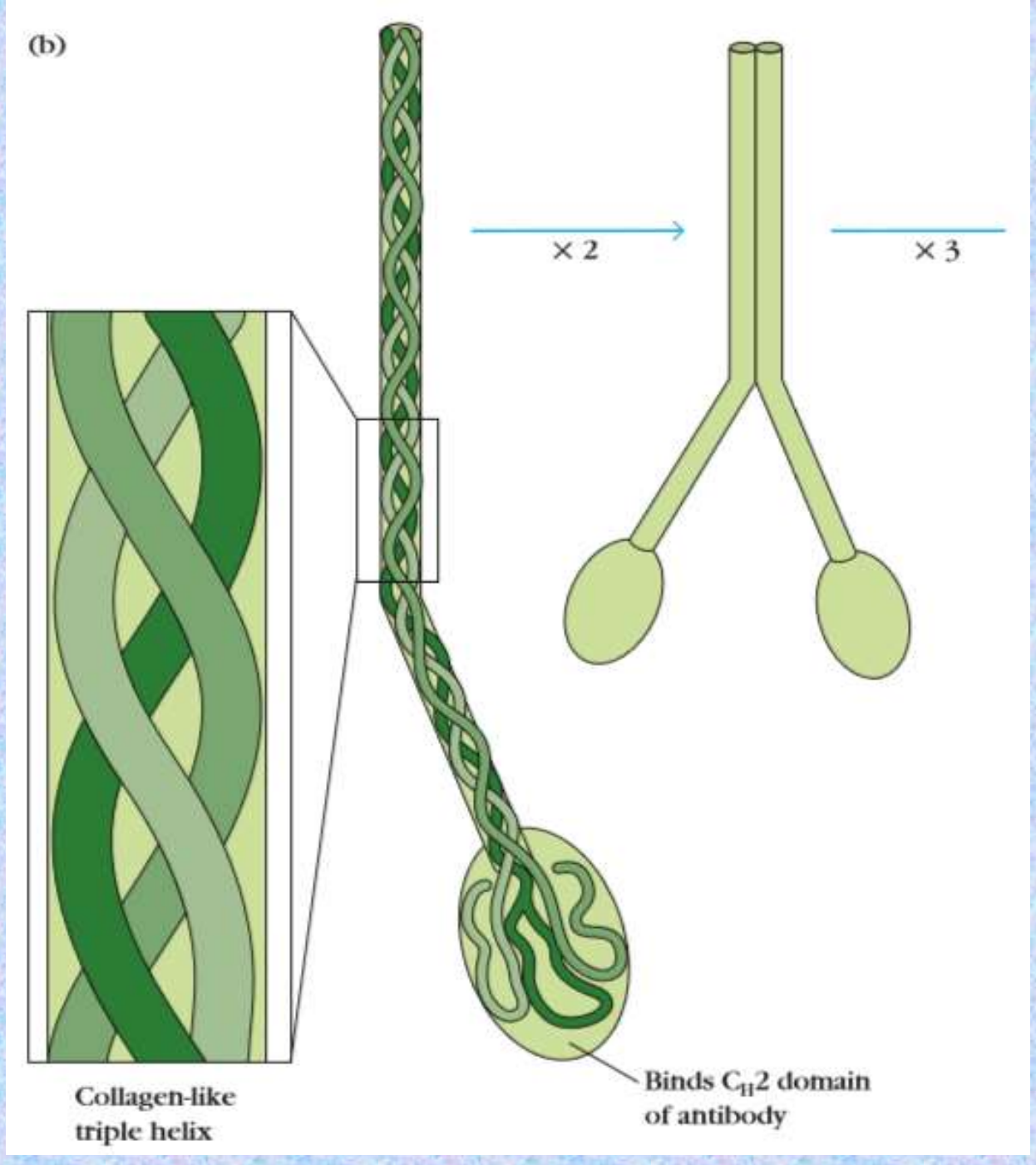
• The formation of an antigen-antibody complex induces conformational changes in the nonantigen-binding (Fc) portion of the antibody molecule.

• This conformational change exposes a binding site for the C1 component of complement.

• In serum, C1 exists as a macromolecular complex consisting of one molecule of C1q and two molecules each of the serine proteases, C1r and C1s, held together in a Ca^{2+} -stabilized complex ($C1qr_2s_2$).

• The C1q molecule itself is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, the tips of which bind the CH_2 domain of the antigen-bound antibody molecule


(a)
C1q
C1
complex
$C1r_2s_2$
s
r
IgG
antibody


(b)
× 2
× 3
Collagen-like
triple helix
Binds C_H2 domain
of antibody

• Binding of C1q to the CH_2 domains of the Fc regions of the antigen-complexed antibody molecule induces a conformational change in one of the C1r molecules that converts it to an active serine protease enzyme.
• This C1r molecule then cleaves and activates its partner C1r molecule.
• The two C1r proteases then cleave and activate the two C1s molecules .
• C1s has two substrates, C4 and C2.
• C4 is activated when C1s hydrolyzes a small fragment (C4a) from the amino terminus of one of its chains

• The C4b fragment attaches covalently to the target membrane surface in the vicinity of C1, and then binds C2.
• On binding C4b, C2 becomes susceptible to cleavage by the neighboring C1s enzyme, and the smaller C2b fragment diffuses away, leaving behind an enzymatically active C4b2a complex.
• In this complex, C2a is the enzymatically active fragment, but it is only active when bound by C4b.
• This C4b2a complex is called C3 convertase, referring to its role in converting C3 into an active form. The smaller fragment generated by C4 cleavage, C4a, is an anaphylatoxin.

• The membrane-bound C3 convertase enzyme, C4b2a, now hydrolyzes C3, generating two unequal fragments; the small anaphylatoxin C3a and the pivotal fragment C3b.

• The generation of C3b is centrally important to many of the actions of complement. Deficiencies of complement components that act prior to C3 cleavage leave the host extremely vulnerable to both infectious and autoimmune diseases.

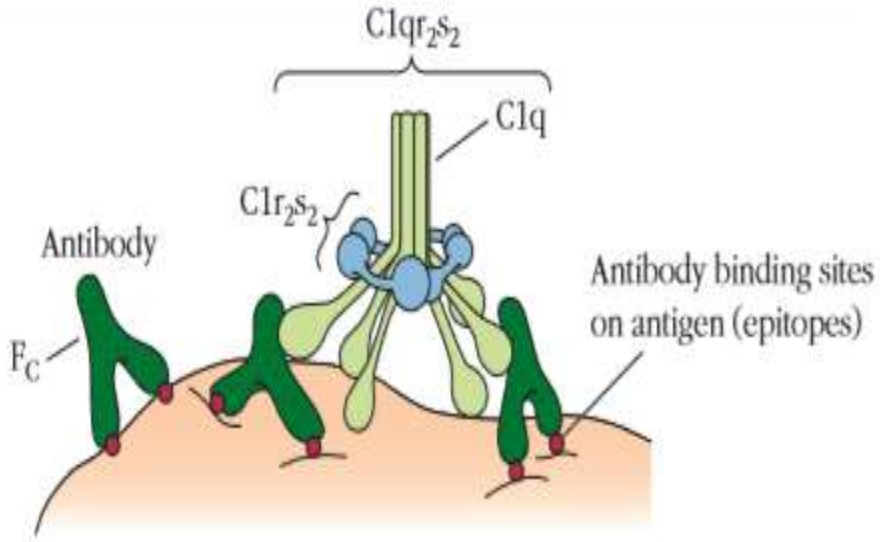
• This is because C3b acts in three important and different ways to protect the host.

• First, in a manner very similar to that of C4b, C3b binds covalently to microbial surfaces, providing a molecular “tag” and thereby allowing phagocytic cells with C3b receptors to engulf the tagged microbes. This process is called opsonization.
• Second, C3b molecules can attach to the Fc portions of antibodies participating in soluble antigen-antibody complexes.
• These C3b-tagged immune complexes are bound by C3b receptors on phagocytes or red blood cells, and are either phagocytosed, or conveyed to the liver where they are destroyed.

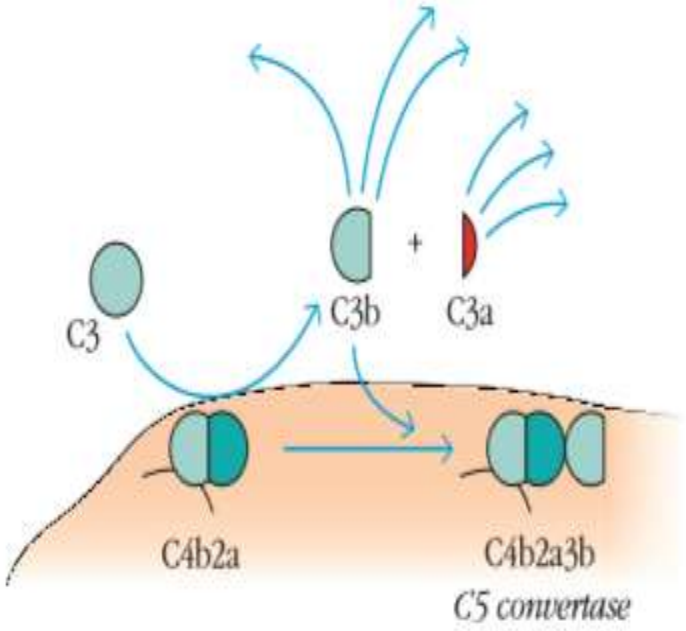
• Finally, some molecules of C3b bind the membrane localized C4b2a enzyme to form the trimolecular, membrane bound, C5 convertase complex C4b2a3b.
• The C3b component of this complex binds C5, and the complex then cleaves C5 into the two fragments: C5b and C5a.
• C4b2a3b is therefore the C5 convertase of the classical pathway.
• This trio of tasks accomplished by the C3b molecule places it right at the center of complement attack pathways.
• As we will see, C5b goes on to form the MAC with C6, C7, C8, and C9.

Intermediates in the Classical Pathway of Complement Activation up to the Formation of the C5 Convertase

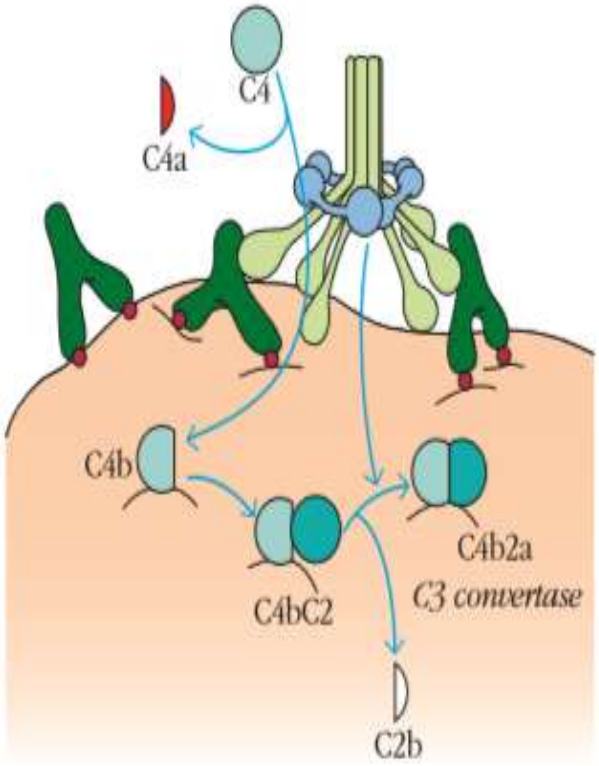
1. C1q binds antigen-bound antibody, and induces a conformational change in one C1r molecule, activating it. This C1r then activates the second C1r and the two C1s molecules.



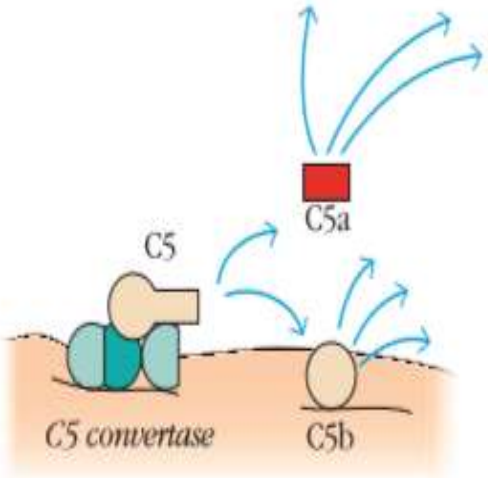
3. C3 convertase hydrolyzes many C3 molecules. Some combine with C3 convertase to form C5 convertase.



2 C1s cleaves C4 and C2. C4 is cleaved first and C4b binds to the membrane close to C1. C4b binds C2 and exposes it to the action of C1s. C1s cleaves C2, creating the C3 convertase, C4b2a.



4 The C3b component of C5 convertase binds C5, permitting C4b2a to cleave C5.



Antigenic determinants are shown in dark red, initiating components (antibodies and C1q) are shown in green, active enzymes are shown in blue, and anaphylatoxins in bright red.

AUGUST 2009

11 TUE

① Classical pathway :-

Antigen + Antibody (Fc portion)
↓
provides a binding site for C_1q complex.
↓
C_1q complex ($C_1q r_2 s_2$)
↓
Ag + Ab + C_1q → conformational change in one of the C_1r
↓
further activated 2nd C_1r → cleaves C_1s.
↓

12 WED C_1s → 2 substrates [C_4 and C_2]
↓
C_4 → C_4a (small) → anaphylaxis
→ C_4b (large)

C_4b binds C_2 and cleaves it
↓
** C_2 → C_2a (large)
→ C_2b (small)

C_4b2a (C_3 convertase)

AUGUST 2009

$C_3 \rightarrow C_3a$ (small)

$C_3 \rightarrow C_3b$ (large)

13 THU

C_4b2a3b (C_5 convertase)

② Lectin pathway :- (antibody independent)

Lectin present on the microbial surfaces + MBL (mannose binding lectin)

(resembles C_1q)

↓

MBL associated in the serum with MASP proteins (mannose associated serine proteases)

[resembles ~~C_1r and~~ C_1s which cleaves C_4 and C_2]

14 FRI

↓

Specifically, MASP-2 functions

$C_4 \rightarrow C_4a$ + C_4b (large)

$C_2 \rightarrow C_2a$ + C_2b

C_4b2a (C_3 convertase)

$C_3 \rightarrow C_3a$ + C_3b

C_4b2a3b (C_5 convertase)

Sun	Mon	Tue	Wed	Thu	Fri	Sat
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

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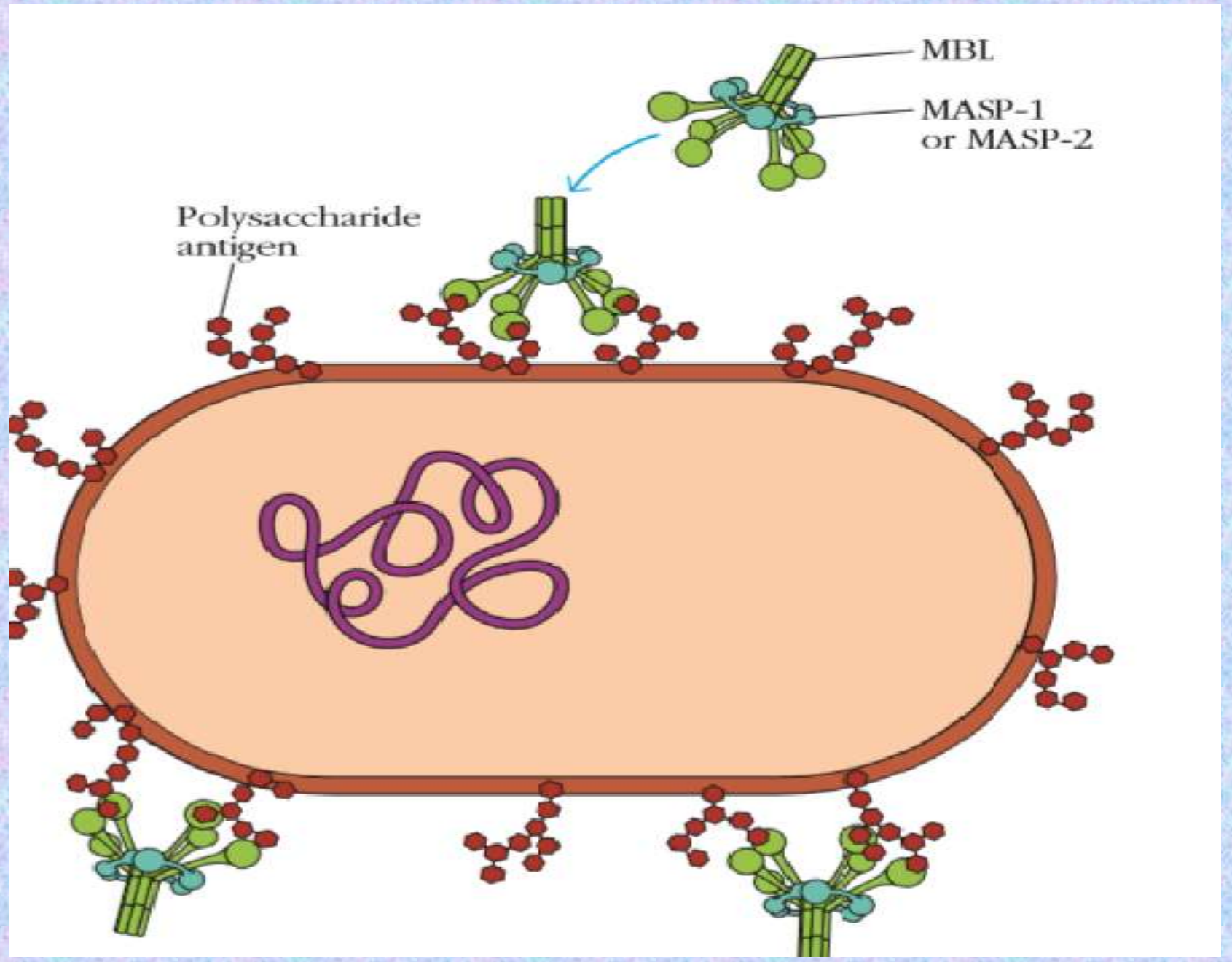
NOTES

❑ Lectin pathway:

• The lectin pathway, like the classical pathway, proceeds through the activation of a C3 convertase composed of C4b and C2a.

• However, instead of relying on antibodies to recognize the microbial threat and to initiate the complement activation process, this pathway uses lectins—proteins that recognize specific carbohydrate components primarily found on microbial surfaces—as its specific receptor molecules


MBL
MASP-1
or MASP-2
Polysaccharide
antigen

• Mannose-binding lectin (MBL), the first lectin demonstrated to be capable of initiating complement activation, binds close-knit arrays of mannose residues that are found on microbial surfaces such as those of Salmonella, Listeria, and Neisseria strains of bacteria; Cryptococcus neoformans and Candida albicans strains of fungi; and even the membranes of some viruses such as HIV-1 and respiratory syncytial virus.

• The complement pathway that it initiates is referred to as the lectin pathway of complement activation.

• MBL is constitutively expressed by the liver and, like C1q, which it structurally resembles, MBL belongs to the subclass of lectins known as collectins.

• MBL is associated in the serum with MBL-Associated Serine Proteases, or MASP proteins.

• Three MASP proteins— MASP1, MASP2, and MASP3—have been identified, but most studies of MASP function point to the MASP2 protein as being the most important actor in the next step of the MBL pathway.

• MASP-2 is structurally related to the serine protease C1s, and can cleave both C2 and C4, giving rise to the C3 convertase, C4b2a, that we first encountered in our discussion of the classical pathway.
• Thus, the lectin pathway utilizes all the same components as the classical pathway with the single exception of the C1 complex.
• The soluble lectin receptor replaces the antibody as the antigen-recognizing component, and MASP proteins take the place of C1r and C1s in cleaving and activating the C3 convertase.

• Once the C3 convertase is formed, the reactions of the lectin pathway are the same as for the classical pathway; the C5 convertase of the lectin pathway, like that of the classical pathway, is also C4b2a3b.

- Alternative pathway

Initiation of the alternative pathway of complement activation is independent of antibody-antigen interactions, and so this pathway, like the lectin pathway, is also considered to be part of the innate immune system.

AUGUST 2009

15 SAT

③ Alternative pathway :-
(independent of antibody-antigen interactions)

Triggered by almost any foreign substance.

↓

C_3 —spontaneous hydrolysis→ C_3a (small) + C_3b (large)

↓

C_3b binds with 'factor B' (C_3bB)

C_3bB acts as a substrate for 'factor D'

C_3bB —factor D→ B → Ba (diffuses away) + Bb

16 SUN

↓

C_3bBb (C_3 convertase) — stabilized by 'properdin'

↓

C_3 → C_3a + C_3b

↓

C_3bBb3b (C_5 convertase)

- This pathway is antibody independent.
- This pathway of complement activation is triggered by almost any foreign substance.
- The most widely studied include lipopolysaccharide from the outer membrane of gram negative bacteria, the cell wall of some yeasts, and a protein present in cobra venom, known as cobra venom factor.
- This major pathway of complement activation involves four serum proteins: C3, factor B, factor D, and properdin also known as factor P.

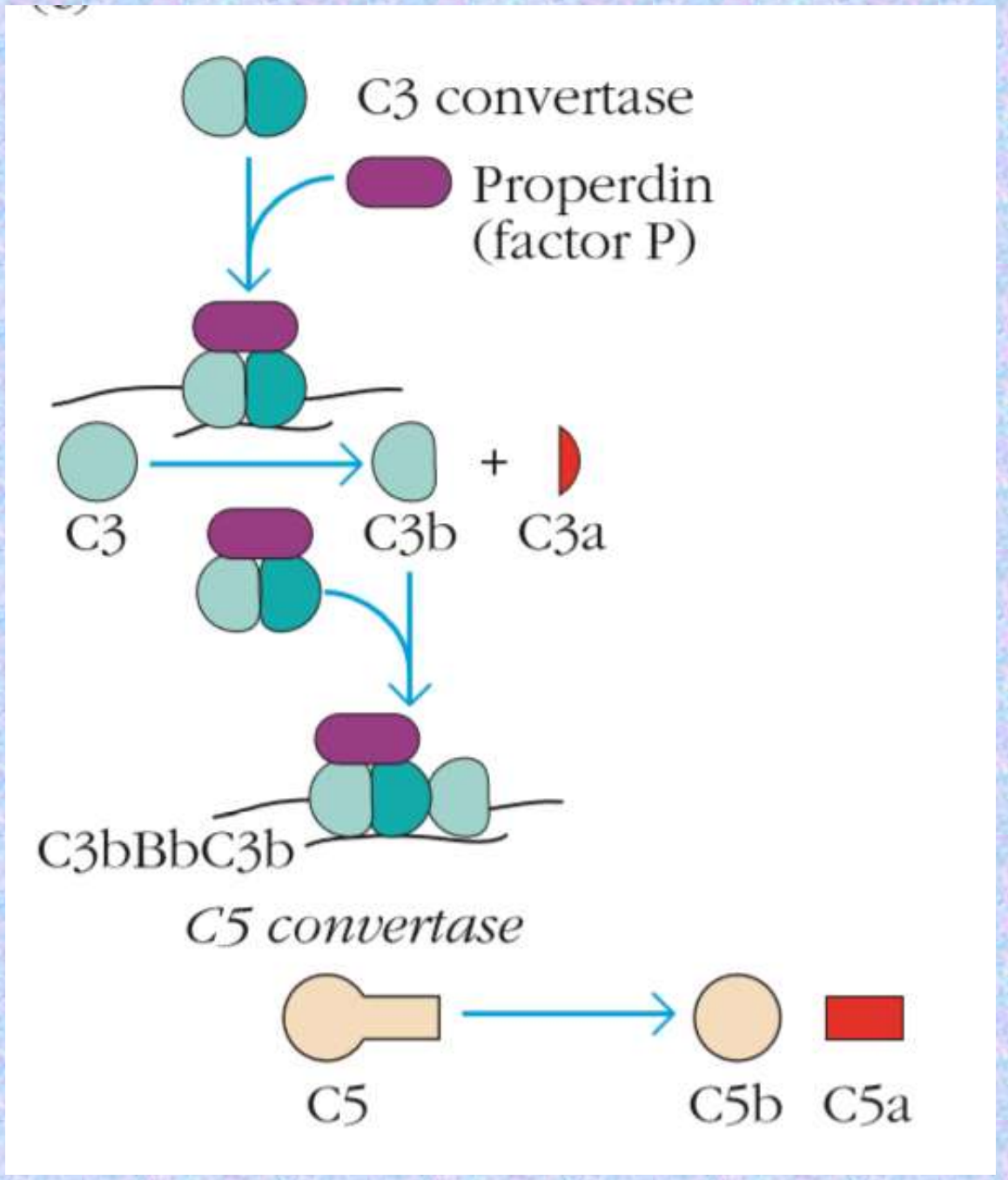
• In the alternative pathway, serum C3 is subject to spontaneous hydrolysis to yield C3a and C3b.
• The C3b component can bind to foreign surface antigens.
• The C3b present on the surface of the foreign cells can bind another serum protein called factor B to form a complex.
• Binding to C3b serves as the substrate for an enzymatically active serum protein called factor D.
• Factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba) that diffuses away and generating C3 convertase.

• The cell-bound C3bBb C3 convertase is unstable until it is bound by properdin, a protein from the serum. in addition to stabilizing the ongoing activity of the alternative pathway, properdin may also serve to initiate it.
• C3 convertase formation is a crucial step in all three complement pathways.
• The C3 convertase cleaves C3 into two fragments: the smaller, C3a and large fragments, C3b.
• Just as the C5 convertase of the classical and lectin pathways was formed by the addition of C3b to the C4b2a C3 convertase complex, so the C5 convertase

of the alternative pathway is formed by the addition of C3b to the alternative pathway C3 convertase complex.

- The C5 convertase complex therefore has the composition C3bBbC3b, and, like the C3 convertase, is also stabilized by binding to factor P.

- Like the classical and lectin pathway C5 convertase, C3bBbC3b cleaves C5, which goes on to form the MAC.


C3 convertase
Properdin
(factor P)
C3
C3b
+
C3a
C3bBbC3b
C5 convertase
C5
C5b
C5a

❖ The three complement pathways converge at the formation of the C5 convertase:

• The end result of the three initiation pathways is the formation of a C5 convertase.
• For the classical and lectin pathways, the C5 convertase has the composition C4b2a3b; for the alternative pathway, the C5 convertase has the formulation C3bBbC3b.
• However, the end result of all types of C5 convertase activity is the same: the cleavage of the C5 molecule into two fragments, C5a and C5b.

• The large C5b fragment is generated on the surface of the target cell or immune complex and provides a binding site for the subsequent components of the MAC.

❖ C5 initiates the generation of MAC:

• Up to this point in the complement cascades, all of the complement reactions take place on the hydrophilic surfaces of microbes or on immune complexes in the fluid phase of blood, lymph, or tissues.

• In contrast, when C5b binds C6 and C7, the resulting complex undergoes a conformational change that exposes hydrophobic regions on the surface of the C7 component capable of inserting into the interior of the microbial membrane.

• Released C5b67 complexes can insert into the membrane of nearby cells and mediate “innocent bystander” lysis.

• C8 is made up of two peptide chains: C8β and C8αγ.

• Binding of C8 to the C5b67 complex induces a conformational change in the C8 dimer such that the hydrophobic domain of C8γ can insert into the interior of the phospholipid membrane.

• The C5b678 complex can create a small pore, 10 Å in diameter, and formation of this pore can lead to lysis of red blood cells, but not of nucleated cells.

• The final step in the formation of the MAC is the binding and polymerization of C9 to the C5b678 complex.

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* Converge at C_5 convertase.

17 MON

Classical, Lectin → C_4b2a3b

Alternative → C_3bBb3b

→ C_5 → C_5a, C_5b.

↓

C_5b generated will provide a binding site for MAC.

* Generation of MAC :-

C_5b binds C_6 and C_7 → C_5b67 complex

↓

C_5b678 complex

18 TUE

↓

it can polymerize C_9 molecules (about 10 to 19)

↓

Thus, C_5b678 + surrounded by poly C_9 complex

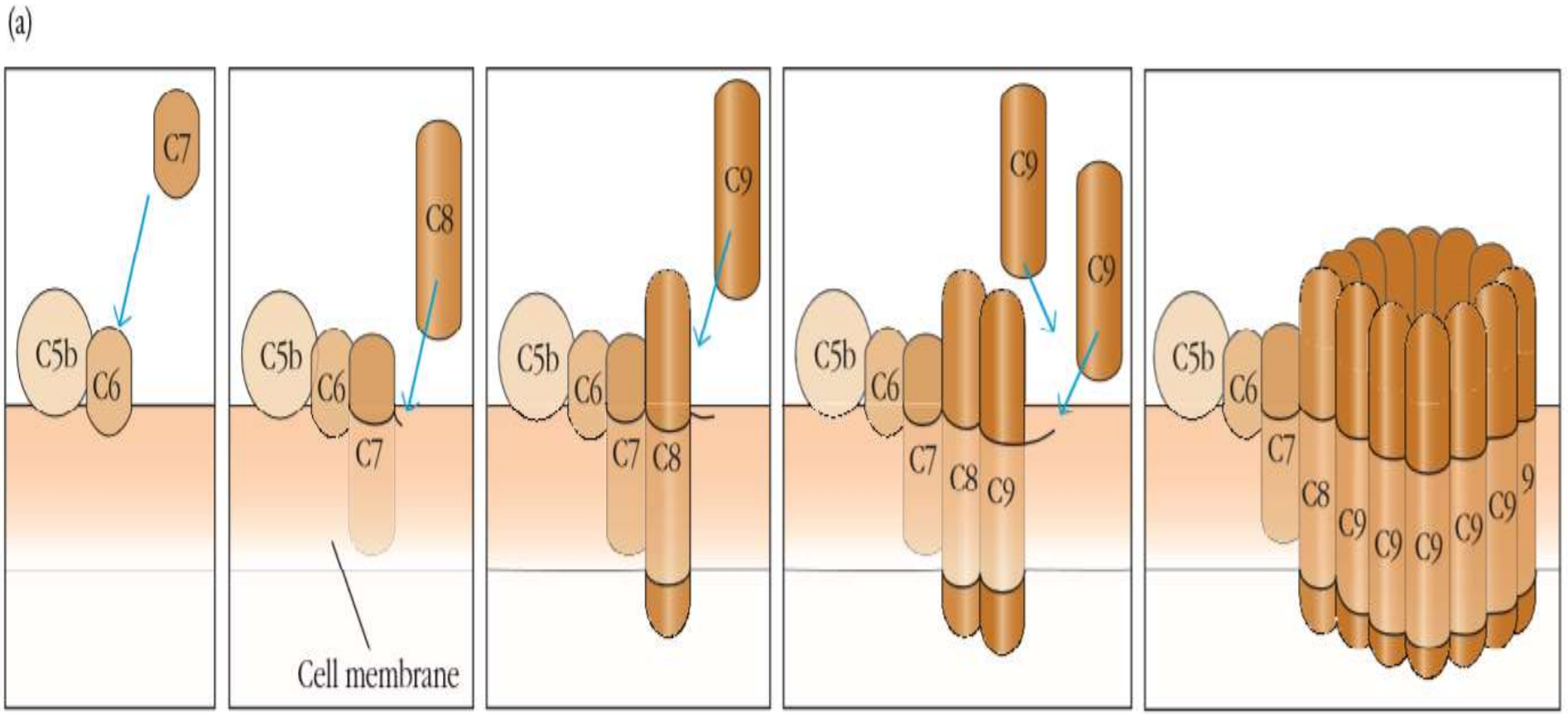
↓

MAC (formation of pore).

↓

lysis. (cell death).

• As many as 10 to 19 molecules of C9 can be bound and polymerized by a single C5b678 complex.
• During polymerization, the C9 molecules undergo a transition, so that they, too, can insert into the membrane.
• The completed MAC, which has a tubular form and functional pore diameter of 70 Å to 100 Å, consists of a C5b678 complex surrounded by a poly-C9 complex .
• Loss of plasma membrane integrity leads irreversibly to cell death.


(a)
C7
C5b
C6
C8
C7
Cell membrane
C9
C7
C8
C9
C9
C7
C8
C9
C7
C8
C9
C9
C9
C9
C9
9

(b)