The Complement System

Dr. Mahmood Altobje

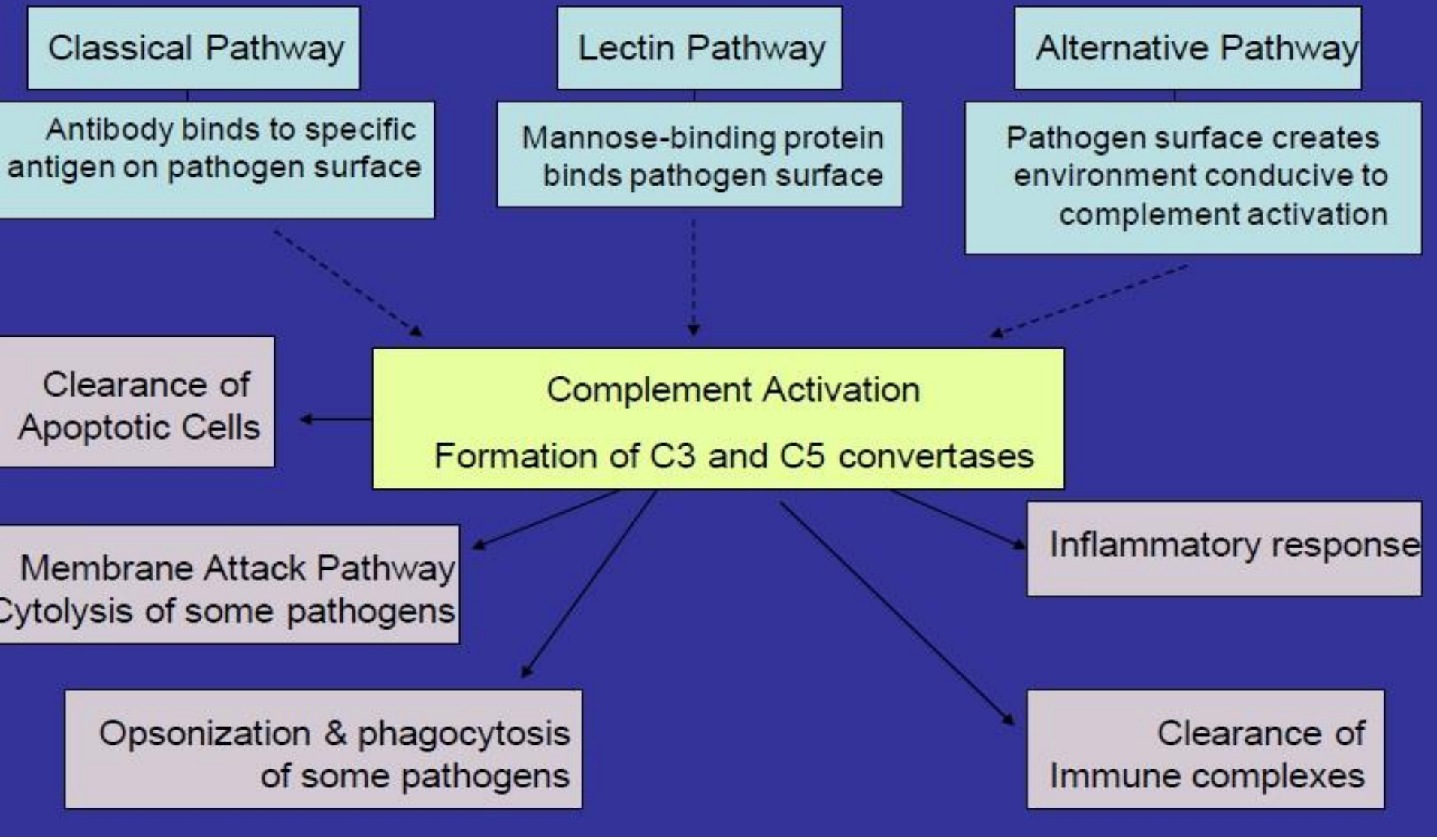
Introduction

•The complement system consists of a group of serum proteins that act in concert and in an orderly sequence to exert their effect

•These proteins are not immunoglobulins and their concentrations in serum do not increase after immunization

•Complement activation (fixation) leads to lysis of cells and to the generation of many powerful biologically active substances

Overview of Complement



Complement Activation Pathways

•The Classical Pathway

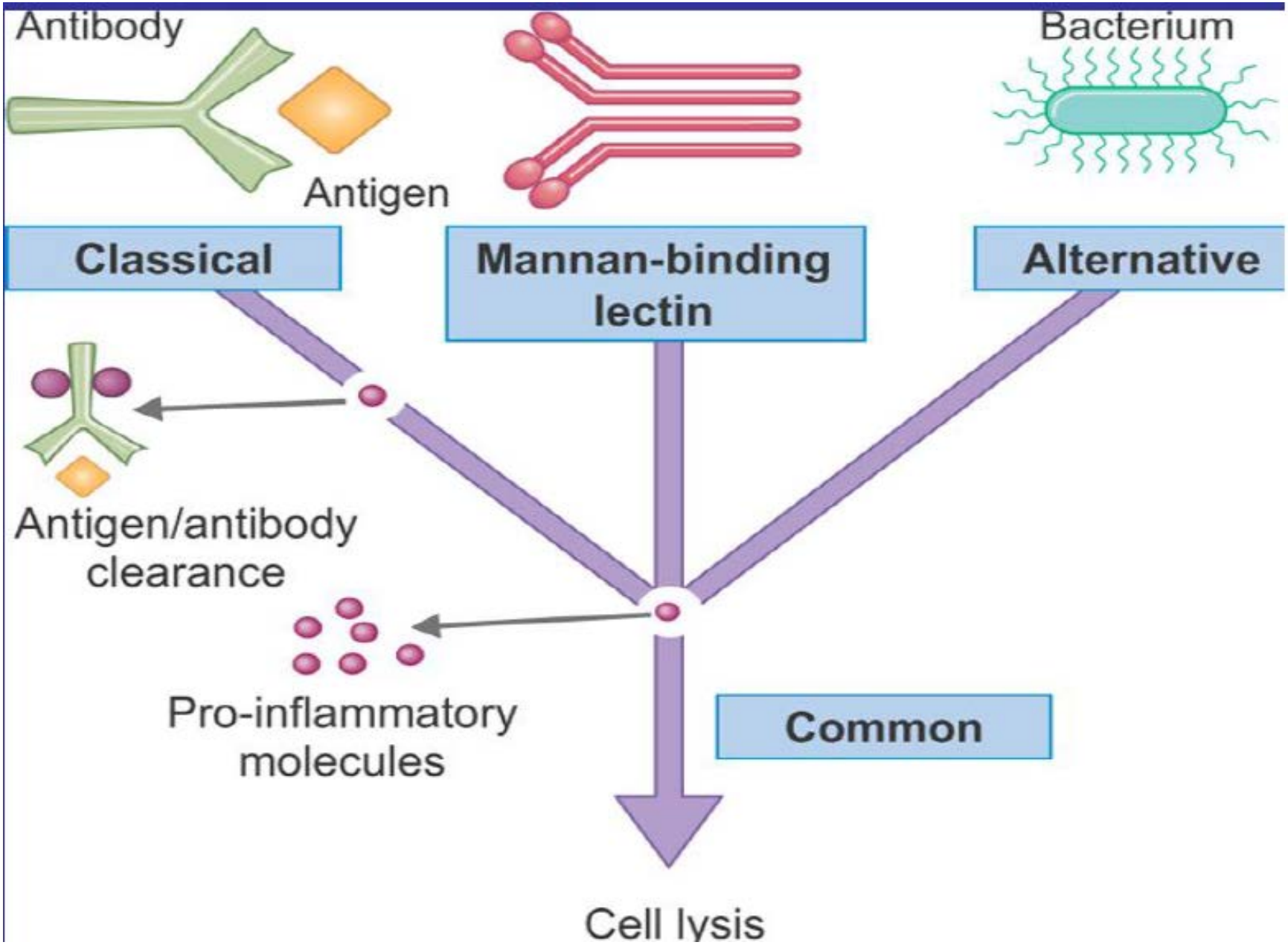
Ag- Ab complexes -

•The Alternative Pathway

- Aggregated immunoglobulins and microbial products

•The Mannan - Binding Lectin Pathway

- Microbial products


Antibody
Antigen
Bacterium
Classical
Mannan-binding lectin
Alternative
Antigen/antibody clearance
Pro-inflammatory molecules
Common
Cell lysis

The Classical Pathway

- Activators: Ag – Ab complexes
- Antibodies involved: IgG and IgM
- Activation in an orderly fashion of nine major protein components; C1 – C9
- Products of activation are enzymes that catalyze the subsequent step

The Classical Pathway

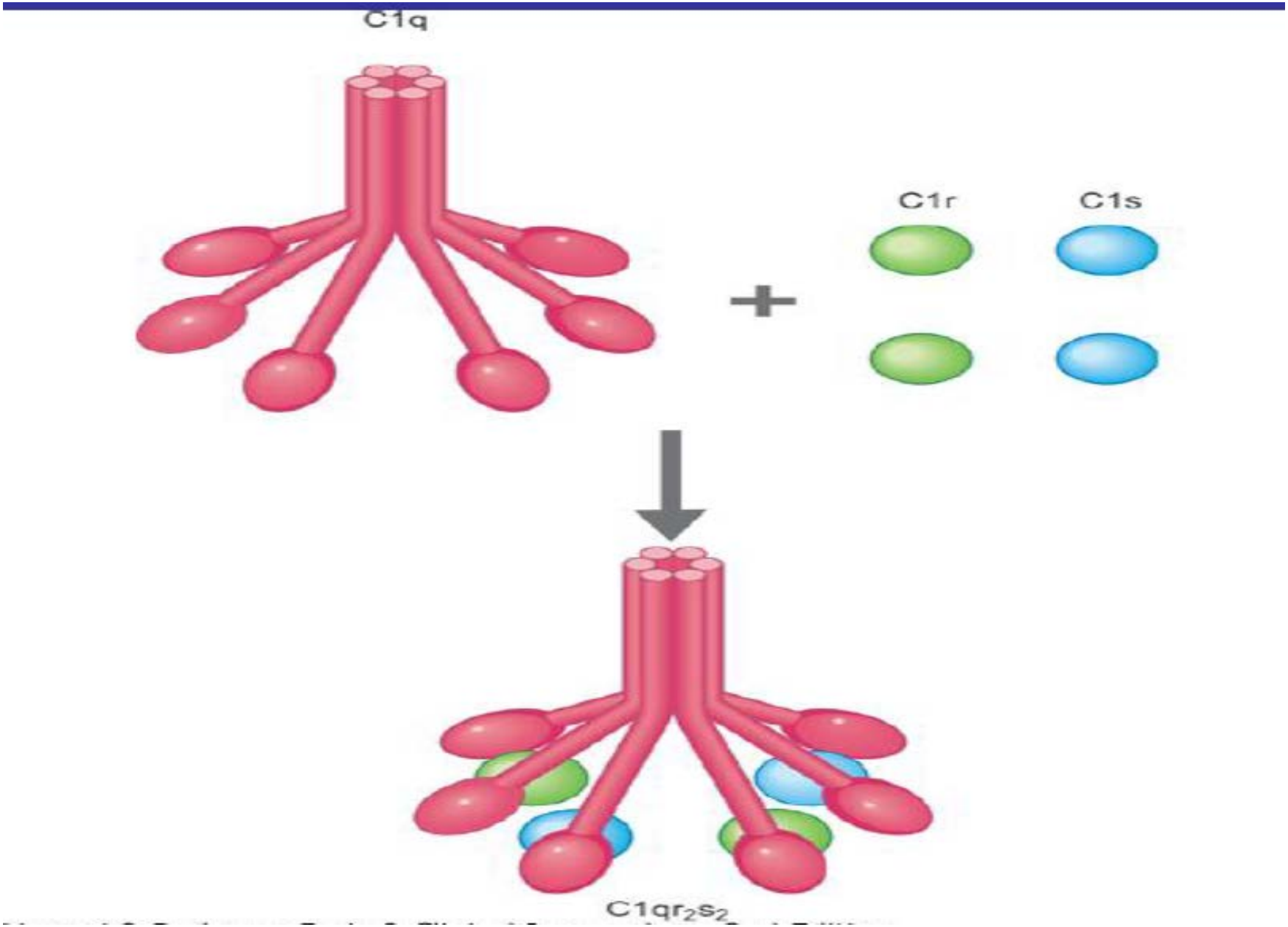
- Activation of C1:

- C1 consists of C1q (400.000 Daltons), C1r (95000 Daltons), and C1s (85000 Daltons)

- Subunits are held together by Calcium ions

- C1q is a polymer of 6 identical units

- C1q activation requires binding to a c1q- specific receptor on the FC region of at least 2 adjacent molecules of IgG or a single molecule of IgM, a reaction that requires Calcium ions


C1q
C1r
C1s
$C1qr_2s_2$

Molecular structure of C1

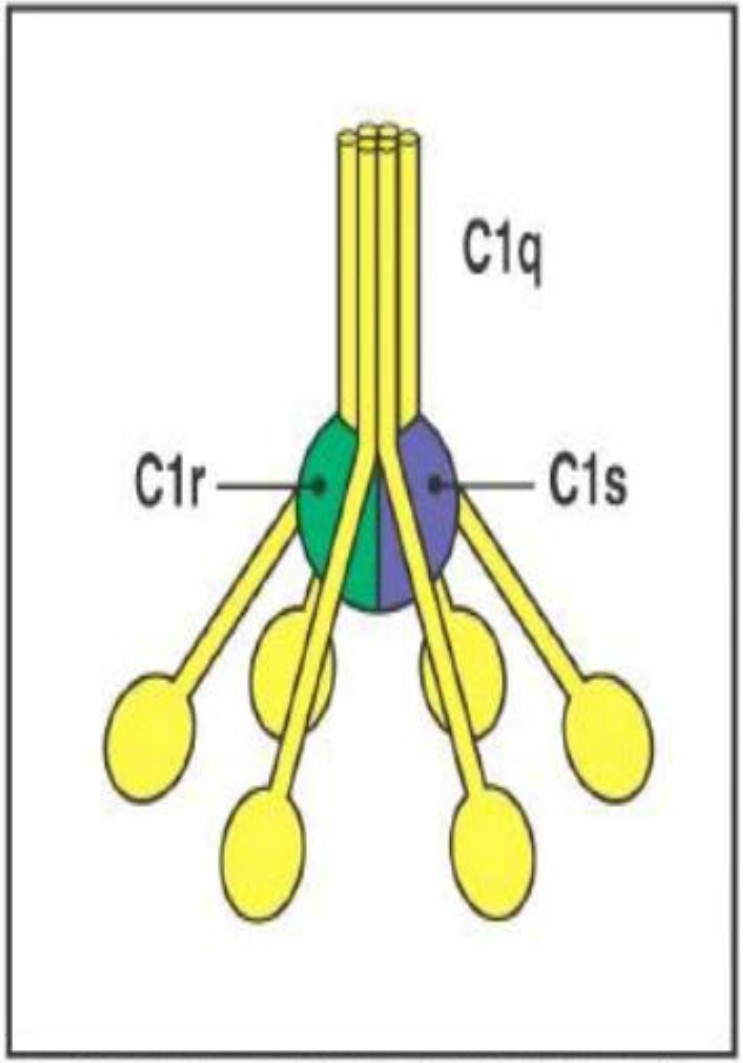
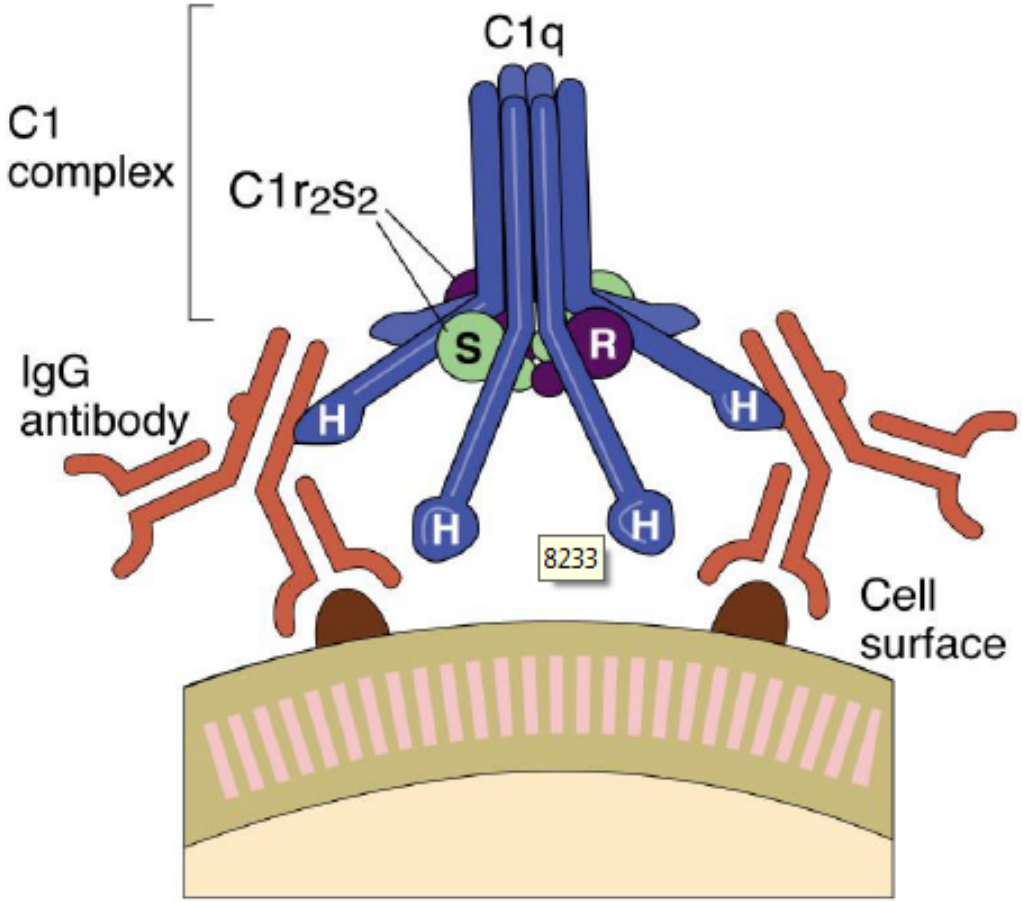


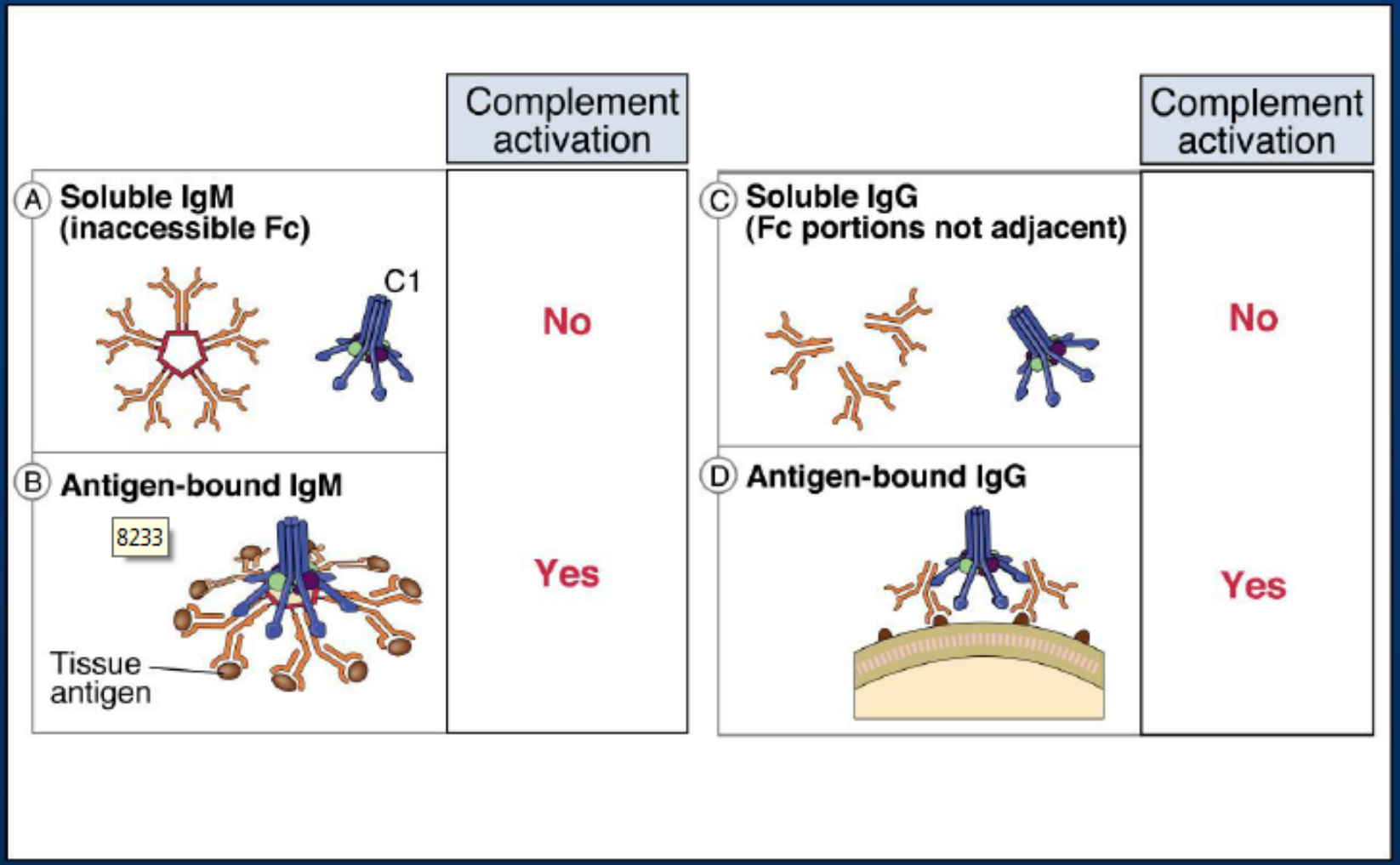
Figure 7-31 The Immune System, 2/e (© Garland Science 2005)

The C1 component of complement



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 14-9

Activation of complement by IgM and IgG antibodies



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 14-10

IgA and IgE cannot activate complement

The Classical Pathway

- IgG4, IgA, and IgE do not have complement receptors
- Activated C1q activates C1r which in turn activates C1s
- Activated C1s has esterolytic and proteolytic properties which acts on C4 splitting it into two fragments; C4a and C4b
- C4b complexes with C1s forming an active component that acts on C2 splitting it into C2a and C2b
- C2a binds to C4b creating a very active complex called the C3 convertase, where a single molecule can activate hundreds of C3 molecules

The Classical Pathway

- C3 is split by C4b2a into C3a and C3b

- C3b binds to cells and to C4b2a to generate C5 convertase which splits C5 into C5a and C5b

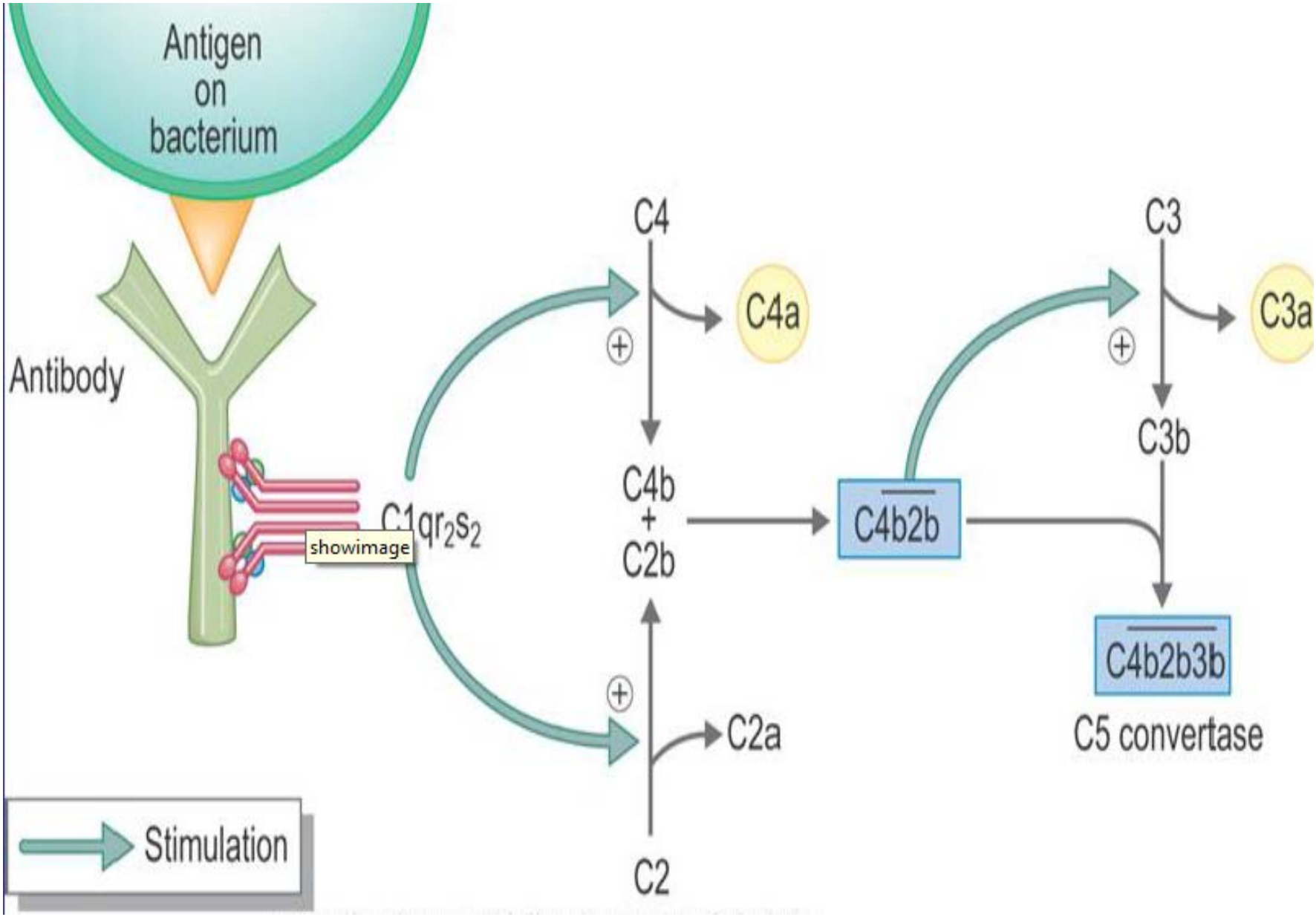
- C5b binds to cells and activates C6 and C7

- The complex C5b67 activates C8 and C9 forming a giant molecule with a molecular weight of 106 Daltons called the membrane attack complex (MAC)

The Classical Pathway

- C5b6789 bound to cells insert themselves into the cell membrane and produce transmembrane channels allowing ions to pass through

- The osmotic equilibrium of the cell is disturbed with rapid influx of water into the cell which swells and lyses


Antigen on bacterium
Antibody
C1qr2s2
showimage
C4
C4a
C4b + C2b
C2
C2a
C3
C3a
C3b
C4b2b
C4b2b3b
C5 convertase
Stimulation

The Alternative (properdin) Pathway

- Activators: Bacterial LPS, cell wall of some bacteria, some yeast cells, aggregated IgA, and a factor present in cobra venom

- Components: C3 – C9, factor B, factor D, and Properdin

- C3b present in trace amounts in serum combines with factor B forming C3bB

1. C3 hydrolyzes spontaneously; C3b fragment attaches to foreign surface.

2. Factor B binds C3a, exposes site acted on by factor D. Cleavage generates C3bBb, which has C3 convertase activity.

figure 7-07

3. Binding of properdin stabilizes convertase.

4. Convertase generates C3b; some binds to C3 convertase, activating C5' convertase. C5b binds to antigenic surface.

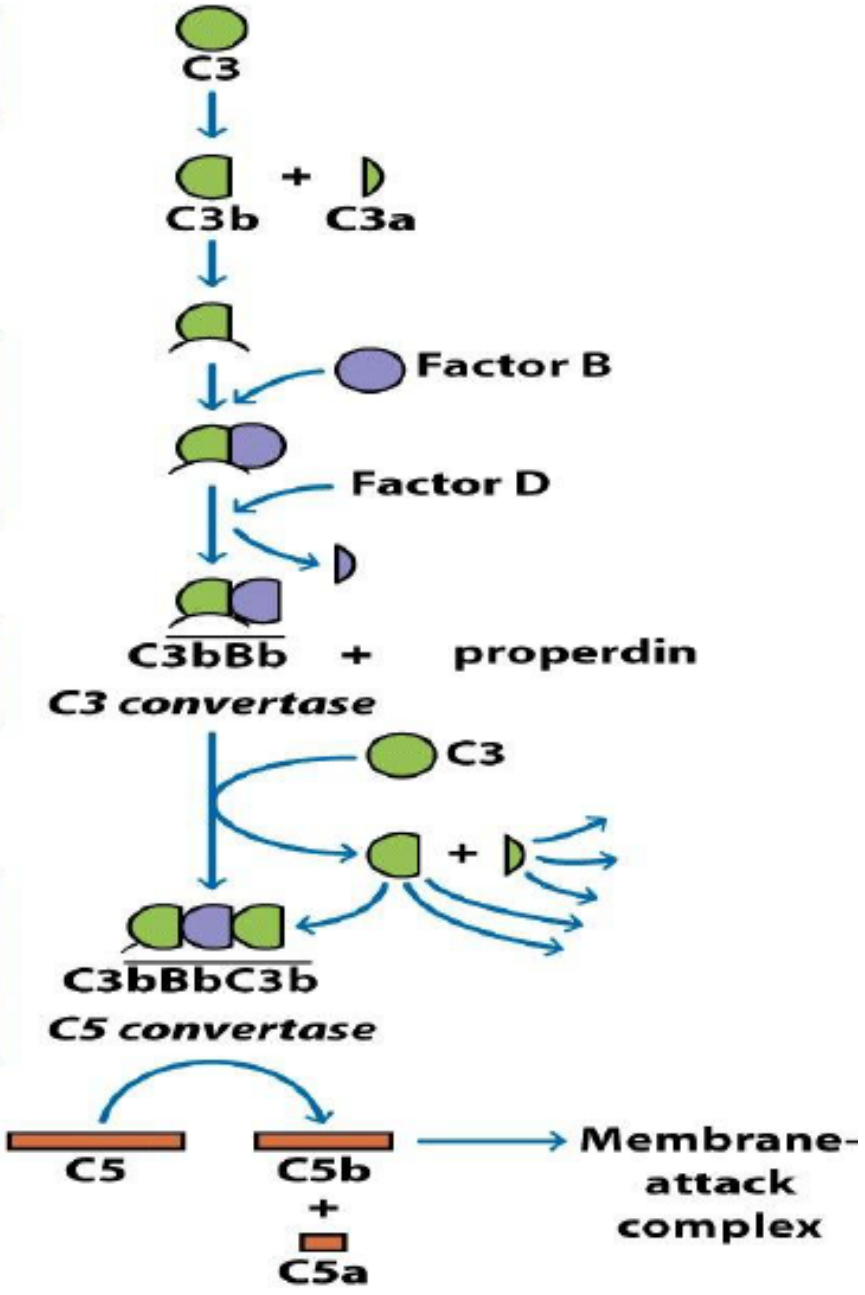
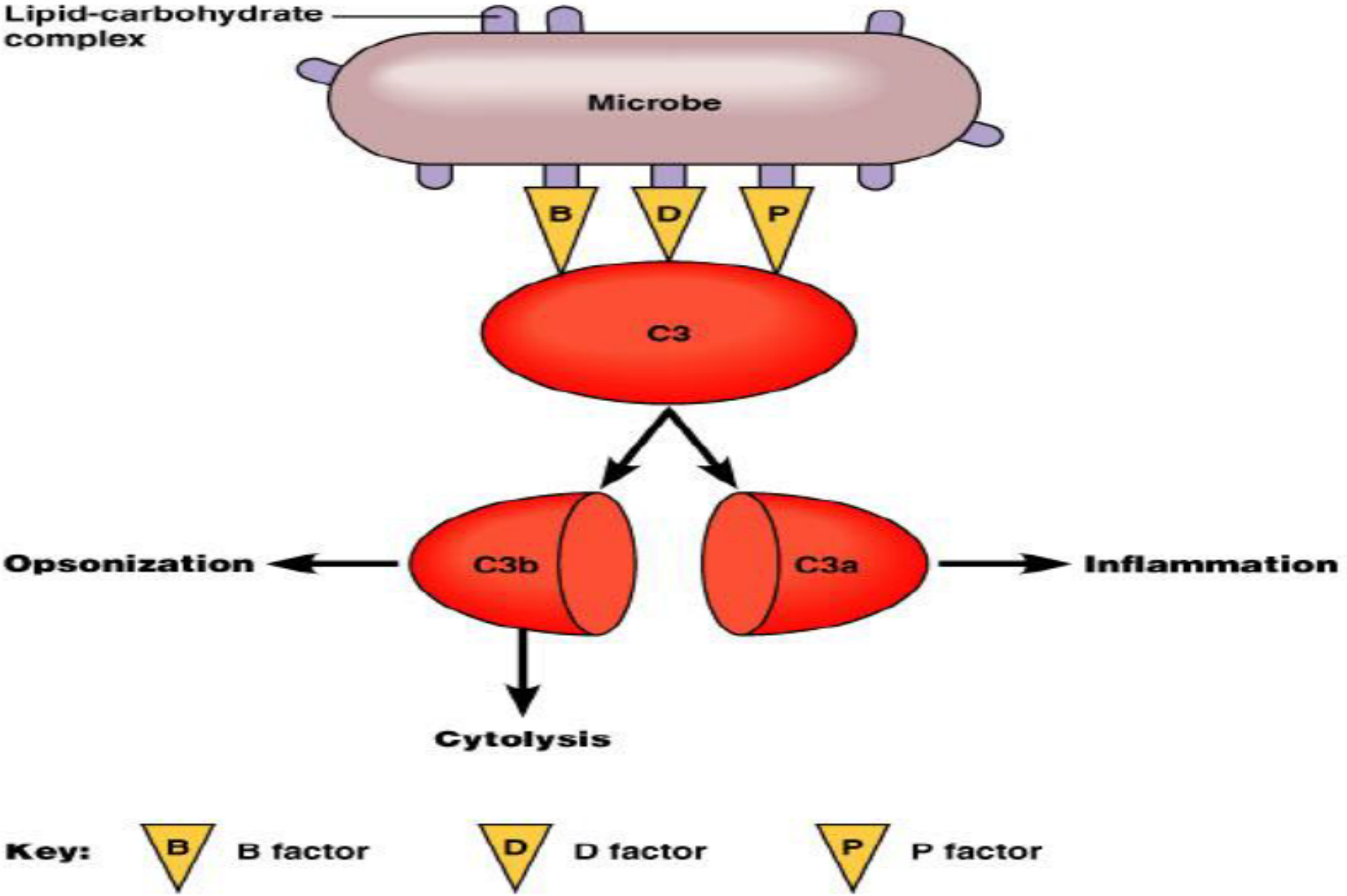


Figure 7-7

Alternative Pathway



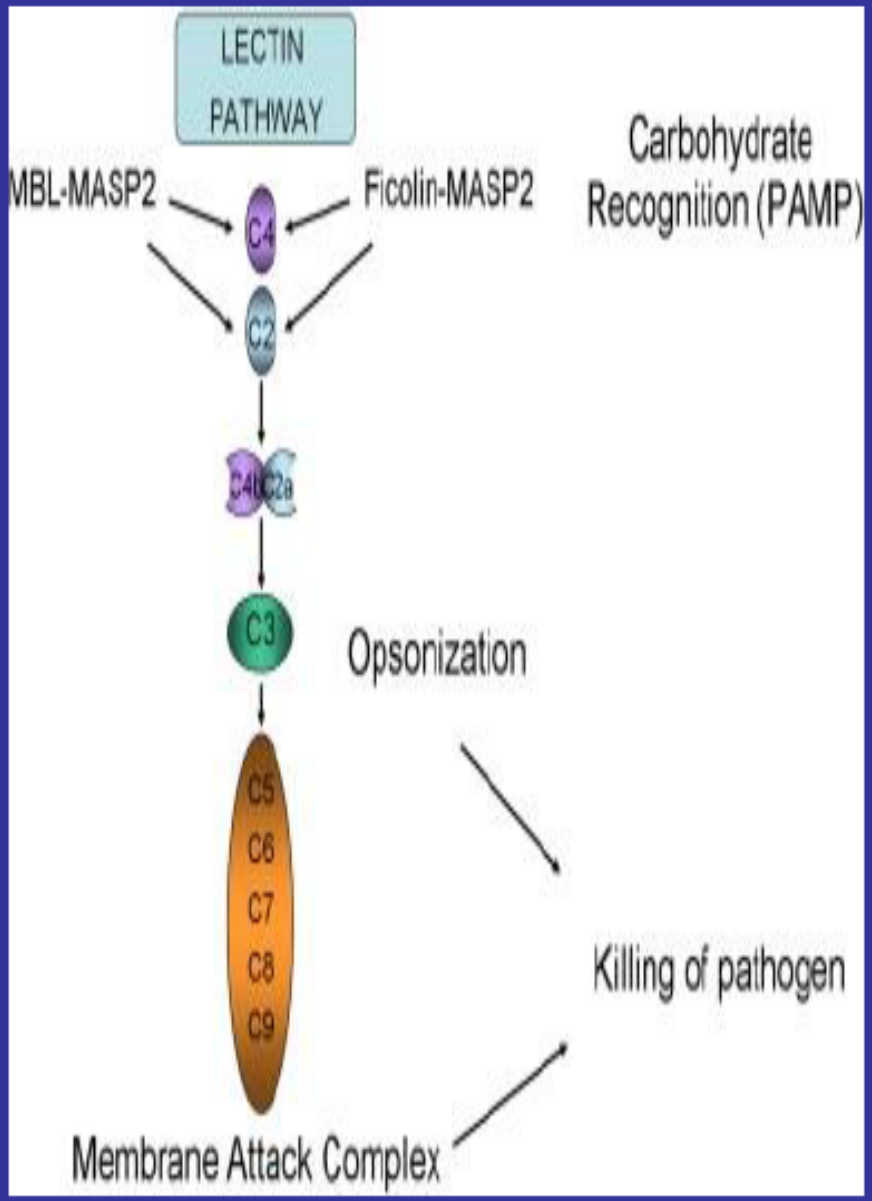
The Mannan Binding Lectin (MBL)

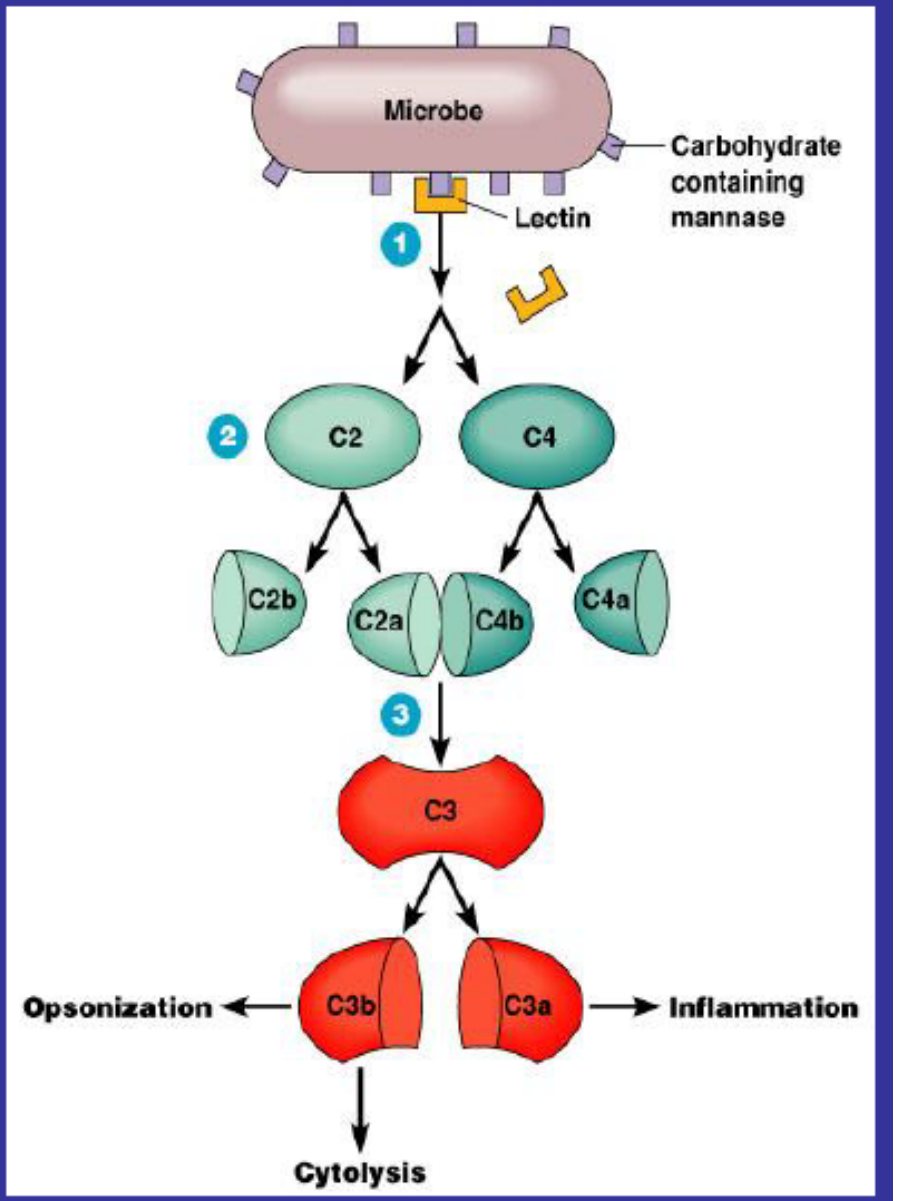
- Activators: microorganisms and foreign invaders

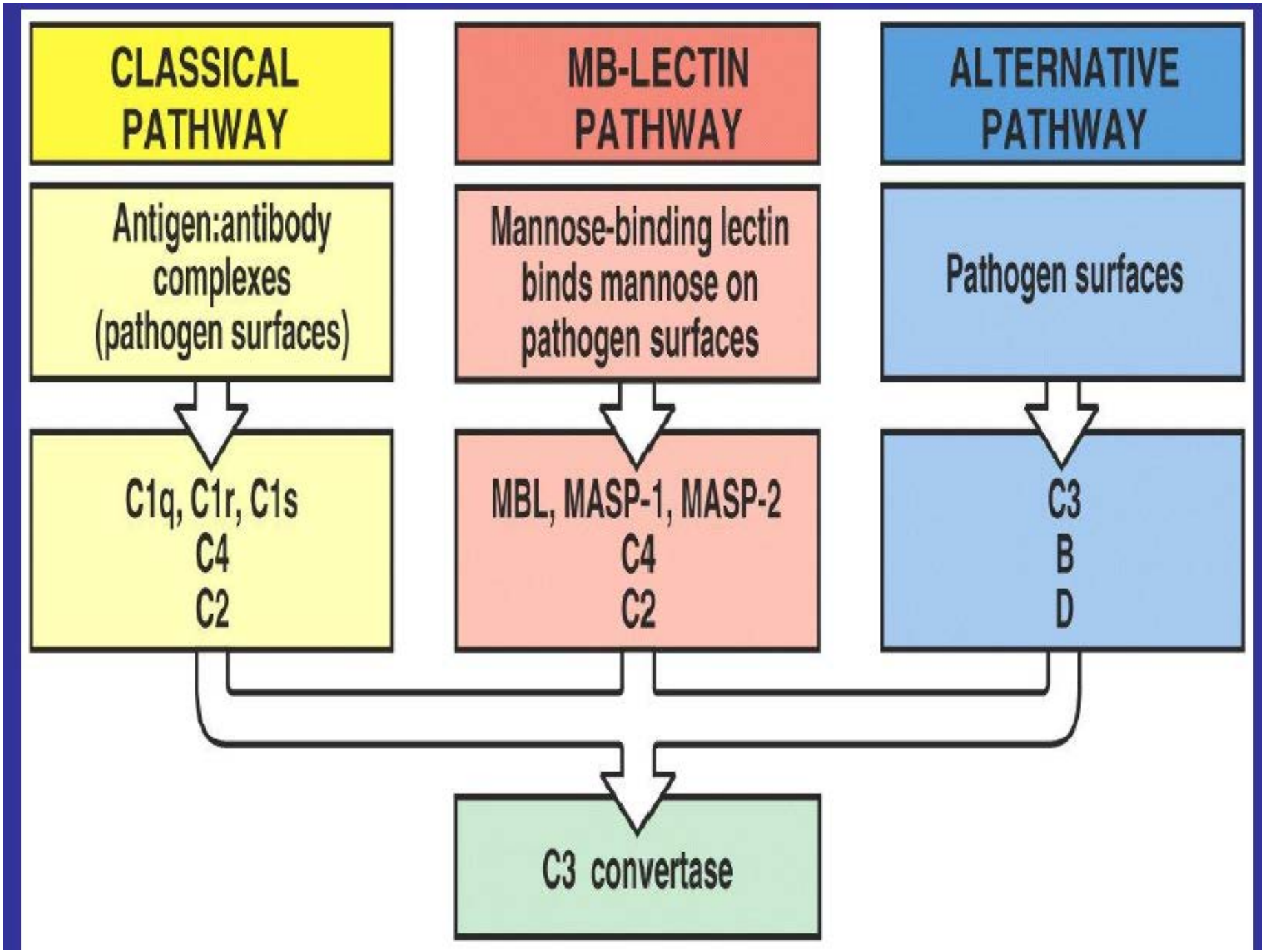
- Components: C2 – C9, MASP

- MBL recognizes carbohydrate structures through its carbohydrate – recognizing domain (CRD) and then it can interact with an enzyme called MBL – activated serine protease (MASP)

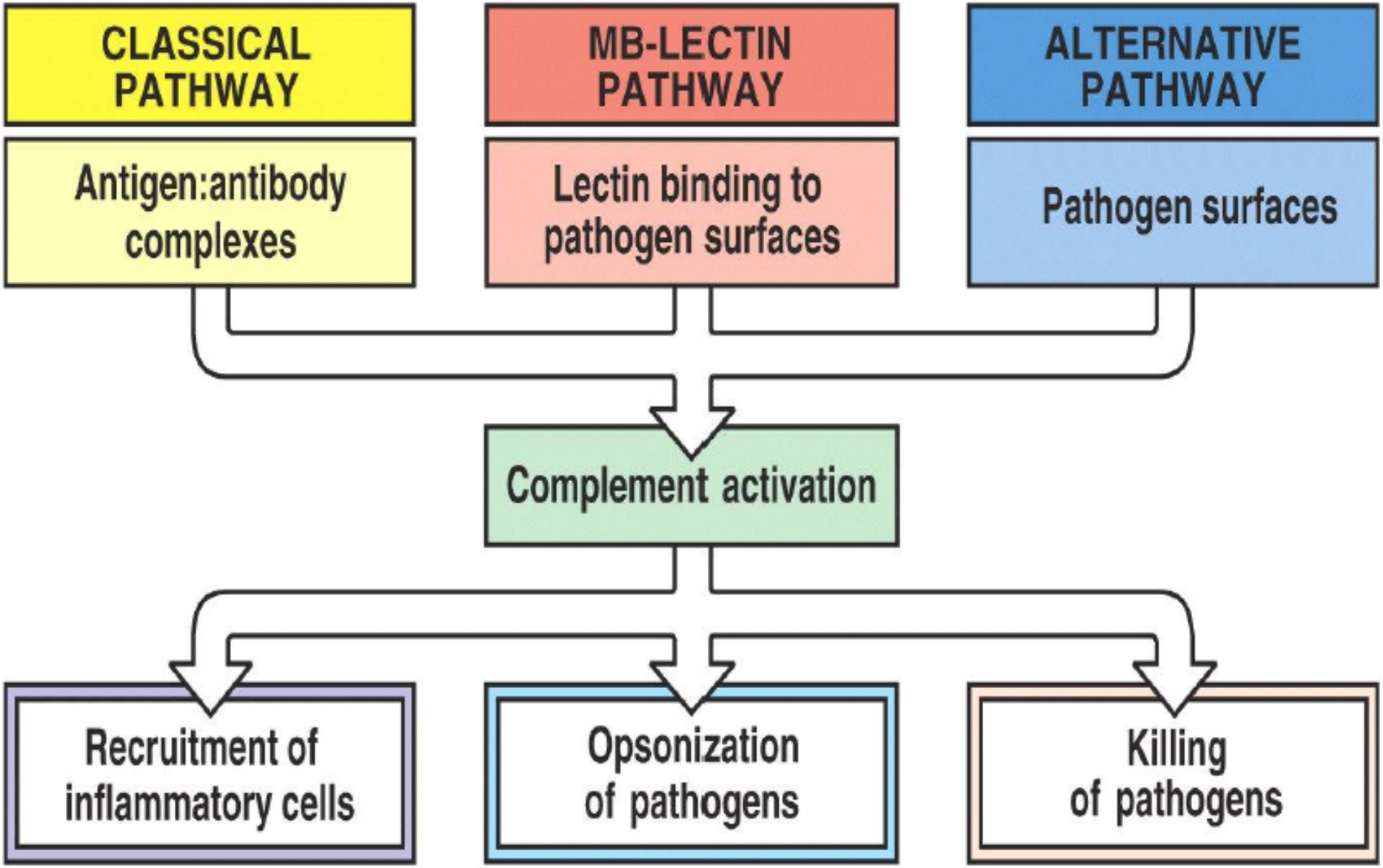
Lectin Pathway



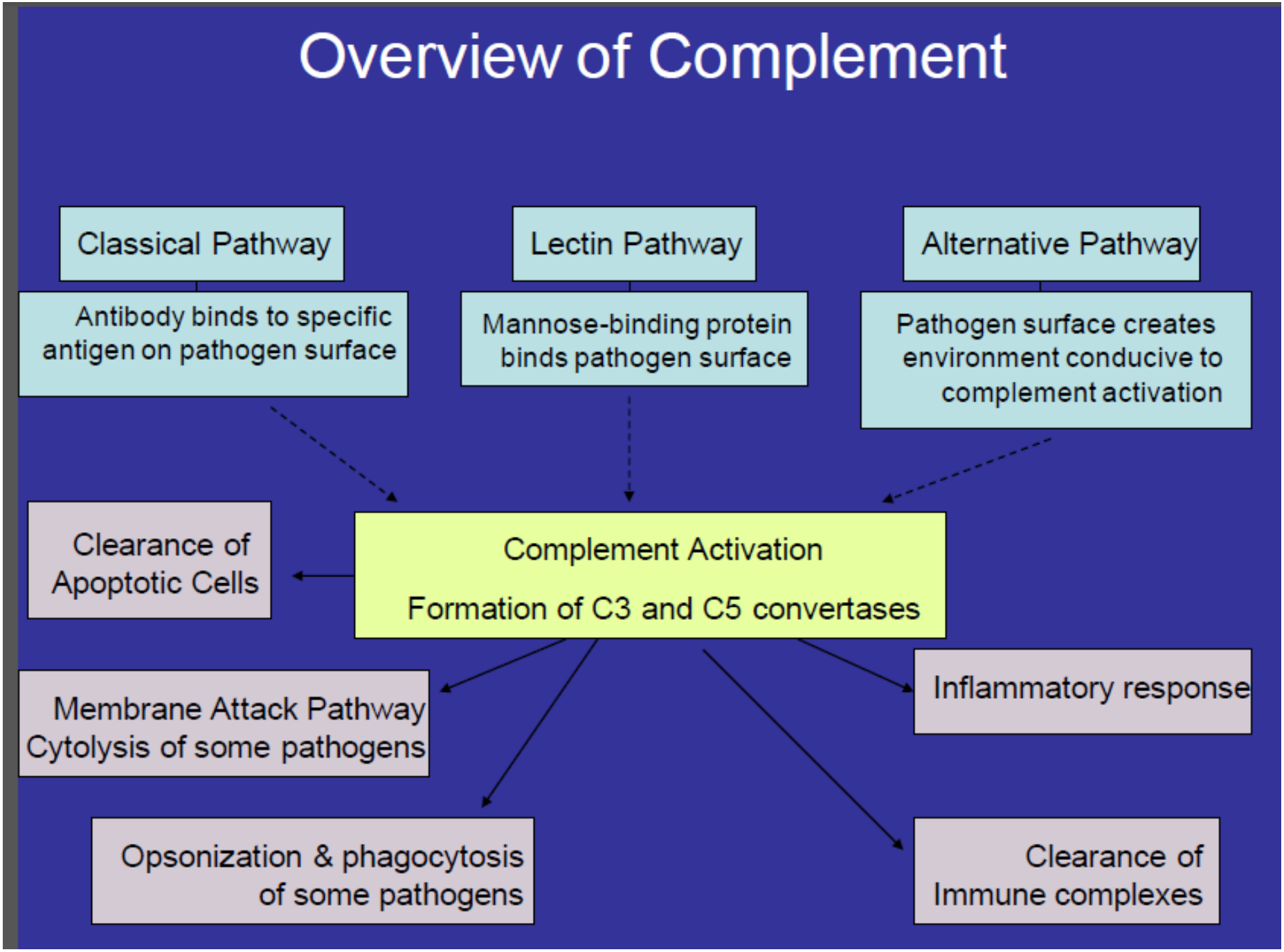



CLASSICAL PATHWAY
MB-LECTIN PATHWAY
ALTERNATIVE PATHWAY
Antigen:antibody complexes (pathogen surfaces)
Mannose-binding lectin binds mannose on pathogen surfaces
Pathogen surfaces
C1q, C1r, C1s
C4
C2
MBL, MASP-1, MASP-2
C4
C2
C3
B
D
C3 convertase

General Functions of Complement



Overview of Complement



Complement functions related to immune defense

- Lysis of cells: This is the original function identified and causes hypotonic cell death by making holes. It is not effective against organisms with rigid cell walls such as fungi

Terminal complement components and the formation of the membrane attack complex

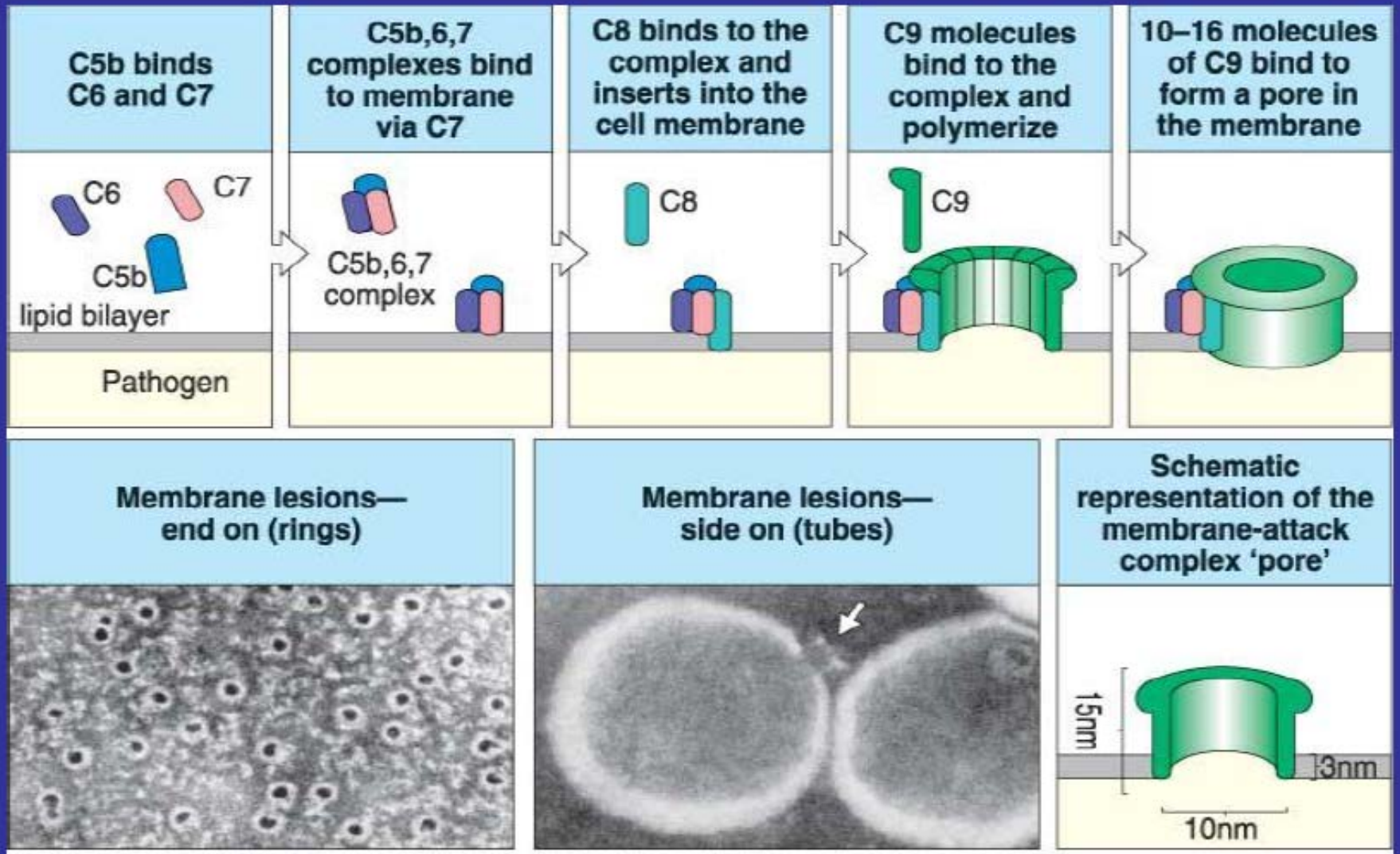
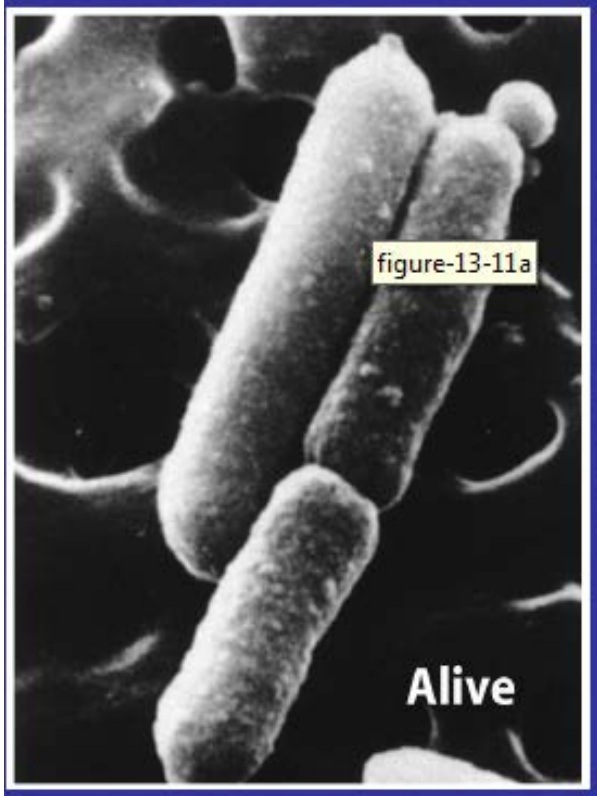
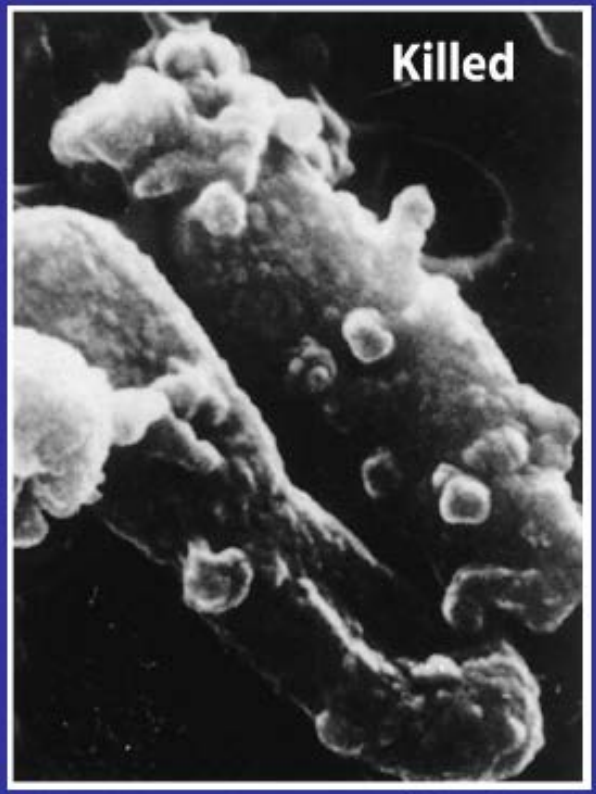
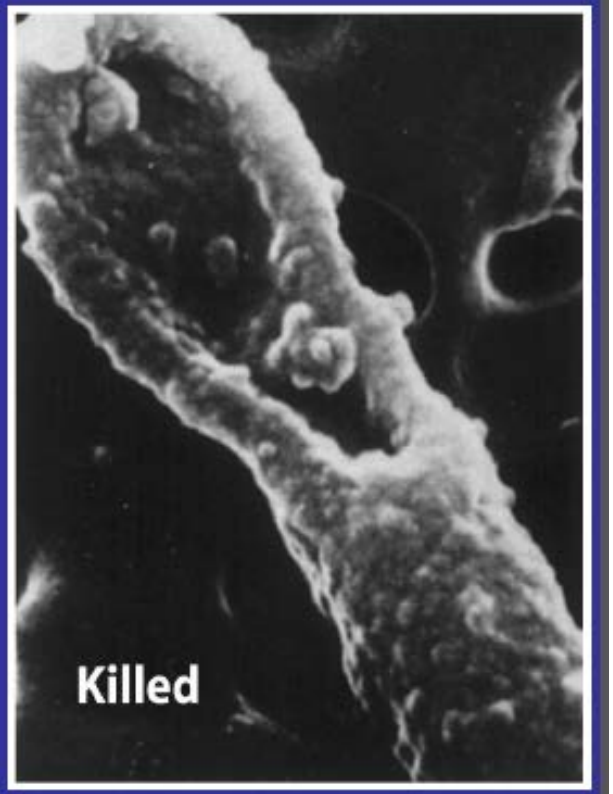


Fig 2.24 © 2001 Garland Science

The contents of the cell leak out through the MAC pore and the cell dies







Before complement

After complement treatment

Opsonization: Antigen coated with C3b binds to cells bearing complement receptors and if the cell is a phagocyte, the antigen will be phagocytosed.

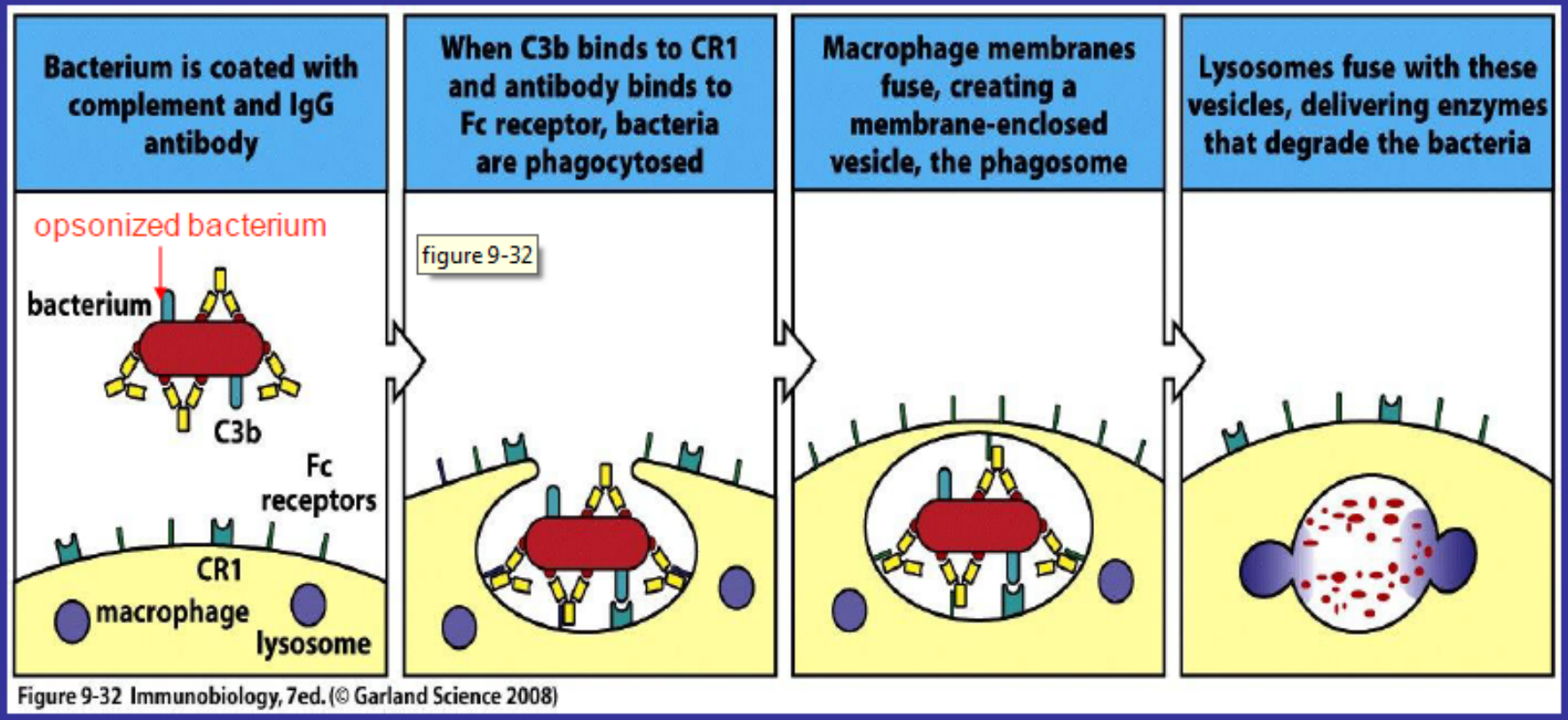


Figure 9-32 Immunobiology, 7ed. (© Garland Science 2008)

•**Inflammation:**

- A naphylatoxins: C5a, C3a, and C4a of which C5a is the most potent bind receptors on mast cells and basophils and cause degranulation with the release of pharmacologically active mediators which induce smooth-muscle contraction and increases in vascular permeability.

- Chemoattractants: C3a, C5a and C5b67 attract and induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillaries and migrate to the site of complement activation in the tissue.

Inflammation

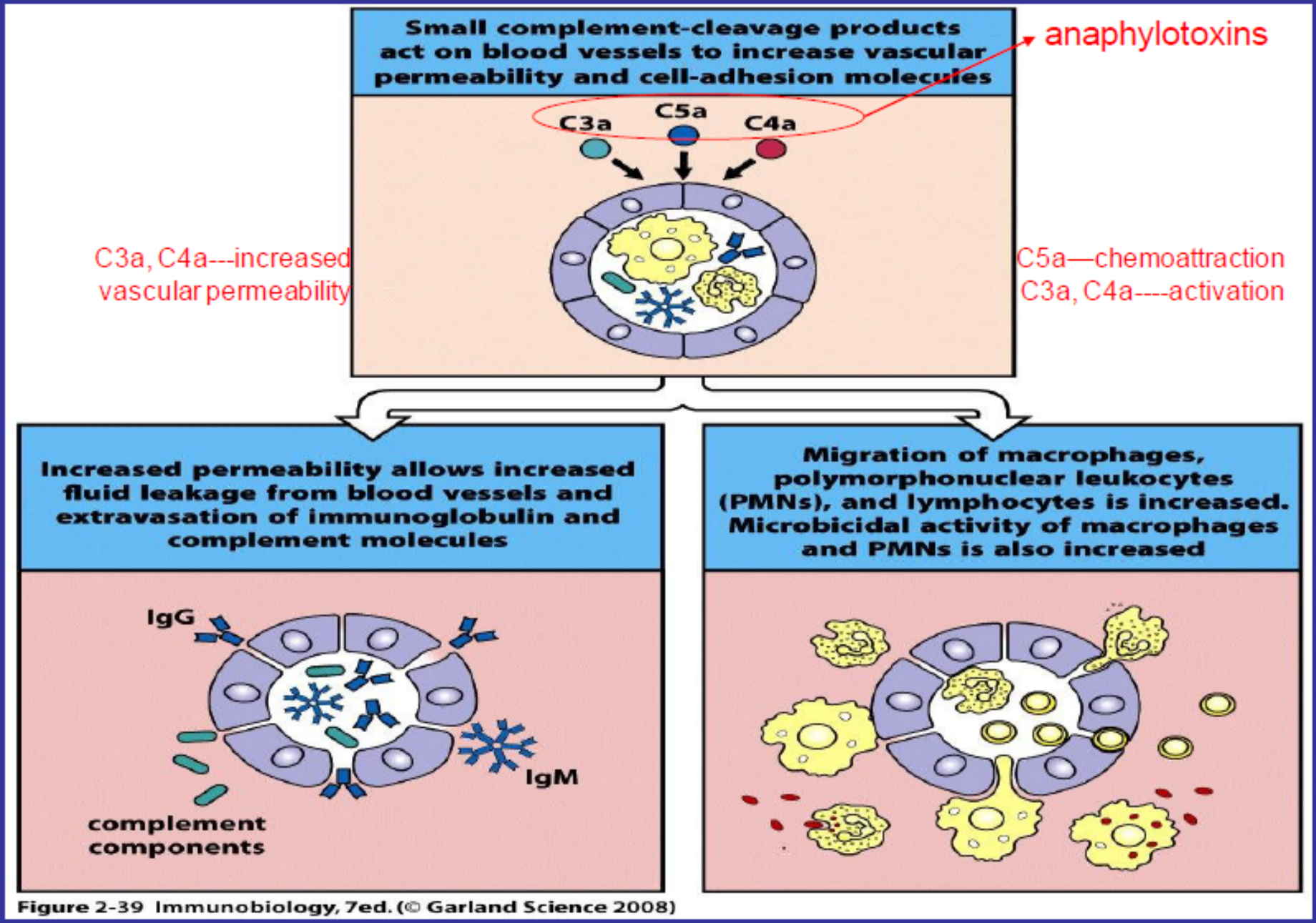


Figure 2-39 Immunobiology, 7ed. (© Garland Science 2008)

- Immune clearance: Removes immune complexes from the circulation and deposits them in the liver where they are degraded.

- Virus neutralization: Complement mediates viral neutralization by facilitating viral aggregation and by coating the viral surface.

Clearance of Immune Complexes

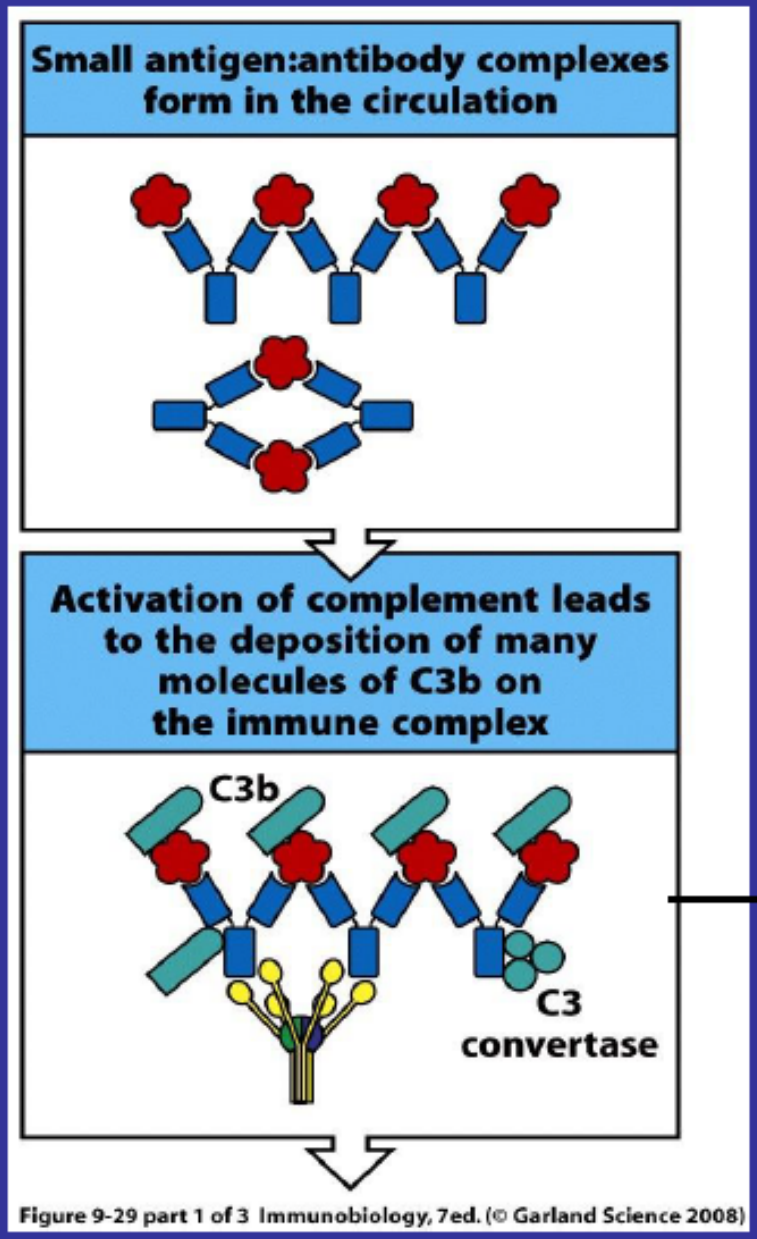


Figure 9-29 part 1 of 3 Immunobiology, 7ed. (© Garland Science 2008)

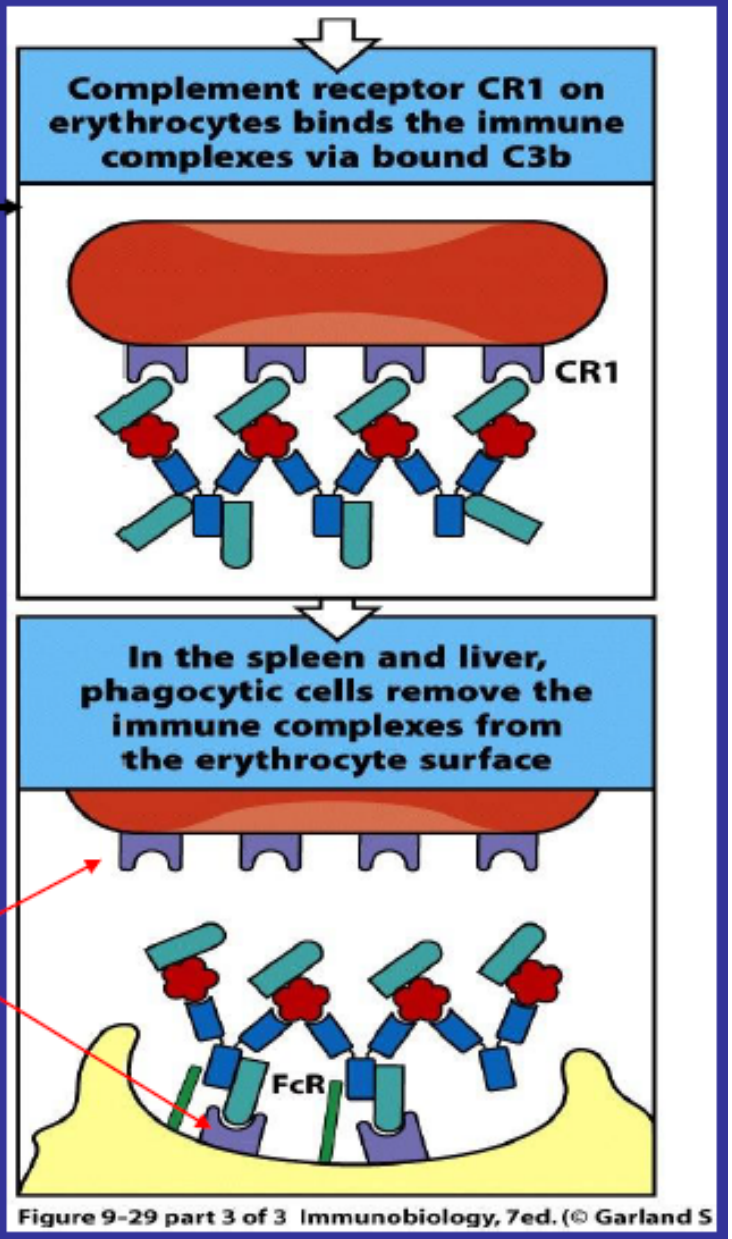
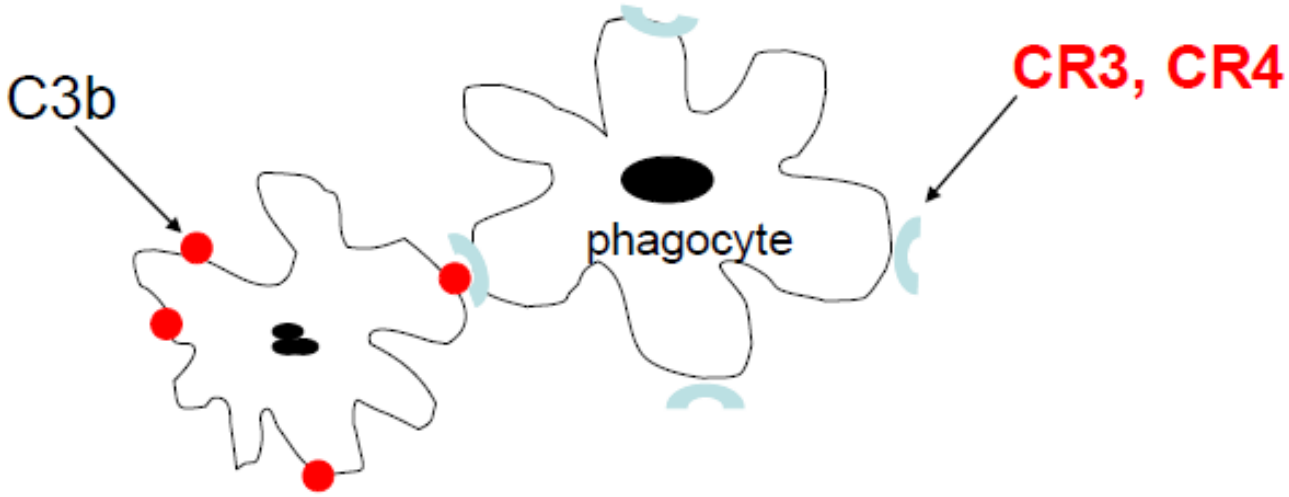
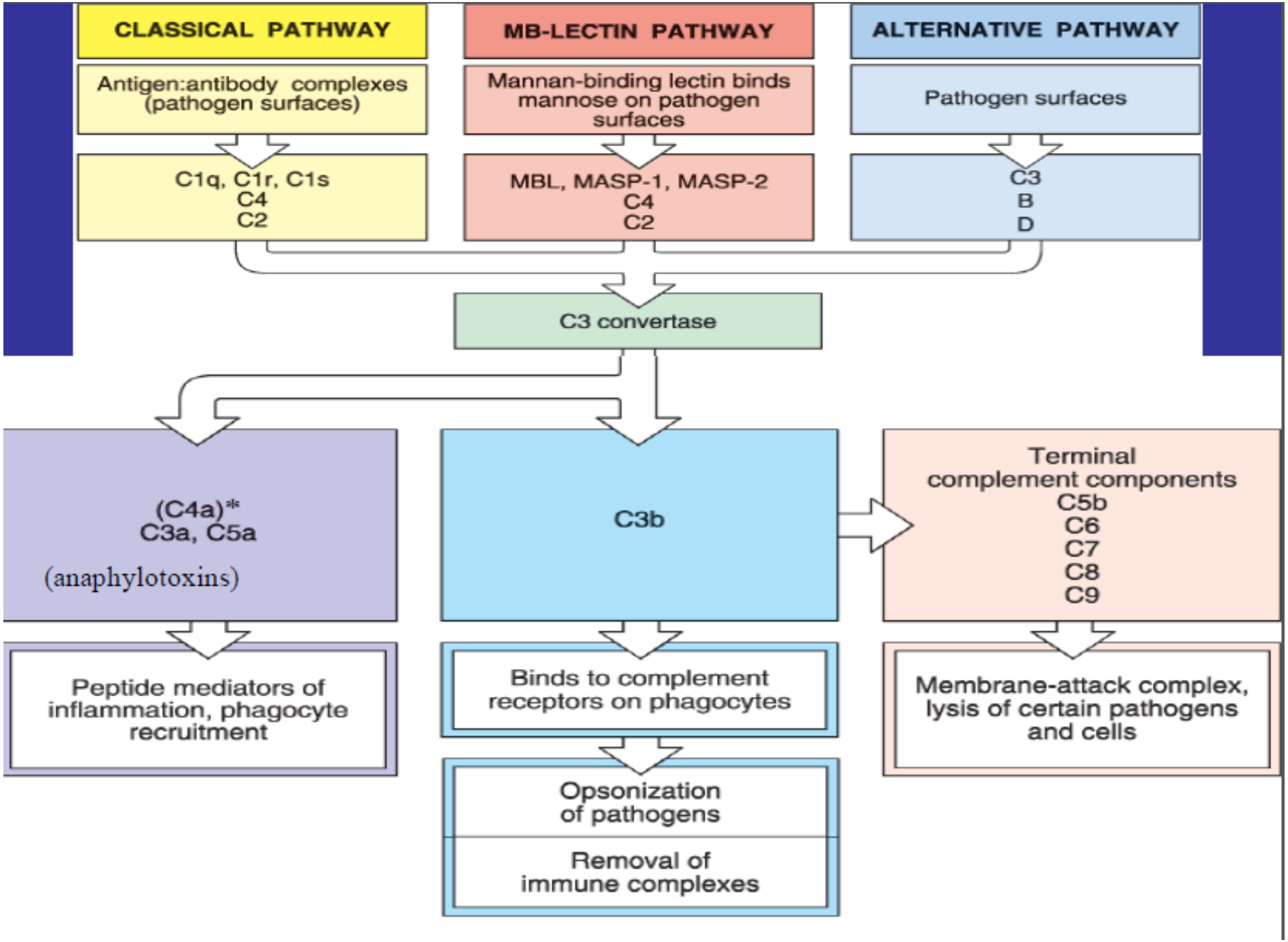


Figure 9-29 part 3 of 3 Immunobiology, 7ed. (© Garland S

Clearance of Apoptotic Cells



- Phagocyte recognizes C3b deposited on the surface of apoptotic cell
- Apoptotic cell is ingested and destroyed by phagocyte
- This is an important mechanism for clearing self antigens and preventing autoimmune responses
- Uptake of apoptotic cell also induces self tolerance, thereby prevents autoimmune response

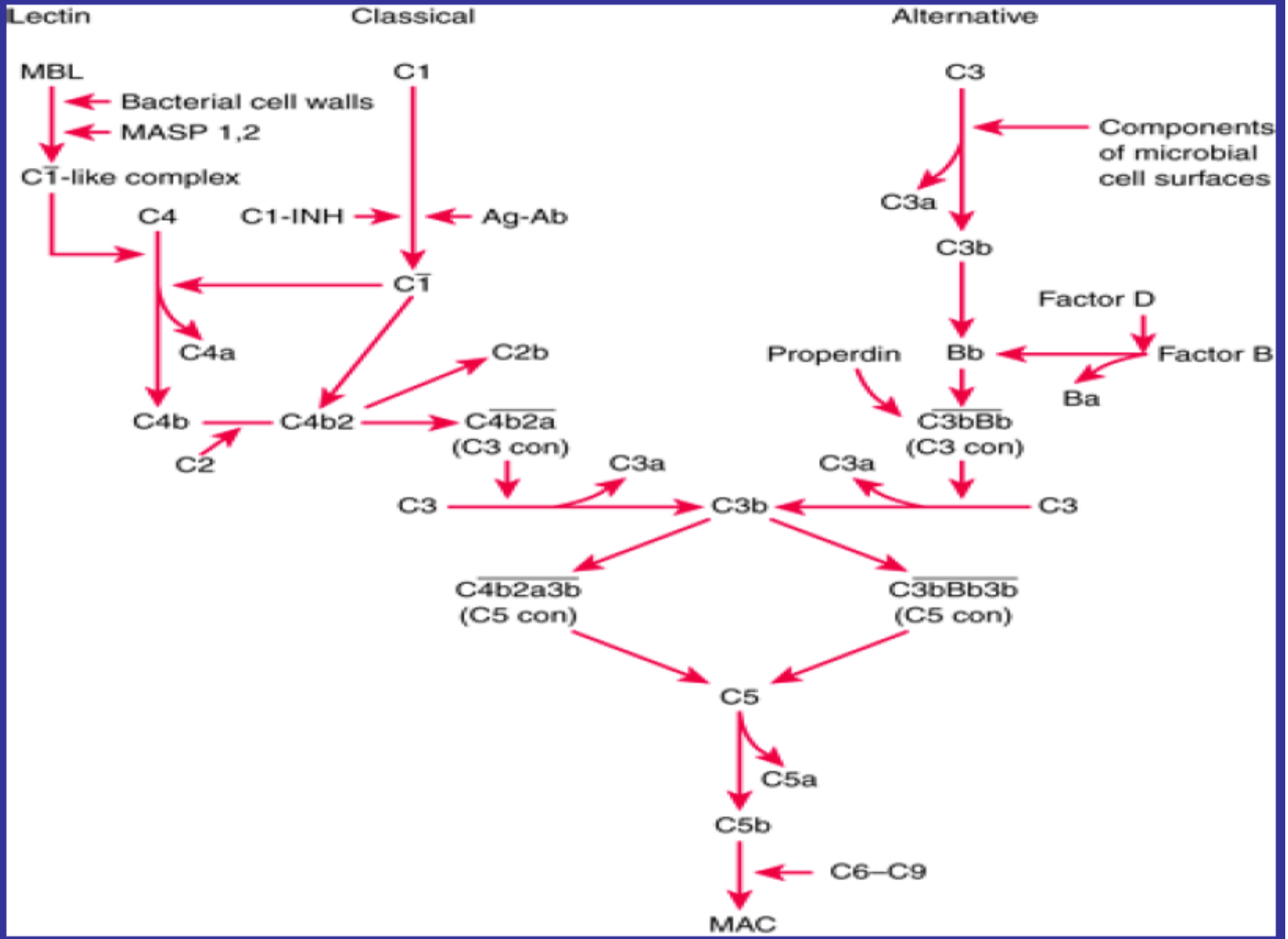

CLASSICAL PATHWAY
MB-LECTIN PATHWAY
ALTERNATIVE PATHWAY
Antigen:antibody complexes (pathogen surfaces)
Mannan-binding lectin binds mannose on pathogen surfaces
Pathogen surfaces
C1q, C1r, C1s
C4
C2
MBL, MASP-1, MASP-2
C4
C2
C3
B
D
C3 convertase
(C4a)*
C3a, C5a
(anaphylotoxins)
C3b
Terminal complement components
C5b
C6
C7
C8
C9
Peptide mediators of inflammation, phagocyte recruitment
Binds to complement receptors on phagocytes
Membrane-attack complex, lysis of certain pathogens and cells
Opsonization of pathogens
Removal of immune complexes

Functional protein classes in the complement system	
Binding to antigen:antibody complexes and pathogen surfaces	C1q
Binding to mannose on bacteria	MBL
Activating enzymes	C1r C1s C2b Bb D MASP-1 MASP-2
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a

Functional protein classes in the complement system	
Membrane-attack proteins	C5b C6 C7 C8 C9
Complement receptors	CR1 CR2 CR3 CR4 C1qR
Complement-regulatory proteins	C1INH C4bp CR1 MCP DAF H I P CD59

Regulatory proteins of the classical and alternative pathways

Name (symbol)	Role in the regulation of complement activation
C1 inhibitor (C1INH)	Binds to activated C1r, C1s, removing them from C1q, and to activated MASP-2, removing it from MBL
C4-binding protein (C4BP)	Binds C4b, displacing C2a; cofactor for C4b cleavage by I
Complement receptor 1 (CR1)	Binds C4b, displacing C2a, or C3b displacing Bb; cofactor for I
Factor H (H)	Binds C3b, displacing Bb; cofactor for I
Factor I (I)	Serine protease that cleaves C3b and C4b; aided by H, MCP, C4BP, or CR1
Decay-accelerating factor (DAF)	Membrane protein that displaces Bb from C3b and C2a from C4b
Membrane cofactor protein (MCP)	Membrane protein that promotes C3b and C4b inactivation by I
CD59 (protectin)	Prevents formation of membrane-attack complex on autologous or allogeneic cells. Widely expressed on membranes

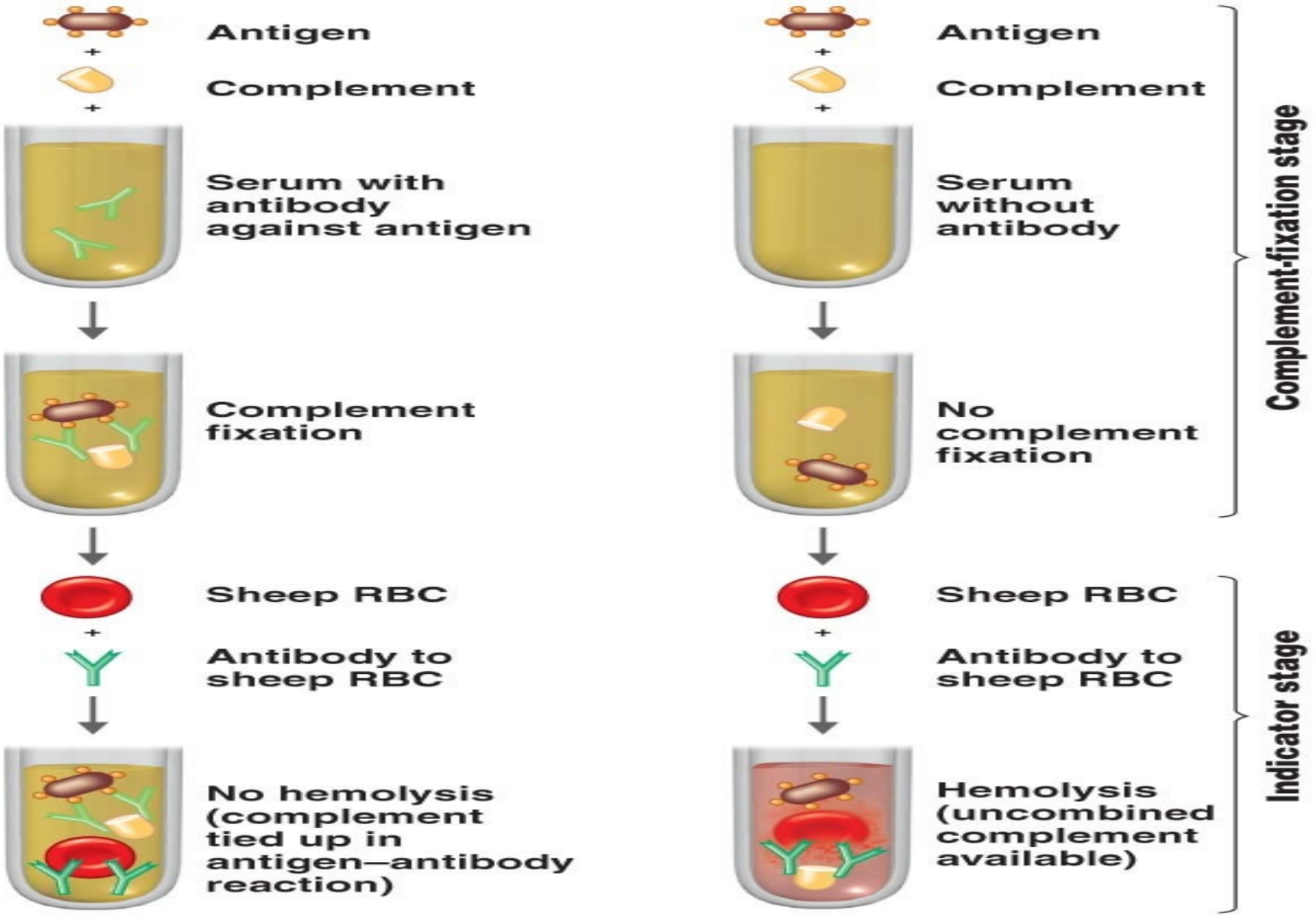

Lectin
Classical
Alternative
MBL
Bacterial cell walls
MASP 1,2
C1
C3
Components of microbial cell surfaces
C1-like complex
C4
C1-INH
Ag-Ab
C3a
C3b
C1
Factor D
C4a
C2b
Properdin
Bb
Factor B
Ba
C4b
C4b2
C4b2a (C3 con)
C3bBb (C3 con)
C2
C3a
C3a
C3
C3b
C3
C4b2a3b (C5 con)
C3bBb3b (C5 con)
C5
C5a
C5b
C6–C9
MAC

The Complement Fixation Test

- Antibody (lysin), antigen, complement, and sensitized sheep RBCs are required

- Complement is fixed to a Ab - Ag-complex

- Fixed complement cannot participate in RBC
- lysis = positive reaction or identification



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Testing for antigen

While detection of antibodies is the more common test format, it is equally possible to test for the presence of antigen. In this case, the patient's serum is supplemented with specific antibody to induce formation of complexes; addition of complement and indicator sRBC is performed as before.

Semi-quantitative testing The test can be made quantitative by setting up a series of dilutions of patient serum and determining the highest dilution factor that will still yield a positive CF test. This dilution factor corresponds to the titer.

- **The following tests are done by Complement Fixation**:
- Adenovirus
- Fungal Panel (Blastomyces, Coccicioides, & Histoplasma)
- Influenza A & B
- Parainfluenza 1, 2, & 3
- Poliovirus 1, 2, & 3
- Respiratory Syncitial Virus (RSV)

Role in disease

Complement deficiency

It is thought that the complement system might play a role in many diseases with an immune component, such as Barraquer–Simons Syndrome, asthma, lupus erythematosus, glomerulonephritis, various forms of arthritis, autoimmune heart disease, multiple sclerosis, inflammatory bowel disease, paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome and ischemia-reperfusion injuries[] and rejection of transplanted organs

The complement system is also becoming increasingly implicated in diseases of the central nervous system such as Alzheimer's disease and other neurodegenerative conditions such as spinal cord injuries

Deficiencies of the terminal pathway predispose to both autoimmune disease and infections (particularly Neisseria meningitidis, due to the role that the membrane attack complex ("MAC") plays in attacking Gram-negative bacteriaInfections with *N. meningitidis* and *N. gonorrhoeae* are the only conditions known to be associated with deficiencies in the MAC components of complement.- 40–50% of those with MAC deficiencies experience recurrent infections with *N. meningitidis*

Deficiencies in complement regulators.

Mutations in the complement regulators factor H and membrane cofactor protein have been associated with atypical hemolytic uremic syndrome. Moreover, a common single nucleotide polymorphism in factor H (Y402H) has been associated with the common eye disease age-related macular degeneration. Polymorphisms of complement component 3, complement factor B, and complement factor I, as well as deletion of complement factor H-related 3 and complement factor H-related 1 also affect a person's risk of developing age-related macular degeneration. Both of these disorders are currently thought to be due to aberrant complement activation on the surface of host cells.

Mutations in the C1 inhibitor gene can cause hereditary angioedema, a genetic condition resulting from reduced regulation of bradykinin by C1-INH.

Paroxysmal nocturnal hemoglobinuria is caused by complement breakdown of RBCs due to an inability to make GPI. Thus the RBCs are not protected by GPI anchored proteins such as DAF.

Modulation by infections

Recent research has suggested that the complement system is manipulated during HIV/AIDS, in a way that further damages the body.

Total complement activity

Total complement activity is a test performed to assess the level of functioning of the complement system.

The terms "CH50 "or "CH100 "may refer to this test. The test is based on the capacity of a serum to lyse sheep erythrocytes coated with anti-sheep antibodies (preferably rabbit IgG.(

In combination with the Alternative pathway hemolytic assay ("AH50 (" it can indicate terminal pathway deficiencies (C3 ،C5-C9 ؛absence of hemolysis in both CH50 and AH50 ،(classic pathway deficiencies (C1 ، C2 ،C4 ؛absence of lysis in CH50 (and alternative pathway deficiencies (Factor I, B, H, D, properdin; absence of lysis in AH50.(

Increased CH50 values may be seen in cancer or ulcerative colitis.

Decreased CH50 values may be seen

in cirrhosis or hepatitis[۲] or Systemic lupus erythematosus.

Complement tests

C4 (C)	FB (A)	C3	CH50	Conditions
·	↓	↓	↓	PSG, C3 NeF AA
↓	·	↓	·	HAE, C4D
·	·	·	↓	TCPD
↓	· /↓	↓	↓	SLE
↑	↑	↑	↑	inflammation

Complement-dependent cytotoxicity

From Wikipedia, the free encyclopedia

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Complement-dependent cytotoxicity (CDC) is an effector function of IgG and IgM antibodies. When they are bound to surface antigen on target cell (e.g. bacterial or viral infected cell), the classical complement pathway is triggered by bonding protein C1q to these antibodies, resulting in formation of a membrane attack complex (MAC) and target cell lysis.

Complement system is efficiently activated by human IgG1, IgG3 and IgM antibodies, weakly by IgG2 antibodies and it is not activated by IgG4 antibodies.

It is one mechanism of action by which therapeutic antibodies or antibody fragments can achieve an antitumor effect

Use of CDC assays

Therapeutic antibodies

Development of antitumor therapeutic antibodies involves *in vitro* analysis of their effector functions including ability to trigger CDC to kill target cells. Classical approach is to incubate antibodies with target cells and source of complement (serum). Then cell death is determined with several approaches:

***Radioactive method*:** target cells are labeled with Cr before CDC assay, chromium is released during cell lysis and amount of radioactivity is measured.

Measuring of the metabolic activity of live cells (live cells staining): after incubation of target cells with antibodies and complement, plasma membrane-permeable dye is added (e.g. calcein-AM or resazurin. Live cells metabolise it into impermeable fluorescent product that can be detected by flow cytometry. This product can't be formed in metabolically inactive dead cells.

Measuring of the activity of released intracellular enzymes: dead cells release enzyme (e.g. LDH or GAPDH) and addition of its substrate leads to color change, that is usually quantified as change of absorbance or luminiscence.

Dead cells staining: a (fluorescent) dye gets inside the dead cells through their damaged plasma membrane. For instance propidium iodide binds to DNA of dead cells and fluorescent signal is measured by flow cytometry.[6]

HLA typing and crossmatch test

CDC assays are used to find a suitable donor for organ or bone marrow transplantation, namely donor with matching phenotype of histocompatibility system HLA. At first, HLA typing is done for patient and donor to determine their HLA phenotypes. When potentially suitable couple is found, crossmatch test is done to exclude that patient produces donor-specific anti-HLA antibodies, which could cause graft rejection.

CDC form of HLA typing (other words serologic typing) uses batch of anti-HLA antibodies from characterised allogeneic antisera or monoclonal antibodies. These antibodies are incubated one by one with patient‘s or donor‘s lymphocytes and source of complement. Amount of dead cells (and thus positive result) is measured by dead or live cells staining. Nowadays CDC typing is being replaced by molecular typing, which can identify nucleotide sequences of HLA molecules via PCR.

CDC assay is usually used for performing crossmatch test. The basic version involves incubation of patient's serum with donor's lymphocytes and second incubation after adding rabbit complement. Presence of dead cell (positive test) means that donor isn't suitable for this particular patient. There are modifications available to increase test sensitivity including extension of minimal incubation time, adding antihuman globulin (AHG), removing unbound antibodies before adding complement, separation of T cell and B cell subset. Besides CDC crossmatch there is flow-cytometric crossmatch available, that is more sensitive and can detect even complement non-activating antibodies.

Complement receptors

Many white blood cells express complement receptors on their surface, particularly monocytes and macrophages. All four complement receptors bind to fragments of complement component 3 or complement component 4 coated on pathogen surface, but the receptors have different functions. Complement receptor (CR) 1, 3, and 4 work as opsonins (stimulate phagocytosis), whereas CR2 is expressed only on B cells as a co-receptor.

Red blood cells (RBCs) also express CR1. With these receptors, RBCs bring antigen-antibody complexes bound to complement fragments in the blood to the liver and spleen for degradation.

CR #	Name	Ligand	CD
CR1	-	C3b, C4b, iC3b	CD35
CR2	-	C3d, iC3b, C3dg, Epstein-Barr virus	CD21
CR3	Macrophage-1 antigen or "integrin $\alpha_M\beta_2$"	iC3b	CD11b+CD18
CR4	Integrin alphaXbeta2 or "p150,95"	iC3b	CD11c+CD18
-	C3a receptor	C3a	-
-	C5a receptor	C5a	CD88

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