## THE TEMPO OF REJECTION

Hyperacute rejection

Acute rejection

Chronic rejection

## PREVENTION OF REJECTION

The rejection response can be reduced by tissue matching

The perfectly matched donor and recipient would be isogeneic, for example monozygotic twins. However, this situation is rare, and in all other cases there will be major and/or minor histocompatibility differences between the donor and recipient. Only the major (MHC, i.e.HLA) antigens can be practicably matched. This can be done by serology which takes only a few hours and can therefore be performed while the donor organ is preserved on ice. Recently, sensitive and accurate typing has been achieved using the polymerase chain reaction (PCR) to identify HLA genes in the DNA of donors and recipients.

Matching for all known HLA antigens is practically impossible, but good organ graft survival is obtained when the donor and recipient share only the same MHC class II antigens, especially HLA-DR because these are the antigens that directly activate the recipient's TH cells.

The lists of known class I (HLA-A, HLA-B and HL-C) and class II (HLA-DP, HLA-DQ and HLA-DR) antigens are long, and the chances of completely matching two individuals at random are extremely remote.

The mixed lymphocyte reaction (MLR) can also be used to test the responsiveness of recipient lymphocytes to antigens expressed on donor cells Low recipient anti-donor MLR responses are associated with excellent transplant survival. However, the 4-5 days required for the MLR test precludes its use in most clinical organ transplantation, because organs from

dead or brain-dead donors cannot be preserved for more than 24-48 hours. In those for when living donors (e.g. relatives) are to be used, MLR can be used. It is especially important in bone marrow transplantation, to assess whether the donor bone marrow cells can respond to recipient antigens and because GVHD DNA typing has now largely superseded these older methods.

## Non-specific immunsuppression can control rejection reactions

There are two main categories of immunosuppressive treatment: antigen-non-specific and antigen-specific. Non-specific immunosupression blunts or abolishes the activity of the immune system regardless of the antigen. This can leave a graft recipient very vulnerable to infections. For instance, a large dose of X-ray prevents rejection but also has man deleterious effects, as well as abolishing antimicrobial immunity. Most non-specific treatments used today are selective for the immune system, or are used in a way which creates some selectivity. The very best treatment would take this further and inactivate only those clones of lymphocytes with specificity for donor antigens, leaving other clones intact, so that the patient does not suffer infections or side-effects. Such highly specific immunosupression remain the 'Holy Grail' of transplantation immunobiology and is described later.

The three non-specific agents that are most widely used in current clinical practice are steroids, cyclosporine and azathioprine

Steroids have anti-inflammatory properties and suppress activated macrophages, interfere with APC function and reduce the expression of MHC antigens. In effect, steroids reverse many of the actions of IFNγ on macrophages and transplanted tissues.

Cyclosporin is a fungal macrolide produced by soil organisms, and has interesting and potent immunosuppressive properties. Its principal action is to suppress lymphoine production by TH cells by interfering with the activation of lymphoine genes and, directly or indirectly, to reduce the expression of the receptors for IL-2 on lymphocytes undergoing activation. Other macrolides such as FK506 suppresses lymphokine production by TH cdells in a way similar to cyclosporine. Rapamycin interferes with the intracellular signaling pathways of the IL-2 receptor and therefore preventsIL-2- dependent lymphocyte activation.

The rejection response involves the rapid division and differentiation – proliferation – of lymphocytes. Azathioprine is an antiproliferative drug, an analogue of 6-mercaptopurine. Its incorporation into the DNA of dividing cells prevents further proliferation. New antiproliferative drugs, such as mycophenolic acid derivatives, are under investigation.

These agents can be effective used alone, although high doses are usually required and the likelihood of adverse toxic effects is increased. Used together in various combinations, they work in synergy because they interfere with different

stages of the same immune pathway. The doses of individual agents can thus be reduced and the adverse effects minimized. The clinical results obtained since the introduction of cyclosporine are very good (85-90% graft acceptance at 1 year for kidneys, hearts and livers). However, the expected half-life of a kidney transplant is 7-8 years because of the problem of chronic rejection, and long-term use of drugs is still associated with adverse effects. Further improvements might be obtained with the introduction of new drugs.

# THE COMPLEMENT SYSTEM

## Introduction

The complement system is a group of more than 30 plasma and membrane proteins that play a critical role in host defense. When activated, complement components interact in a highly regulated fashion to generate products that:

Recruit inflammatory cells (promoting inflammation).

Opsonize microbial pathogens and immune complexes (facilitating antigen clearance).

Kill microbial pathogens (via a lytic mechanism known as the membrane attack complex).

Generate an inflammatory response.

Complement activation takes place on antigenic surfaces. However, the activation of complement generates several soluble fragments that have important biologic activity.

There are three distinct pathways of activation of complement: the Classical, the MB-lectin, and the Alternative Pathways. See **Figure 1**.

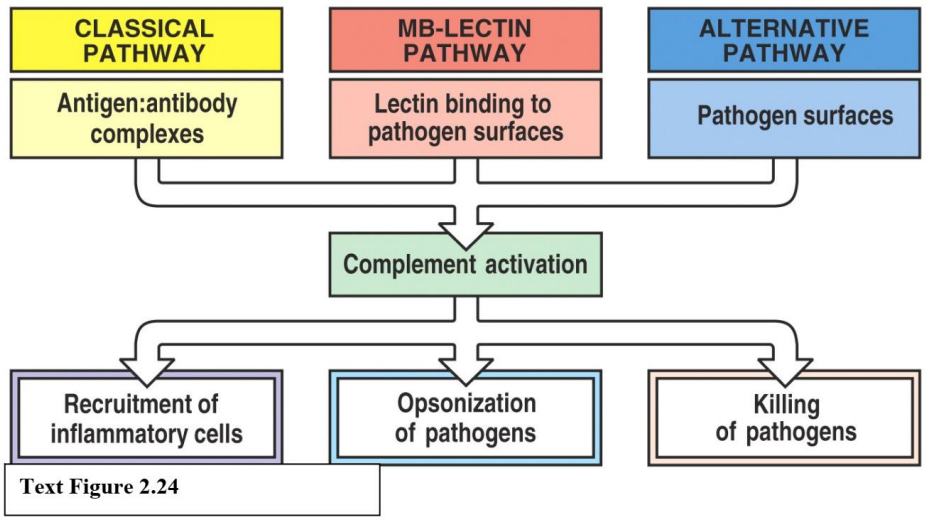



**Figure 1. The three pathways of complement activation.**

## Nomenclature

The components of the classical pathway are designated by the letter C followed by a simple number designation, e.g. C3.

Many complement components are proteases that become active following proteolytic cleavage.

When the components are cleaved during activation, the resulting fragments are given lower case letter designations, such as C3a and C3b.

Components of the alternative pathway are named by capital letters, such as factor B and factor D.

For the MB-lectin pathway, components are designated by acronyms, such as MASP-1 (**M**annan binding lectin-**A**ssociated **S**erine **P**rotease-1)

The lower case "i" is added to indicate that a component is inactive, e.g. iC3b.

## Activation of Complement

Activation of the **CLASSICAL PATHWAY:**

Classical pathway activation is initiated after immune complex formation.

Complement component C1 recognizes the antigen-antibody complex.

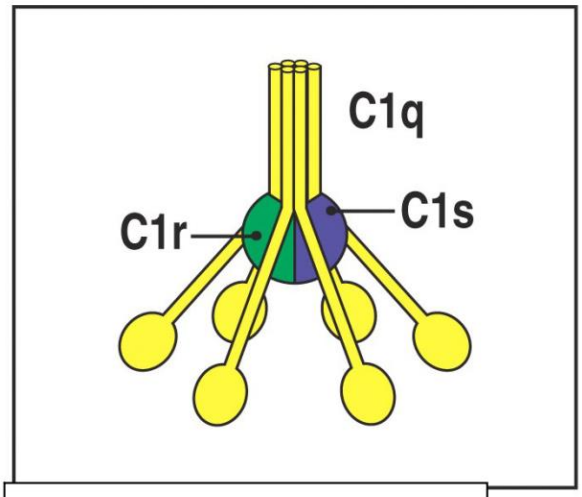
The binding of antibody to antigen induces a conformational change in the antibody constant region. This exposes a site on the Fc portion that can be bound by the first complement component of the classical pathway, C1.

C1 is a macromolecule that consists of C1q (comprised of 6 globular heads and extended tails) in complex with C1r and C1s (the C1qrs complex). See **Figure 2**.

Activation of the C1qrs complex occurs when at least two of the C1q globular heads are simultaneously bound to antibody. See **Figure 2**.

For this to occur, two Fc portions need to be in within close molecular proximity of each other on the antigenic surface.

In contrast to IgG, the pentameric nature of IgM allows a single molecule of antigen bound IgM to activate C1.

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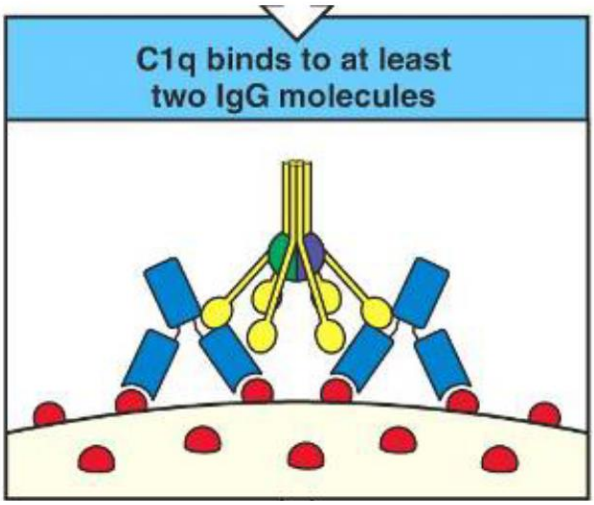


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**Figure 2. Complement component C1.**

Once C1q is bound to antibody, C1r undergoes a conformational change and becomes enzymatically active.

C1r then cleaves C1s, which after cleavage is enzymatically active as well.

Activation of the **MB-LECTIN PATHWAY:**

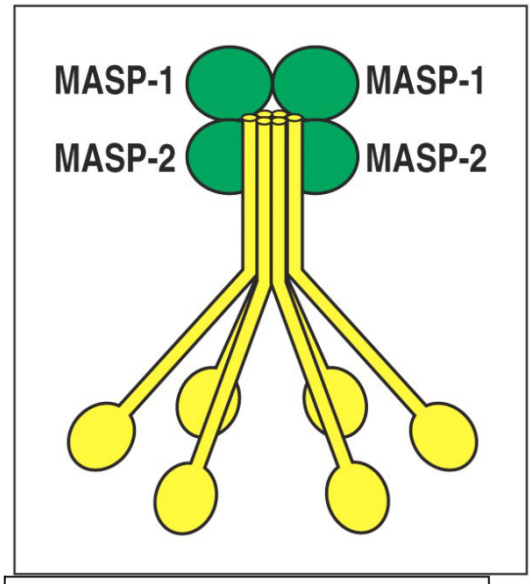
Activation of the mannan binding lectin pathway is similar to the classical pathway.

Except that the MB-lectin pathway is initiated by a protein, Mannan Binding Lectin (MBL), which is homologous to C1q.

MBL binds to mannose and certain other complex carbohydrates that are found on the surface of many microbial pathogens. See **Figure 3**.

MBL is physically associated with two serine proteases, MASP-1 and MASP-2 (mannan binding lectin-associated serine protease-1) that are similar to C1r and C1s.

When MBL binds to the pathogen, MASP-1 and MASP-2 become activated.

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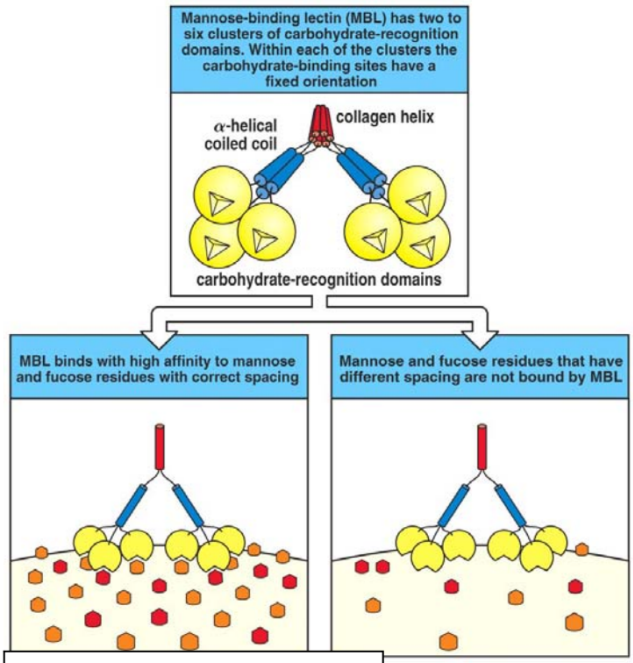


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**Figure 3. Mannan binding lectin (MBL).**

**Further complement sequence progression.**

Activated C1qrs (or separately MBL activated MASP-1 and MASP-2) cleaves C4, and the C4b fragment becomes bound to a cell surface (e.g. microorganism). Bound C4b fragment binds C2. See **Figure 4**.

Once bound to C4b, C2 is also cleaved by C1s, forming the C4b2a complex, which remains bound to the cell surface. (For MB-Lectin Pathway activation, MBL can be substituted for C1q, substitute MASP-1 and MASP-2 for C1r and C1s in **Figure 4**).

C4b2a is a C3 convertase, capable of cleaving C3 into C3b and C3a. This is a major point of amplification of the pathways, since one C3 convertase can cleave up to 1000 molecules of C3.

C3b, bound to the antigenic surface, acts as a powerful opsonin and enhances the uptake of antigenic particle by phagocytes.

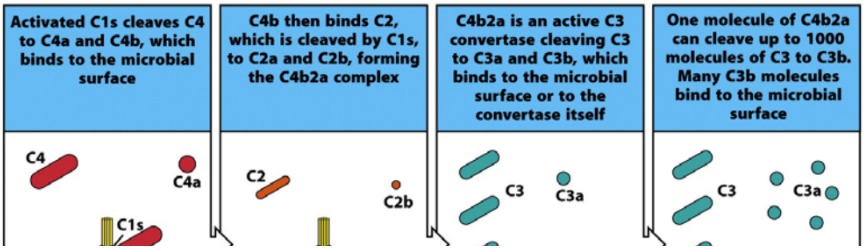


**Figure 4. C3 convertase and C5 convertase generation.**

C4b2a3b complex, also called C5 convertase, cleaves C5 into C5a, which is a soluble inflammatory mediator, and C5b, which is capable of complexing with additional complement components. The generation of C5b initiates the final phase of complement activation, which is the formation of the **M**embrane **A**ttack **C**omplex (MAC). See **Figure 5.**

The MAC is identical for all pathways of complement activation.

C3a and C5a remain soluble and produce local inflammatory effects.

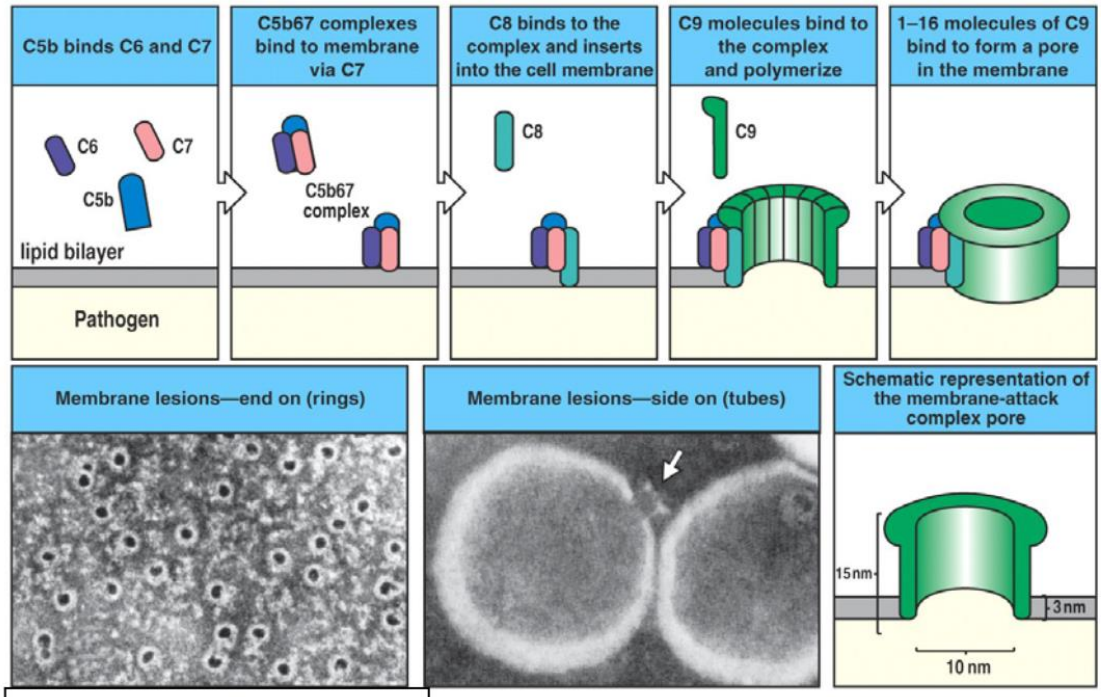


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**Figure 5. Membrane attack complex, C5-C9.**

Activation of the **ALTERNATIVE COMPLEMENT PATHWAY:**

The alternative pathway depends upon the slow hydrolysis of C3, which spontaneously occurs in plasma.

Hydrolyzed C3 can bind and cleave Factor B, and the resulting C3 ($H_2O$) Bb complex is a C3 convertase that generates additional molecules of C3b. See **Figure 6.**

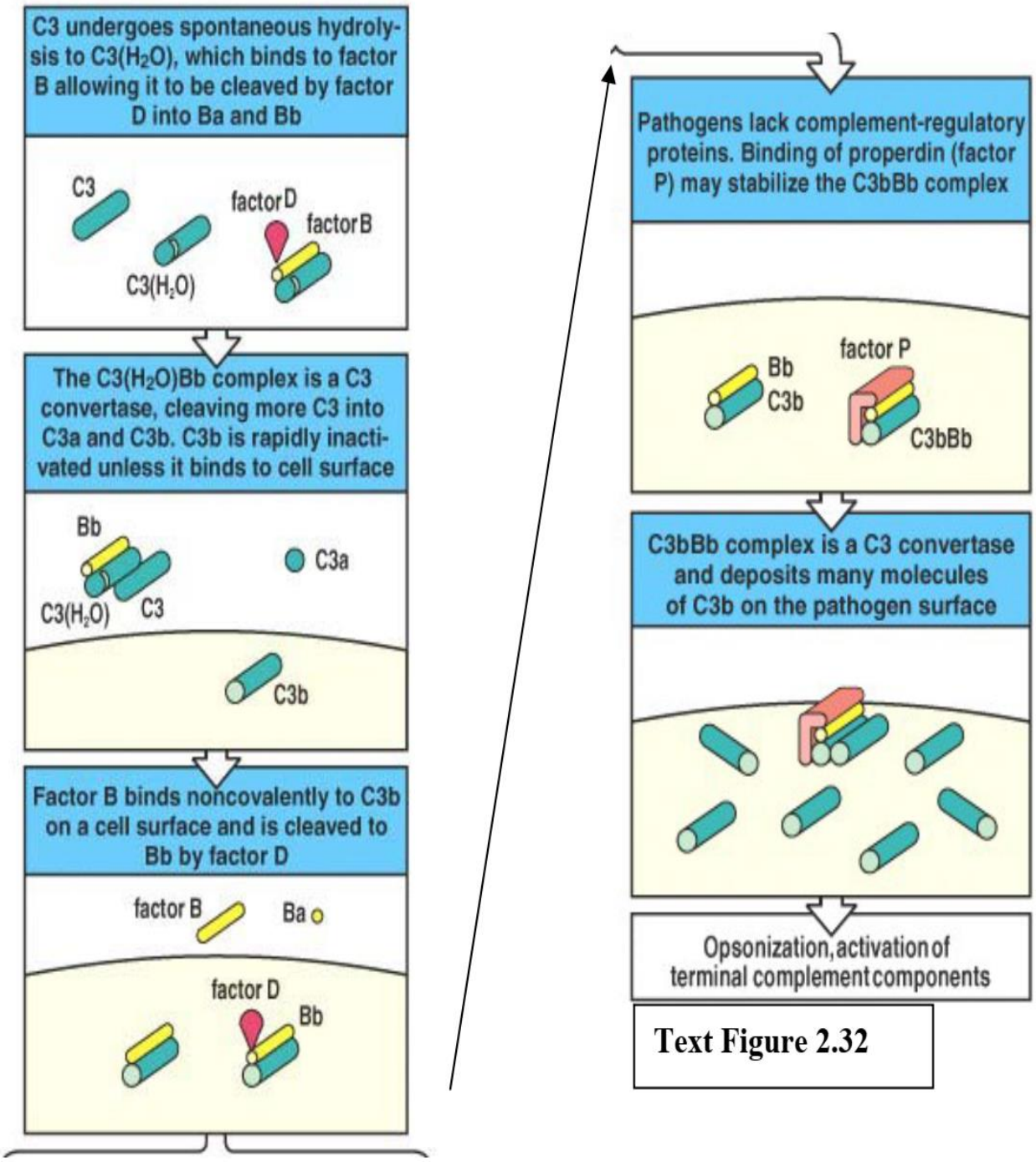


**Figure 6. Alternative complement pathway activation.**

C3b is rapidly degraded, unless stabilized by attachment to certain favorable pathogenic surfaces.

Once attached, C3b binds Factor B, and Factor B is cleaved by Factor D, forming bound C3bBb.

When stabilized by Factor P (properdin), the C3bBb complex acts as a C3 convertase, analogous to C4b2a of the classical pathway.

When another molecule of C3b associates with C3bBb (forming C3bBbC3b), a C5 convertase is formed.

This C5 convertase is analogous to C4b2a3b of the classical pathway. From this point (the cleavage of C5), the alternative and classical pathways converge, leading to the formation of a MAC complex as in **Figure 5**.

The three pathways of complement activation are summarized in **Figure 7**.

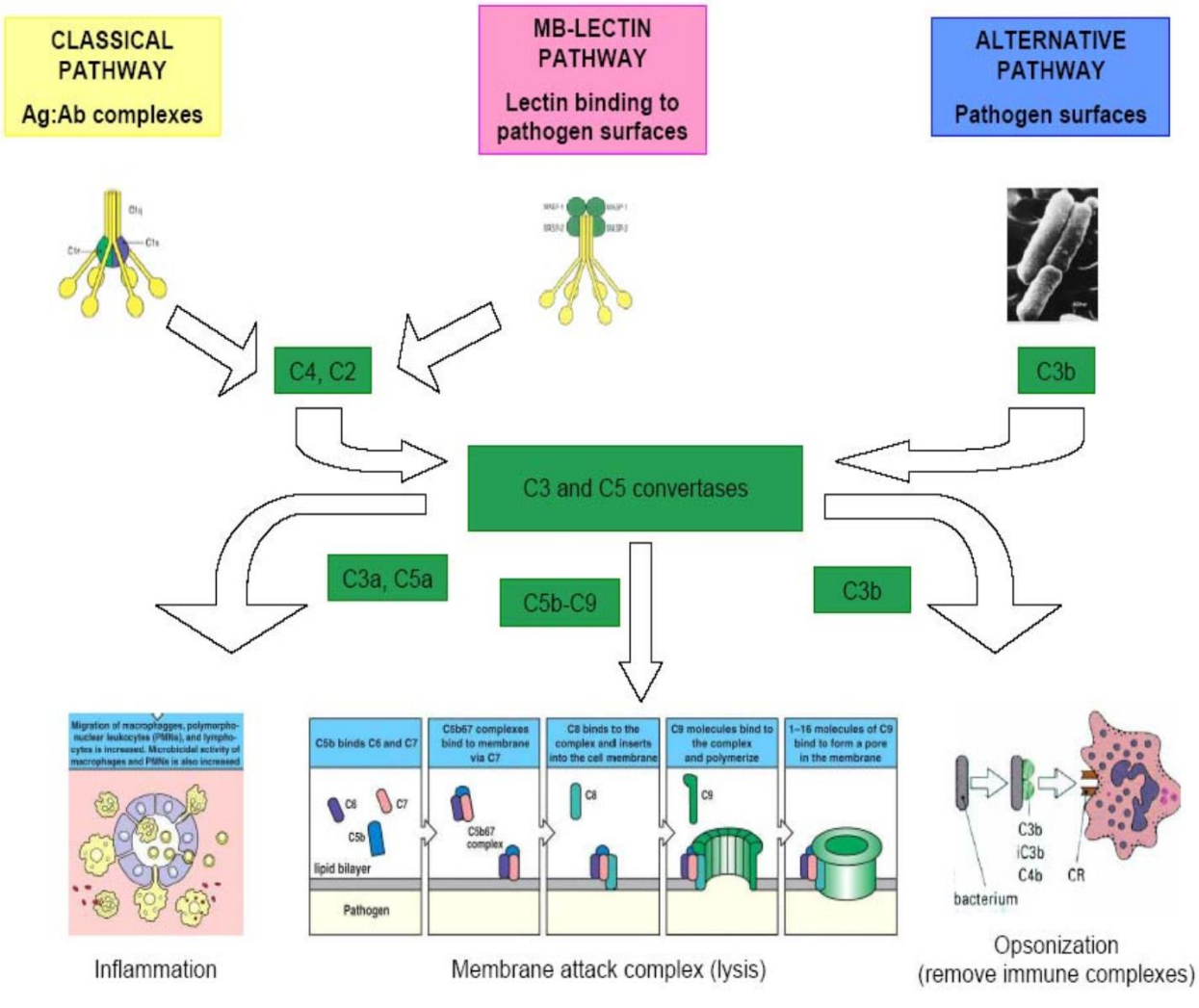


**Figure 7. Integration of the three pathways of complement activation.**

## Biological Consequence of Complement Activation

**Cell lysis and viral neutralization.** The MAC complex (C5b→9) creates a pore in the cell membrane, and disrupts cell homeostasis, by cellular lysis (e.g. Gram-negative bacteria). Certain viruses with a membrane coat can also be lysed in this manner. See **Figure 8**.

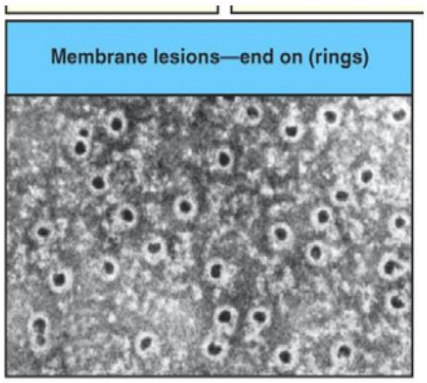


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**Figure 8. Pore in a cell membrane as a consequence of MAC.**

**Opsonization.** Phagocytic leukocytes, including neutrophils and macrophages, carry receptors for C3b (CR1). When an antigenic particle is coated with C3b, C3b (also C4b) assists in the adherence and ultimate ingestion of the particle by the phagocytic cell. The C5a fragment also enhances phagocytosis by stimulating phagocytic cells to ingest C3b coated antigens. See **Figure 9**.

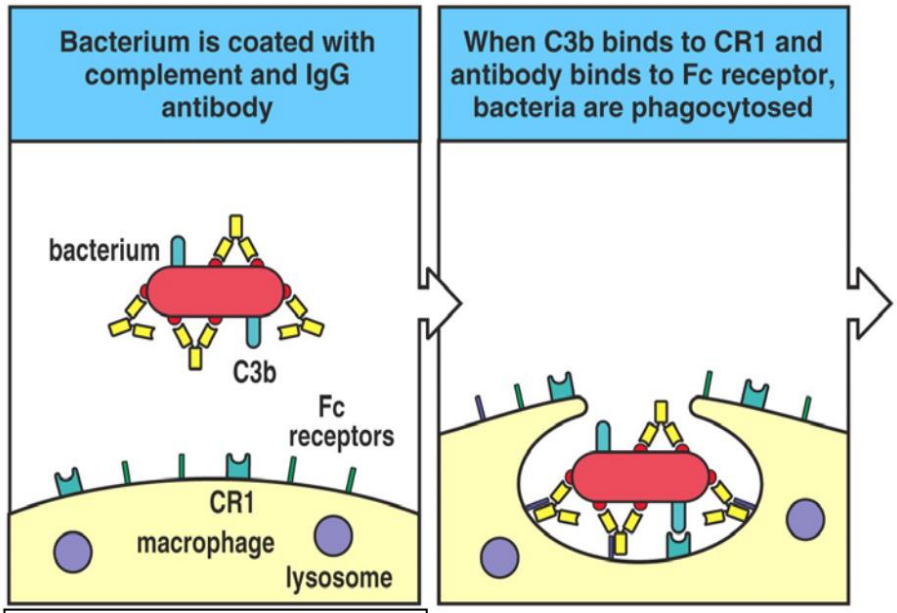


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**Figure 9. Opsonization and phagocytosis via C3b and CR1.**

**Clearance of Immune Complexes.** The removal of antigen-antibody complexes from the circulation depends upon C3b. Via C3b, antigen-antibody complexes bind to complement receptors on circulating red blood cells. As the RBCs pass through the spleen and liver, the coated complexes are stripped off of the RBCs by resident phagocytes. See **Figure 10**.

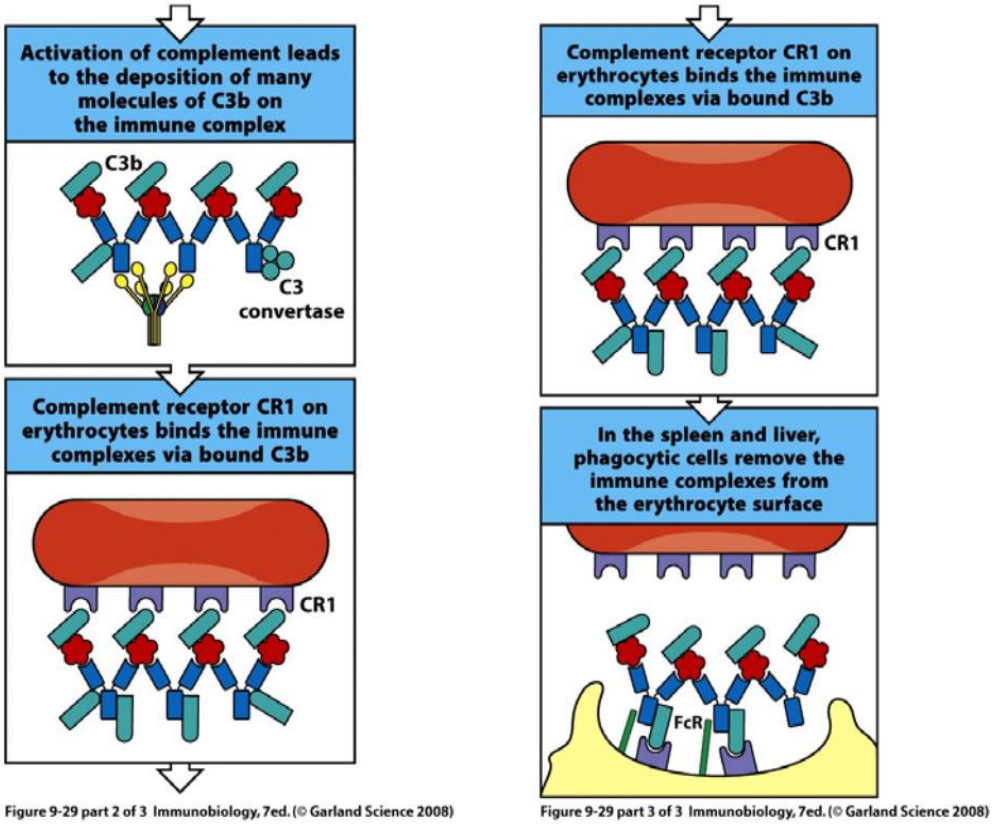



**Figure 10. Clearance of immune complexes.**

**Inflammation.** The soluble fragments that are produced during complement activation play several roles in inflammation. See **Figure 11.**

**Chemotaxis.** C5a is an important chemoattractant for neutrophils, eosinophils, basophils, and monocytes. The development of a C5a gradient at sites of complement activation assists in the recruitment of leukocytes to the area of antigenic challenge.

**Vascular Changes**. The fragments C3a, C4a, and C5a are capable of binding to specific receptors on mast cells and basophils, triggering granule release by these cells. The release of histamine leads to vascular changes, including increased vascular permeability. Because of this property, C3a, C4a and C5a are called anaphylatoxins.

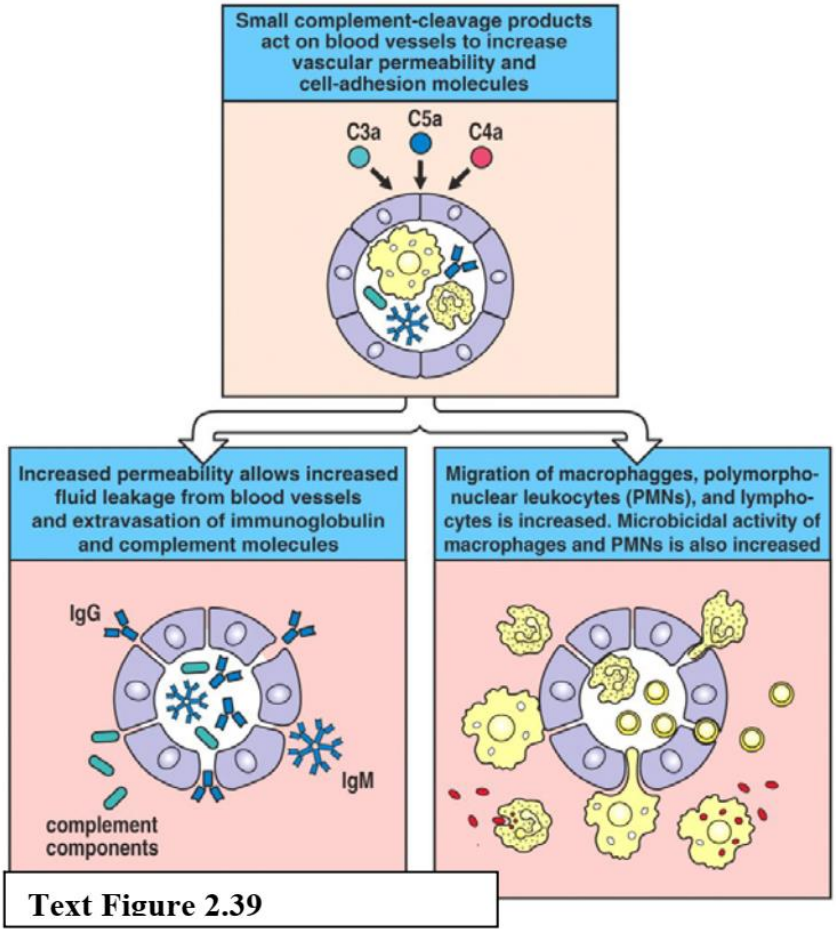



**Figure 11. Inflammation mediated by complement.**

## Regulation of Complement Activation

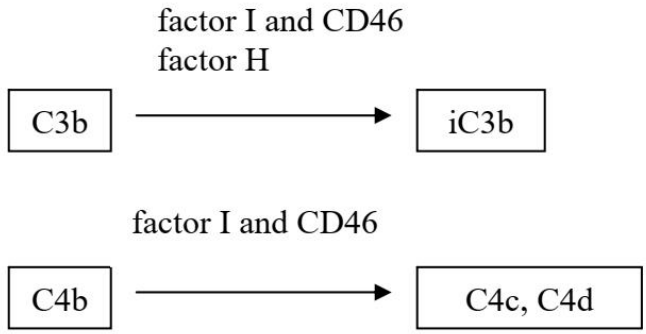
A series of proteins serve to protect host cells from accidental damage by acting at various different stages of complement activation and dissociating complexes or catalyzing enzymatic degradation of covalently bound complement proteins. If not regulated, activated complement can cause excessive inflammation and tissue damage. See below with summaries in **Figures 12, 13 and 14**.

**Regulation by protease inhibition. See Figure 12.**

C1 inhibitor (C1INH)

C1r:C1s ⟶ C1r, C1s

**Regulation by catalytic cleavage. See Figure 12.**




**Factor I:** catalyzes the cleavage of surface bound C3b or C4b.

**CD46:** binds either C3b or C4b and promoting inactivation by Factor I. CD46 therefore functions to limit C3 and C5 convertase activity, and provides regulation for the classical, MBL and alternative pathways.

**Factor H:** binds C3b and serves as a cofactor for the cleavage of surface bound C3b by Factor I.

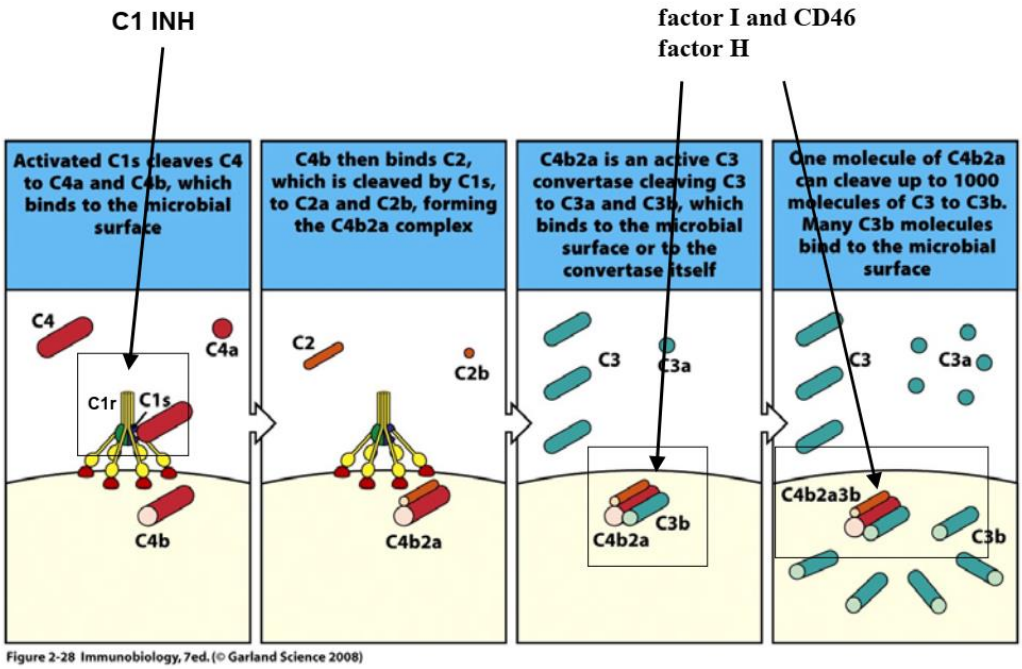
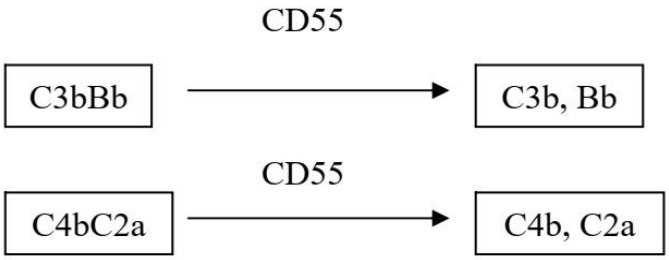



**Figure 12. Complement regulation.**

**Regulation by decay acceleration. See Figure 13.**




**CD55:** a membrane protein that serves to disengage C2a from C4b in the classical and MBL pathways and Bb from C3b for the alternative pathway. In both cases, CD55 inhibits convertase activity.

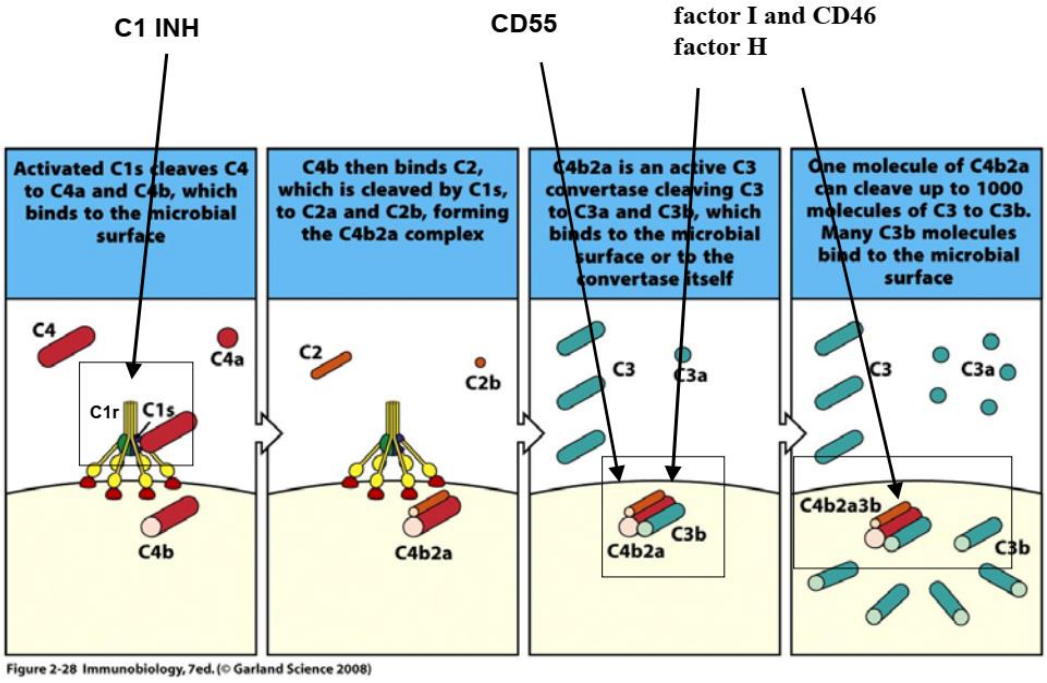
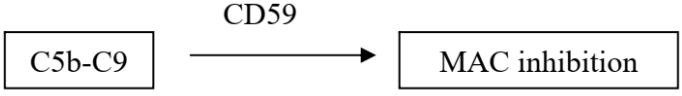



**Figure 13. Complement regulation.**

**Regulation by inhibition of lysis. See Figure 14.**



**CD59:** prevents the assembly of C5b-9 at the final C8/C9 stage.

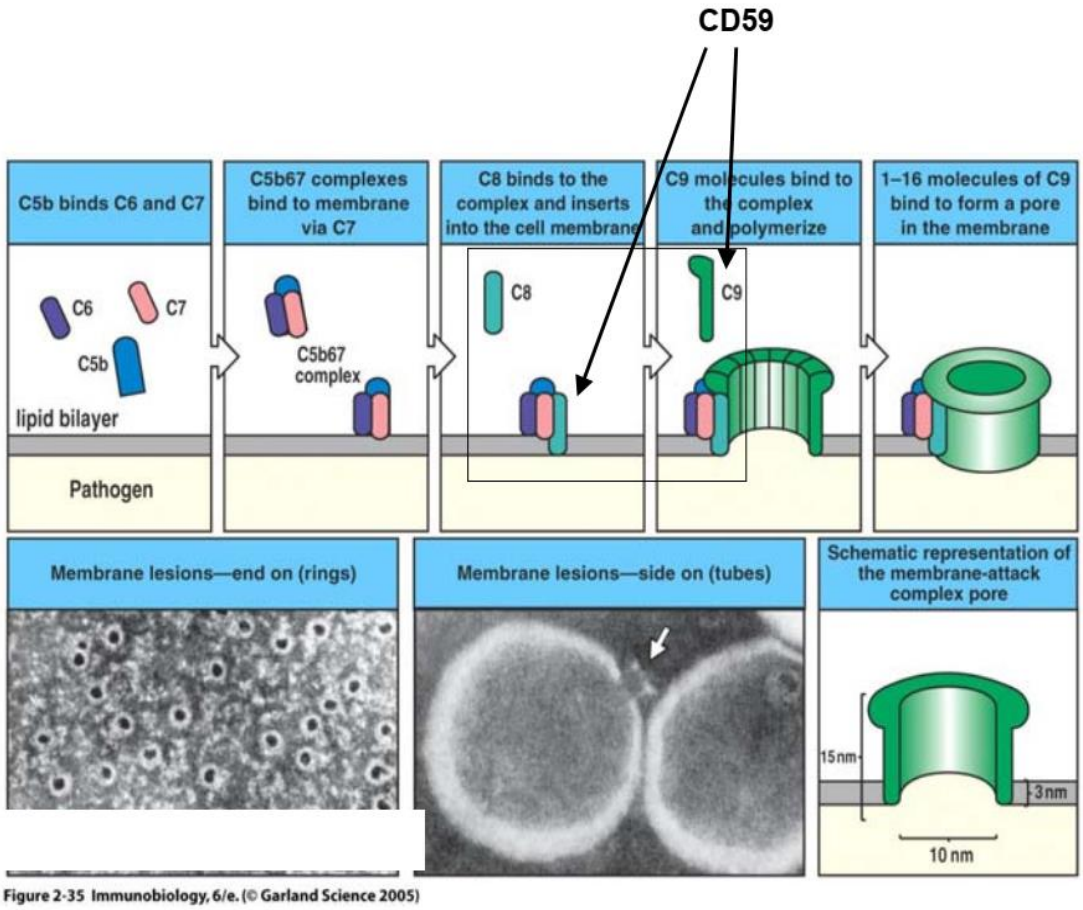



**Figure 14. Complement regulation.**

## Human Complement Component Deficiencies

**Deficiencies of components.** Deficiencies of complement components are very rare. Defects in the early components of the classical pathway do not lead to overwhelming infection, as the MBL and alternative pathways can bypass this defect. See **Figure 13**.

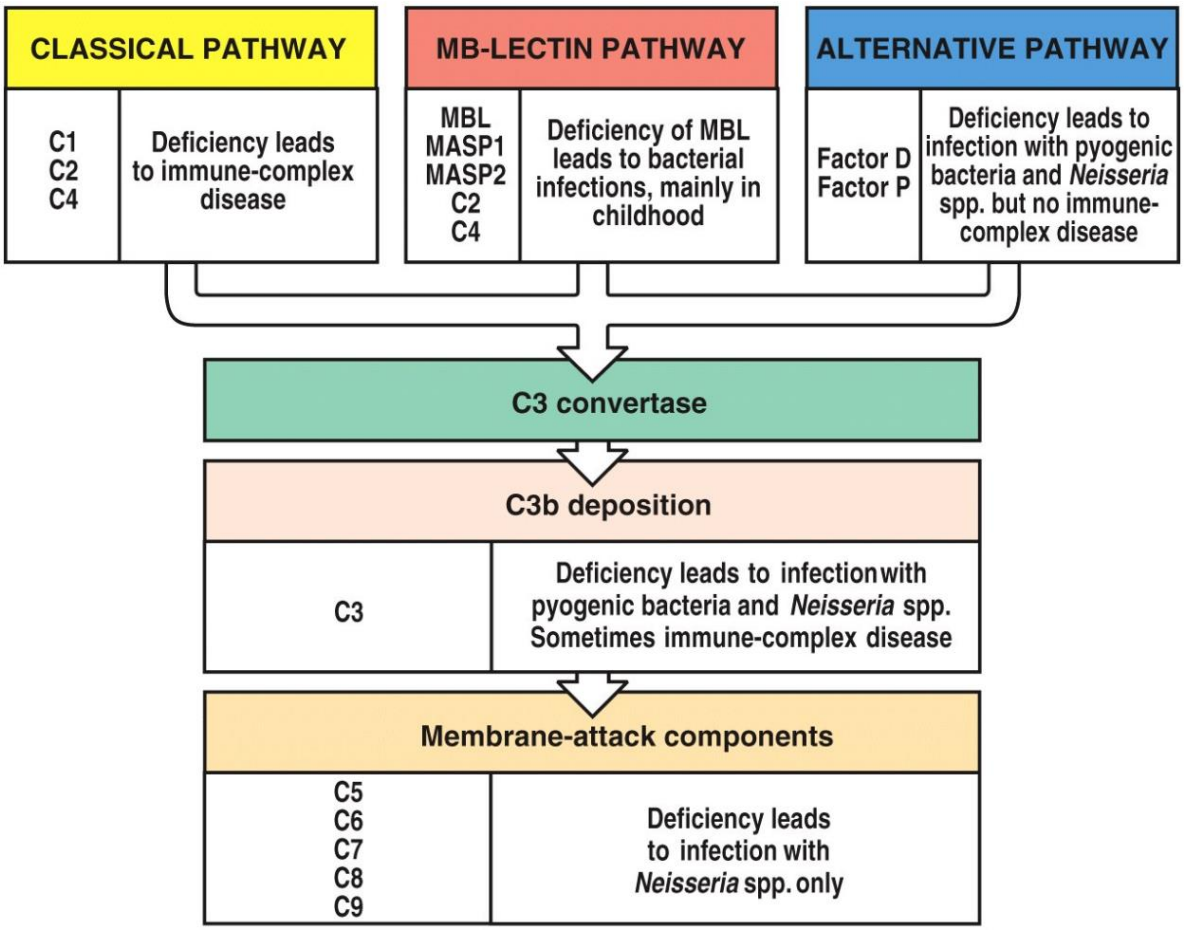



**Figure 15. Complement deficiency consequences.**

Reference:

Cutting Edge: Atopy Promotes Th2 Responses to Allo-antigens and Increases the Incidence and Tempo of Corneal Allograft Rejection by - Clay Beauregard, Christina Stevens, Elizabeth Mayhew and Jerry Y. Niederkorn

The complement system in regulation of adaptive immunity by Michael C Carroll (2004)

Cellular and Molecular Immunology, Updated Edition: With STUDENT CONSULT Online Access, 5e (Cellular and Molecular Immunology, Abbas) 5th Edition by Abul K. Abbas MBBS, Andrew H. H. Lichtman MD PhD