

# Teaching unit 8

## Effector mechanisms of humoral immunity

*Elimination of extracellular microorganisms and toxins*

*Effector functions of antibodies*

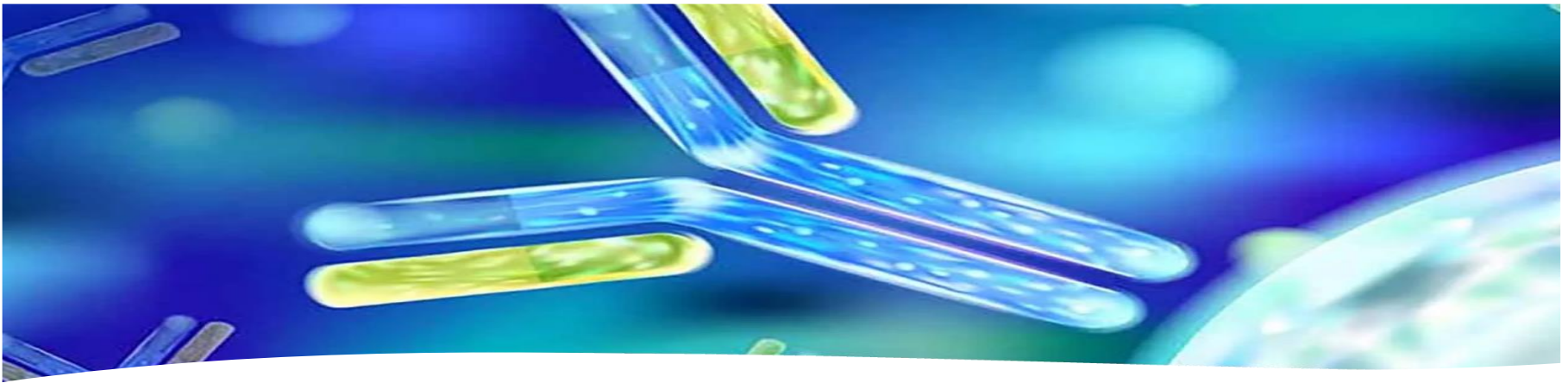


***... we learned about humoral immunity ...***

**... it is a type of acquired immune response in which antibodies play a major role**

**... it is a defense mechanism against extracellular microorganisms and their toxins**

**... antibodies cannot enter the cells**



*... we have to learn ...*

**... that prevention of infection is performed only by antibodies, which disable microorganisms to bind to and enter host cells**

**... that antibodies bind to toxins and thus neutralize them**

**... that antibodies eliminate microorganisms, toxins and infected cells from the body**

**... that antibodies also play an important role in the defense against intracellular microorganisms, because they can bind to them before they enter the cell or during the transition from cell to cell**

# PROPERTIES OF ANTIBODIES THAT DETERMINE EFFECTOR FUNCTION

Antibodies work, throughout the body, in places far from the place of formation.

Protective antibodies that are produced during the **primary** response are produced in greater quantity and during each subsequent (**secondary**) response to the same antigen (long-lived plasma cells and memory B lymphocytes).

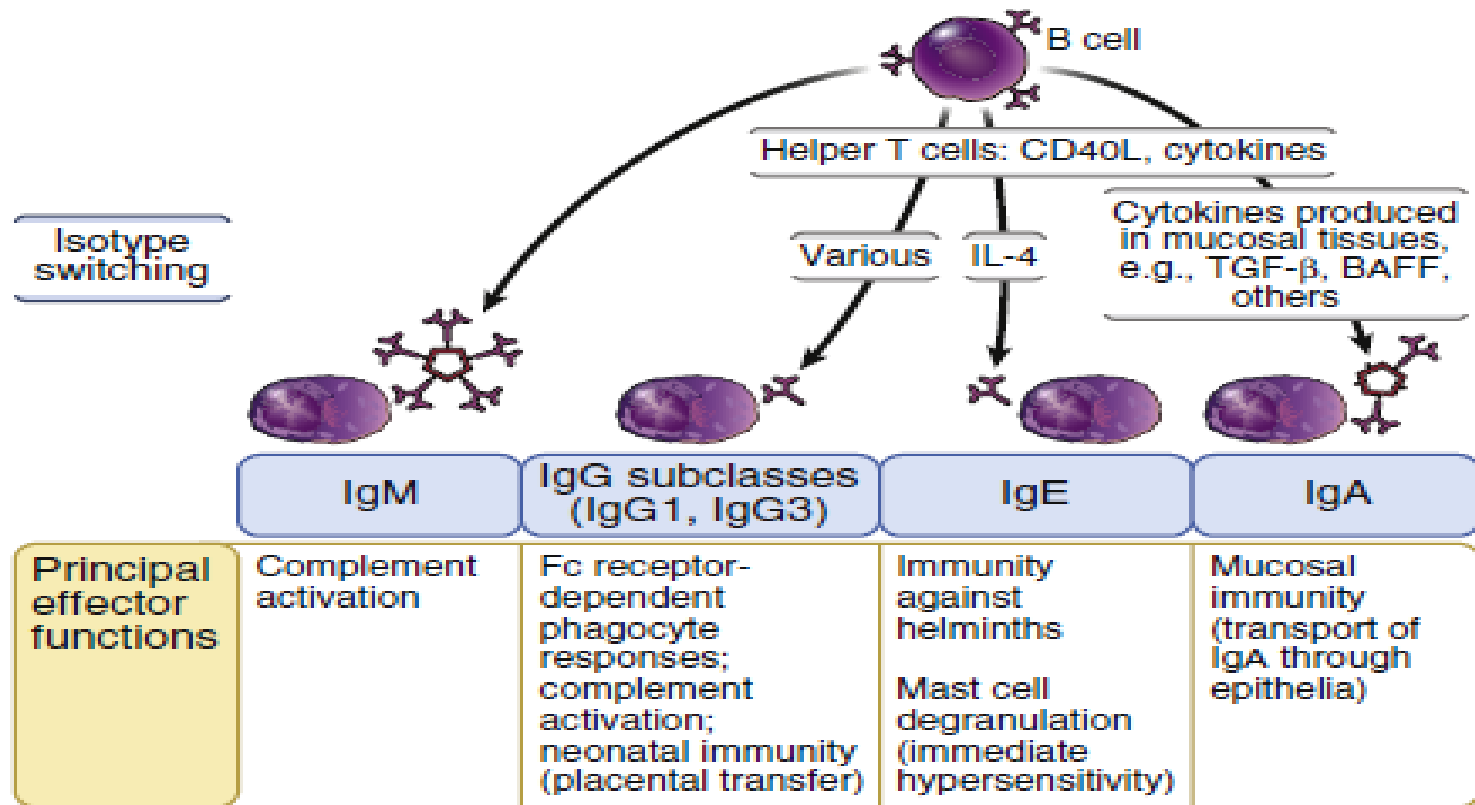
# PROPERTIES OF ANTIBODIES THAT DETERMINE EFFECTOR FUNCTION

The active sites of the **Fab** region by binding to antigens block (neutralize) their harmful effects, while **Fc** fragments activate various effector mechanisms for the elimination of microorganisms and toxins.

**In order to express the effect of the Fc region, it is necessary to pre-attach the active site to the epitope.**

Changing the class of antibodies as well as maturation of affinity enhance the protective role of antibodies.

# CD4+ Th lymphocyte induces the CLASS CHANGE of antibodies synthesized by B lymphocytes

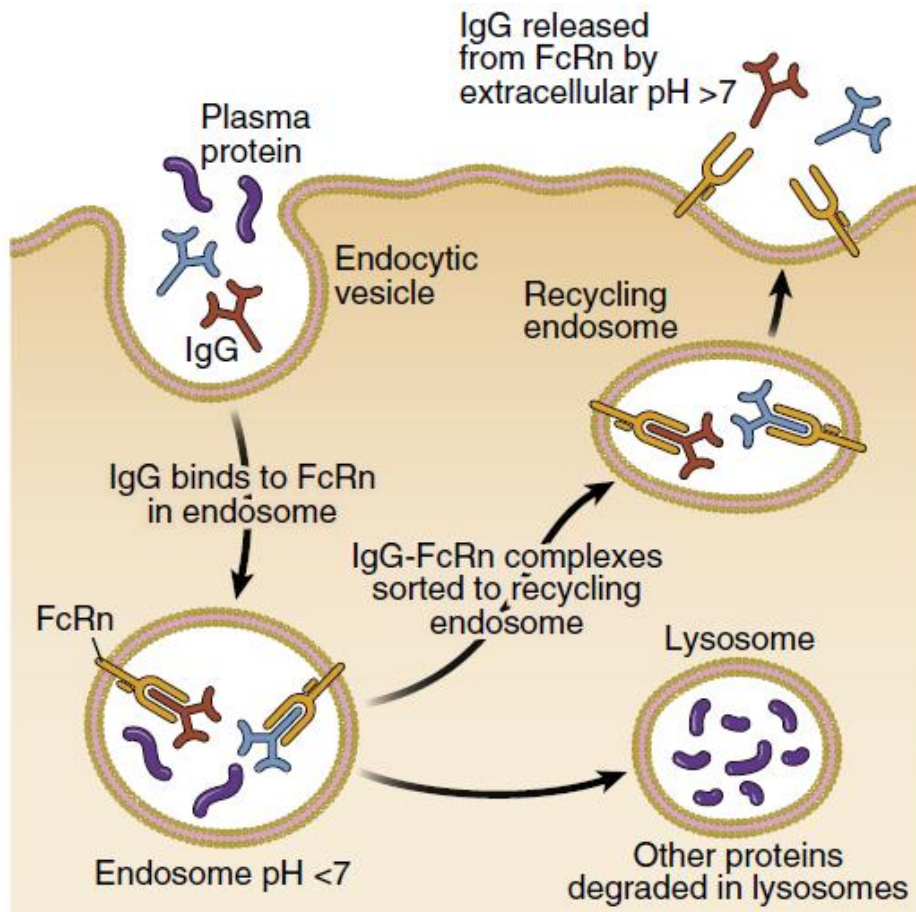


**Class change allows the adaptation of humoral immune response to the type of microorganism.**

**The class change initiates contact CD40 (B)-CD40 ligand (T) and is directed by different cytokines.**



# The neonatal Fc receptor (**FcRn**) contributes to the long half-life of IgG

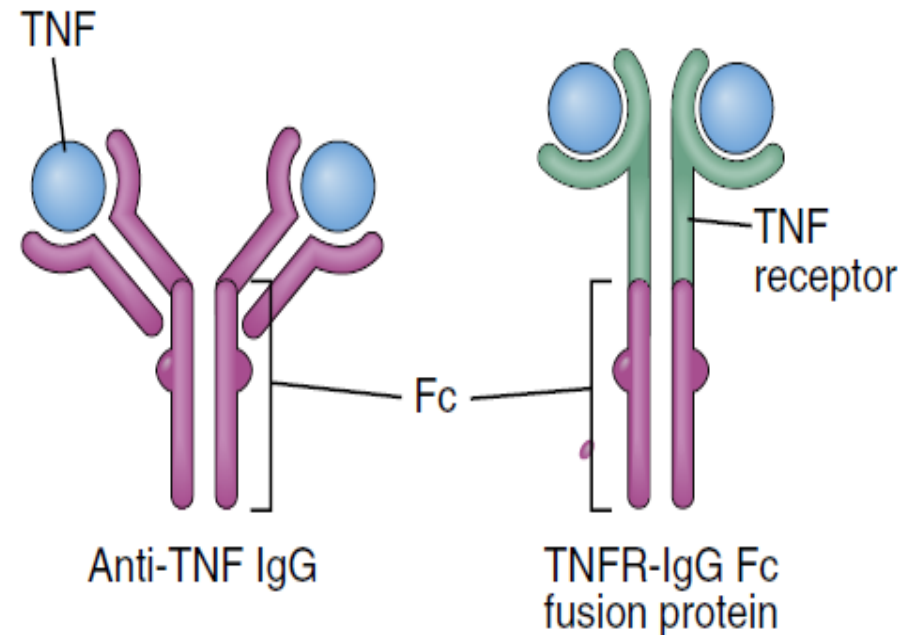


The change to the IgG isotype increases the time during which the antibody remains in the blood and thereby increases the functional activity of the antibody.

*This property of the Fc region of IgG has been exploited to extend the half-life of other proteins by binding to the Fc region of IgG*

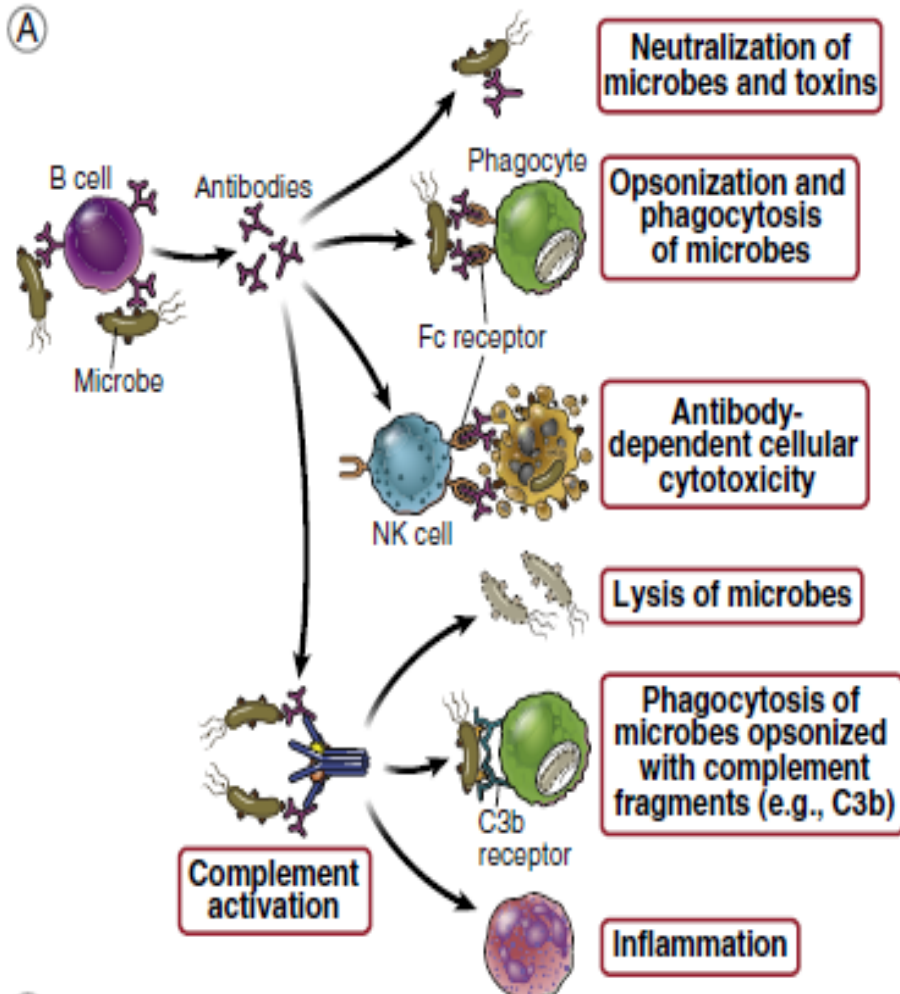
An antibody specific for TNF can bind to the cytokine, block its activity and persist in the circulation for a long time (weeks) thanks to recycling via FcRn.

The extracellular domain of the receptor for TNF is an antagonist of this cytokine, and the binding of the soluble receptor to the Fc region of IgG prolongs its half-life in the blood.





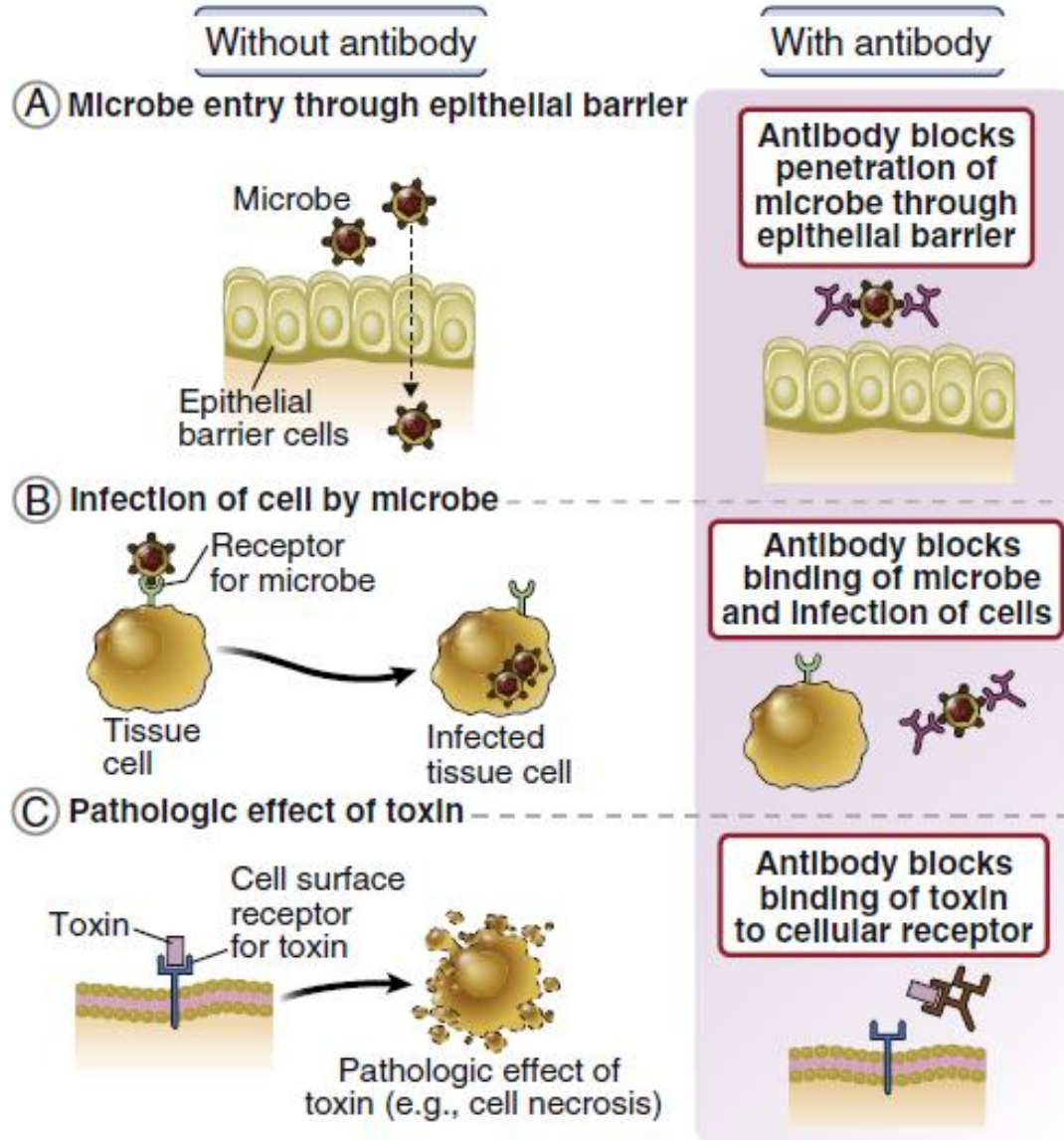
# EFFECTOR FUNCTIONS OF ANTIBODIES



**B**

Antibody isotype	Effector functions
IgG	<ul style="list-style-type: none"> <li>Neutralization of microbes and toxins</li> <li>Opsonization of antigens for phagocytosis by macrophages and neutrophils</li> <li>Activation of the classical pathway of complement</li> <li>Antibody-dependent cellular cytotoxicity mediated by NK cells</li> <li>Neonatal immunity: transfer of maternal antibody across placenta and gut</li> <li>Feedback inhibition of B cell activation</li> </ul>
IgM	<ul style="list-style-type: none"> <li>Activation of the classical pathway of complement</li> </ul>
IgA	<ul style="list-style-type: none"> <li>Mucosal immunity: secretion of IgA into lumens of gastrointestinal and respiratory tracts, neutralization of microbes and toxins</li> </ul>
IgE	<ul style="list-style-type: none"> <li>Eosinophil- and mast cell-mediated defense against helminths</li> </ul>

# NEUTRALIZATION OF MICROBES AND MICROBIAL TOXINS

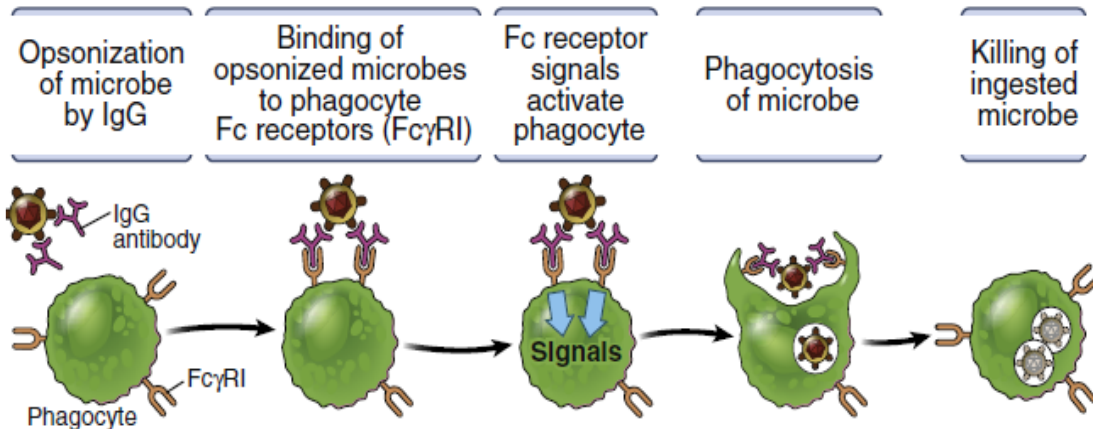


Antibodies prevent the binding of microbes to cells, thereby **blocking the ability of the microbes to infect host cells.**

Antibodies can neutralize the microbes during their transit from cell to cell and thus also **limit the spread of infection.**

**Antibodies block the binding of toxins to cells,** thereby inhibiting the pathologic effects of the toxins.

# OPSONIZATION



**Opsonization** - the process of coating particles for subsequent phagocytosis.

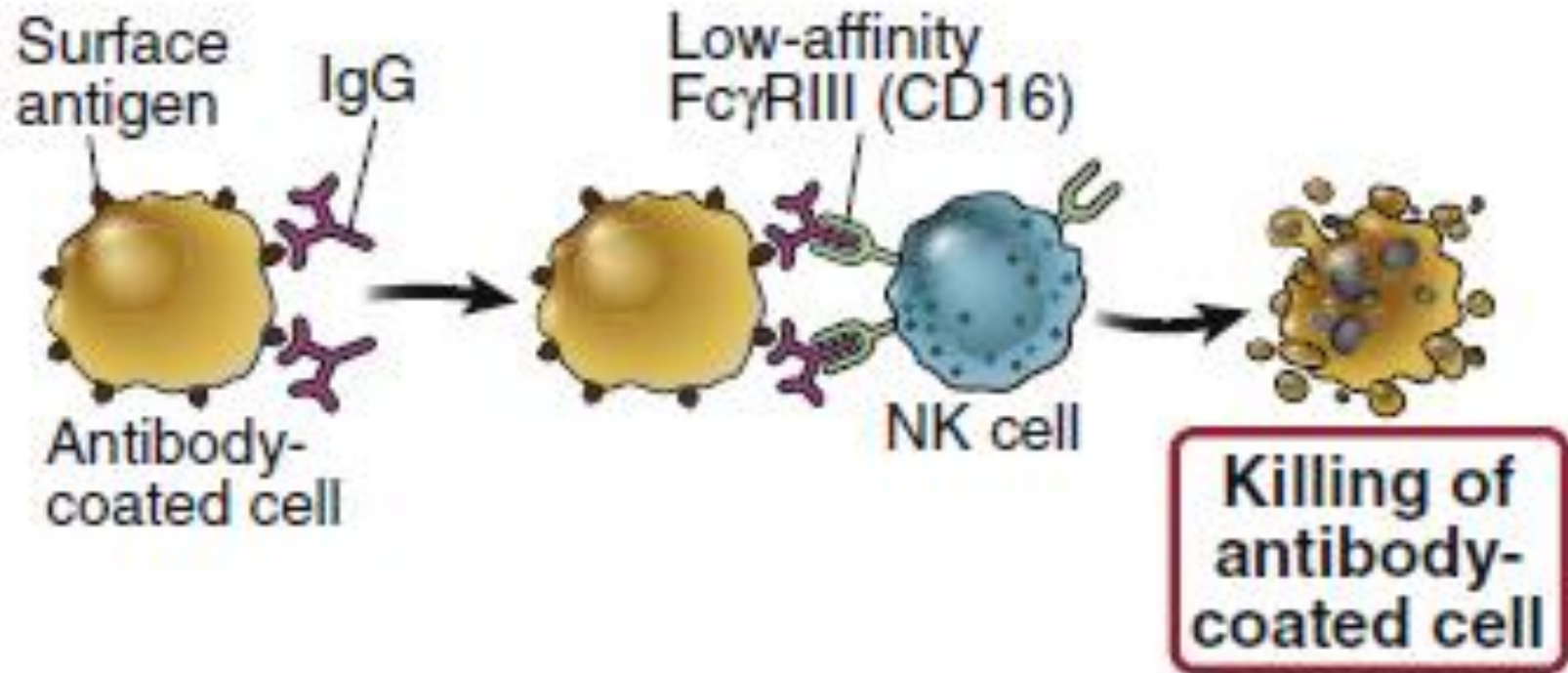
The molecules that coat microbes and enhance their phagocytosis are **opsonins**.

Fc regions of IgG1 and IgG3 bind to a high affinity receptor Fc $\gamma$ RI (CD64), expressed on neutrophils and macrophages.

Fc Receptor	Affinity for Ig	Cell distribution	Function
Fc $\gamma$ RI (CD64)	High; binds IgG1 and IgG3	Macrophages, neutrophils	Phagocytosis; activation of phagocytes
Fc $\gamma$ RIIB (CD32)	Low	B lymphocytes, DCs, mast cells, neutrophils, macrophages	Feedback inhibition of B cells, attenuation of inflammation
Fc $\gamma$ RIIA (CD16)	Low	NK cells	Antibody-dependent cellular cytotoxicity (ADCC)
Fc $\epsilon$ RI	High; binds IgE	Mast cells, basophils, eosinophils	Activation (degranulation) of mast cells and basophils

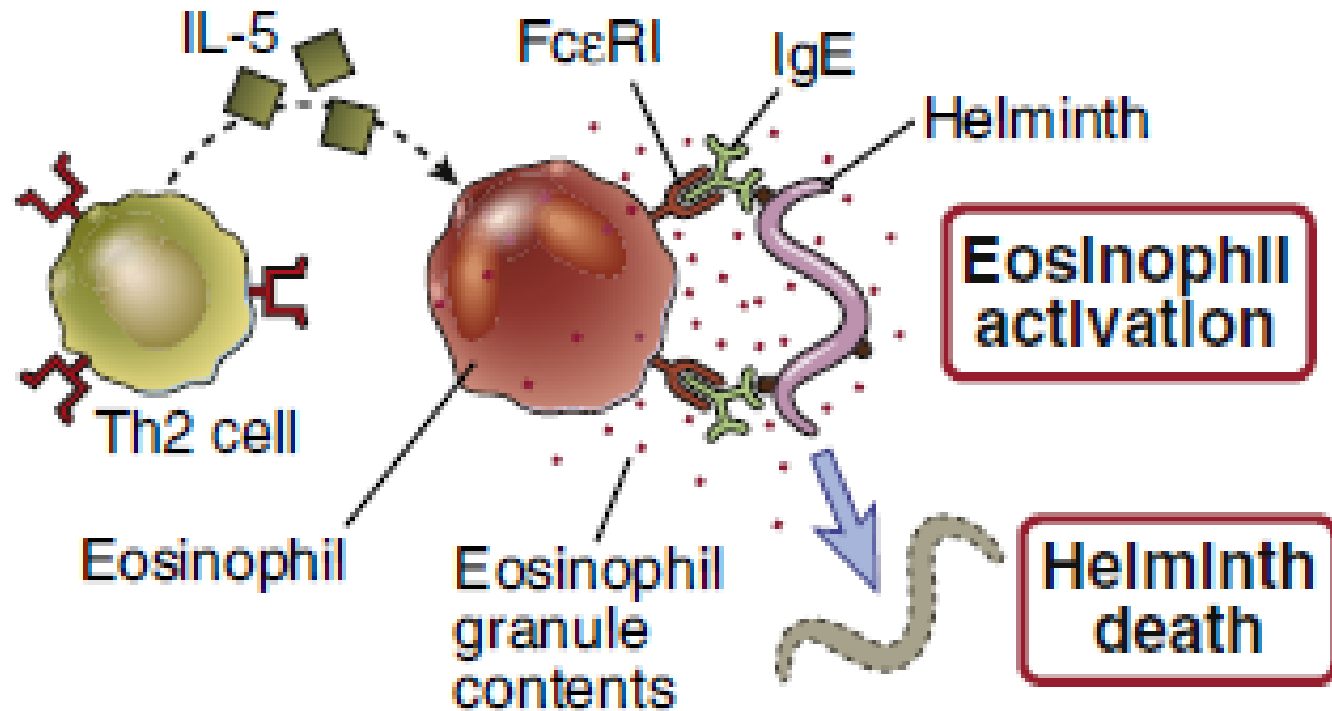
Antibody-mediated phagocytosis is the major mechanism of defense against encapsulated bacteria. As the spleen contains large numbers of phagocytes, patients with splenectomy are susceptible to disseminated infections by encapsulated bacteria.

# ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)



**Natural killer (NK) cells bind to antibody-coated cells and destroy these cells. NK cells express the  $Fc\gamma$  receptor called  $Fc\gamma RIII$  (CD16).**

# IgE- AND EOSINOPHIL/MAST CELL-MEDIATED REACTIONS



Most helminths are too large to be phagocytosed, and their thick integument makes them resistant to many of the microbicidal substances produced by neutrophils and macrophages.

High-affinity Fc receptor for IgE, FcεRI, is expressed on eosinophils and mast cells.



# THE COMPLEMENT SYSTEM

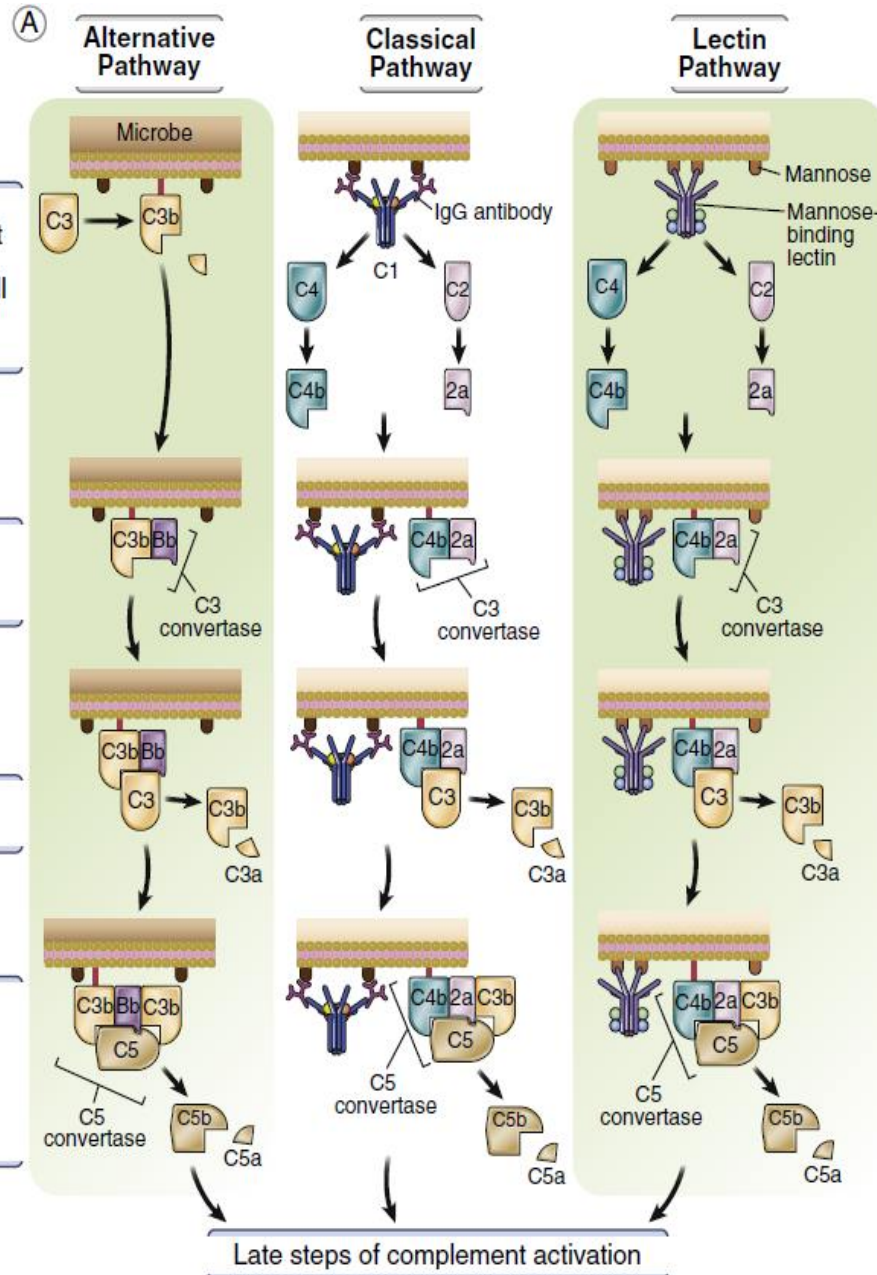
Term **complement** refers to the ability of these proteins to assist, or complement, the activity of antibodies.

The complement system is a collection of circulating and cell membrane proteins that play important roles in host defense against microbes and in antibody-mediated tissue injury.

Features of complement system activation:

- **Sequential proteolytic cleavage of proteins** - generation of enzyme complexes with proteolytic activity
- **Amplification** - even a small number of activated complement molecules produced early in the cascade may generate a large number of effector molecules
- Activated complement proteins become covalently attached to the cell surface where the activation occurs - **complement effector functions are limited to the correct sites**
- **Prevention of complement-mediated damage of healthy cells** - normal host cells have regulatory mechanisms that inhibit the activation of complement and the deposition of activated complement proteins

# Pathways of complement system activation (early phase)



**Alternative pathway** - as a product of spontaneous hydrolysis of C3, C3b is deposited on the surface of a microbe by covalent binding to microbial proteins or polysaccharides.

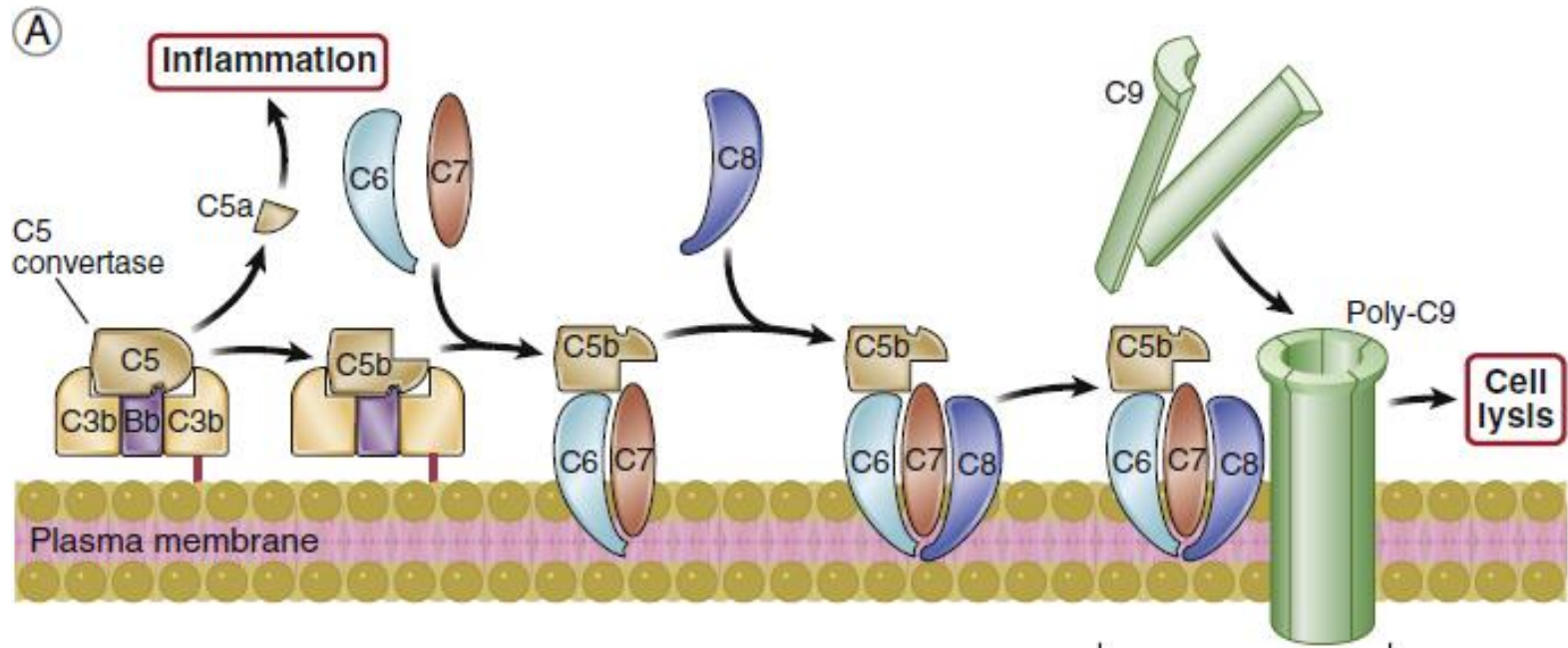
**Lectin pathway** - MBL (mannose-binding lectin) binds to the surface of a microbe. Serine proteases, structurally related to C1s of the classical pathway, activate C4...

**Classical pathway** - triggered by IgM and IgG (1 and 3) bound to the antigen. Adjacent Fc regions of the antibodies bind C1 complement system protein...

The result of early steps of complement system activation is that microbes become coated with covalently attached C3b.



# Late phase of complement system activation



The late phase of complement system activation is initiated by proteolysis of C5 and subsequent generation of C5b.

C6, C7, C8, and C9, bind to C5b. C9 polymerizes to form a pore in the cell membrane through which water and ions can enter, causing death of the microbe (MAC).

# Important properties of proteins involved in the early phase of complement system activation by alternative, classical and lectin pathways

**B**

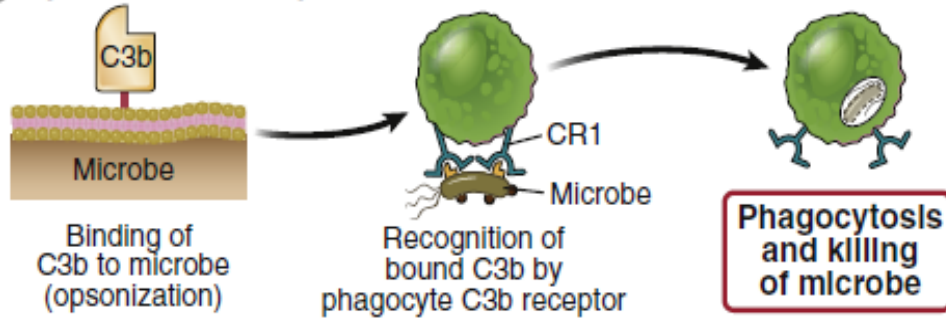
Alternative pathway proteins		
Protein	Serum conc. (µg/mL)	Function
C3	640–1660	C3b binds to the surface of microbes, where it functions as an opsonin and as a component of C3 and C5 convertases C3a stimulates inflammation
Factor B	200	Bb is a serine protease and the active enzyme of C3 and C5 convertases
Factor D	1–2	Plasma serine protease that cleaves Factor B when it is bound to C3b

**C**

Classical and lectin pathway proteins		
Protein	Serum conc. (µg/mL)	Function
C1 (C1qr <sub>2</sub> s <sub>2</sub> )		Initiates the classical pathway; C1q binds to Fc portion of antibody; C1r and C1s are proteases that lead to C4 and C2 activation
C4	150–450	C4b covalently binds to surfaces of microbes or cells where antibody is bound and complement is activated C4b binds to C2 for cleavage by C1s C4a stimulates inflammation
C2	20	C2a is a serine protease functioning as an active enzyme of C3 and C5 convertases
Mannose binding lectin (MBL)	0.8–1	Initiates the lectin pathway; MBL binds to terminal mannose residues of microbial carbohydrates. MBL-associated proteases activate C4 and C2, as C1r and C1s do in the classical pathway.

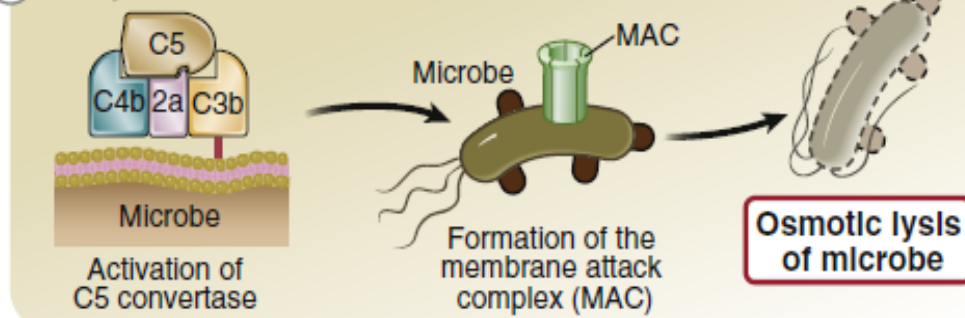
# The functions of complement system in defense against microbes

## A Opsonization and phagocytosis



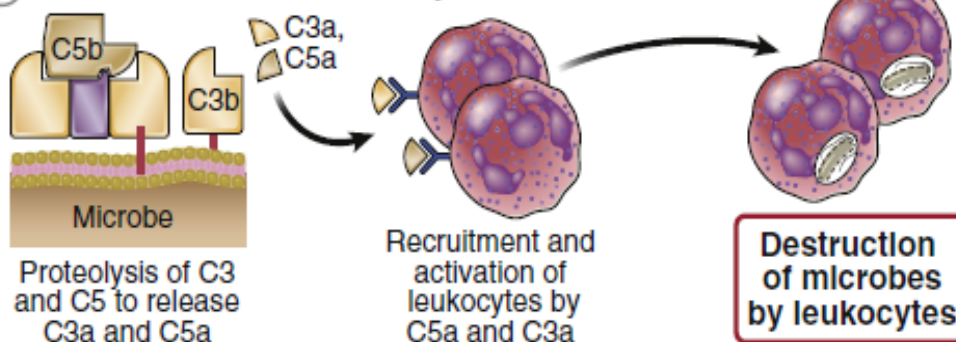
Phagocytes express a receptor for C3b – CR1 (CD35) - **opsonization**.

## B Complement-mediated cytotoxicity



MAC induces osmotic lysis of microbes, effective only against microbes that have thin cell walls, such as the *Neisseria* species of bacteria.

## C Stimulation of inflammatory reactions



C3a, C4a, C5a act chemotactically on neutrophils, release mediators of inflammation from leukocytes and act on the endothelium, which enables the entry of leukocytes and plasma proteins into the tissue - **inflammation**.

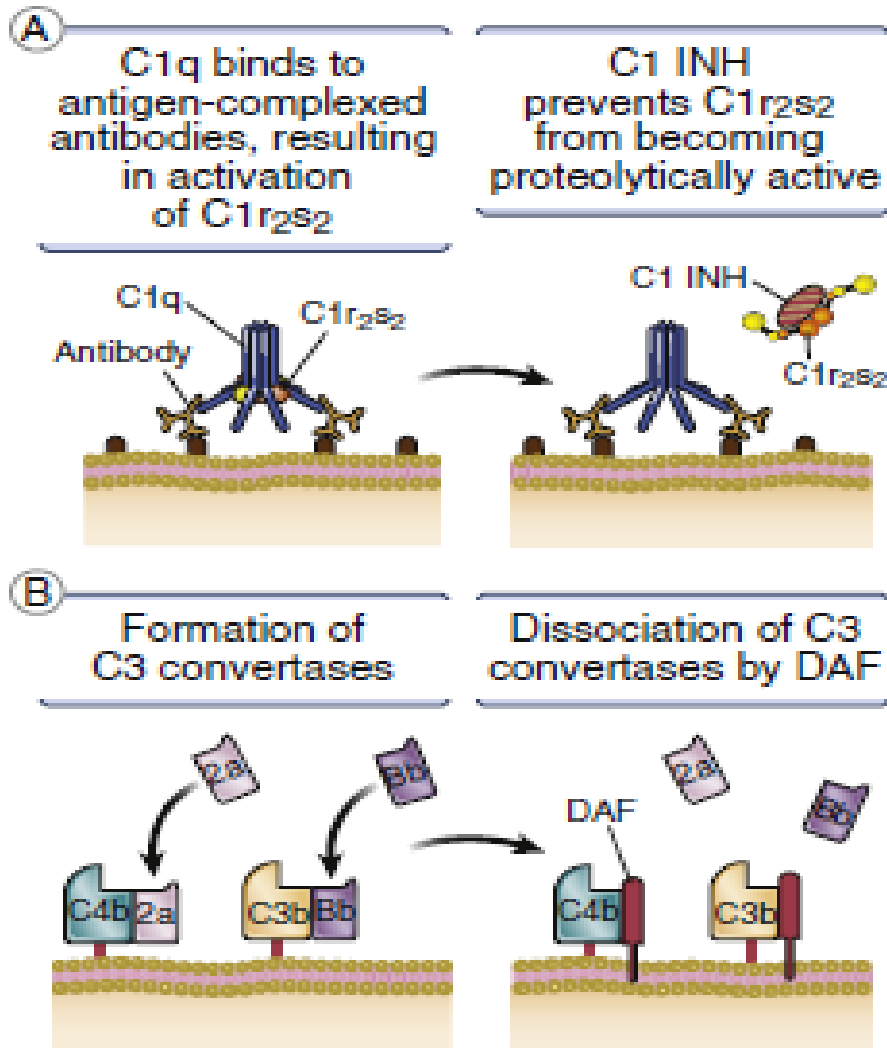
# Complement system deficiencies

**Deficiency of C3:** high susceptibility to infections fatal already in the first years of life.

**Deficiency of C2 and C4:** surprisingly no major consequences (points to the greater importance of an alternative activation pathway). This is where immune complex disease occurs because complement system is important for their elimination.

**C9 deficiency:** increased susceptibility to *Neisseria* spp.

# Regulation of complement system activation



Mammalian cells express regulatory proteins that prevent complement system activation and tissue damage (microorganisms on membranes do not have regulatory proteins).

# The major regulatory proteins of the complement system and their functions

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## Plasma proteins

Protein	Plasma concentration	Function
C1 inhibitor (C1 INH)	200 µg/ml	Inhibits C1r and C1s serine protease activity
Factor I	35 µg/ml	Proteolytically cleaves C3b and C4b
Factor H	480 µg/ml	Causes dissociation of alternative pathway C3 convertase subunits Co-factor for Factor I-mediated cleavage of C3b
C4 binding protein (C4BP)	300 µg/ml	Causes dissociation of classical pathway C3 convertase subunits Co-factor for Factor I-mediated cleavage of C4b

## Membrane proteins

Protein	Distribution	Function
Membrane co-factor protein (MCP, CD46)	Leukocytes, epithelial cells, endothelial cells	Co-factor for Factor I-mediated cleavage of C3b and C4b
Decay accelerating factor (DAF)	Blood cells, endothelial cells, epithelial cells	Blocks formation of C3 convertase
CD59	Blood cells, endothelial cells, epithelial cells	Blocks C9 binding and prevents formation of the MAC
Type 1 complement receptor (CR1, CD35)	Mononuclear phagocytes, neutrophils, B and T cells, erythrocytes, eosinophils, FDCs	Causes dissociation of C3 convertase subunits Co-factor for Factor I-mediated cleavage of C3b and C4b



# Hereditary deficiencies of complement system regulatory proteins - clinical syndromes caused by uncontrolled complement activation

- **Deficiency of C1 INH:** hereditary angioedema
- **DAF (*Decay Accelerating Factor*) and CD59 deficiency:** paroxysmal nocturnal hemoglobinuria

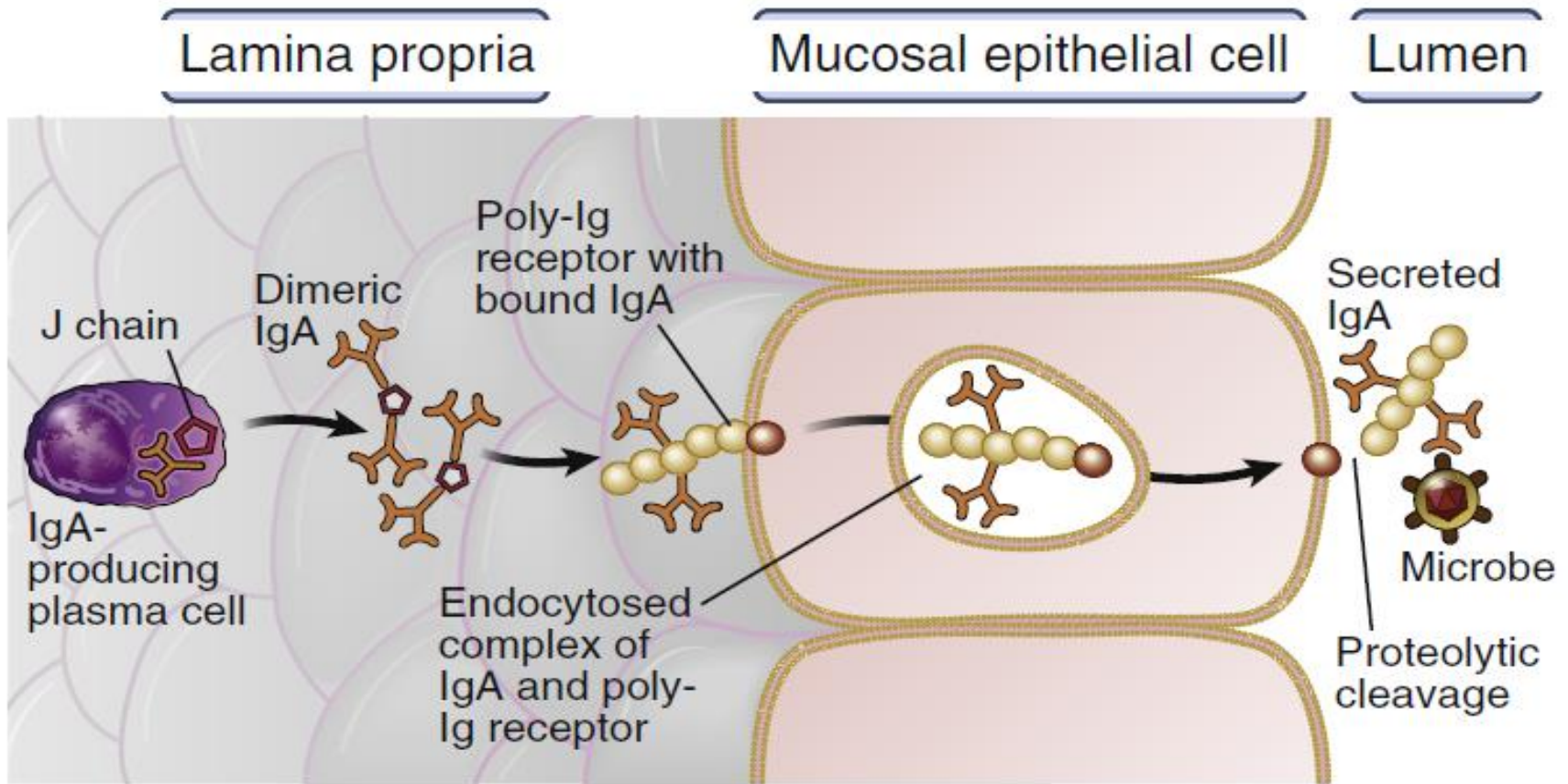


# **Humoral immunity in special anatomical locations**

**Mucosal immunity**

**Fetal and neonatal immunity**

# Mucosal immunity

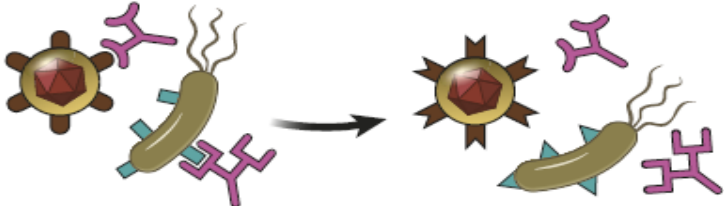
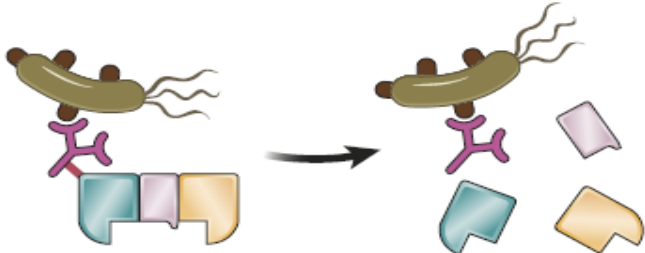
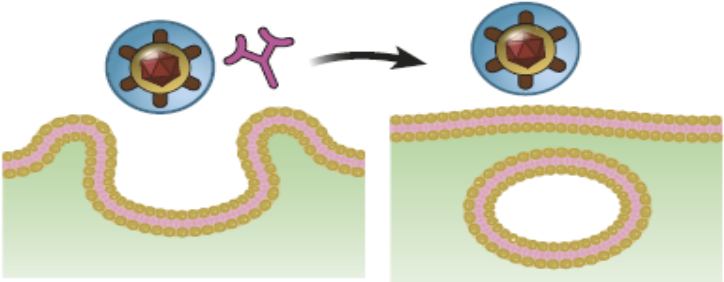


**In the mucosa of the gastrointestinal and respiratory tracts, plasma cells in the lamina propria produce IgA that is actively transported through epithelial cells. IgA recognizes ingested and inhaled microorganisms and blocks their penetration through the epithelium.**

# Fetal and neonatal immunity

- Neonatal Fc receptor (FcRn) on syncytiotrophoblast cells, endothelial cells and enterocytes
- Physiological immunodeficiency of neonates
- Physiological selective IgA immunodeficiency in childhood
- Which vaccines can be given immediately after birth, and which cannot?

# How do microorganisms evade humoral immunity?

Mechanism of immune evasion	Example(s)	
Antigenic variation	<p>Many viruses (e.g., influenza, HIV)</p> <p>Bacteria (e.g., <i>Neisseria gonorrhoeae</i>, <i>Escherichia coli</i>)</p> <p>Protozoa (e.g., <i>Trypanosoma cruzi</i>)</p>	
Inhibition of complement activation	Many bacteria	
Blocking by hyaluronic acid capsule	Streptococcus	

## **Literature:**

Basic Immunology: Functions and Disorders of the Immune  
System, 6th edition

Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai  
Datastatus, Belgrade, 2019