TEACHING UNIT 5

Humoral immune response

Effector mechanisms of humoral immunity

Humoral immune response

Activation of B lymphocytes and production of antibodies

We know that humoral immunity...

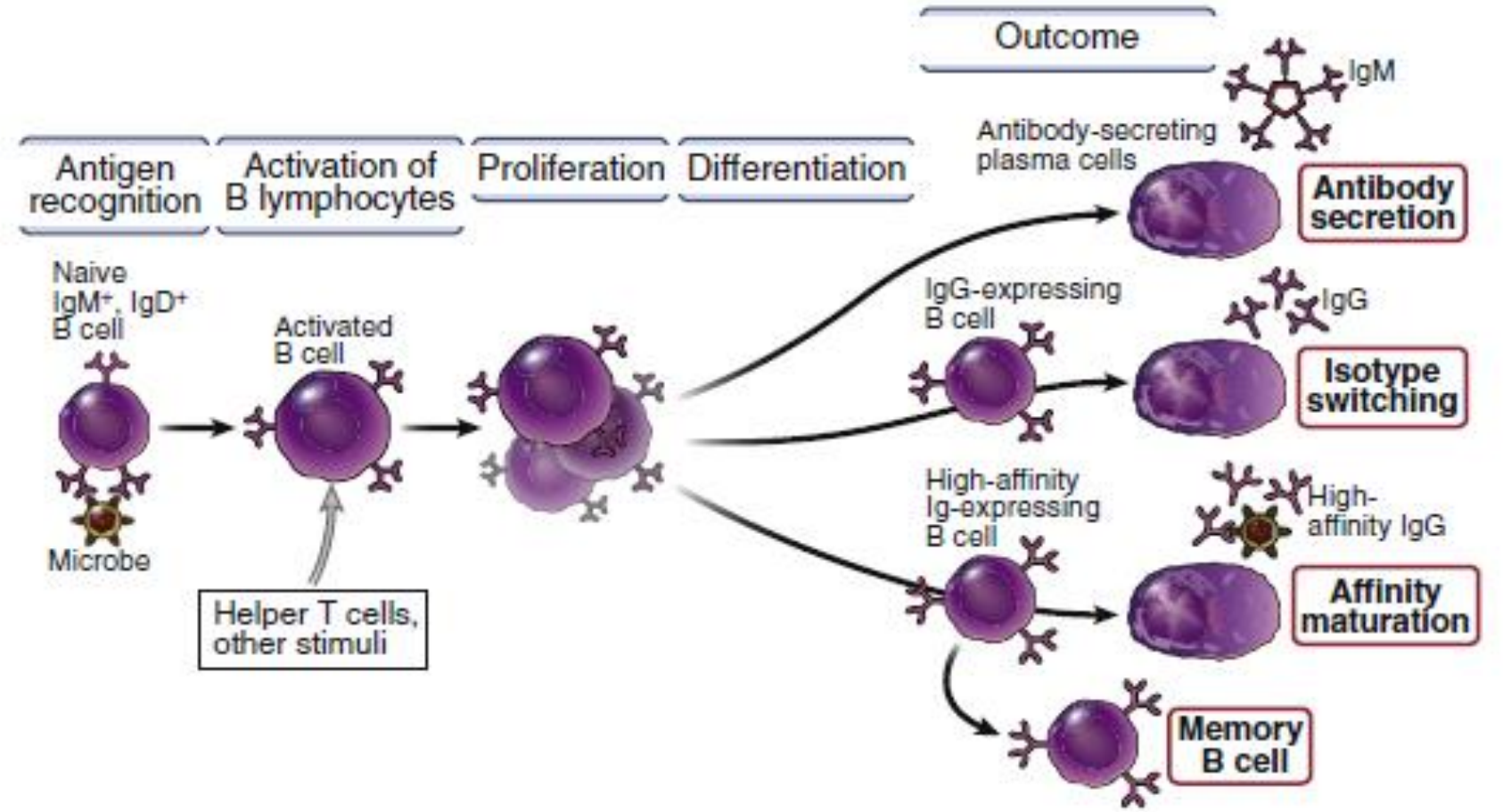
... action of B lymphocytes and antibodies in acquired immunity...

... effective in protection against extracellular microorganisms and their toxins…

... more important than cellular immunity in defence against microorganisms that have membrane rich in polysaccharides and lipids

PHASES OF HUMORAL IMMUNE RESPONSE

The activation of B lymphocytes results in their proliferation, leading to expansion of antigen-specific clones, and their differentiation into plasmocytes which secrete antibodies.



TYPES OF HUMORAL IMMUNE RESPONSE

Antibody responses to different antigens are classified as T-dependent or T independent, based on the requirement for T cell help

Humoral response to T-dependent antigens:

PROTEIN

Change of antibody class

Maturation of antibody affinity

Formation of memory B lymphocytes

Humoral response to T-independent antigens:

NOT PROTEINS

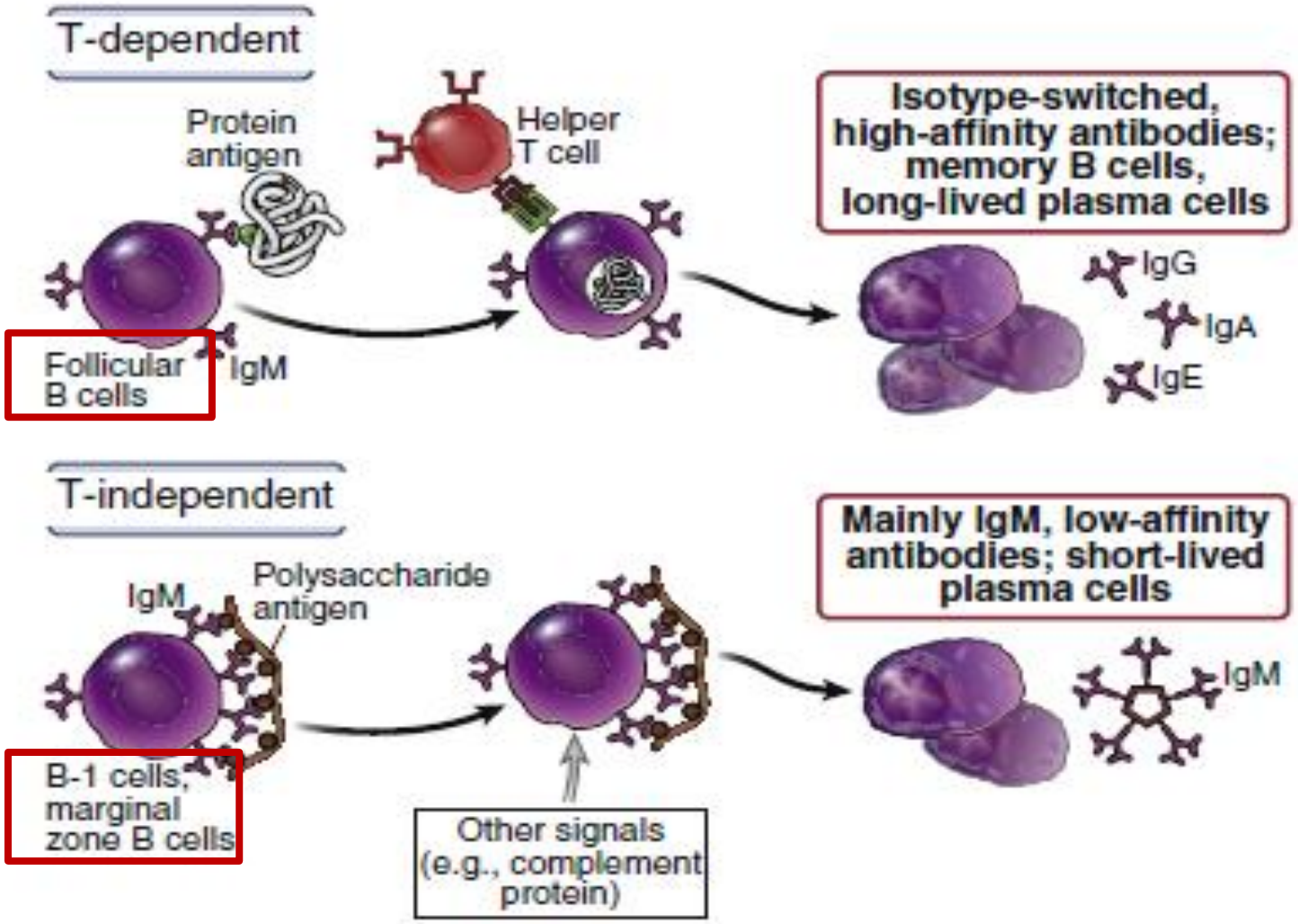
There is no change in the class of antibodies

There is no, or poorly expressed, maturation of antibody affinity

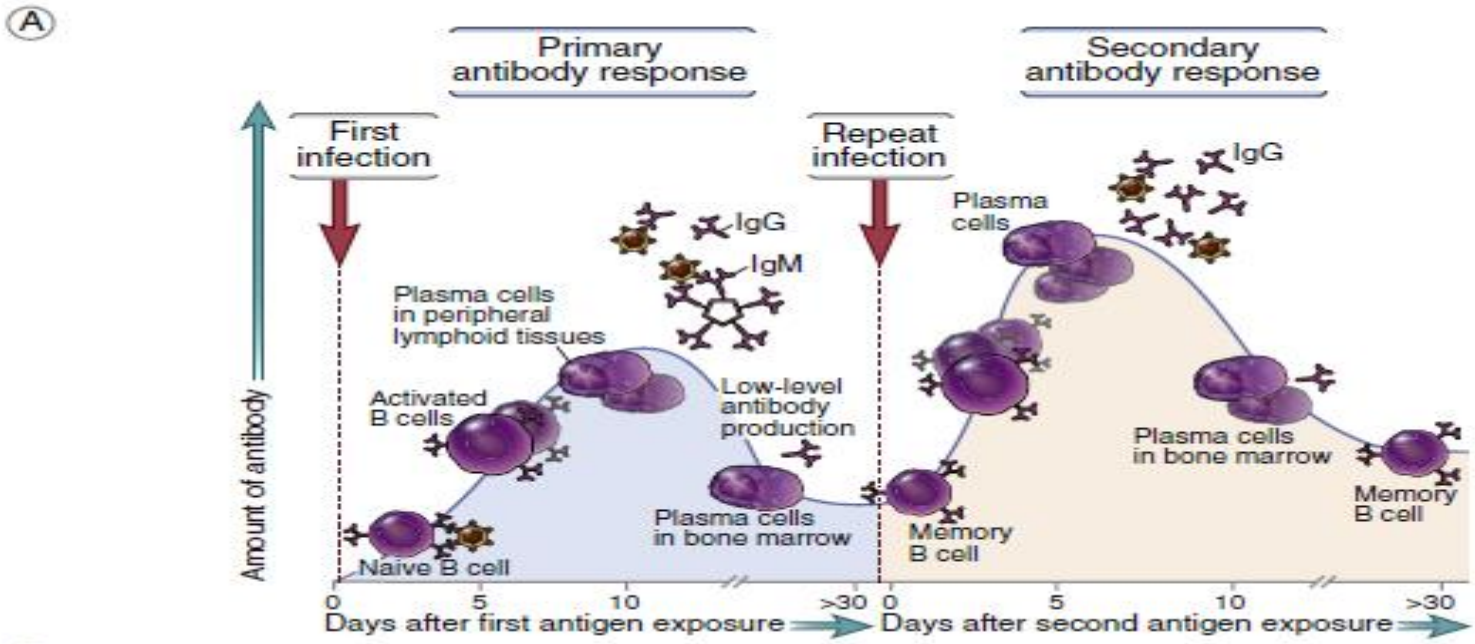
There are no memory B lymphocytes

THERE IS NO SECONDARY IMMUNE RESPONSE

Different subsets of B cells respond preferentially to T-dependent and T-independent antigens:



Features of primary and secondary antibody responses



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	Primary response	Secondary response
Lag after immunization	Usually 5–10 days	Usually 1–3 days
Peak response	Smaller	Larger
Antibody isotype	Usually IgM>IgG	Relative increase in IgG and, under certain situations, in IgA or IgE (heavy-chain isotype switching)
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)

Antibody responses generated during the first exposure to an antigen, called **primary responses**, differ quantitatively and qualitatively from responses to subsequent exposures, called **secondary responses.**

Humoral response to T–dependent antigens...

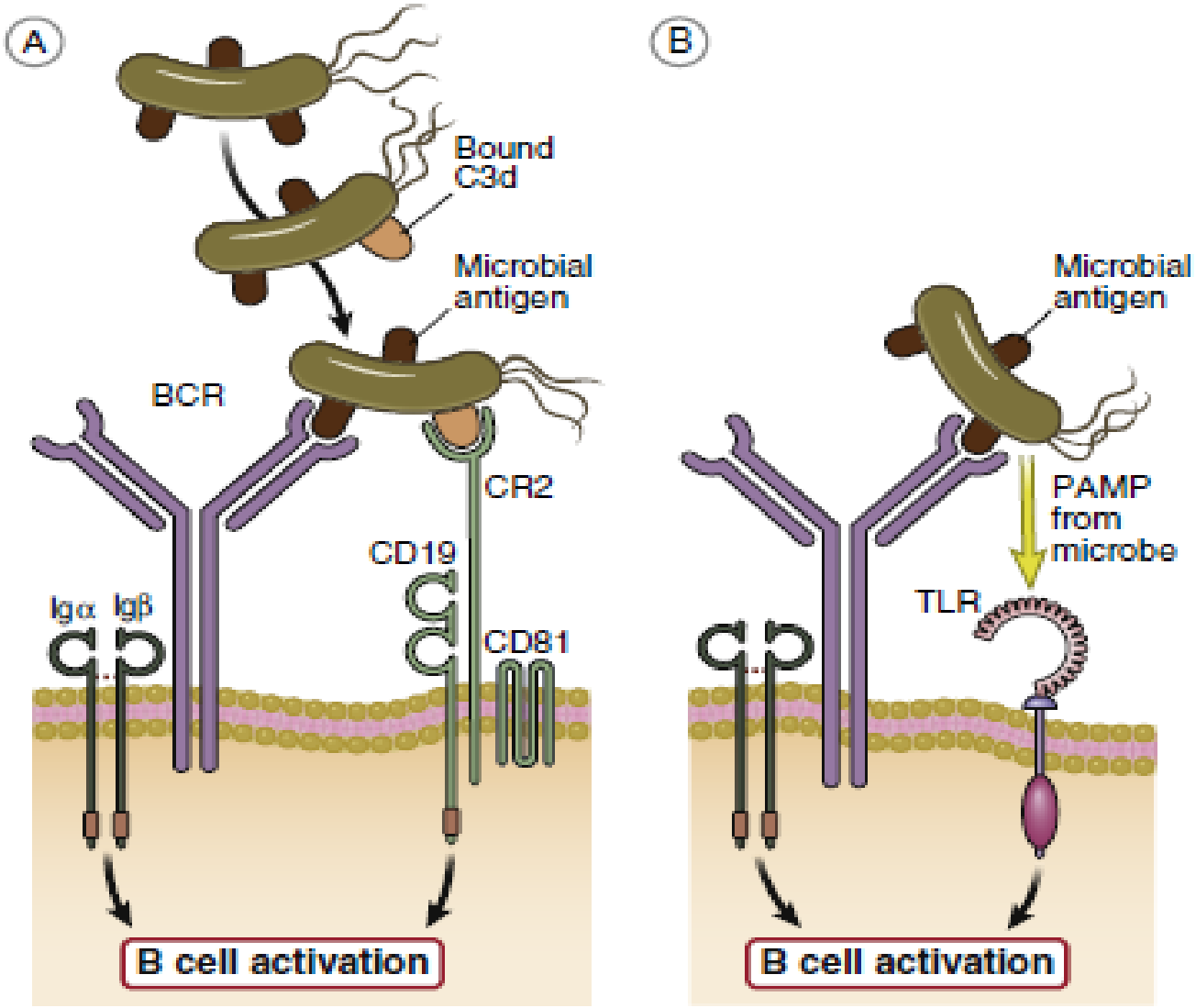
... begins in the lymphatic tissue, when the B lymphocyte recognizes the **NATIVE** antigen.

Antigens of microorganisms from tissue are transported through lymph or through the blood and concentrated in the follicles and marginal zones of peripheral lymphatic organs...

... This is where antigen-specific B lymphocytes recognize antigens....

... which triggers the signaling pathways, the activation of the cell...

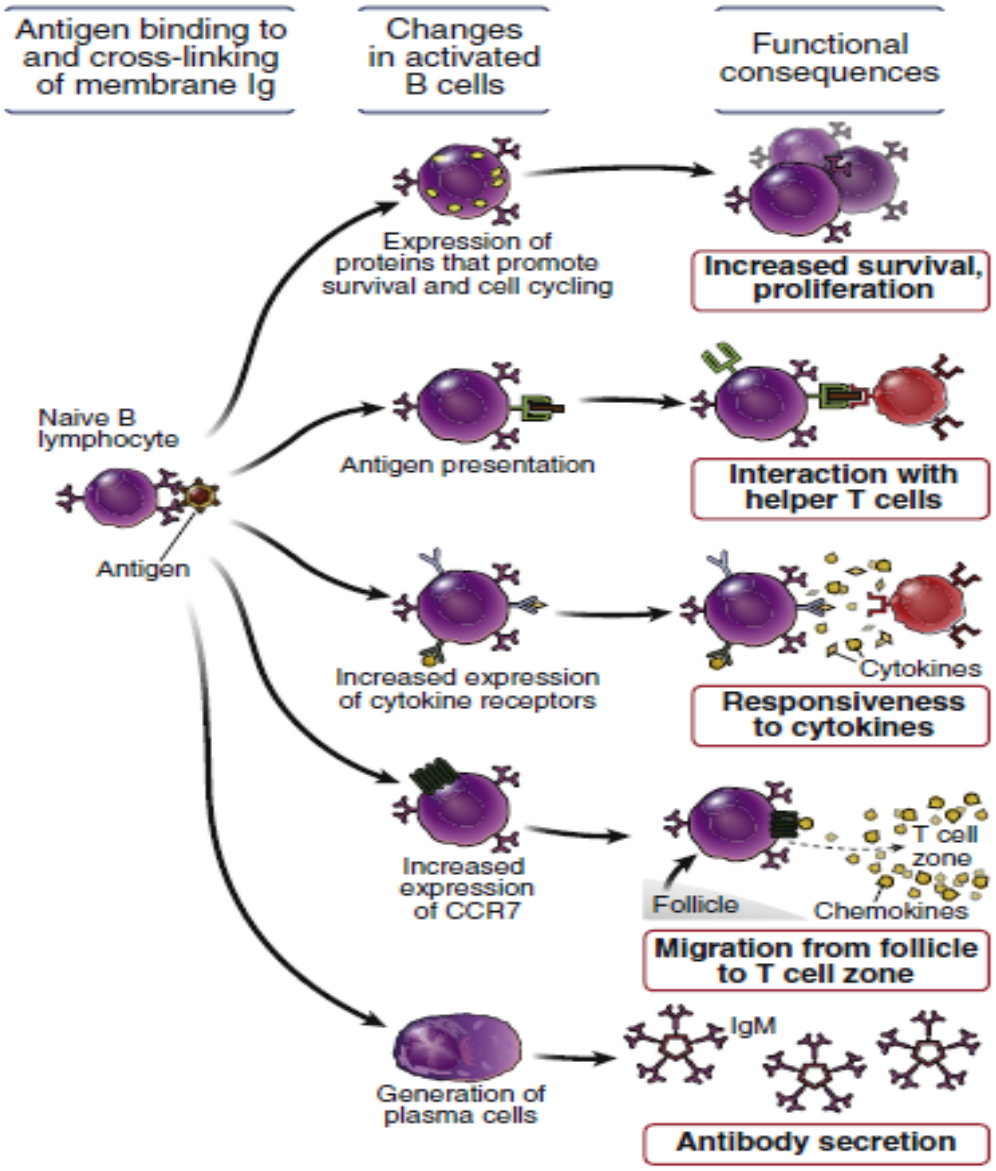
Both in response to protein and in response to non-protein antigens, B lymphocyte activation requires additional (other) signals.



Functional consequences of B lymphocytes activation by antigen: PROLIFERATION and DIFFERENTIATION

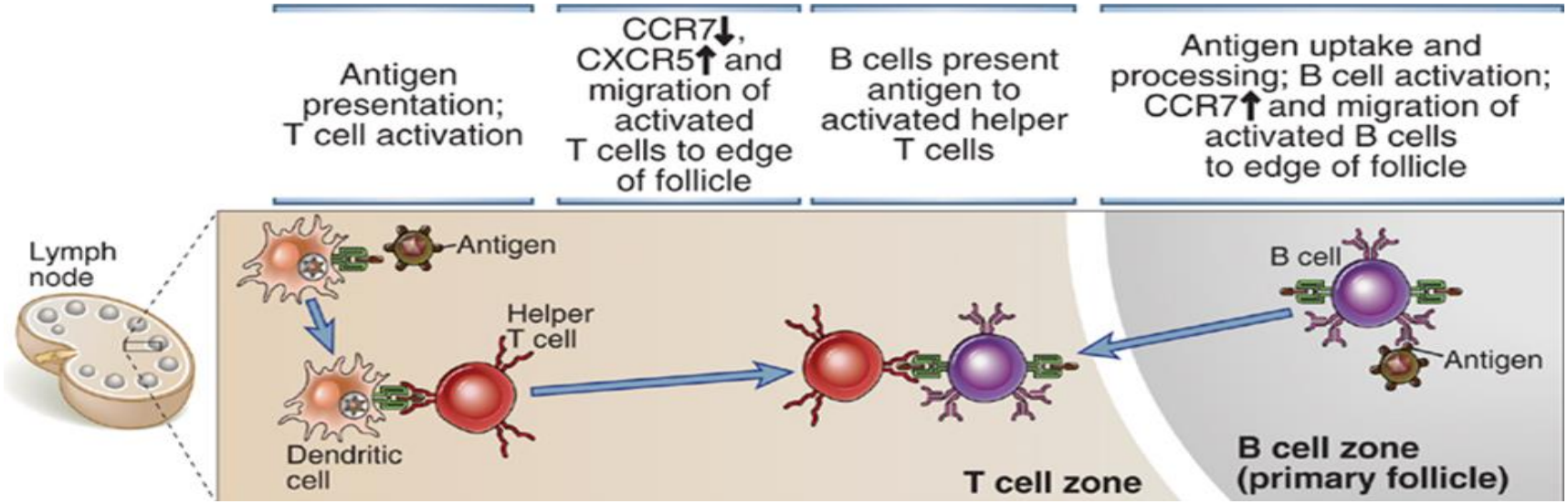
In response to T-independent antigens B lymphocytes begin to synthesize more IgM and to produce some of this IgM in a secreted form - the early phase of the humoral immune response.

Besides proliferation and differentiation, B lymphocytes prepares to interact with helper T lymphocytes if the antigen is a protein.



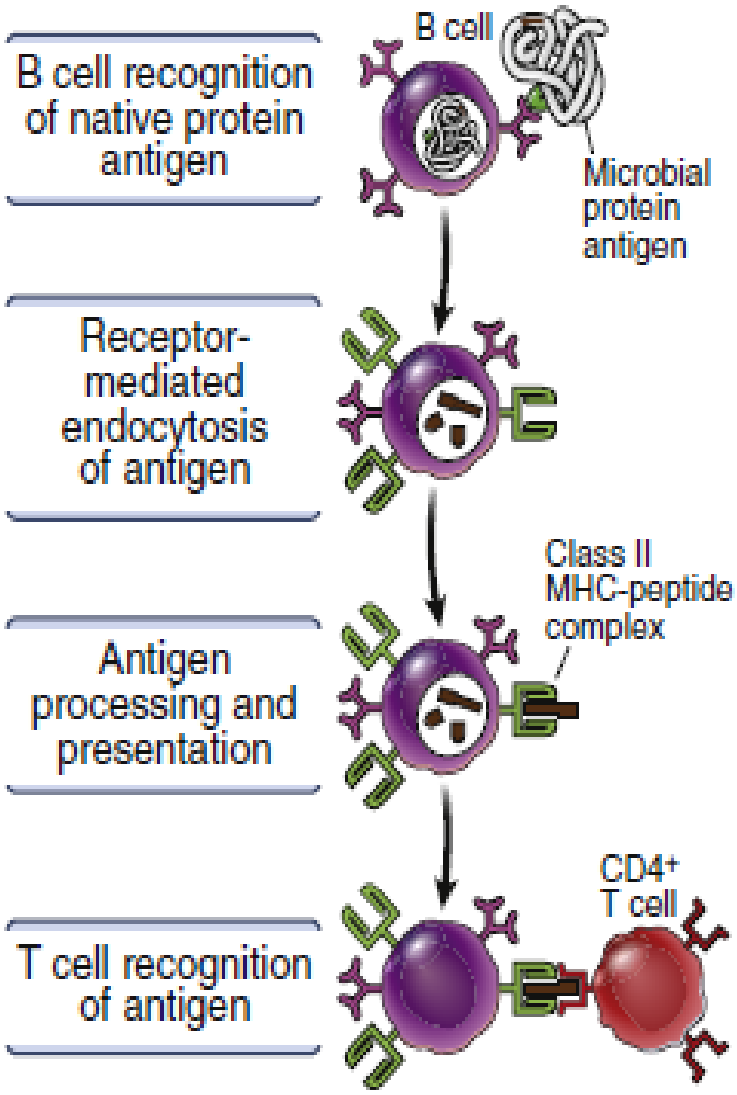
Activation and migration of helper T cells and B cells in response to T-dependent antigens

- Helper T cells that have been activated by dendritic cells migrate toward the B cell zone and interact with antigen-stimulated B lymphocytes in parafollicular areas of the peripheral lymphoid organs.
- Directed migration of activated B and T lymphocytes to each other depends on changes in the expression of certain chemokine receptors.



- **T lymphocytes: expression of CD40L, CXCR5, decrease in CCR7 expression**
- **B lymphocytes: increase in CCR7 and decrease in CXCR5 expression; increase in the expression of CD69 (it is impossible to get out of the lymph node)**

Presentation of antigens by B lymphocytes to helper T cells



- B lymphocytes are very effective APCs, capable of activating previously differentiated effector T cells, but are inefficient at initiating the responses of naive T cells.

- B lymphocytes bind protein antigens by their membrane Ig antigen receptors, endocytose these antigens, process them in endosomal vesicles, and display class II MHC–associated peptides for recognition by CD4+ helper T cells.

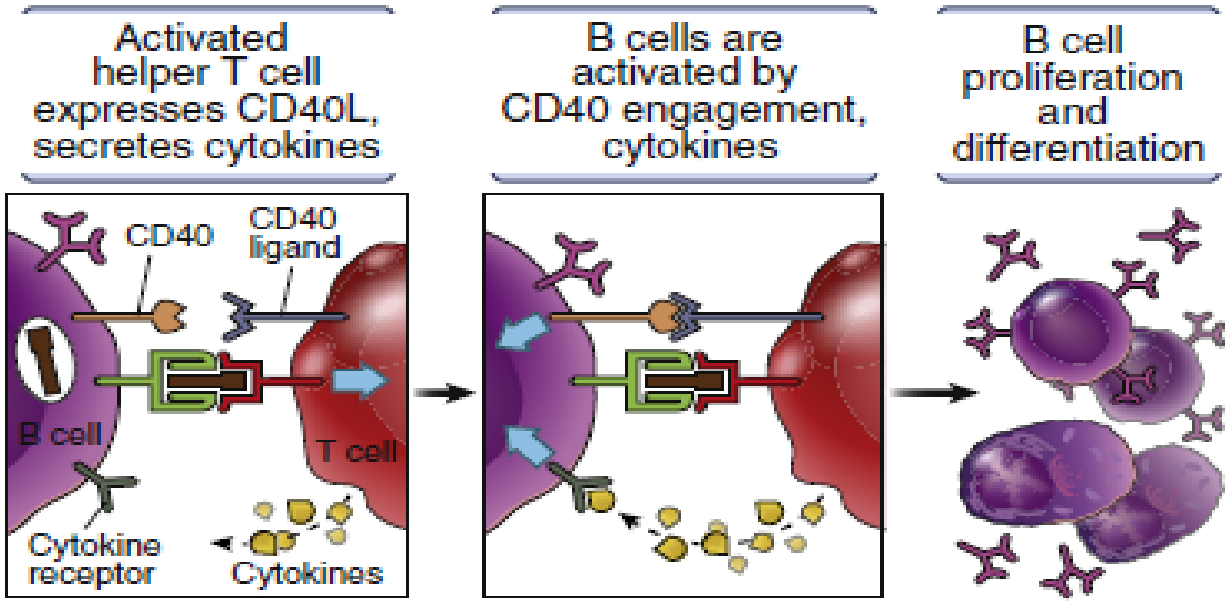
CD4 + Th lymphocyte by co-stimulators and cytokines ADDITIONALLY ACTIVATES B lymphocyte...

... By expressing CD40L

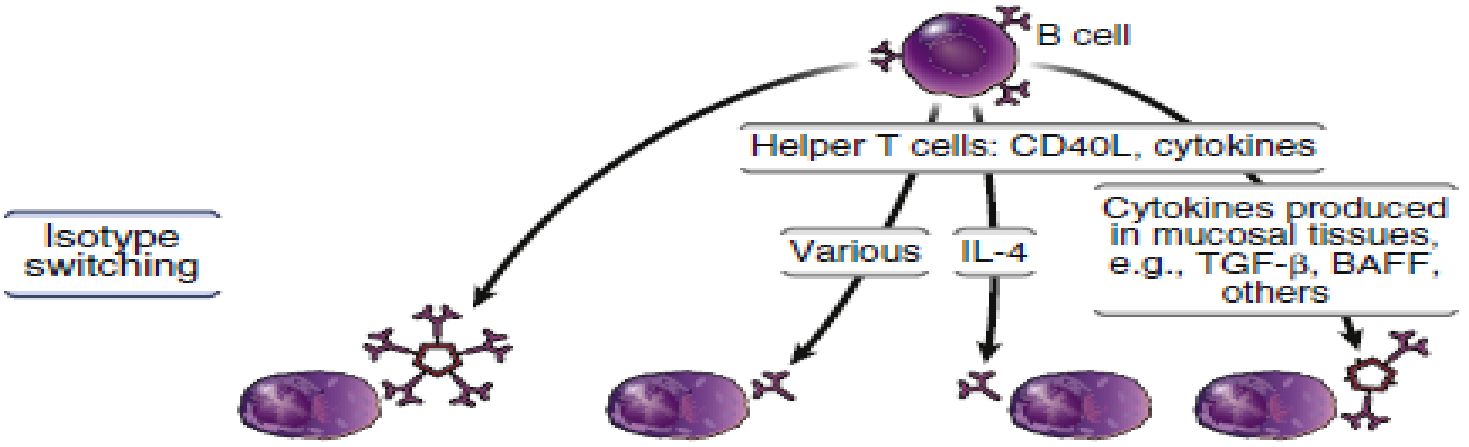
...and also with secretion of cytokines.

CD40 engagement delivers B lymphocytes a proliferative signal as well as a signal for antibody synthesis, class change and affinity maturation.

Cytokines amplify this signal but also modulate it by affecting the class of antibodies and maturation of affinity.



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



	IgM	IgG subclasses (IgG1, IgG3)	IgE	IgA
Principal effector functions	Complement activation	Fc receptor-dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)	Immunity against helminths Mast cell degranulation (immediate hypersensitivity)	Mucosal immunity (transport of IgA through epithelia)

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.

MATURATION OF ANTIBODY AFFINITY…

It is a process by which the affinity of antibodies produced in response to proteins is increased during repeated or prolonged exposure to the same antigen.

It is therefore important for the persistent and recurrent infections.

The basis of this process is the selection of somatic point hypermutations of genes encoding CDR regions.

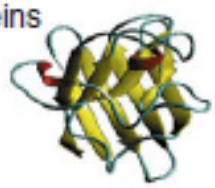
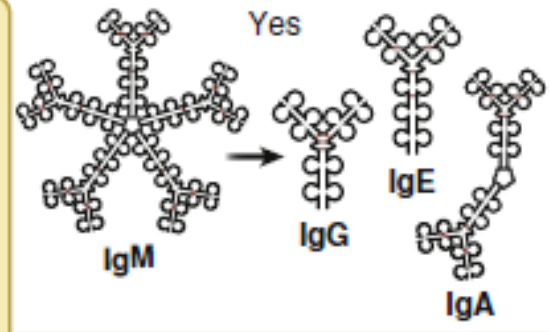
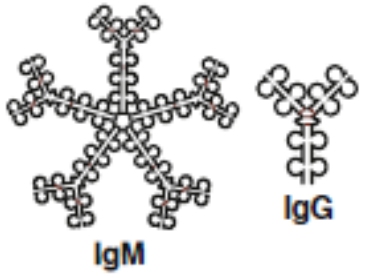
Mutations of these regions depend on Th lymphocytes.

ANTIBODY RESPONSE TO T-INDEPENDENT ANTIGENS

Polysaccharides, lipids, and other non-protein antigens elicit antibody responses without the participation of helper T cells (these non-protein antigens cannot bind to MHC molecules, so they cannot be seen by T cells).

Many bacteria contain polysaccharide-rich capsules, and defense against such bacteria is mediated primarily by antibodies that bind to capsular polysaccharides and target the bacteria for phagocytosis and activate complement system.

FEATURES OF ANTIBODY RESPONSE TO T-INDEPENDENT ANTIGENS

	Thymus-dependent antigen	Thymus-independent antigen
Chemical nature	Proteins 	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids 
Features of anitbody response		
Isotype switching		Low-level switching to IgG 
Affinity maturation	Yes	Little or no
Plasma cells	Long-lived	Short-lived
Secondary response (memory B cells)	Yes	Only seen with some polysaccharide antigens

B lymphocytes of the marginal zone of the spleen are responsible for the humoral immune response to T – independent antigens originating in the blood.

B-1 lymphocytes respond to T – independent antigens in the mucous membranes and peritoneum.

Effector mechanisms of humoral immunity

Elimination of extracellular microorganisms and toxins

Effector functions of antibodies

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).

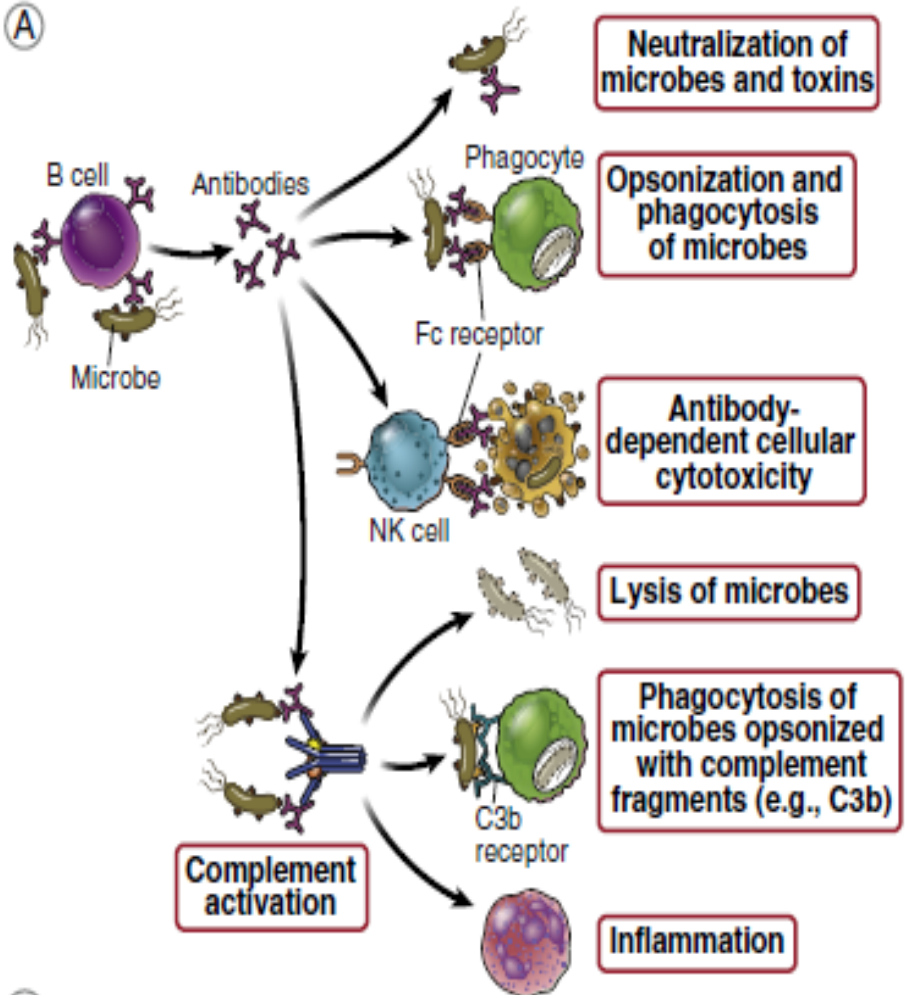
PROPERTIES OF ANTIBODIES THAT DETERMINE EFFECTOR FUNCTION

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.

EFFECTOR FUNCTIONS OF ANTIBODIES



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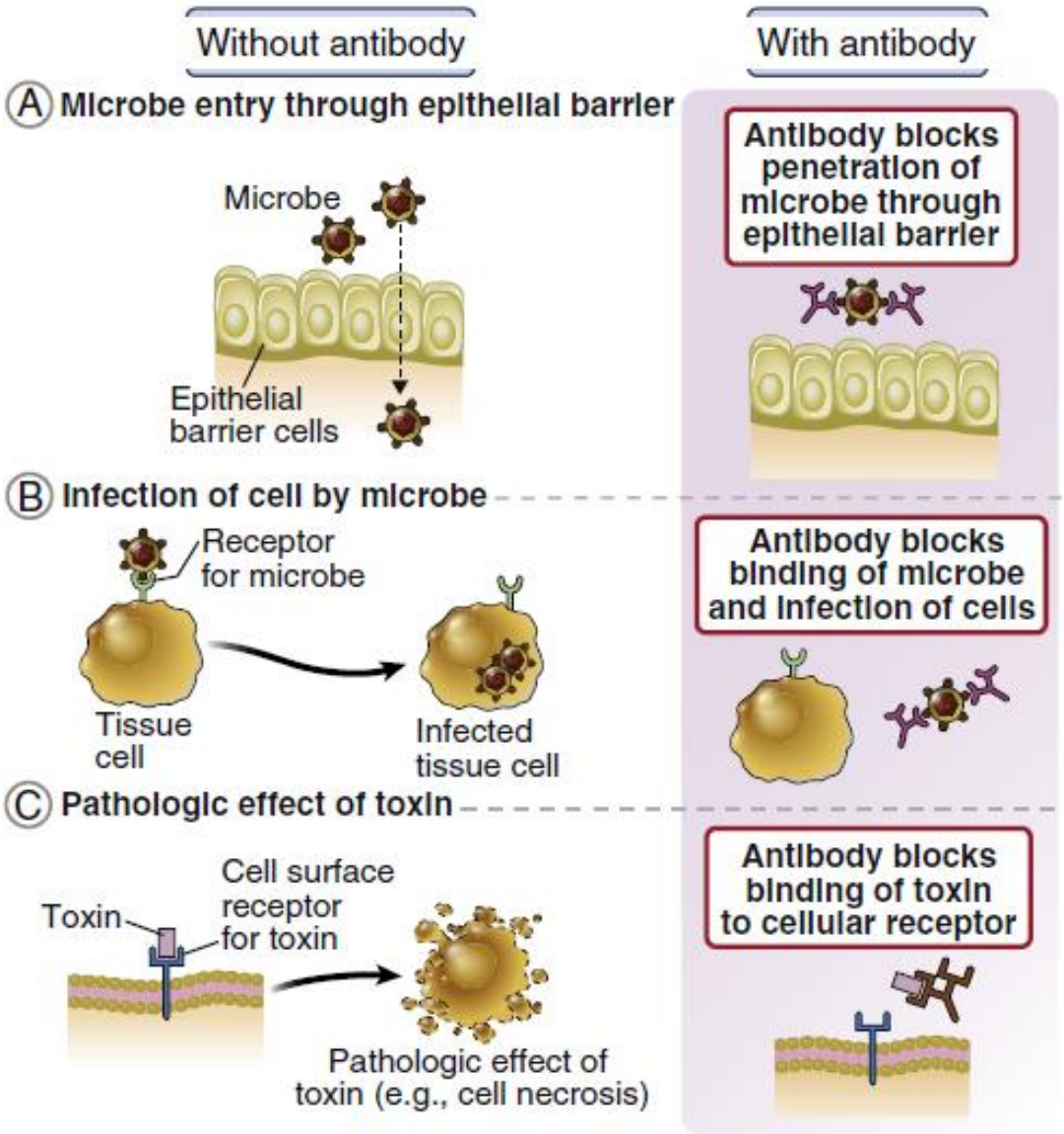
Antibody isotype	Effector functions
IgG	Neutralization of microbes and toxins Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cellular cytotoxicity mediated by NK cells Neonatal immunity: transfer of maternal antibody across placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement
IgA	Mucosal immunity: secretion of IgA into lumens of gastrointestinal and respiratory tracts, neutralization of microbes and toxins
IgE	Eosinophil- and mast cell-mediated defense against helminths

NEUTRALIZATION OF MICROBES AND MICROBIAL TOXINS

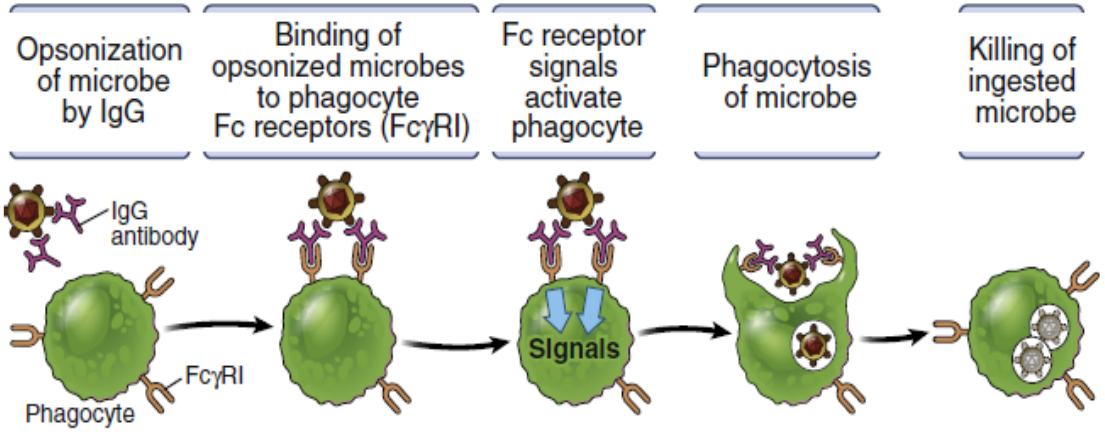


Antibodies prevent the binding of microbes to cells, thereby **blocking the ability of the microbes to infect host cells**.

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



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

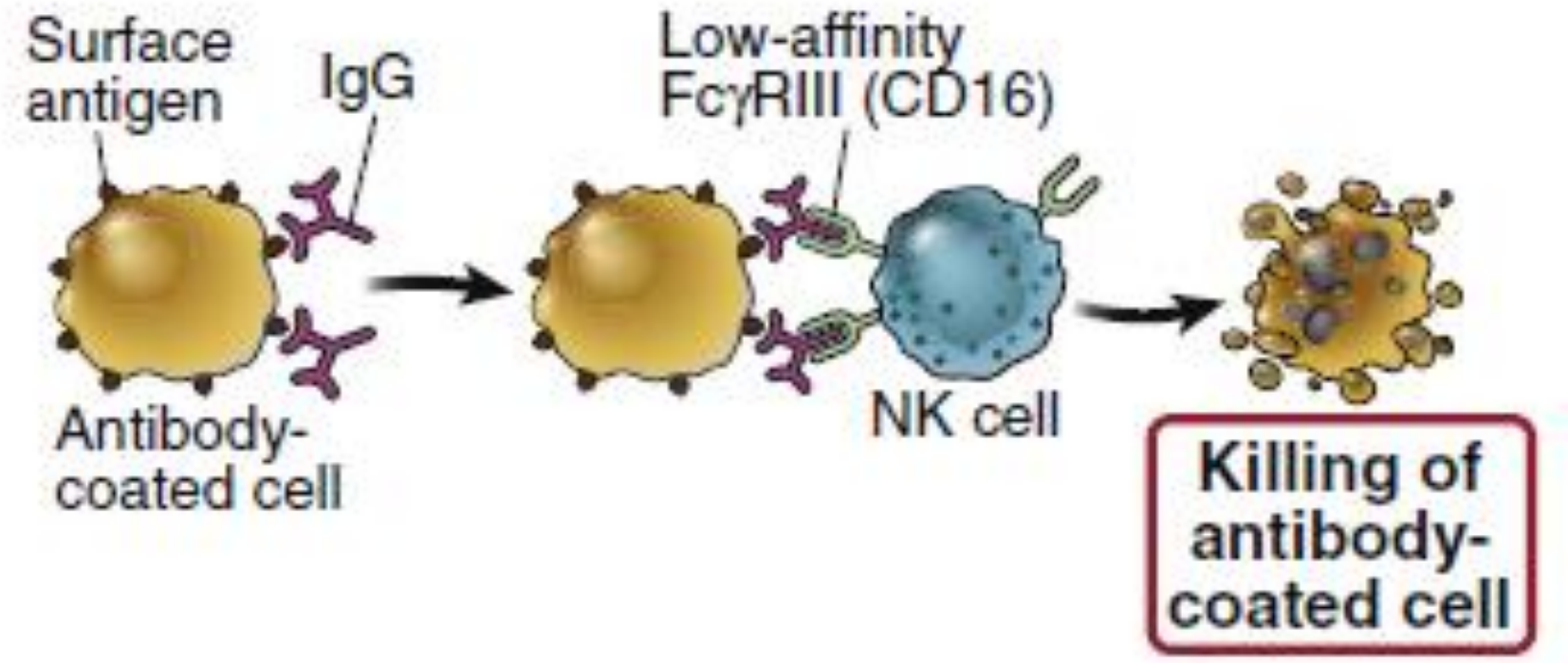
Opsonization - the process of coating particles for subsequent phagocytosis.

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

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

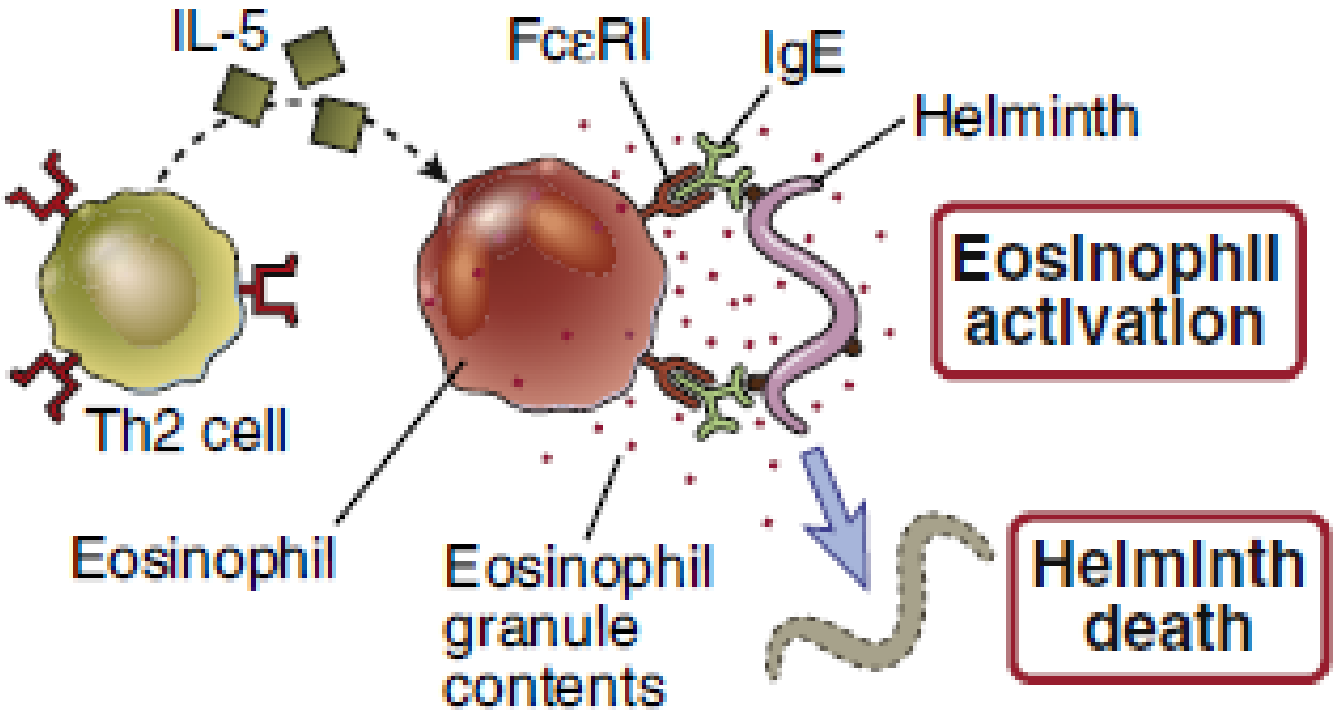
Antibody-mediated phagocytosis is the major mechanism of defense against encapsulated bacteria. As the spleen contains large numbers of phagocytes, patients with splenectomy are susceptible to disseminated infections by encapsulated bacteria.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)



Natural killer (NK) cells bind to antibody-coated cells and destroy these cells. NK cells express the Fcγ receptor called FcγRIII (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

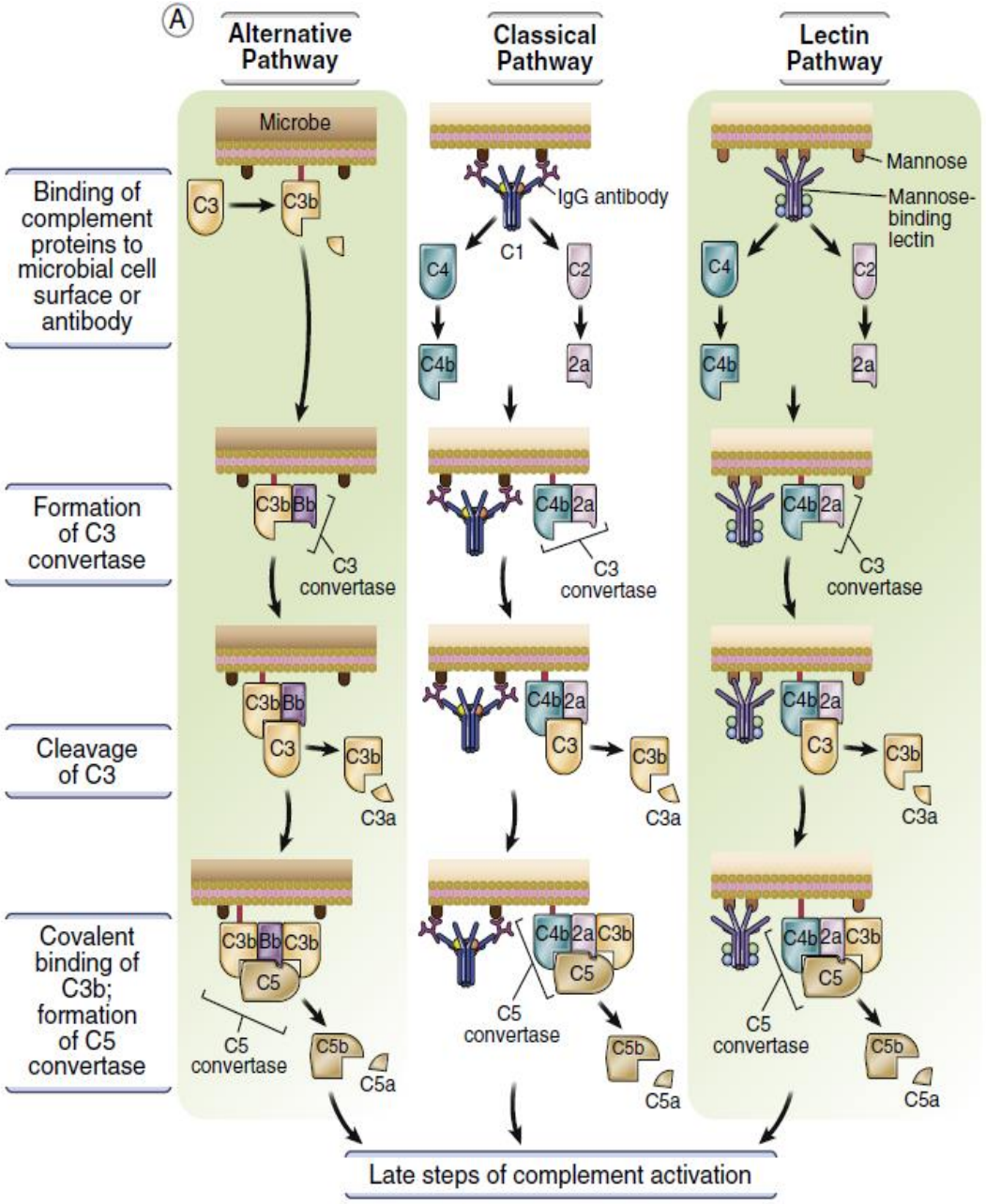
Term **complement** refers to the ability of these proteins to assist, or complement, the activity of antibodies.

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 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 activation (early phase)



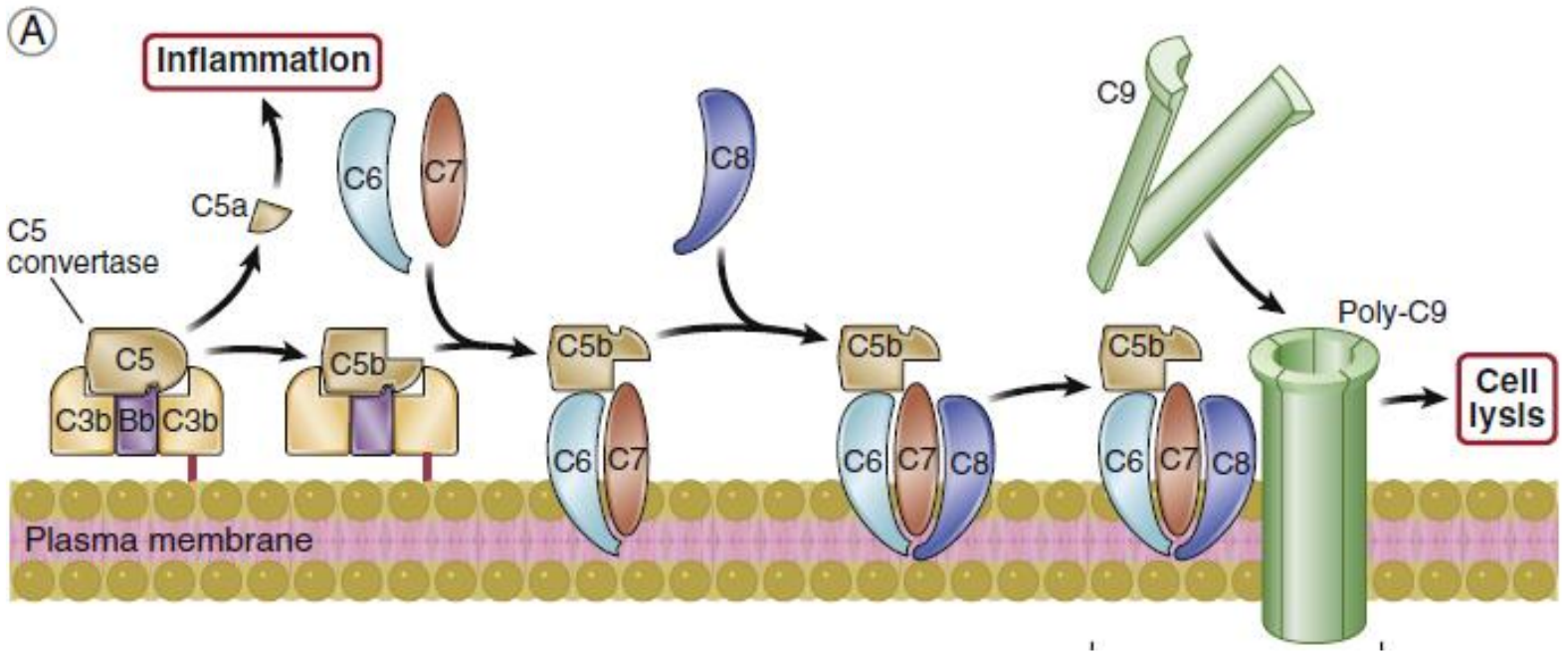
Alternative pathway - as a product of spontaneous hydrolysis of C3, C3b is deposited on the surface of a microbe by covalent binding to microbial proteins or polysaccharides.

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 protein…

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

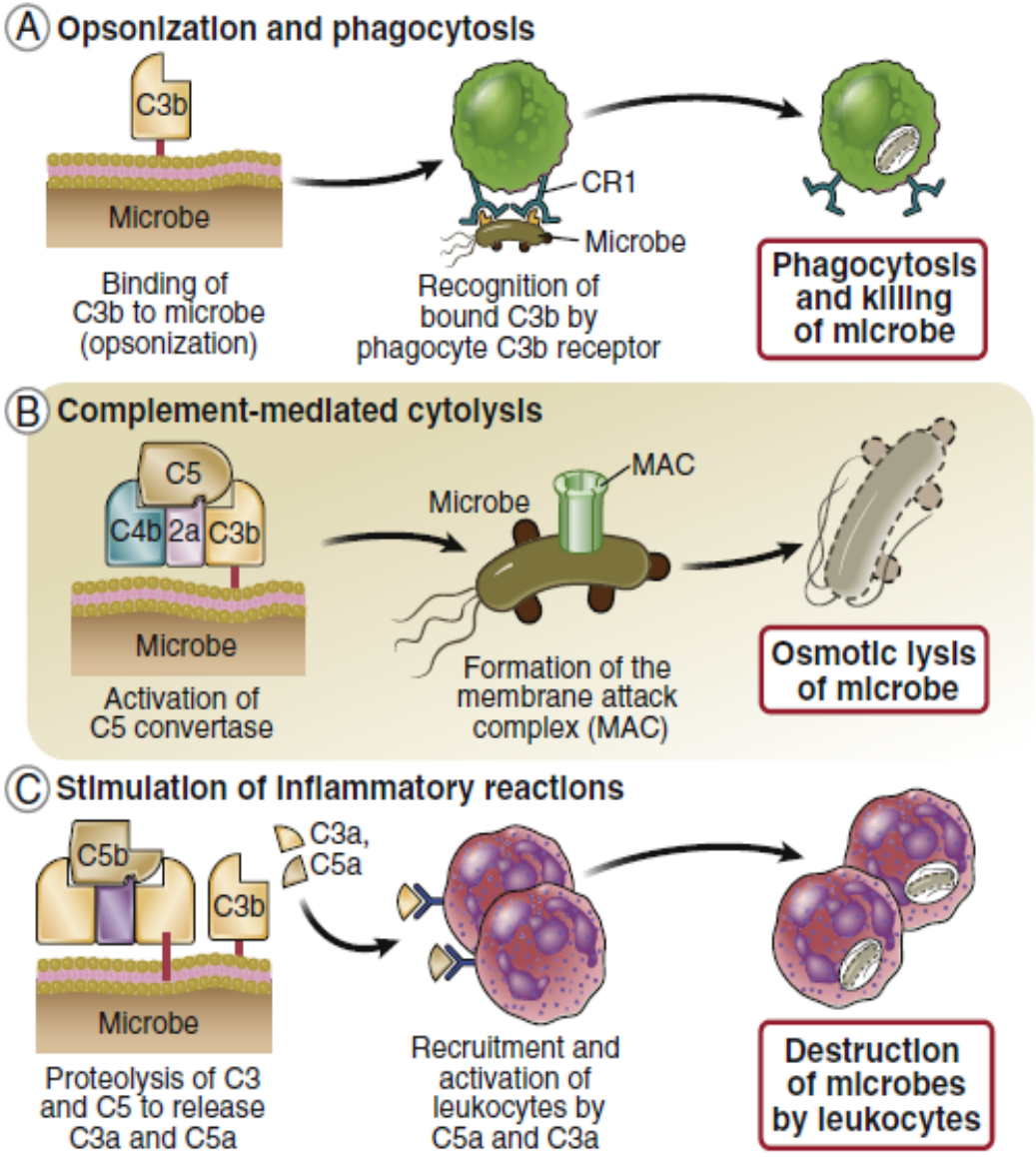
Late phase of complement activation



The late phase of complement 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).

The functions of complement in defense against microbes



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

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

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.

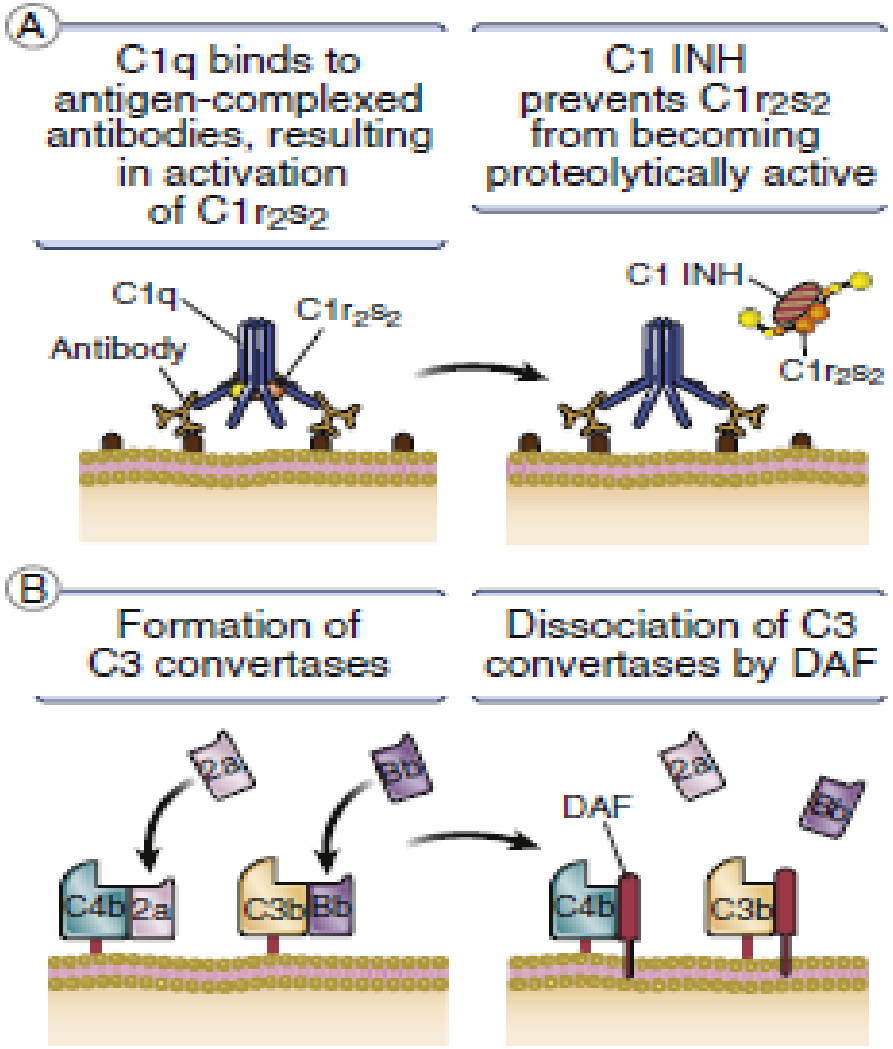
Complement deficiencies

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 is important for their elimination.

C9 deficiency: increased susceptibility to *Neisseria* spp.

Regulation of complement activation



Mammalian cells express regulatory proteins that prevent complement 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 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**

Literature:

Basic Immunology: Functions and Disorders of the Immune System, 6th edition
Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai
Datastatus, Belgrade, 2019