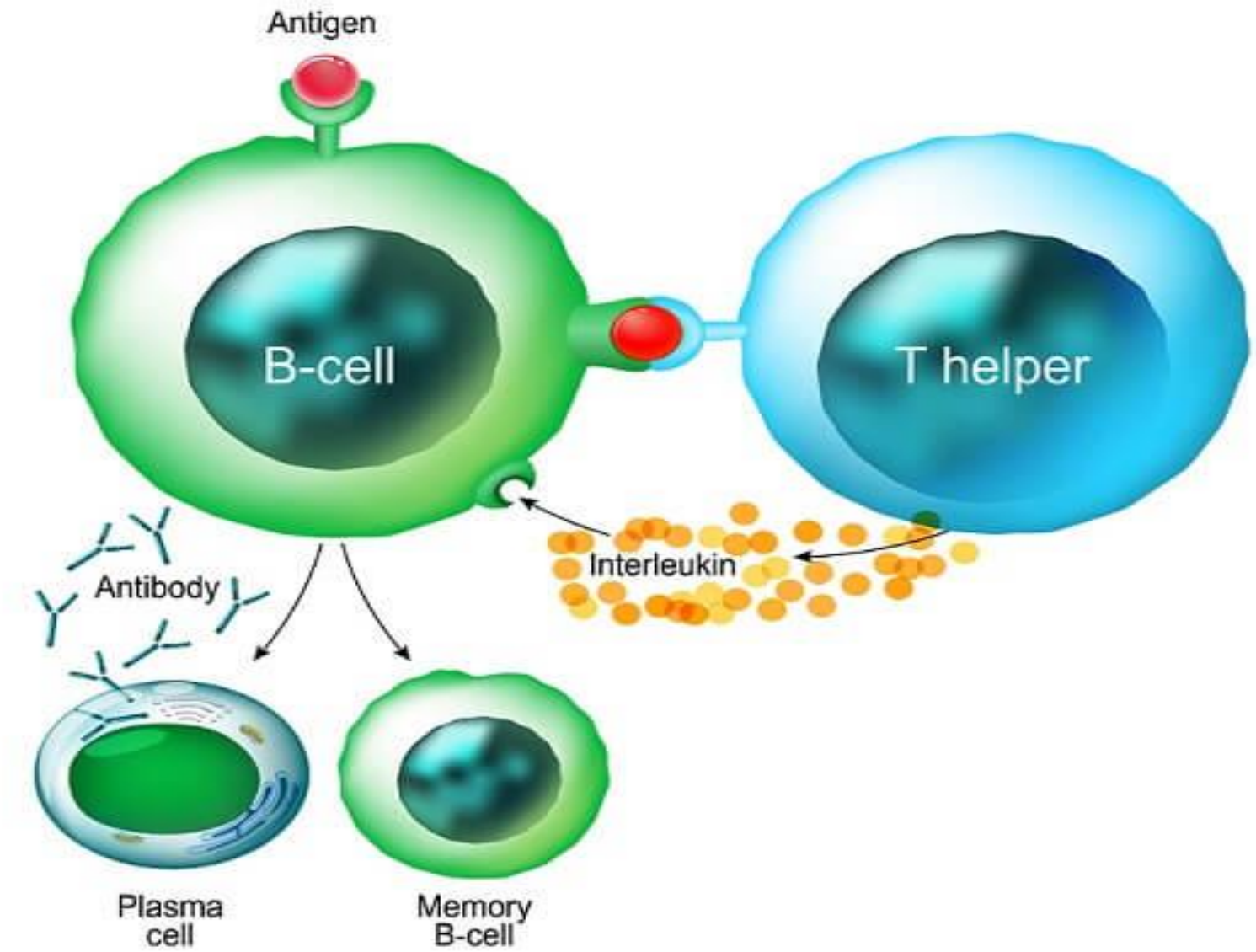
Humoral immune response



HUMORAL IMMUNE RESPONSE

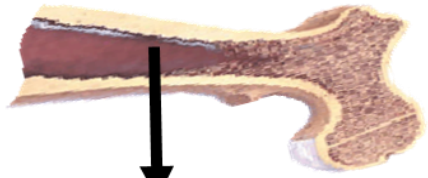
Humoral immune response represents a component of the acquired immunity which is highly effective against non-invasive bacteria (extracellular bacteria), viruses which resides outside of the cells, intestinal worms as well as against toxins of biological agents.

This type of immunity is preferentially performed by B lymphocytes.

Other involved cells in humoral immunity can be:

- antigen presenting cells
- CD4 –T cells

Hematopoietic Cells



Bone Marrow

Hematopoietic Stem Cell

Myeloid Progenitor Cell

Lymphoid Progenitor Cell

RBC

Platelet

Natural Killer Cell

Neutrophil

Eosinophil

Basophil

Monocyte

Dendritic Cell

Macrophage

Inflammation

Innate

T Lymphocytes

- Helper cells
- Regulatory cells
- Cytotoxic cells

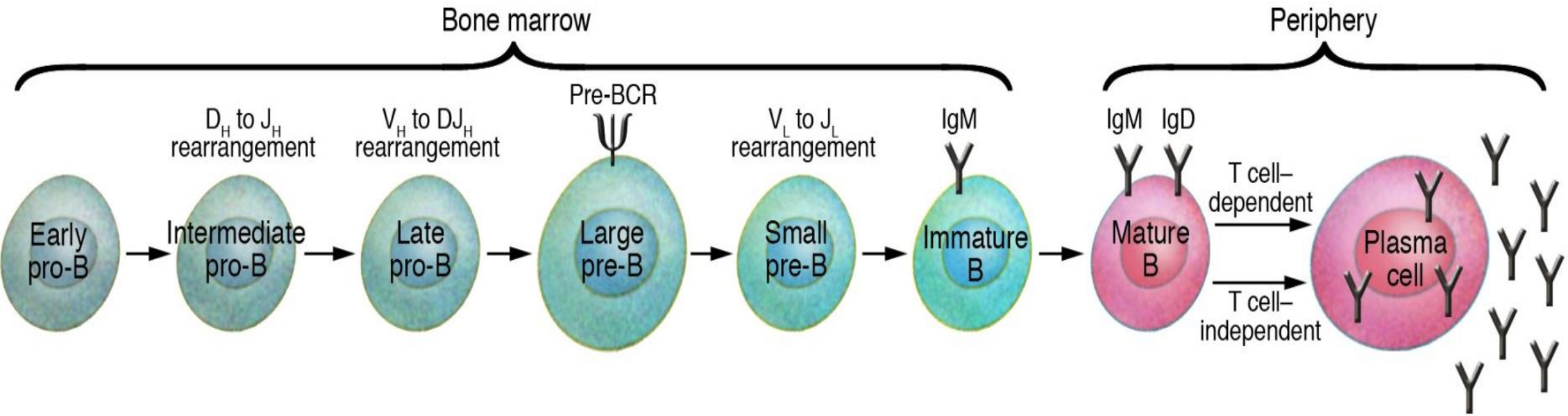
B Lymphocyte

Plasma Cell

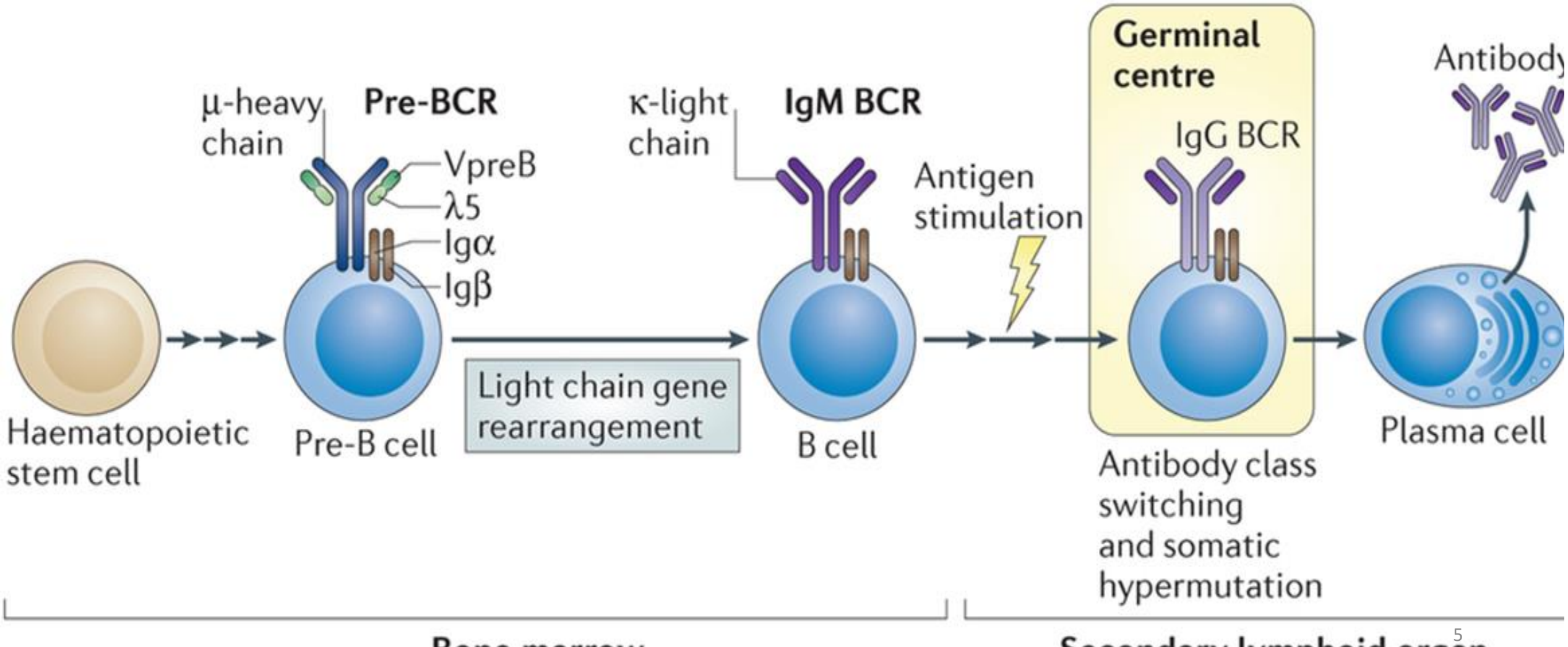
Antibodies

Adaptive

Maturation of B lymphocytes

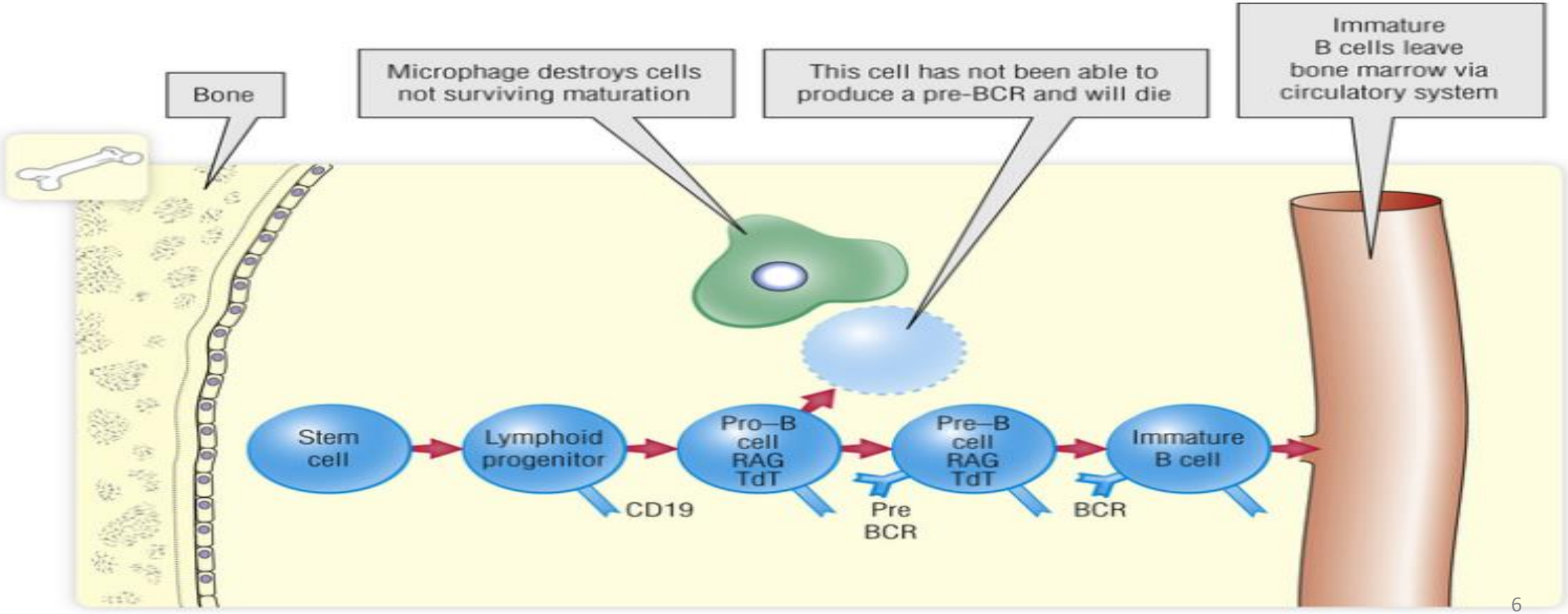


Maturation of B lymphocytes = expesion of receptors for recognition of antigen in the bone marrow. This happen due to direct contact with stromal cells and at late stages with involvement of cytokines (especially IL-7), which are secreted by stromal cells.



- **In the bone marrow, B lymphocytes can undergo a process of negative selection:**

  - destruction of cells which will not survive during maturation process;
- destruction of cells, which exactly like T lymphocytes, show a tight connection with self antigen (self-Ag)- there is the risk for autoimmune response.
- destruction of cells that are not able to produce pre-BCR



At the level of lymphoid organs, B lymphocytes are localized:

- follicles of the extern cortex in the lymph nodes,

- follicles of Peyer patches (gastrointestinal tract),

- follicles of the peripheral zone at the level of white splenic pulp (thymus-independent areas)

B lymphocyte population represents about 10 - 15% from all lymphocytes. Lifespan is short (average 3 – 5 days).

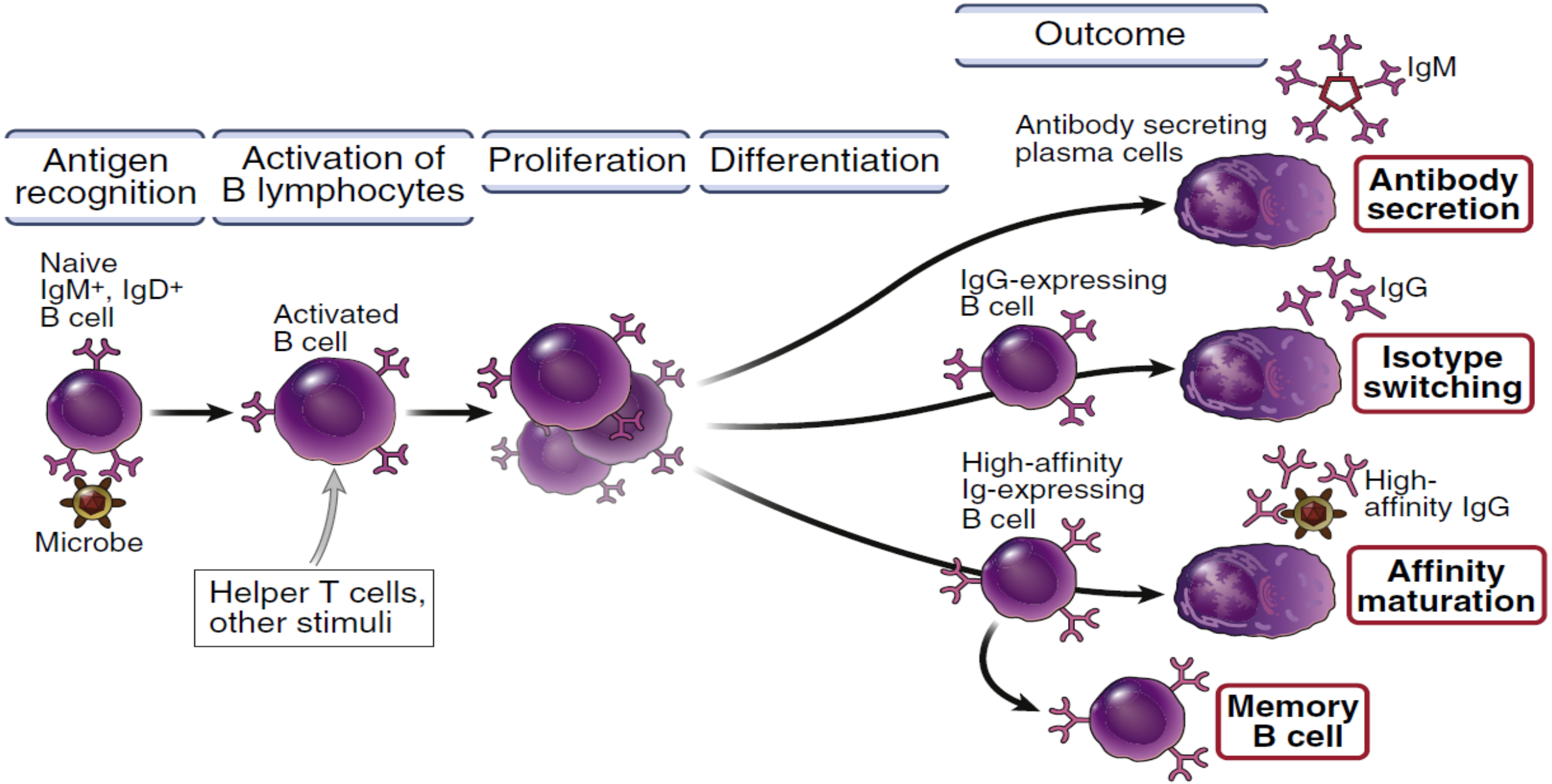
Exactly like T lymphocytes, **naïve B lymphocytes** are cells, which did not meet the antigen.

B lymphocytes resides in peripheral lymphoid organs, and for short periods of time exit in the blood stream to meet the antigen.

After interaction with antigen, there is B lymphocyte activation and proliferation, and differentiation in **effector lymphocytes**:

- **B Lymphocytes – plasma cells,** which secrete antibodies.
- **B Lymphocytes** – memory cells.

Stages of humoral immune response



Stages of the humoral immune response

I. Meeting of the antigen with APC

This happens at the level of suite of entry (macrophages, dendritic cells) as well as at the level of secondary lymphoid organs (B lymphocytes).

- **If the antigen enters directly into the blood, the antigen recognition is at the level of the spleen.**
- **if the antigen enters through the respiratory system, antigen recognition is at the level of amygdales as well as lymphoid tissue of the bronchi and mucosal layer.**
- **if the antigen enters through the gastrointestinal system, antigen recognition is at the level of Peyer's patchers and solitary lymphoid follicles.**
- **if the antigen enters by skin – in the dermal lymphoid tissue.**

Stages of the immune response

2. **Specific recognition of the antigen.** This happens at the level of secondary lymphoid organs and is performed by naïve B which involves receptors for antigens (BCR as two classes of membrane-bound antibodies, immunoglobulins M and D (IgM and IgD).

Important remarks:

- B lymphocytes can recognize superficial conformational epitopes of the native molecules of the antigen
- T lymphocytes are able to recognize only linear epitopes of the protein antigens which are presented by APC in association with MHC-II - Thelper-CD4.

Stages of the humoral immune response

3. Activation, proliferation and differentiation of B lymphocytes in effectors cells as well as B memory cells.

Coordination of these processes is performed by direct cell contacts as well as by cytokines, which are released by different cells.

One activated B cell may generate a few thousand plasma cells, each of which can produce copious amounts of antibody molecules, in the range of several thousand per hour. In this way, humoral immunity can keep pace with rapidly proliferating microbes.

During their differentiation, some B cells may begin to produce antibodies of different heavy-chain isotypes (or classes), which mediate different effector functions and are specialized to combat different types of microbes.

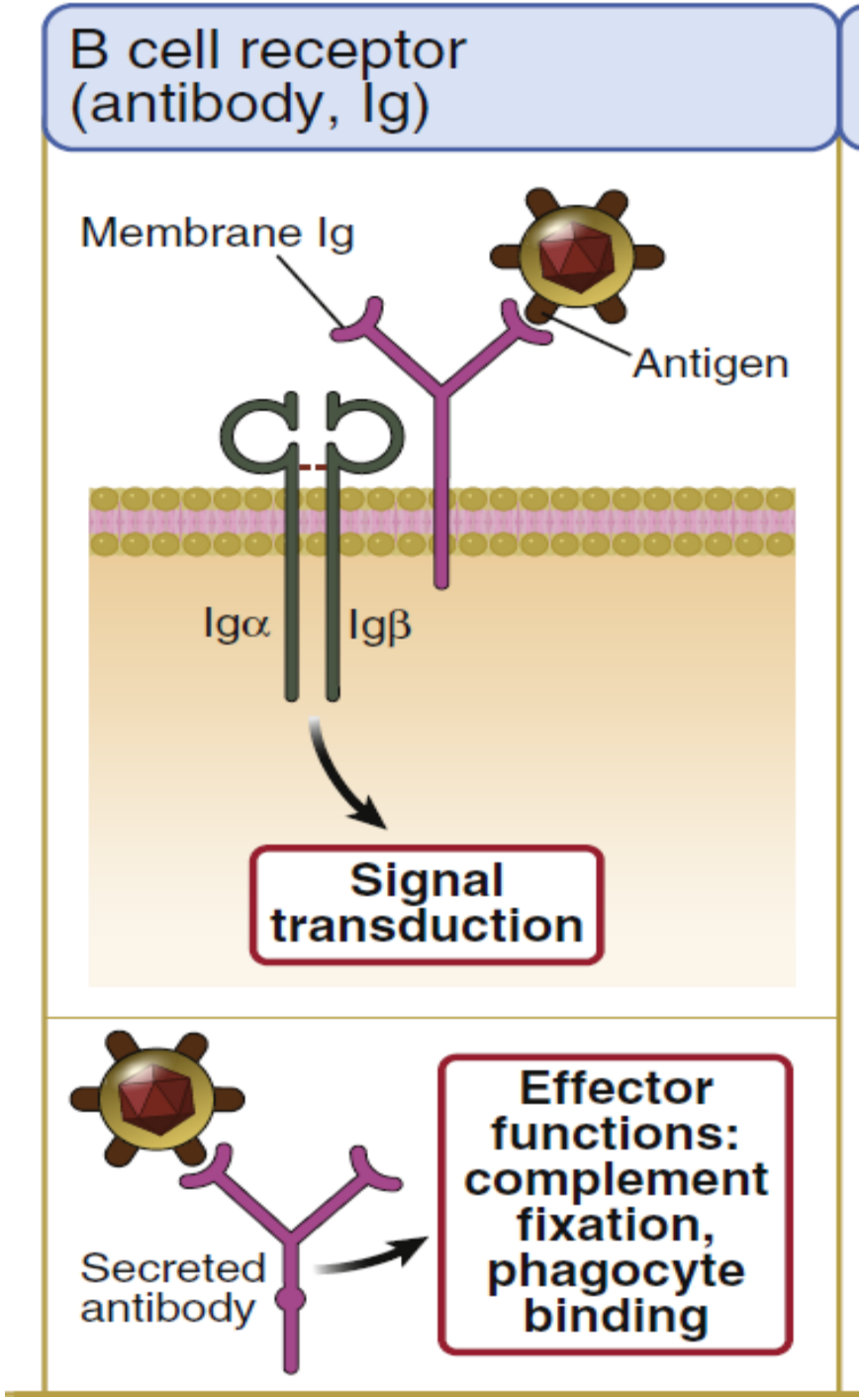
Repeated exposure to a protein antigen results in the production of antibodies with increasing affinity for the antigen. This process is called affinity maturation, and it leads to the production of antibodies with improved capacity to bind to and neutralize microbes and their toxins.

4. Final biological effect (neutralization lysis, opsonisation). Final effect is done at the site of infection.

Stages of the humoral immune response
2. Antigen recognition by B lymphocytes

Receptors of the mature B lymphocytes

- ♦ Receptor for antigen – BCR – B Cell Receptor
- ● CD79, CD19
- ● Receptor for Fc fragment of IgG
- ● Receptor for C3d fragment of the complement system (CR2 or CD21)
- ■ Receptors for interleukins (IL)
- ◙ Proteins of the Major Histocompatibility Complex II



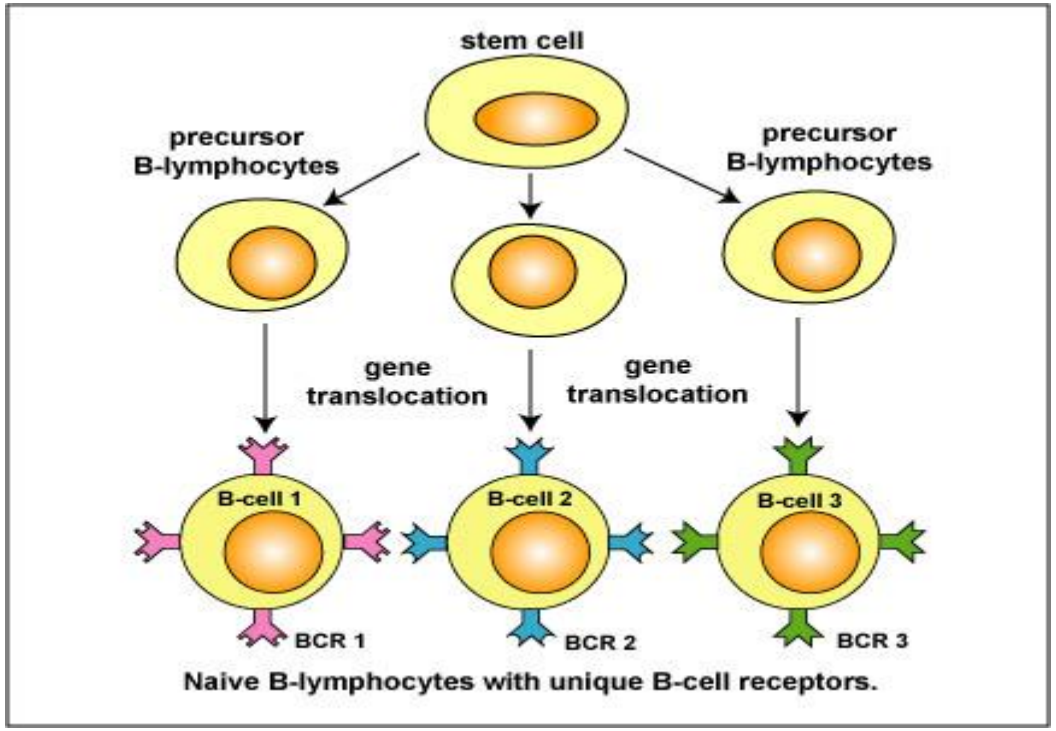
Receptor for antigen (BCR)

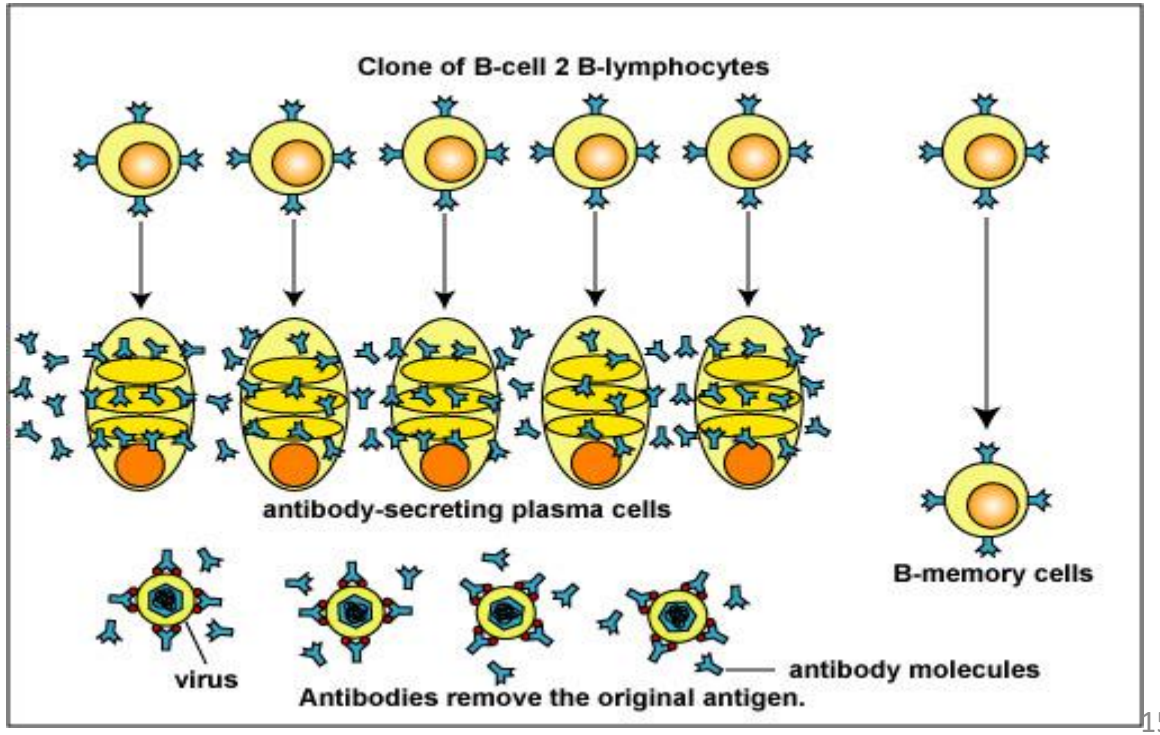
BCR is represented by immunoglobulin molecules – **IgM** and **IgD** monomers (a membranary type of antibodies), associated with 2 proteins **Igα** and **Igβ** (CD79).

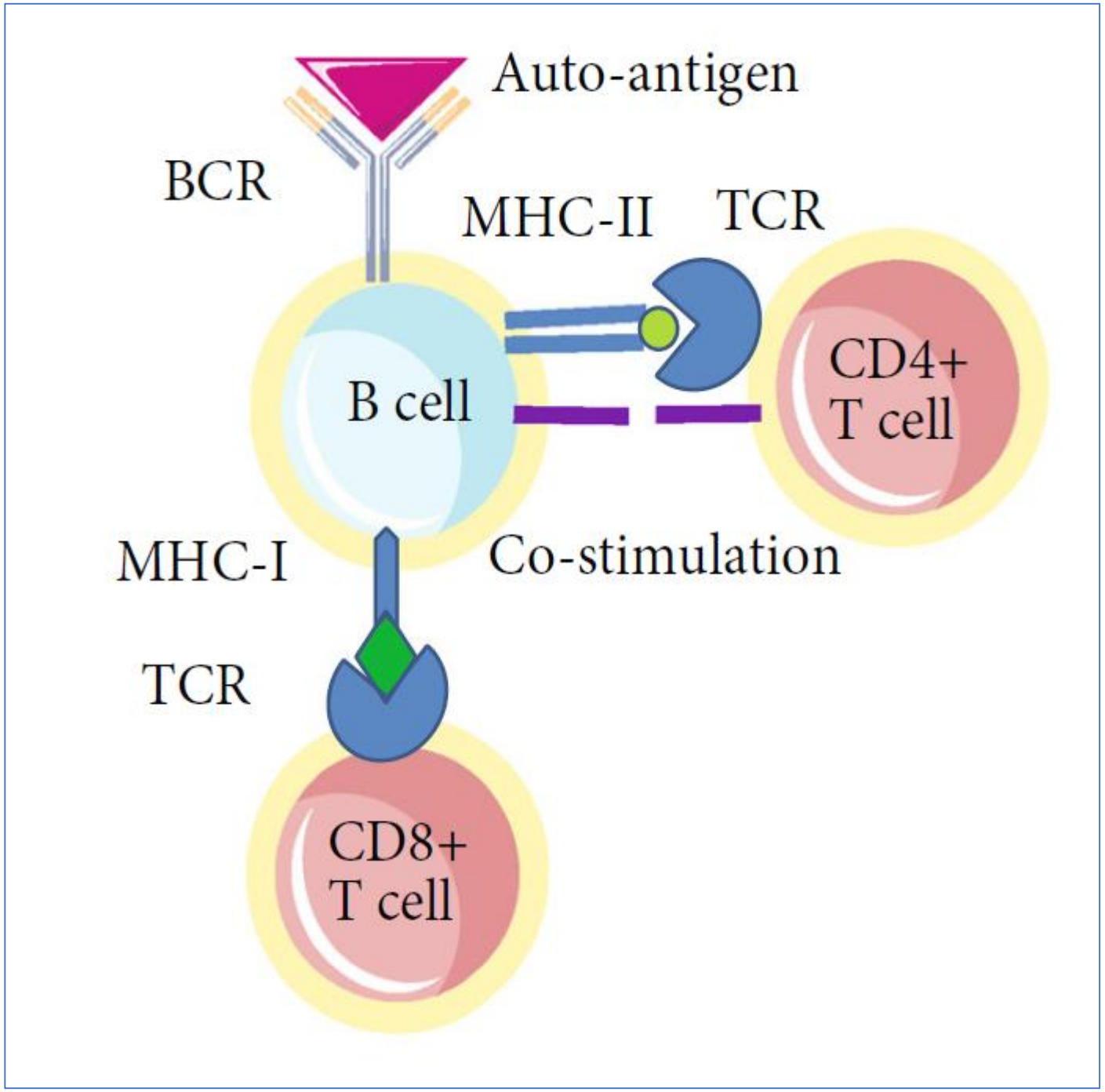
BCR interact with antigen molecules/epitopes from fluids as well as epitopes fixed on cell membrane (native macromolecules – proteins, lipids, carbohydrates, small chemical groups), and thus naive B Lymphocytes recognize the antigen.

In the human body there are 10^7 – 10^9 different clones of B lymphocytes (naïve B lymphocytes), every clone with unique BCR, suitable for different antigens.

BCR will recognize antigens (soluble and corpuscular) according to their configuration.







B lymphocyte is a professional antigen presenting cell.

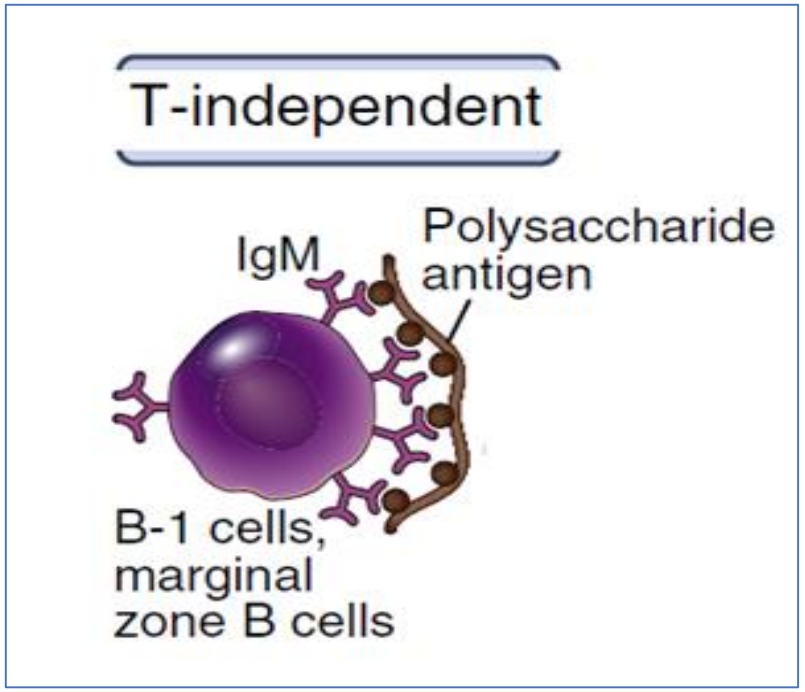
- **uses MHC II for CD4**
- **uses MHC I for CD8**

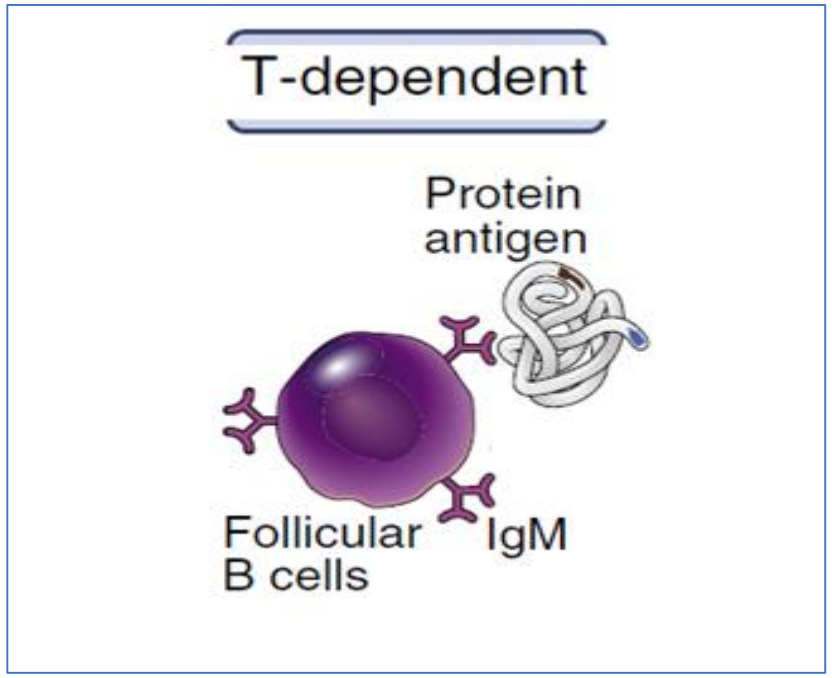
Fixation of protein antigen on B lymphocytes by Ig D and Ig M

Consequences:

- Expression of receptors for cytokines
- Expression of co-stimulatory molecules (CD80/CD86)
- Expression of MHC-II molecules
- Expression of adhesion molecules (favour interaction of B lymphocytes with T lymphocytes)
- Internalization of antigen, antigen processing and presentation of peptides in association with MHC-II)

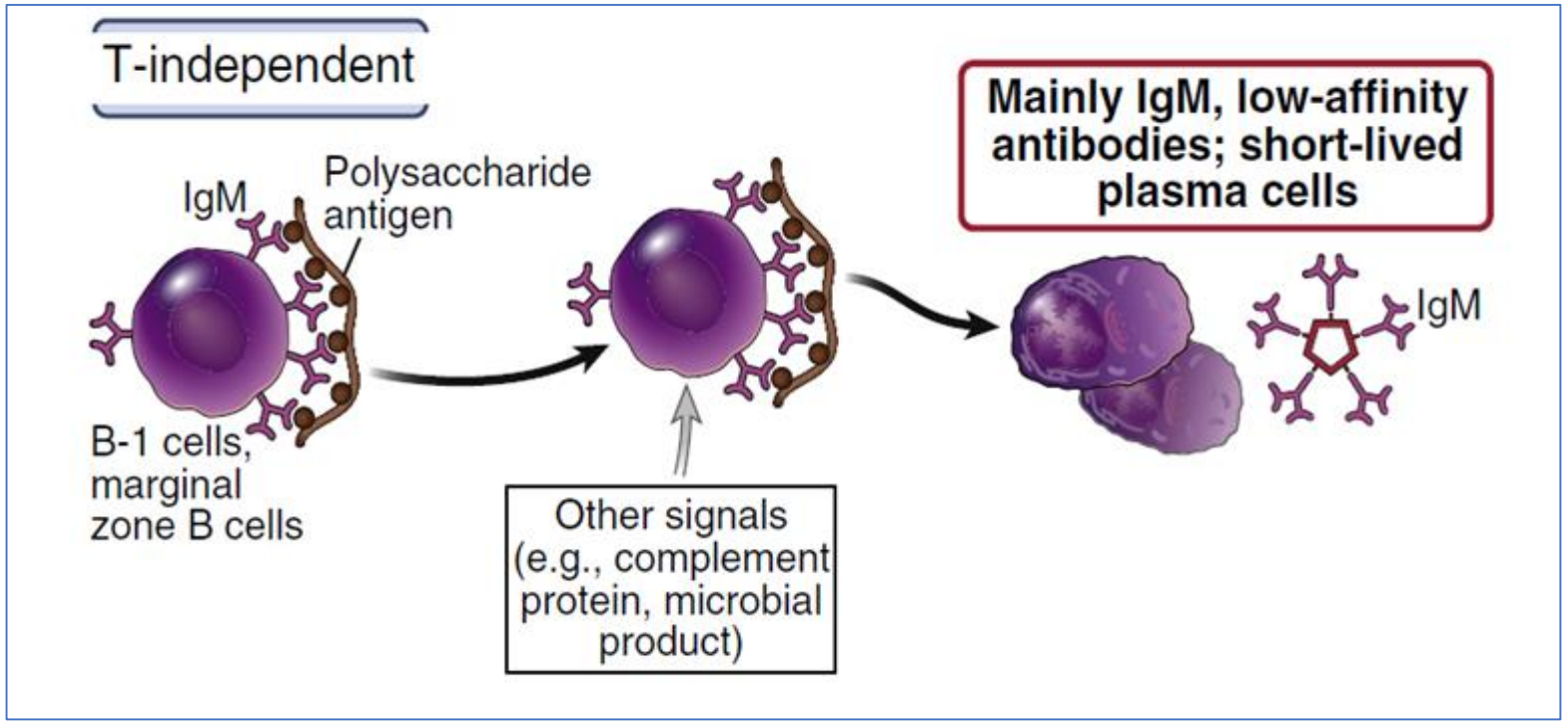
3. Stage of humoral immune response. Activation stage.





- **Different subsets of B cells respond preferentially to protein and nonprotein antigens.**
- **Protein antigens activate B lymphocytes via T – dependent mechanism**
- **Polysaccharides, lipids, and other nonprotein antigens activate B lymphocytes via T – independent mechanism**

3. Stage of humoral immune response. Activation stage.



- **Polysaccharides, lipids, and other nonprotein antigens stimulate antibody production without the involvement of helper T cells, direct via BCR.**
- **Therefore, these nonprotein antigens and the antibody responses to them are called T-independent.**
- **In this case – are not produced memory B cells**

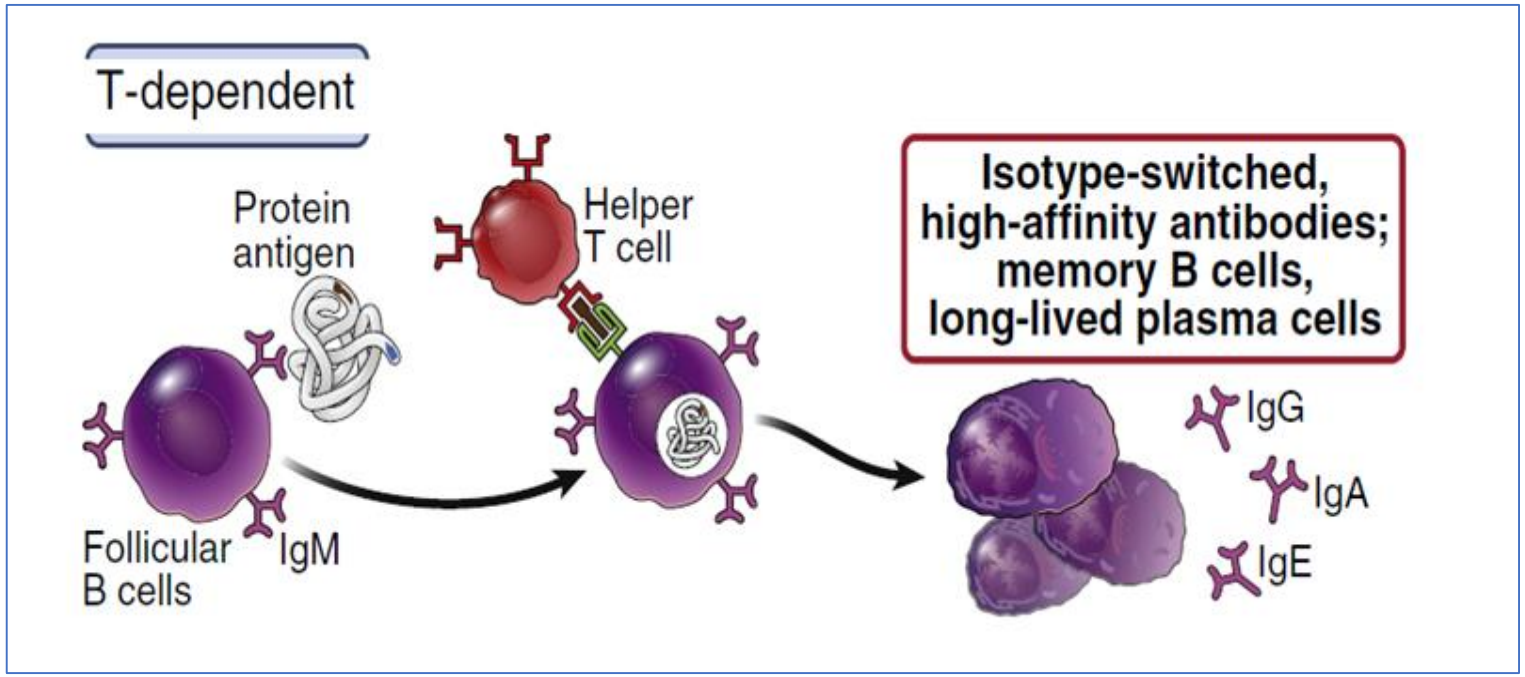
Activation of B lymphocytes by a T-independent manner

After activation of BL via T-independent mechanism (Ag non-protein) follows the proliferation of BL into a clone of identical cells (clonal expansion) and differentiation into plasma cells, which will synthesize and secrete Ab with low affinity from class (isotype) Ig M, being identical to BCR.

B-memory lymphocytes are not produced.

One plasma cell can secrete 100 - 2000 antibodies per second.

3. Stage of humoral immune response. Activation stage.



- **Protein antigens are processed and presented by antigen-presenting cells (APCs) inclusive B Cell via Ig G and Ig M, and are recognized by helper T lymphocytes, which activate B follicular cells, that reside in and circulate through the follicles of lymphoid organs, which induce heavy-chain isotype switching and affinity maturation.**
- **In the absence of Th cells, protein antigens elicit weak or no antibody responses.**

Important rules to remember

- **If B lymphocyte was activated by a thymus dependent antigen (TD-Ag) as protein, then the proliferation will be activated after interaction of B lymphocyte with Th-lymphocytes, which was activated by the same TD-antigen.**
- **T and B lymphocyte have the same antigen specificity, but:**

- **B lymphocytes mainly recognize native epitopes (*conformational epitopes*),**
- **Th lymphocytes recognize mainly the peptide fragments of the antigen, presented by APC - MHC II.**

Important rules to remember

- **Naïve B lymphocytes will meet the TD-Ag in the secondary lymphoid tissues (B zones), such mimicking an APC and performing next eminent stages:**

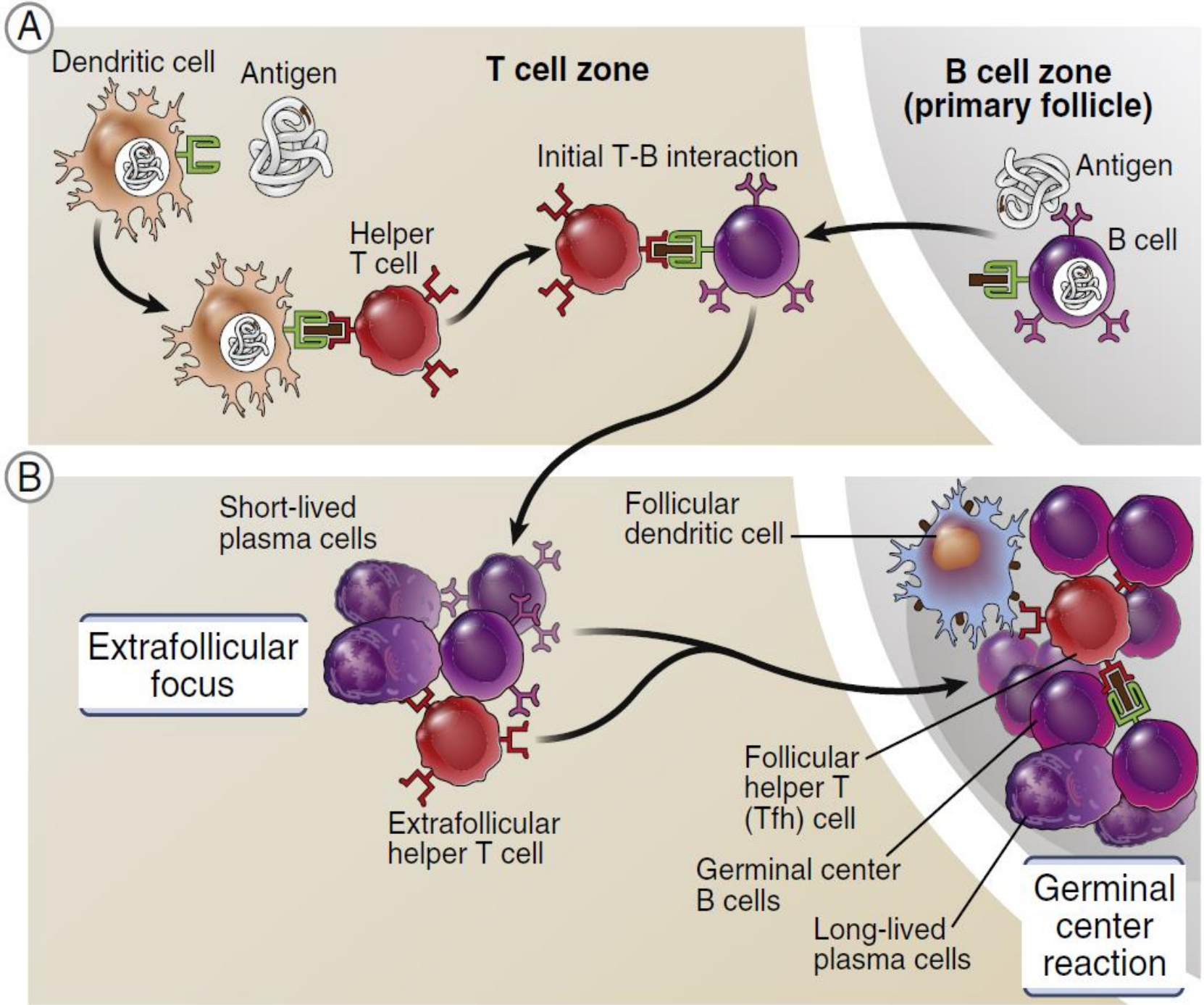
- up-take of antigen,

- procesing of antigen,

- Prezentation of the complex Ag-MHC-II on the membrane for next contact with lymphocytes Th-CD4.

Important rules to remember

- **Lymphocytes Th-CD4 migrate to lymphoid follicles where they meet B lymphocytes, which were activated by the same antigen.**
- **The complex TCR/CD4 on the surface of lymphocytes Th CD4 will recognize the complex Ag/MHC II on the surface of activated B lymphocytes.**
- **Next, another co-stimulatory molecules will be involved, mainly co-stimulation trigger by B7/CD28 and CD40R/CD40L.**
- **Activated Th lymphocytes will start to secrete cytokines, which will act on B lymphocytes, previously activated by antigen (secondary activator signal).**

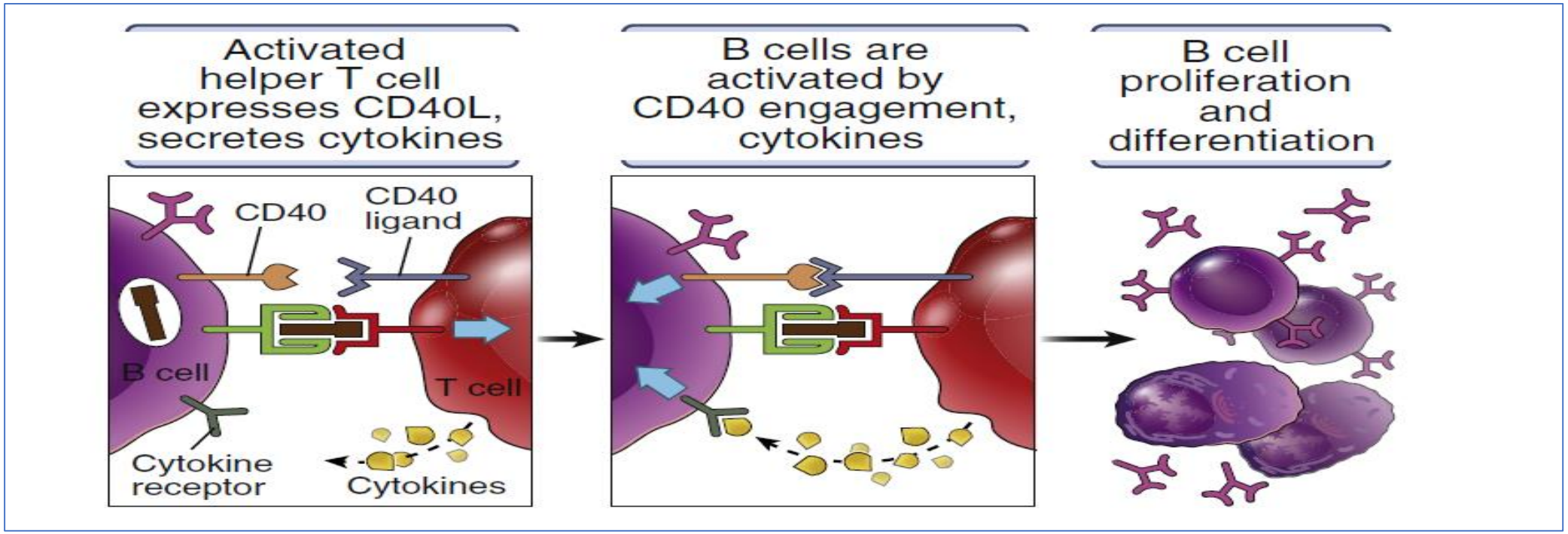


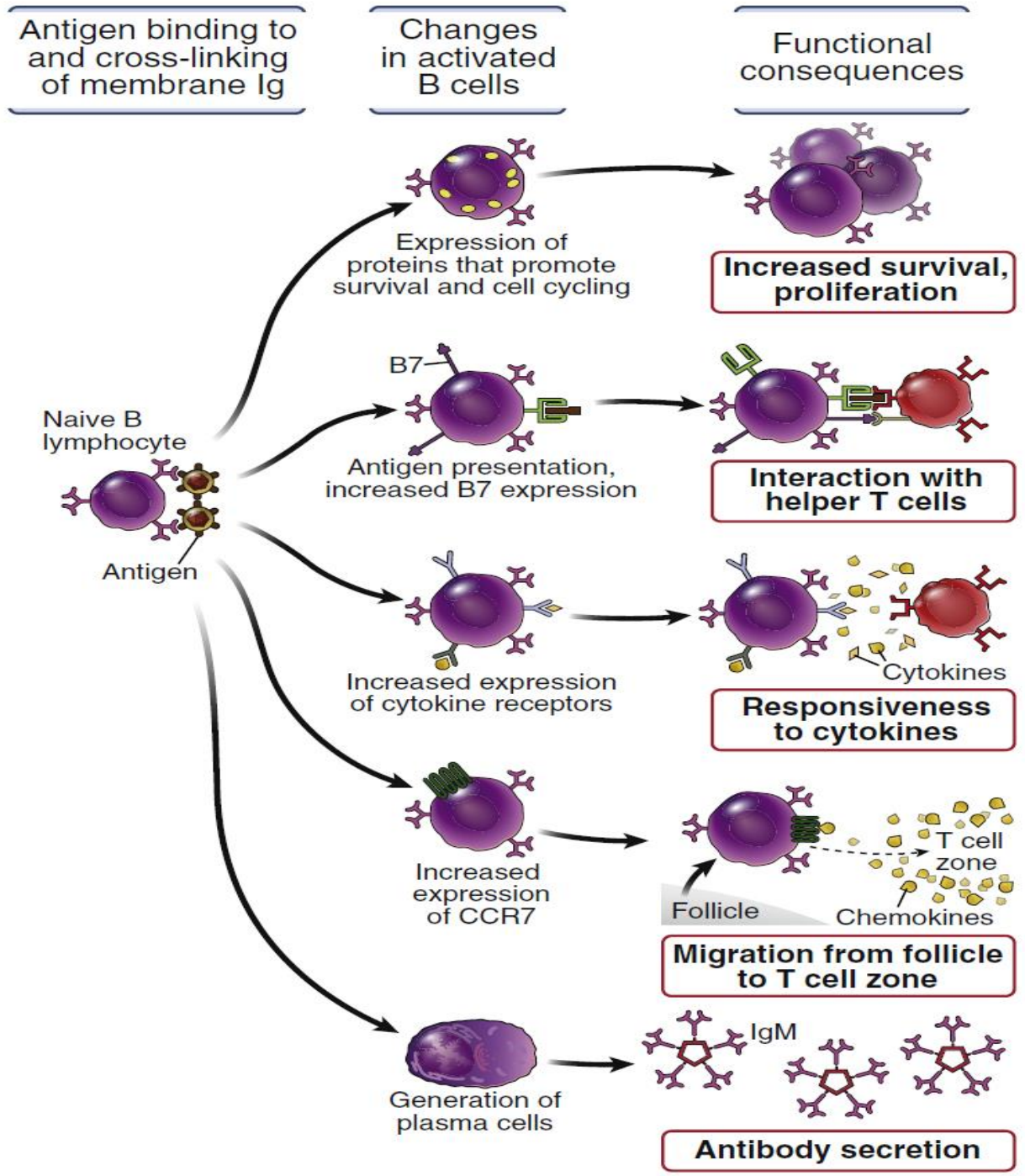
A. T and B lymphocytes independently recognize the antigen in different regions of peripheral lymphoid organs and are activated. The activated cells migrate toward one another and interact at the edges of lymphoid follicles.

B. Some of the activated B and T cells migrate back into the follicle to form the germinal center, where they are differenciated into plasma cells to produce antibodies.

Activation of the B lymphocytes by Ag-T-dependent proteins is performed via several stages:

1. For complete B lymphocyte activation there is need for co-stimulatory signal, derived from T helper Lymphocytes.
2. This activation will involve several co-stimulatory systems (CD21/CR2 for C3d fraction fixation, CD40R-CD40L, IL-4).





Consequences of B lymphocyte activation

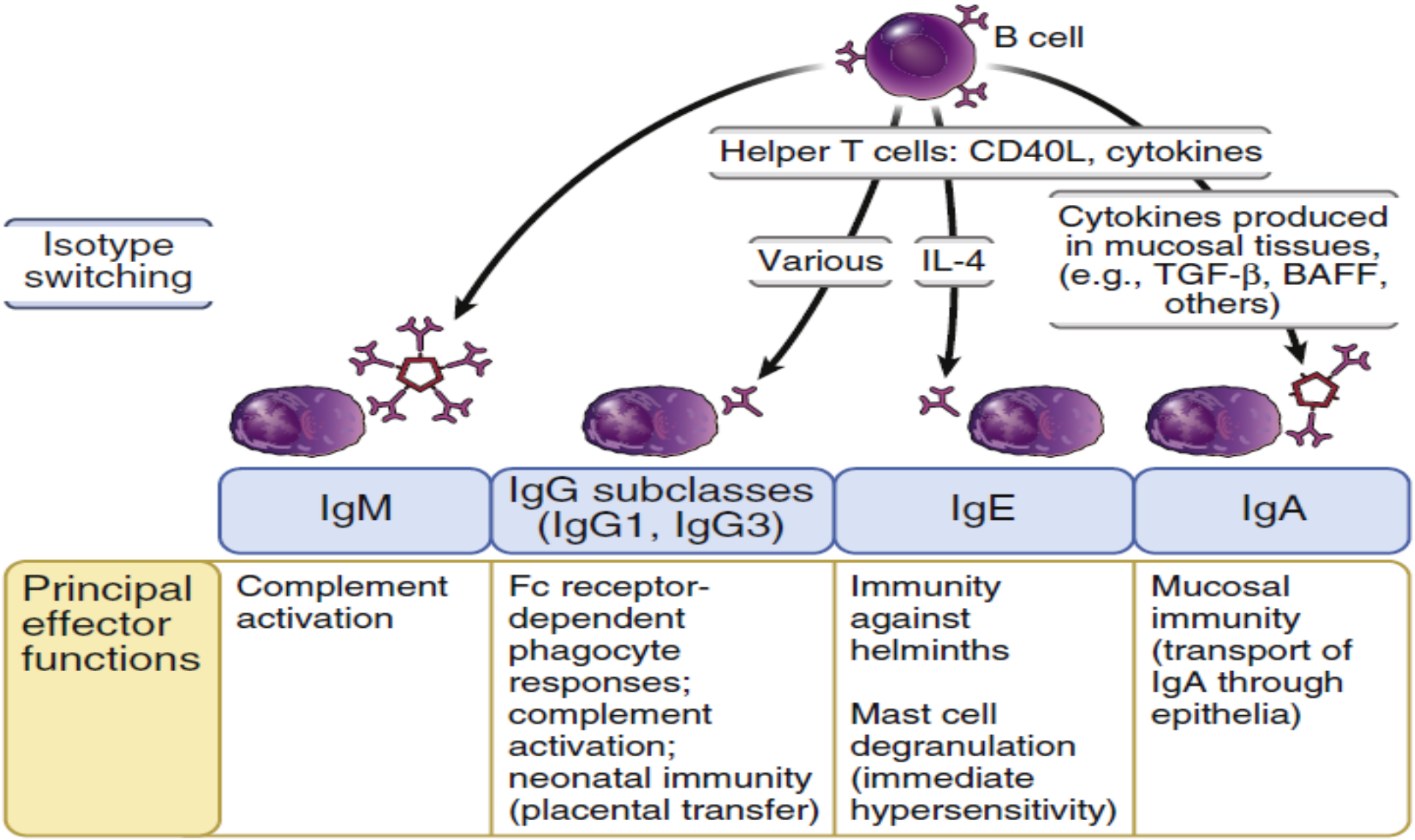
1. **Clonal expansion (proliferation) of B lymphocytes**
2. **Differentiation in plasma cells**
3. **Secretion of Ig**
4. **B cells prepare them to interact with helper T lymphocytes if the antigen is a protein.**

Some B lymphocytes return to lymphoid follicles and under the influence of cytokines produced by Th lymphocytes will differentiate in plasma cells which will secrete Ig from other classes (*heavy chain isotypes).*

- **IFNγ → IgG1, IgG3**
- **IL-4 → IgG4, IgE**
- **TGF β → IgA**

- **In this case there is development of immunological memory.**

Immunoglobulin heavy-chain isotype (class) switching



BAFF is a B cell–activating cytokine that may be involved in switching to IgA, especially in T-independent responses.

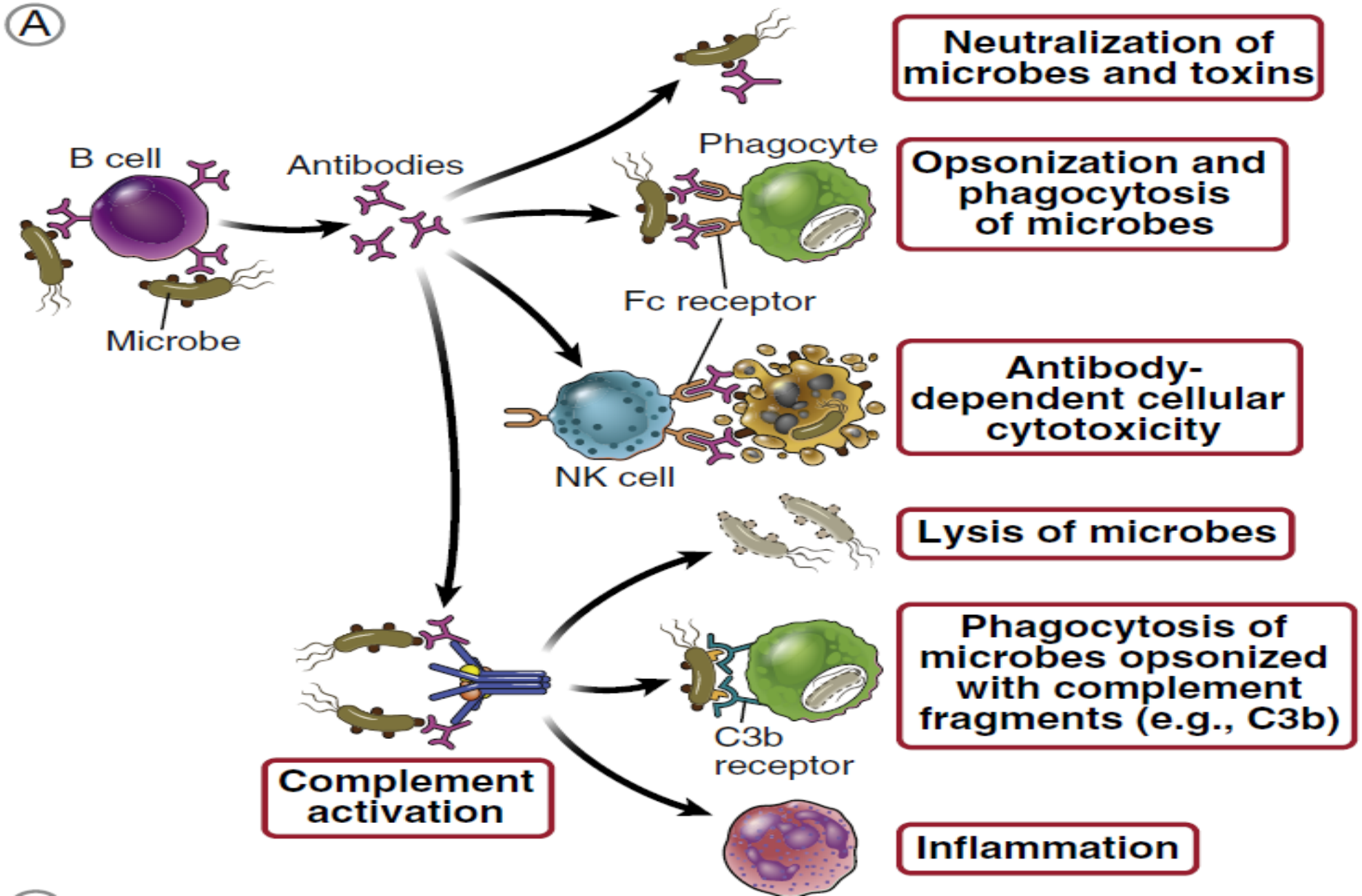
- **Plasma cell will remain to reside in lymphoid tissue, while antibodies leak into systemic circulation.**

- **Some plasma cells can migrate in the bone marrow and can secrete antibodies for long periods of time (months or even years), even if the antigen was removed from the body.**

- **In clinical cases when infection is localized at the level of mucosal layer, plasma cells will resides in the *lamina propria* of the skin, where they will continue to secrete antibodies (mainly IgA).**

- **A part of B lymphocyte will convert to memory cells.**
- ***Memory B lymphocytes* have no abilities to secrete antibodies. They circulate through the blood and reside for months or years, being ready to react to repeated exposure to the same antigen.**

Important events during interaction Ag-Ab



Important events during interaction Ag-Ab

1. Agglutination of bacteria (IgM, IgG) for further phagocytosis.
2. Opsonization of bacteria and enhanced phagocytosis (IgG, IgA).
3. Activation of the complement by classical pathway and formation of C3b fraction, which represents an opsonin.
4. Stimulation of inflammatory reaction by production of C3a, C5a (IgM, IgG).

Important event during interaction Ag-Ab

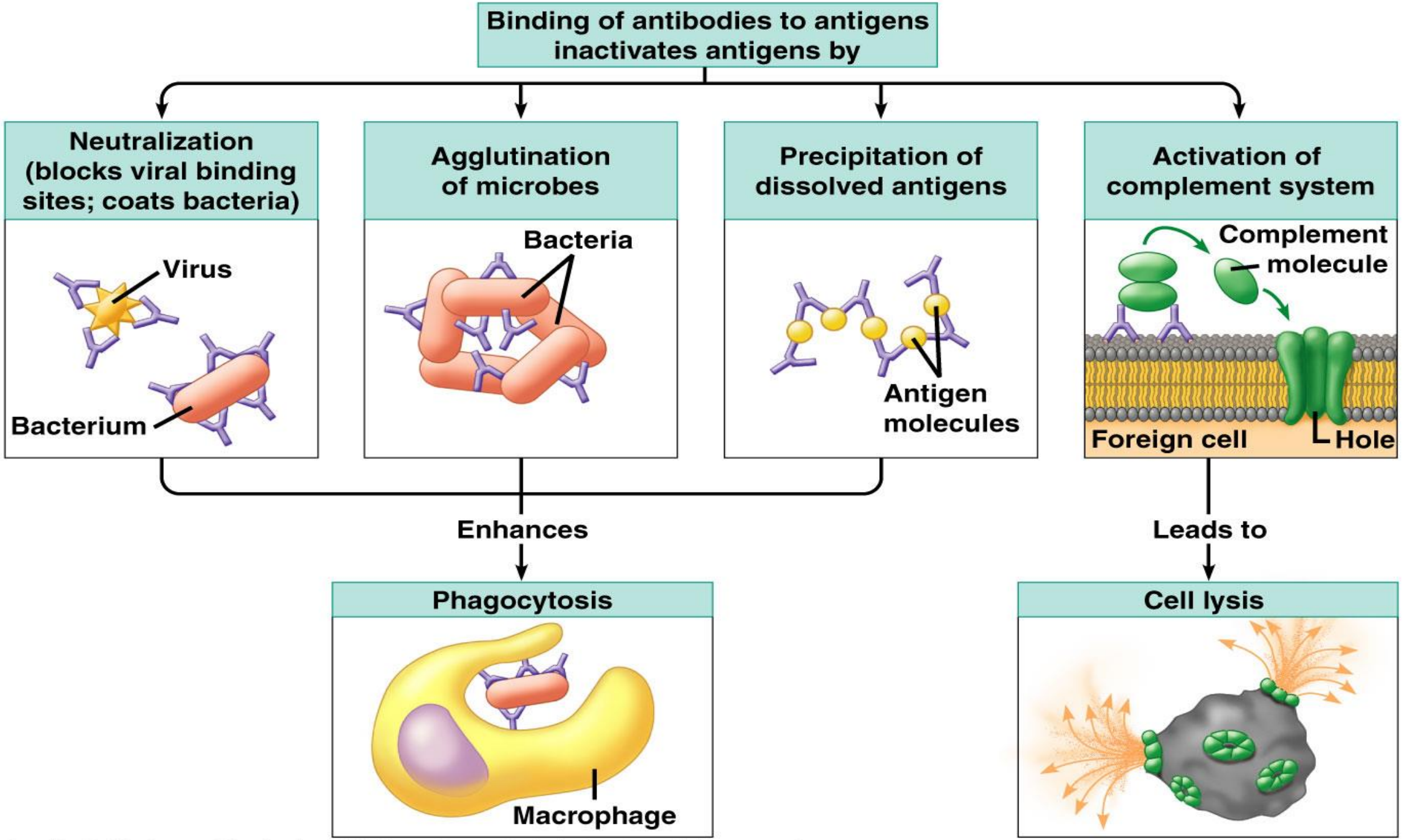
5. Neutralization – blockage of bacterial and viral adhesion to cell receptors, blockage of bacterial toxins and enzymes (IgM, IgG, IgA).

6. Cell mediated cytotoxicity or antibodies-mediated cytotoxicity.

7. IgG stimulate degranulation of NK lymphocytes, such leading to destruction of cells infected with viruses or tumoral cells.

8. IgE stimulate degranulation of eosinophils, leading to worm destruction.

9. Mast cells degranulation – by this way IgE trigger allergic reaction type I.



Primary humoral response (at the first contact with antigen)

Stage 1 **– *latent phase* – first 4 – 7th days till Ab production. During this time there is recognition, processing of antigen and differentiation of B lymphocytes.**

Stage 2 – *logarithmic phase* – concentration of Ab will enhance, till a maximum level at 10 – 15th day after antigen contact. Initially after 4-5 days- secretion of IgM. After, another group of antibodies as IgG will be secreted (under the influence of cytokines produced by Th lymphocytes).

Primary humoral response (at the first contact with antigen)

Stage 3 - there is maximal production of antibodies. Duration of this stage is different in function of antigen.

Stage 4 – there is decline of the antibodies concentration. When the antigen has a protein structure this stage can last several weeks, when the antigen has a polysaccharide structure – several months, viral antigen – several years.

Primary immune response trigger development of memory B lymphocytes.

Secondary humoral response (to repeated contact with the same Ag).

It is provided by memory B cells: they reside mainly in the spleen, where they occupy a rate of about 45% of the entire population of B lymphocytes (CD27) in this organ

- Short latency period (hours).
- Rapid rise of the Ab concentration to the action of a low-level Ag.
- Increased affinity of Ab to Ag.
- Maximum concentration of Ab are maintained for longer duration.
- Much faster production of antibodies (ex, Ig G) triggered by memory B cells - derived plasmocytes.

Effector functions of antibodies.

(B)

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

How long do memory BL live?

♦ The rest of the life of the host organism.

♦ They are renewed upon repeated contact with Ag under the action of IL-2, IL-15.

♦ On secondary contact with Ag, memory B lymphocytes behave as antigen-presenting cells:

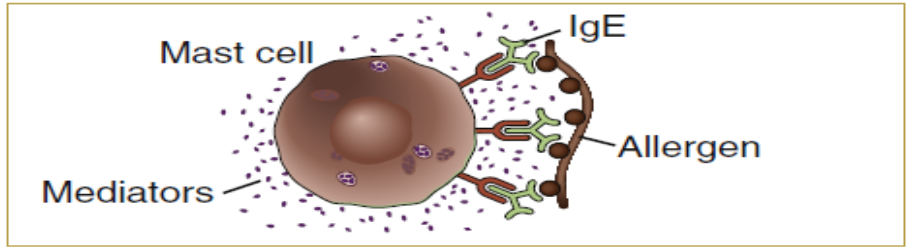
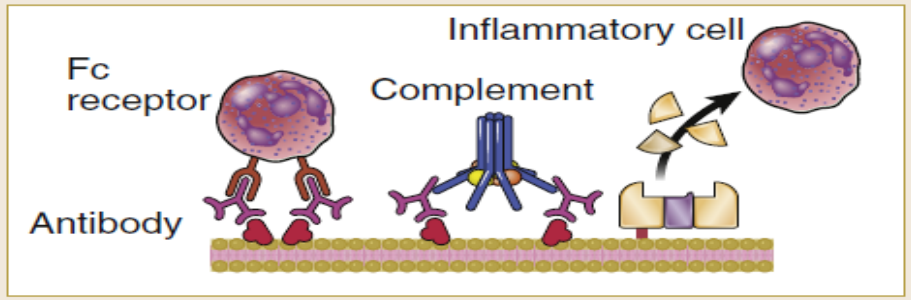
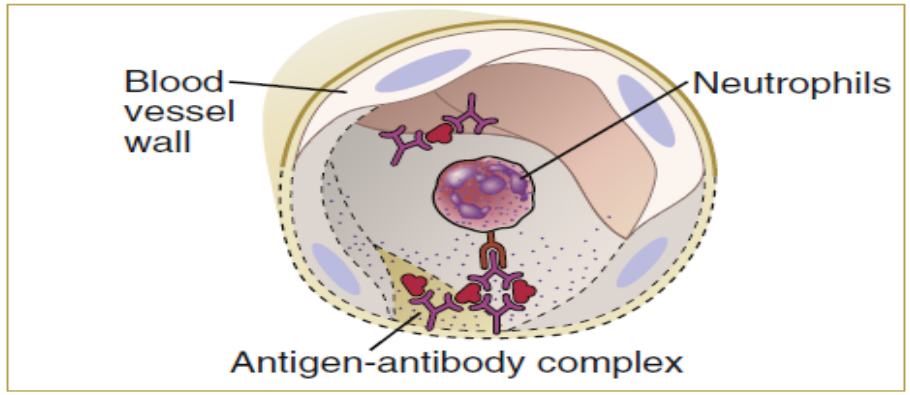
— they also incorporate and process Ag in the MHC II funnel;

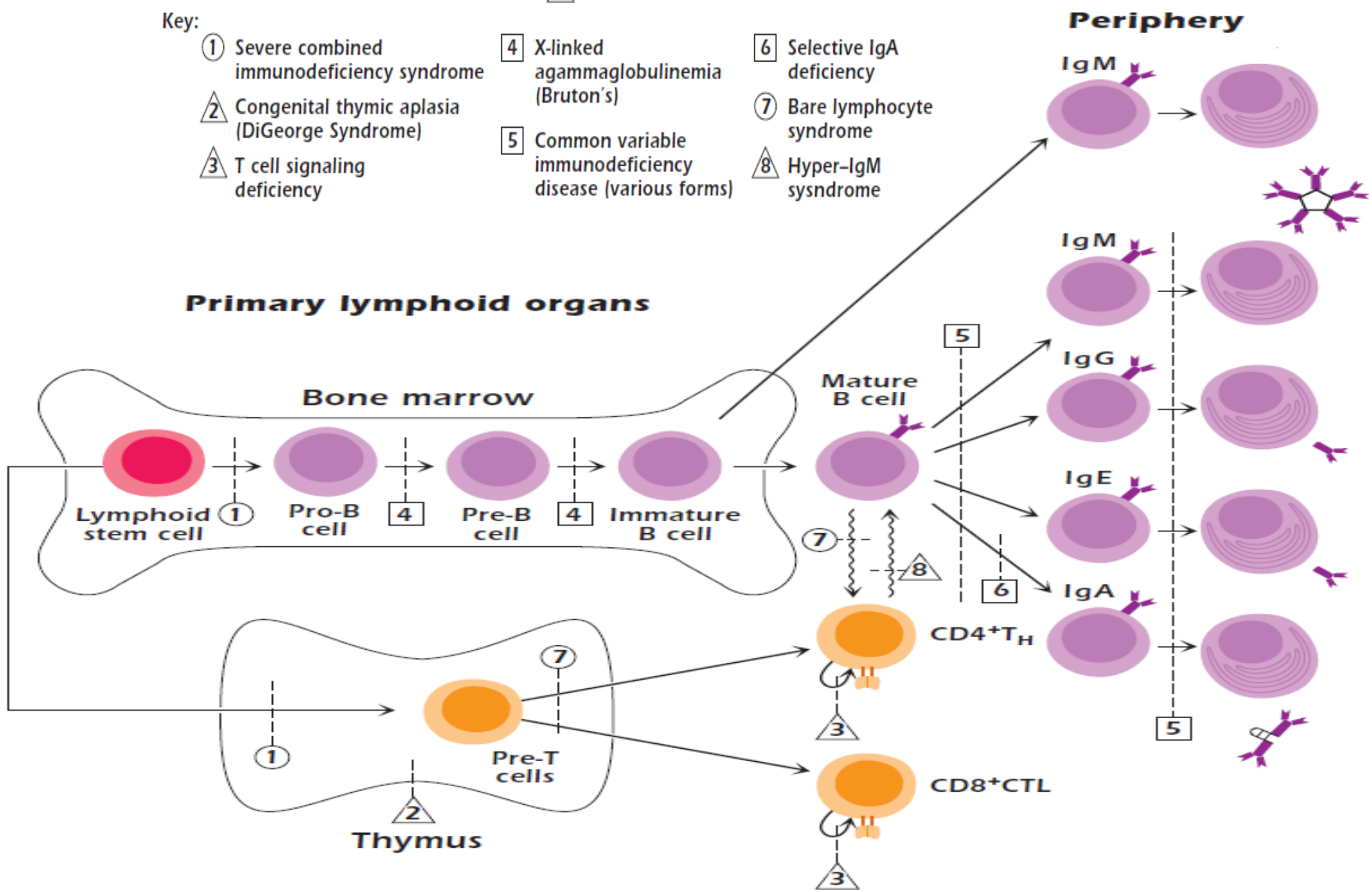
— presents it to naïve T lymphocytes, and under the action of cytokines released by TL will differentiate into Ab-producing plasmocytes.

Disorders related with B cell dusfunction

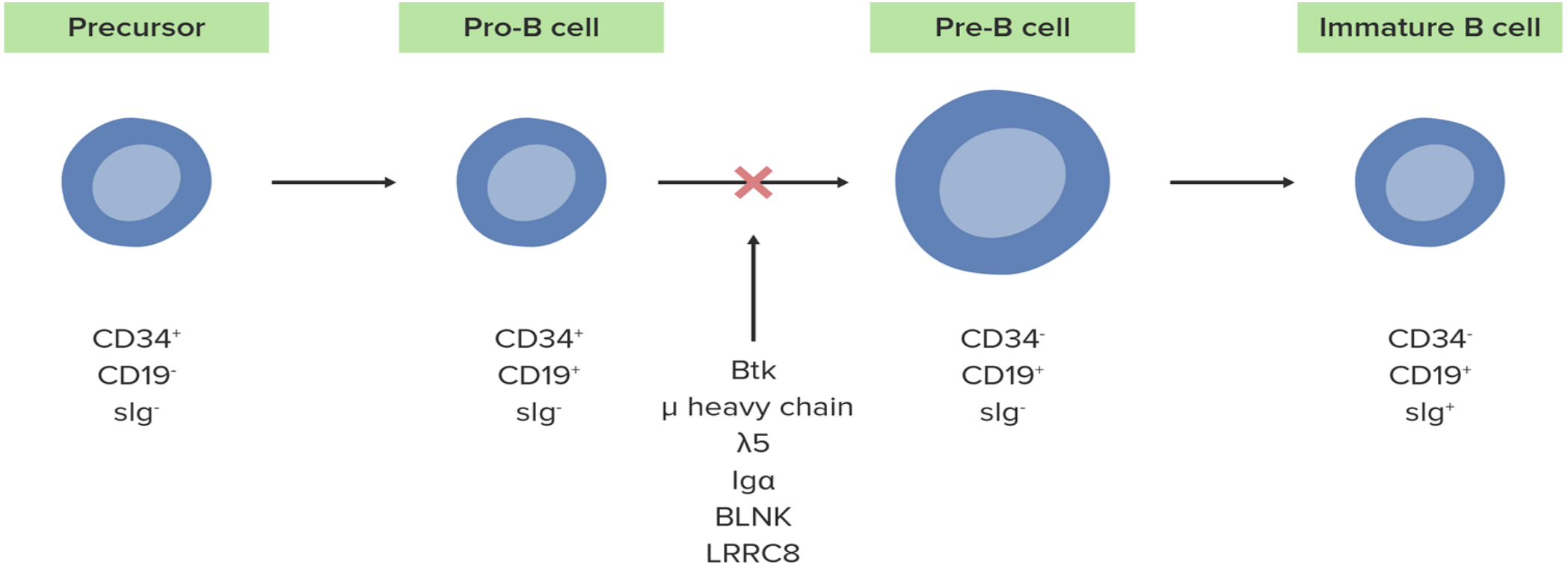
- **Hypersensitivity reactions responses to foreign antigens (microbes and non-infectious environmental antigens).**
- **autoimmune diseases - responses against self antigens**

- **X-linked agammaglobulinemia (Bruton's agammaglobulinemia)**
- **Common variable immunodeficiency (CVID)**
- **X linked hypogammaglobulinemia**
- **Transient hypogammaglobulinemia of infancy**

Type of hypersensitivity	Pathologic immune mechanisms	Mechanisms of tissue injury and disease
Immediate hypersensitivity (Type I)	Th2 cells, IgE antibody, mast cells, eosinophils 	Mast cell-derived mediators (vasoactive amines, lipid mediators, cytokines) Cytokine-mediated inflammation (eosinophils, neutrophils, lymphocytes)
Antibody-mediated (Type II)	IgM, IgG antibodies against cell surface or extracellular matrix antigens 	Complement- and Fc receptor-mediated recruitment and activation of leukocytes (neutrophils, macrophages) Opsonization and phagocytosis of cells Abnormalities in cellular function (e.g., hormone or neurotransmitter receptor signaling)
Immune complex-mediated (Type III)	Immune complexes of circulating antigens and IgM or IgG antibodies deposited in vascular basement membrane 	Complement- and Fc receptor-mediated recruitment and activation of leukocytes, and tissue damage secondary to impaired blood flow

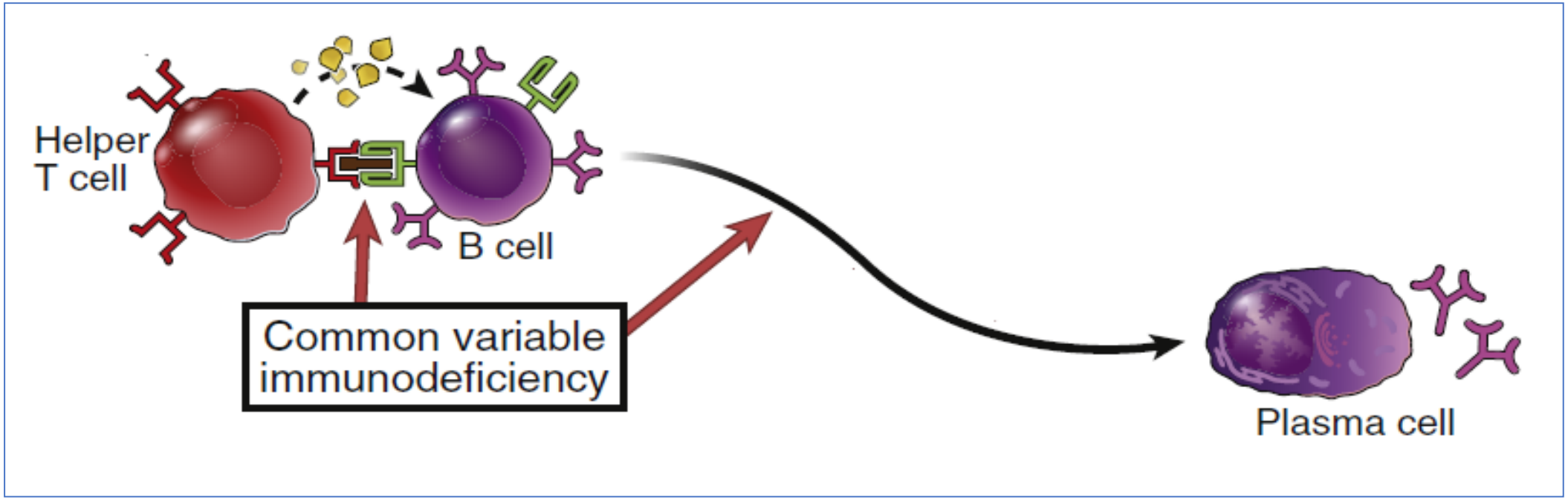

Key:
1 Severe combined immunodeficiency syndrome
2 Congenital thymic aplasia (DiGeorge Syndrome)
3 T cell signaling deficiency
4 X-linked agammaglobulinemia (Bruton's)
5 Common variable immunodeficiency disease (various forms)
6 Selective IgA deficiency
7 Bare lymphocyte syndrome
8 Hyper-IgM sysndrome
Periphery
IgM
IgM
IgG
IgE
IgA
Primary lymphoid organs
Bone marrow
Lymphoid stem cell
Pro-B cell
Pre-B cell
Immature B cell
Mature B cell
Pre-T cells
Thymus
CD4+TH
CD8+CTL

X-linked agammaglobulinemia (Bruton's agammaglobulinemia)



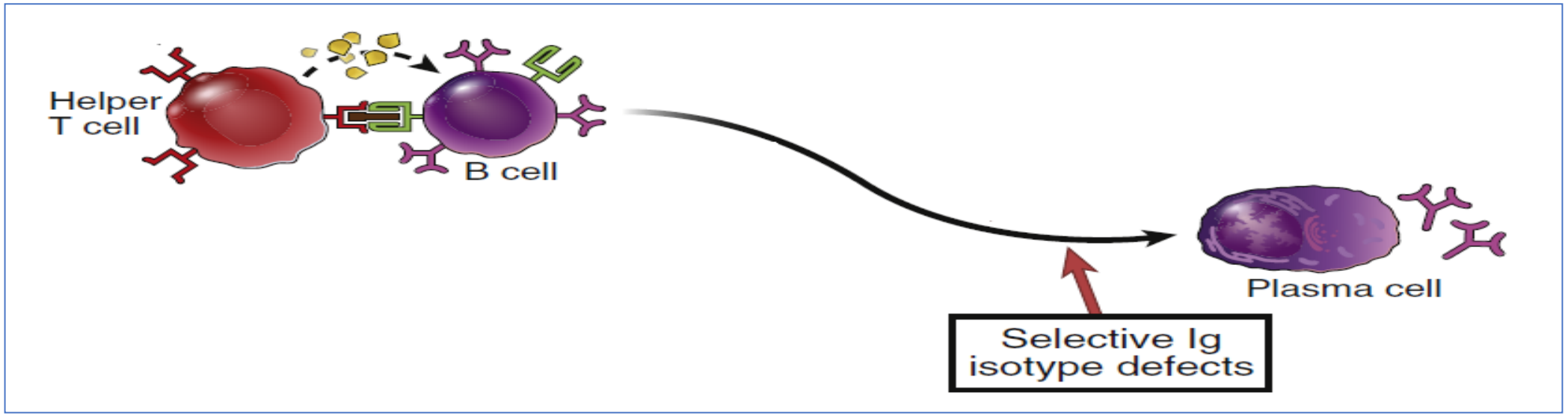
- **Affects male**
- **Genetic mutations of Btk (Bruton tyrosine kinase)**
- **Absence of mature B cells and plasma cells**
- **Failure of infection to respond to antibiotic therapy**

Common variable immunodeficiency (CVID)



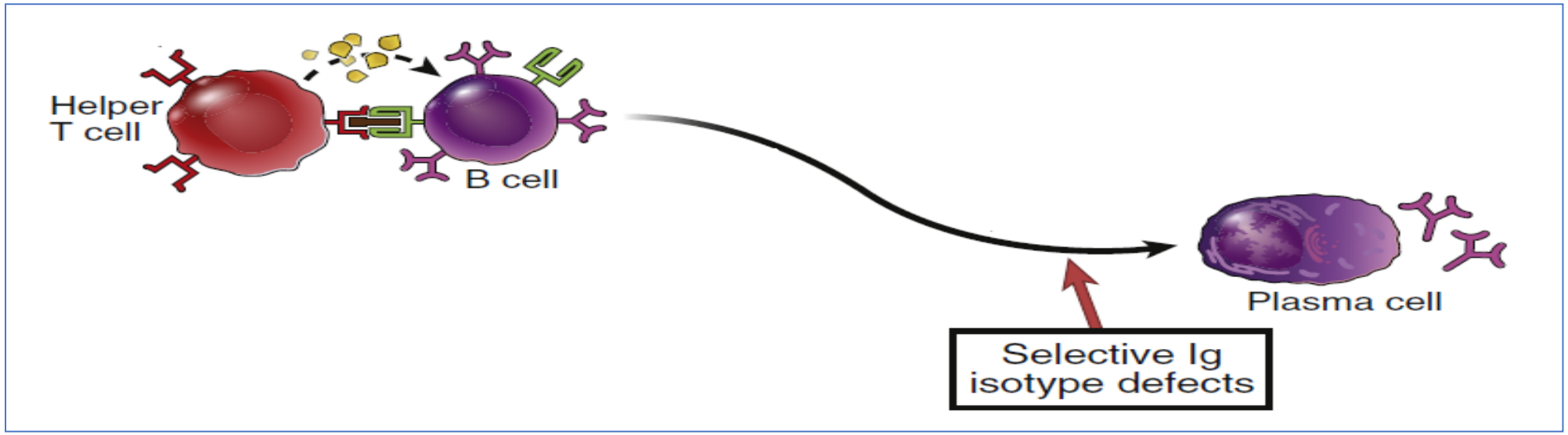
- **Problem in terminal differentiation of mature B cells into the plasma cells**
- **Markedly reduced Ig with normal number of B cells**
- **Between 15 and 35 years, both genders**
- **Patients have recurrent otitis, pulmonary infections**
- **Tendency to: chronic lung disease, autoimmune disorders, hepatitis, gastric carcinoma, etc**

X linked hypogammaglobulinemia
Deficiency of IgA



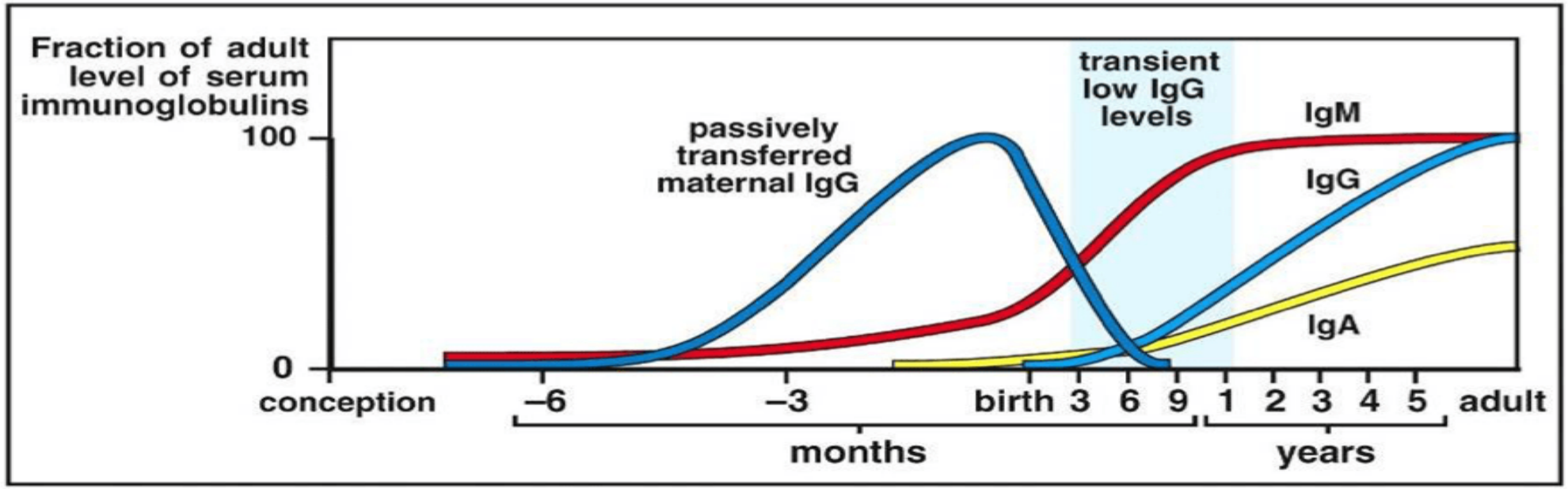
- ***Selective IgA deficiency*** **(1 in 400 up to 1in 1000)**
- **In male and female**
- **In person treated with phenytoin, sulfasalazine**
- **Mostly are asymptomatic, because the Ig G and Ig M are normal.**
- **Severe reduced level of IgA – repeated respiratory and GIT infection, allergic diseases, asthma, autoimmune disorders**

X linked hypogammaglobulinemia
Deficiency of IgG



- **Ig G1 (70%) and IgG2 (20%)**
- **Great risk for: sinusitis, otitis media, pneumonia caused by S . pneumonia, H. influenzae type b and N. meningitis**
- **IgG_1 and IgG_3 are Ab against protein antigens**
- **IgG_2 subclass are Ab against carbohydrate and polysaccharide antigens.**
- **Patients with low IgG_2 can be at greater risk for development of sinusitis, otitis media, and pneumonia caused by polysaccharide-encapsulated microorganisms such as S. pneumoniae, H. influenzae type b, and N. meningitidis.**

Transient hypogammaglobulinemia of infancy



- **delay in the maturation process of B cells that leads to a prolonged deficiency in IgG levels (IgM and IgA levels are normal) beyond 6 months of age.**
- **The total number and antigenic response of circulating B cells is normal, but the chemical communication between B and T cells that leads to clonal proliferation of antibody-producing plasma cells seems to be reduced.**
- **repeated infection of upper respiratory and middle ear infections.**
- **This condition usually resolves by the time the child is 2 to 4 years of age.**

Thank you for your attention

From left: B lymphocyte; Eosinophil; Platelet; Neutrophil; Macrophage; T lymphocyte; Monocyte; NK cell; Dendritic cell; Basophil; Mast cell