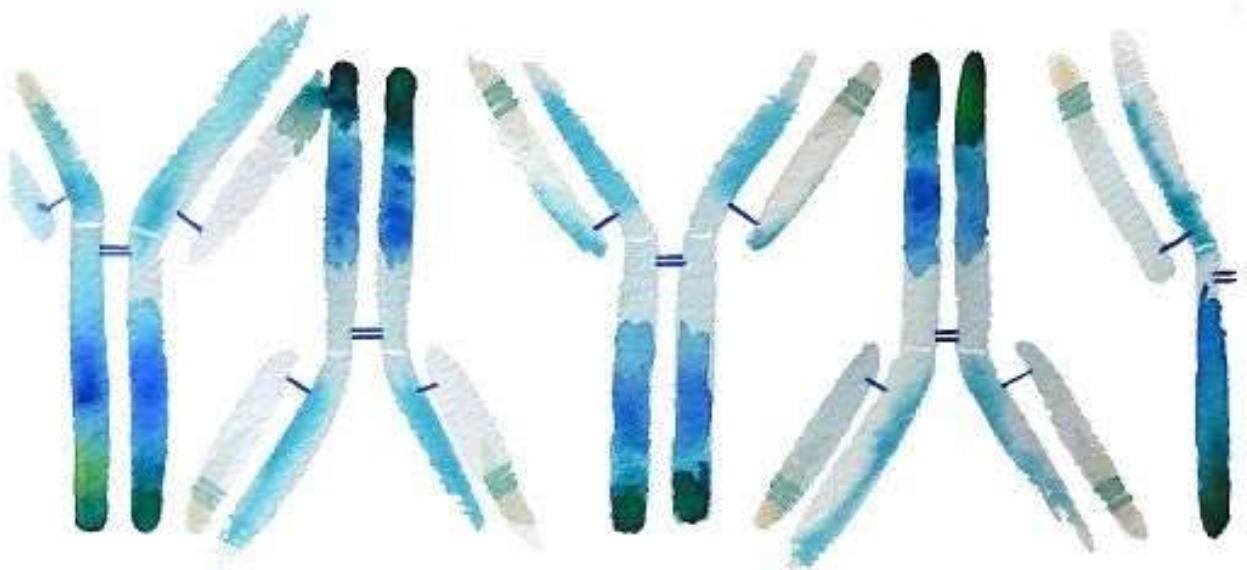
Medical Immunology



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Lecture 7

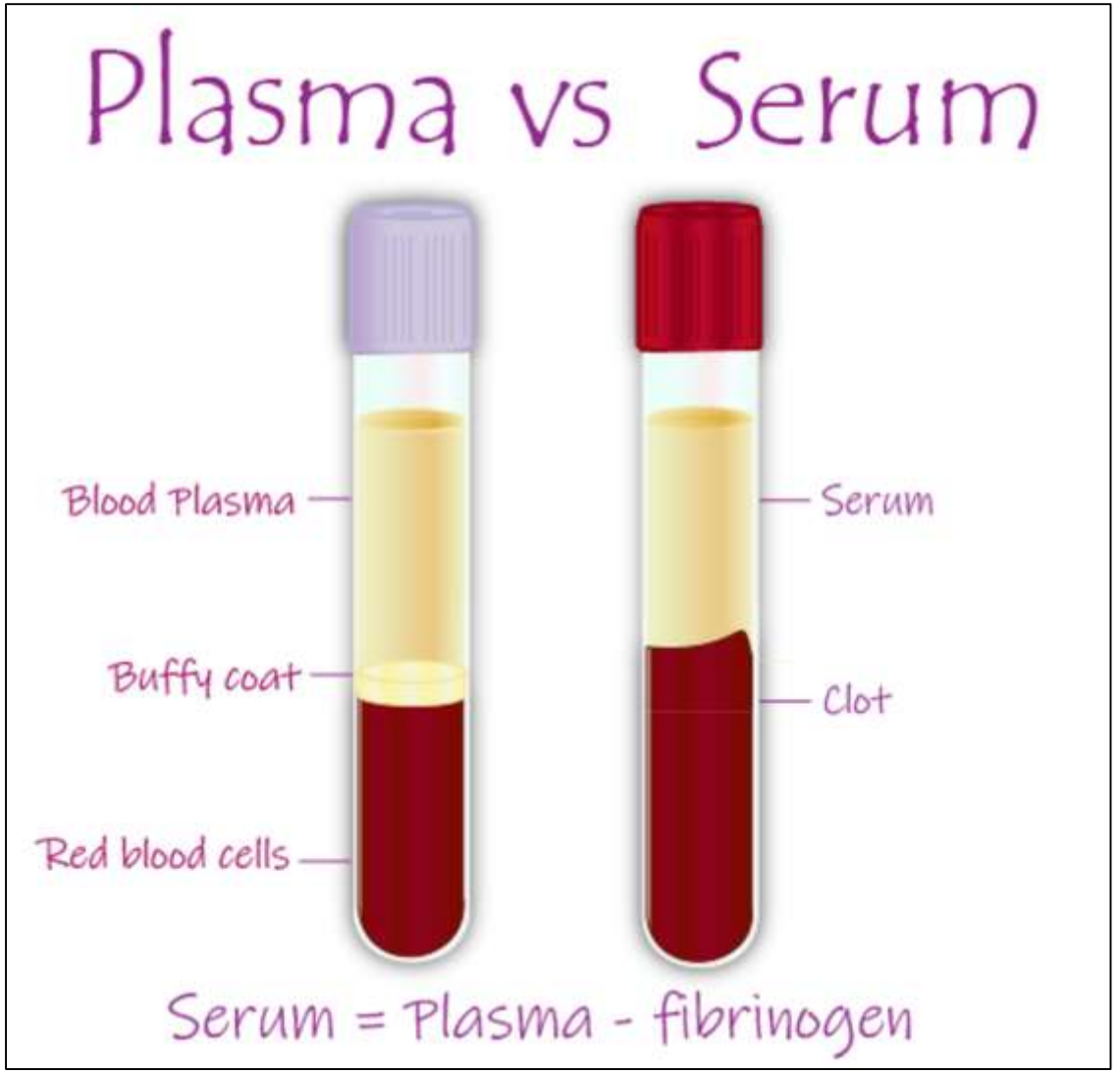
Antibodies and antigens

In this lecture we will discuss:

- Antibodies, their structure, types and function.

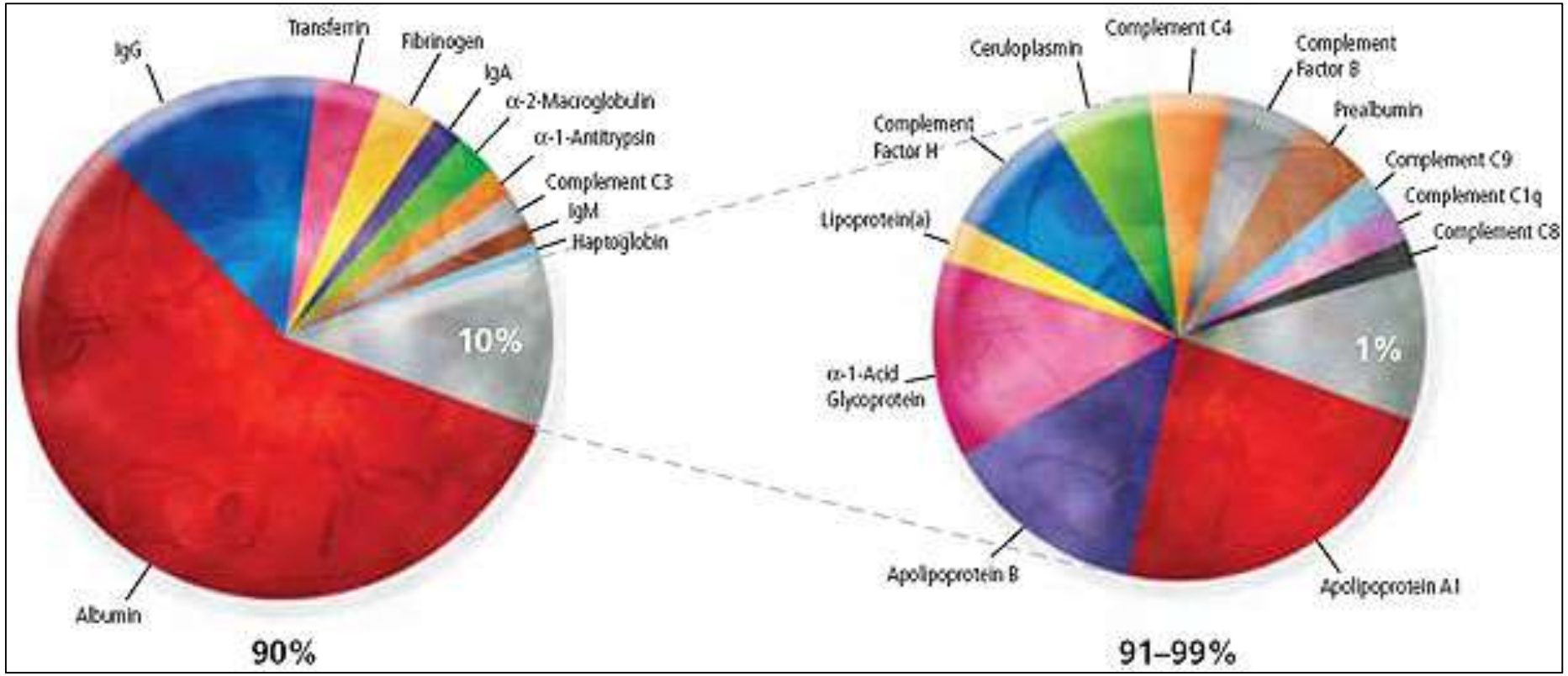
Serum/ overview

- The study of antibodies and their reactions with antigens is classically called **serology**.
- **Serum** lacks coagulation factors but otherwise contains all the proteins found in plasma. Any serum sample that contains detectable antibody molecules that bind to a particular antigen is commonly called an **antiserum**.
- A healthy 70-kg adult human produces about 2 to 3 g of antibodies every day.



Serum/ overview

- Plasma proteomics holds great promise for the future of biomarker discovery, as well as *in vitro* diagnostics. Although plasma is readily accessible for analysis, the study of the plasma proteome is fundamentally limited by its vast dynamic range (10 orders of magnitude).



Antibodies and antigens

- **Antibodies** are circulating proteins that are produced in vertebrates in response to exposure to foreign structures known as **antigens**

- The **strength of the binding** between a single site of an **antibody** and an **epitope** of an antigen is called the **affinity** of the antibody.

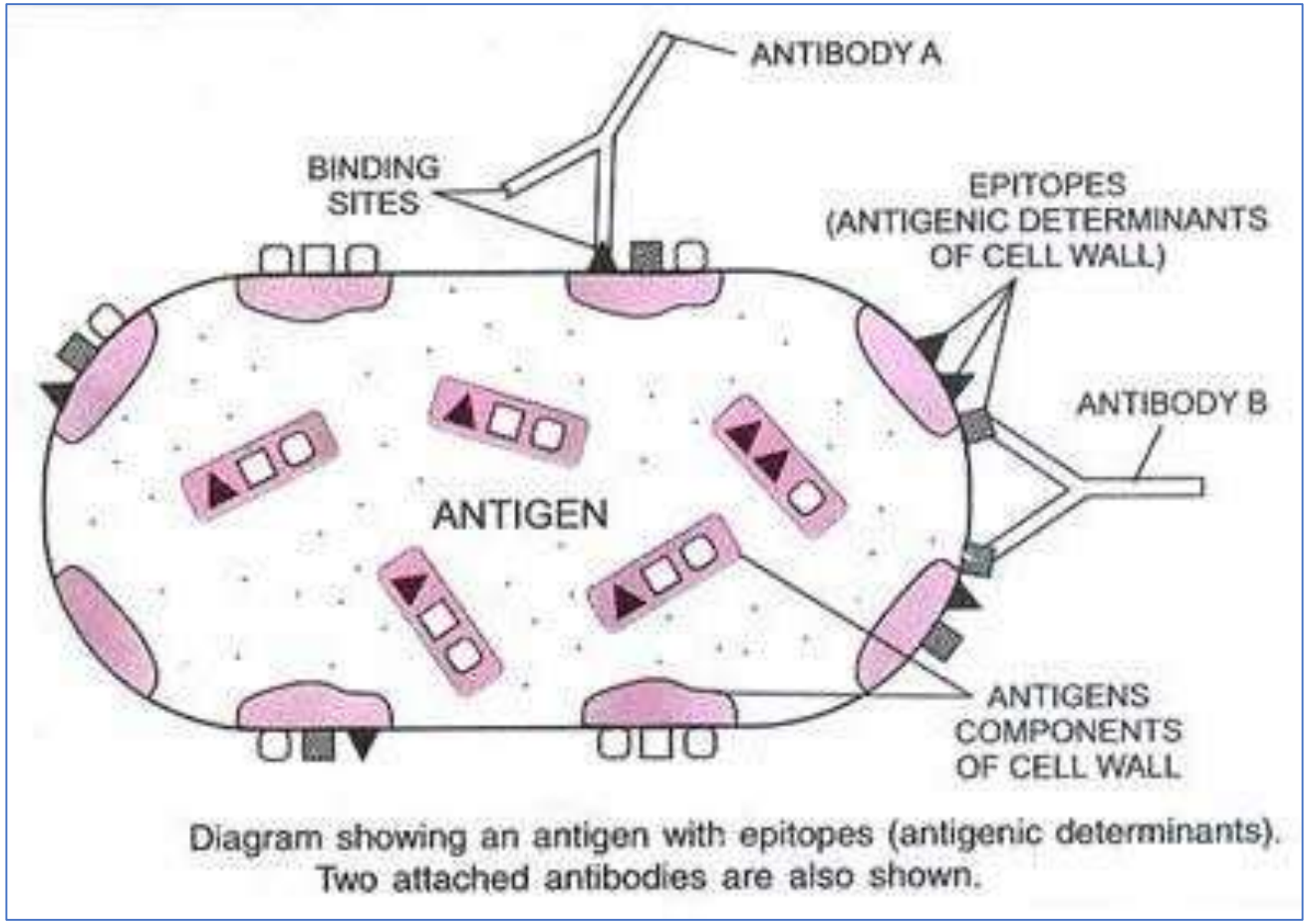


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.

Antibodies and antigens

- Because the **hinge** region of antibodies gives them **flexibility**, a **single antibody** may attach to a single multivalent antigen **by more than one binding site**. the strength of attachment of the antibody to the antigen must take into account binding of all the sites to all the available epitopes. This overall strength of attachment is called the **avidity.**

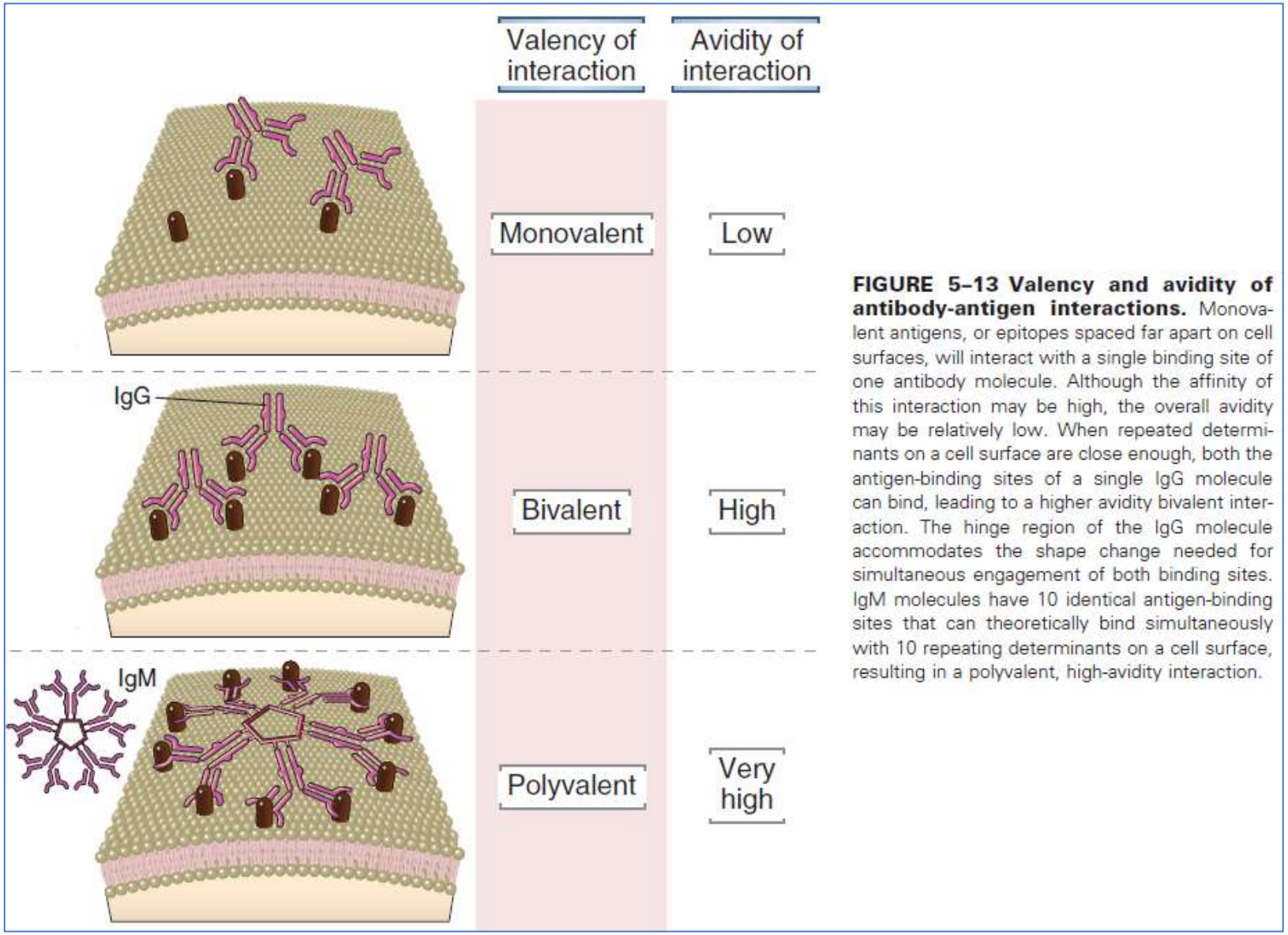
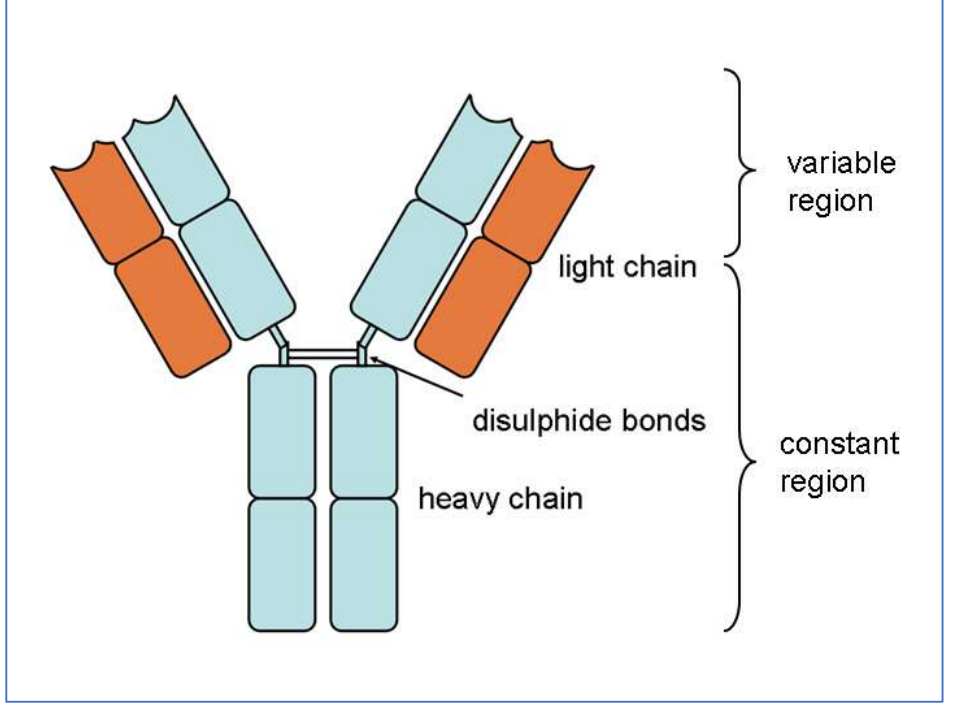


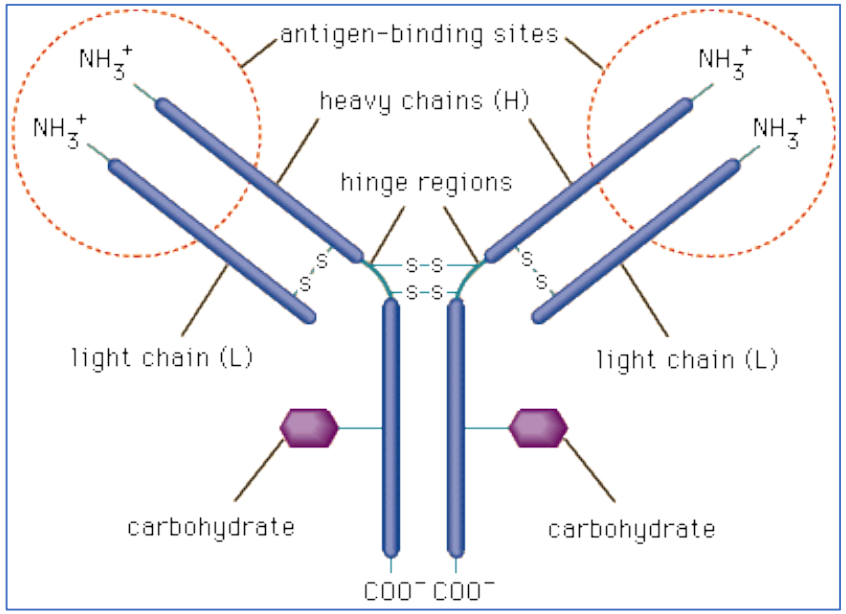
FIGURE 5–13 Valency and avidity of antibody-antigen interactions. Monovalent antigens, or epitopes spaced far apart on cell surfaces, will interact with a single binding site of one antibody molecule. Although the affinity of this interaction may be high, the overall avidity may be relatively low. When repeated determinants on a cell surface are close enough, both the antigen-binding sites of a single IgG molecule can bind, leading to a higher avidity bivalent interaction. The hinge region of the IgG molecule accommodates the shape change needed for simultaneous engagement of both binding sites. IgM molecules have 10 identical antigen-binding sites that can theoretically bind simultaneously with 10 repeating determinants on a cell surface, resulting in a polyvalent, high-avidity interaction.

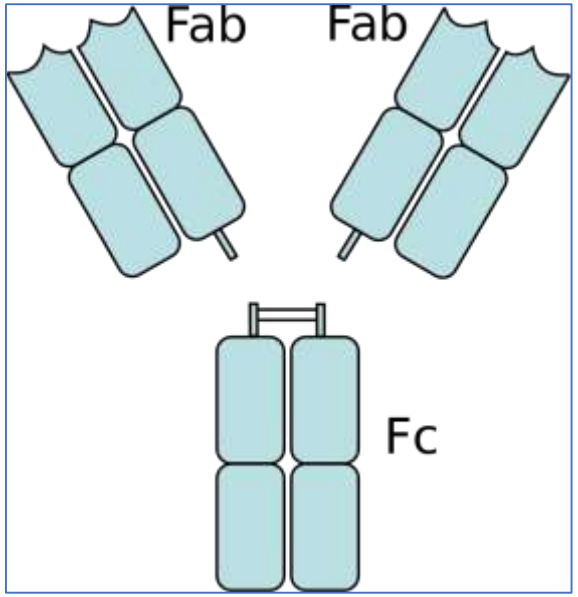
Antibodies/ structure

- Antibodies (immunoglobulins) are made of 2 heavy chains and 2 light chains, those chains combined give us an antibody binding region **(Fab)** and the fragment crystallizable region (**Fc region**) which is the tail region of an antibody that interacts with **cell surface receptors** called **Fc receptors** and some proteins of the **complement** system.

- Immunoglobulins are divided into different classes (isotypes).



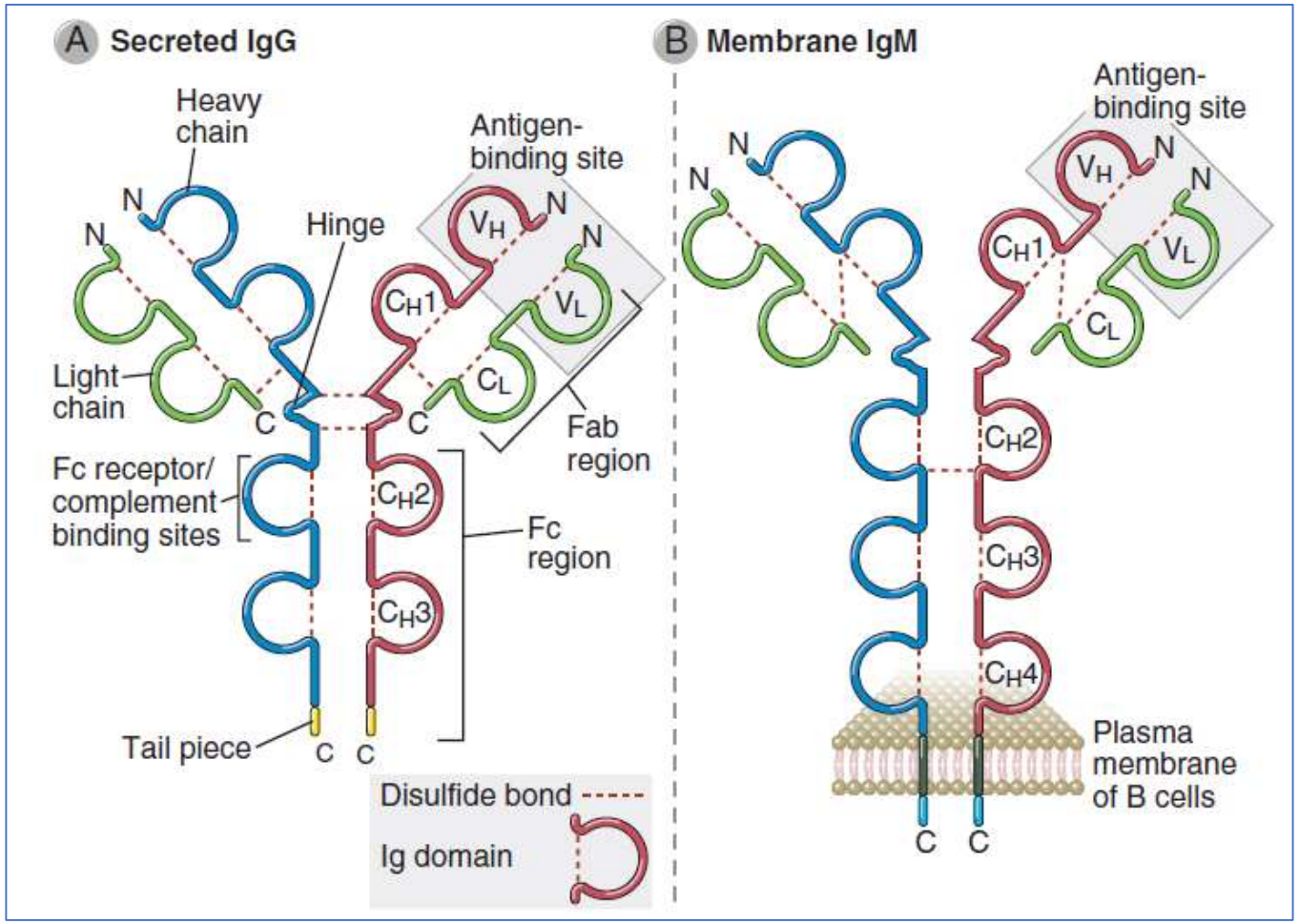




Antibodies/ structure

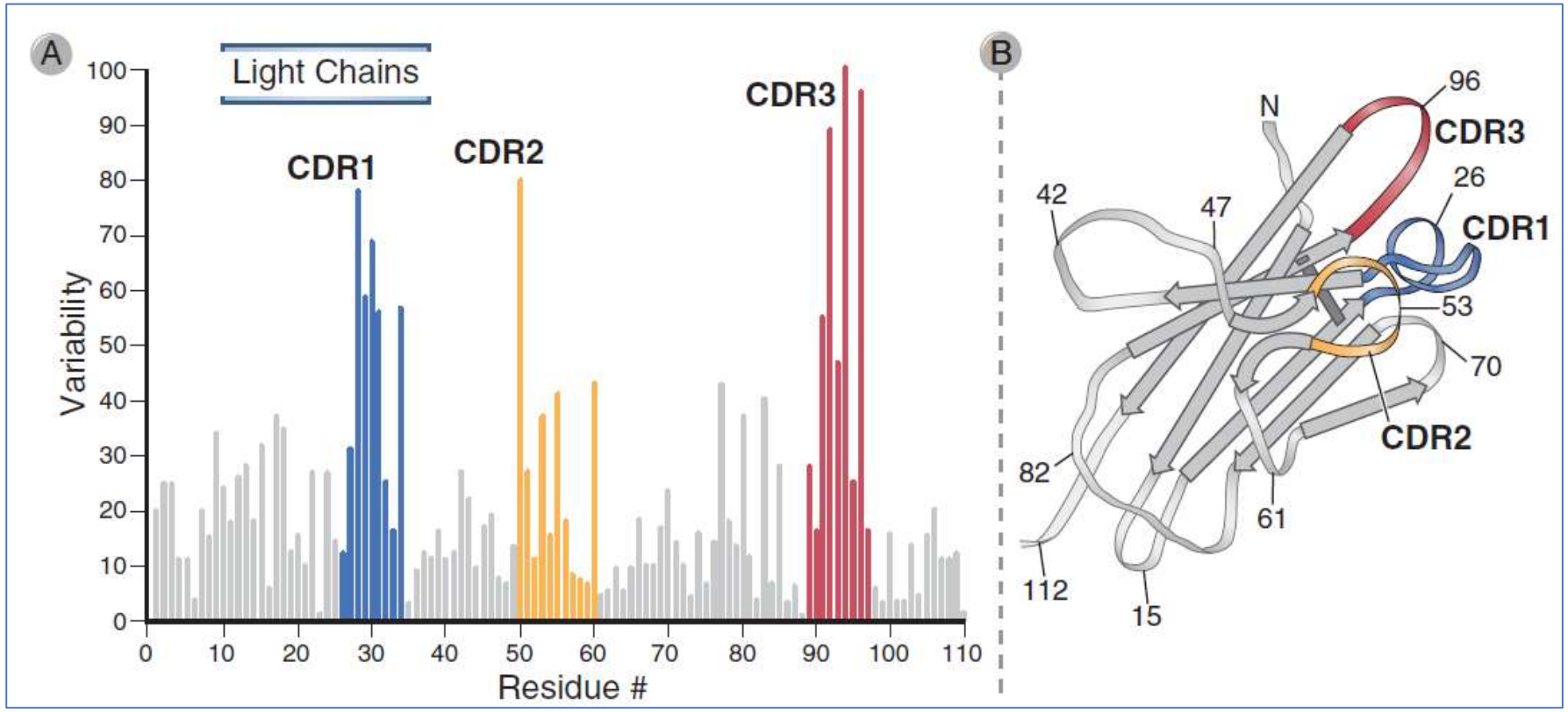
- Antibodies can exist in two forms: **membrane-bound** antibodies **on the surface of B lymphocytes** function as receptors for antigen, and **secreted antibodies** that reside in the circulation, tissues, and mucosal sites

- Both heavy chains and light chains consist of amino terminal **variable** (V) regions that participate in antigen recognition and carboxyl-terminal **constant** (C) regions; the C regions of the heavy chains mediate effector functions.



Antibodies/ structure

- Most of the sequence differences and variability among different antibodies are confined to **three short stretches in the V region** of the **heavy chain** and to **three stretches in the V region of the light chain**. These diverse stretches are known as hypervariable segments, Because these sequences form a surface that is complementary to the three-dimensional structure of the bound antigen, the **hypervariable regions** are also called complementarity-determining regions (**CDRs**).

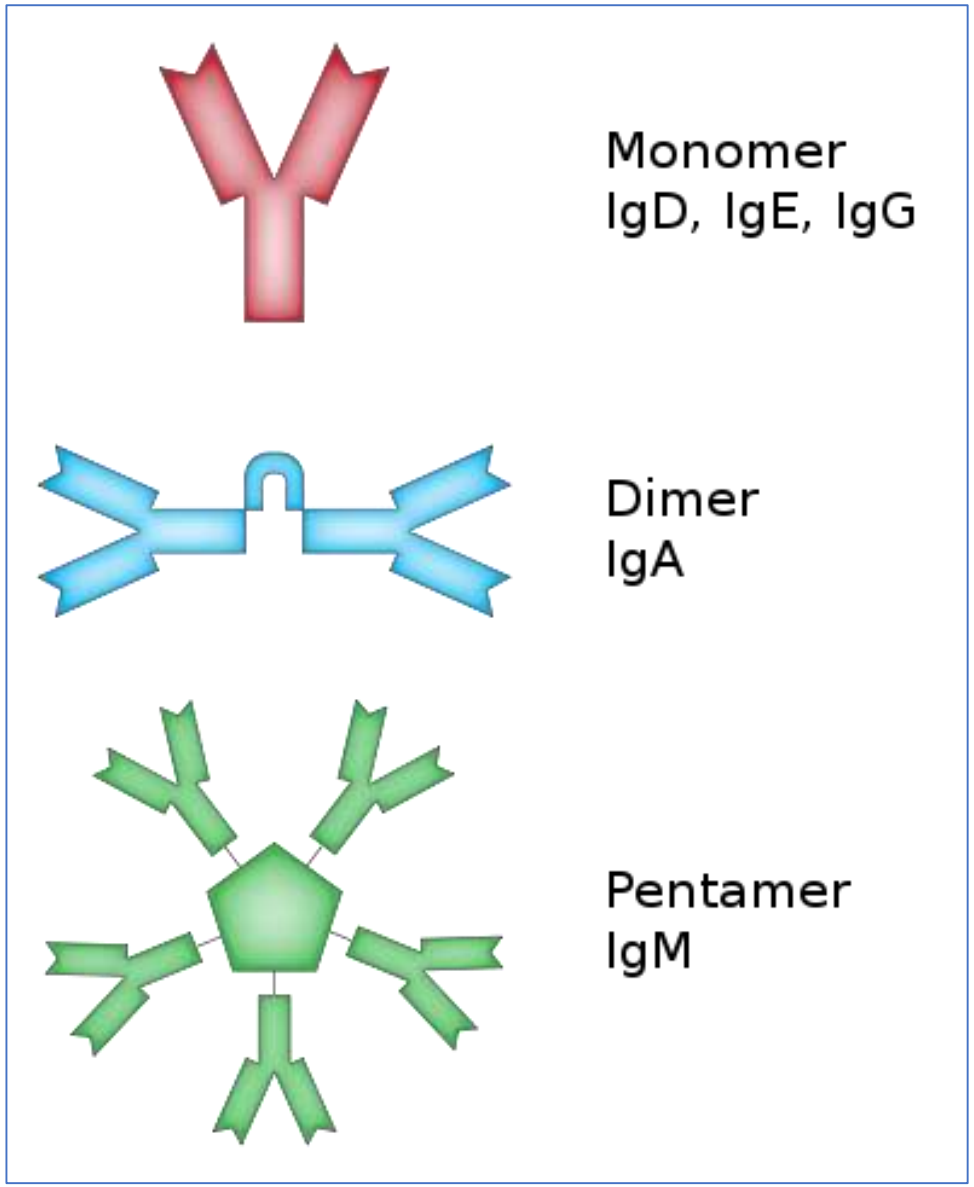


Antibodies

- There are **five immunoglobulin classes (isotypes**) of antibody molecules found in serum: **IgG, IgM, IgA, IgE and IgD**. They are distinguished by the **type of heavy chain** they contain

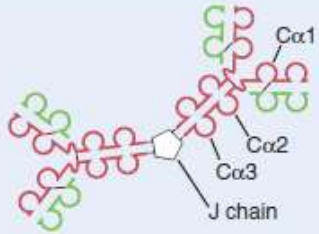
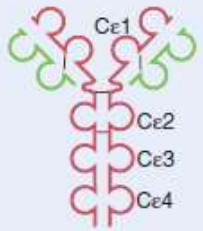
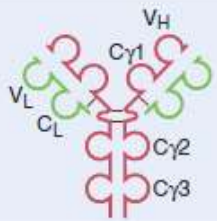
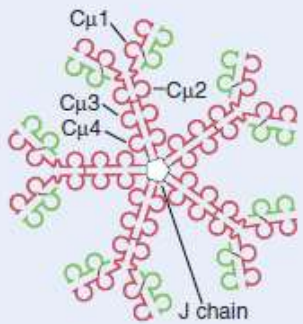
- Antibodies of different classes **differ** in their **location** around the body, appear at **different stages of an adaptive immune response**.

- The **heavy chain C** regions of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence. This sequence is different in antibodies of other isotypes or subtypes.



Antibodies/ structure

TABLE 5–2 Human Antibody Isotypes

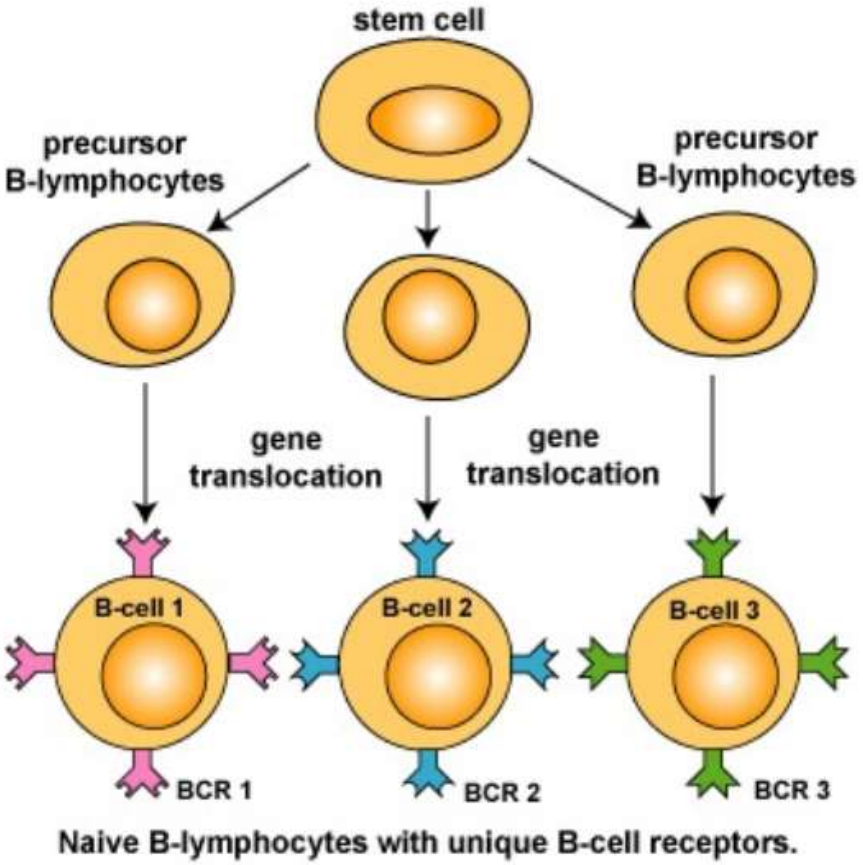
Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form		Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	IgA (dimer) Monomer, dimer, trimer		Mucosal immunity
IgD	None (δ)	Trace	3	None		Naive B cell antigen receptor
IgE	None (ε)	0.05	2	IgE Monomer		Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	IgG1 Monomer		Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	IgM Pentamer		Naive B cell antigen receptor, complement activation

B CELLS AND ANTIBODY RESPONSES

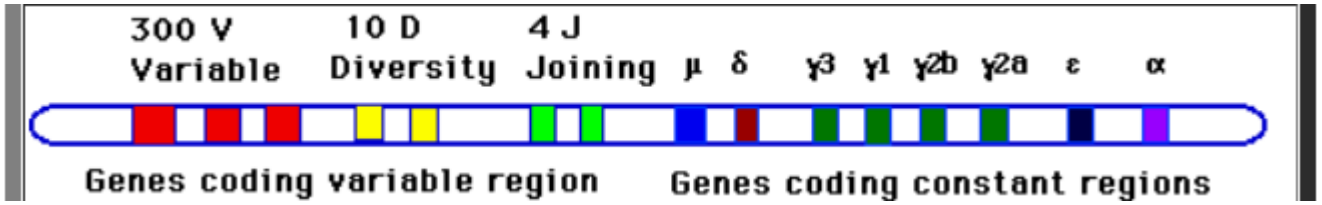
- B lymphocytes are the cells responsible for antibody responses.

- During its development, each B-lymphocyte becomes genetically programmed through a series of **gene-splicing reactions** to produce an antibody molecule with a **unique specificity**

- It is estimated that the human body has the ability to recognize 10^7 or more different epitopes, due to the wide range of possible combinations during gene splicing.



Naive B-lymphocytes with unique B-cell receptors.



Antibodies/ Antibody Production

- Initial contact with a new antigen evokes the **primary response**, which is characterized by a lag phase of approximately 1 week between the challenge and the detection of circulating antibodies.

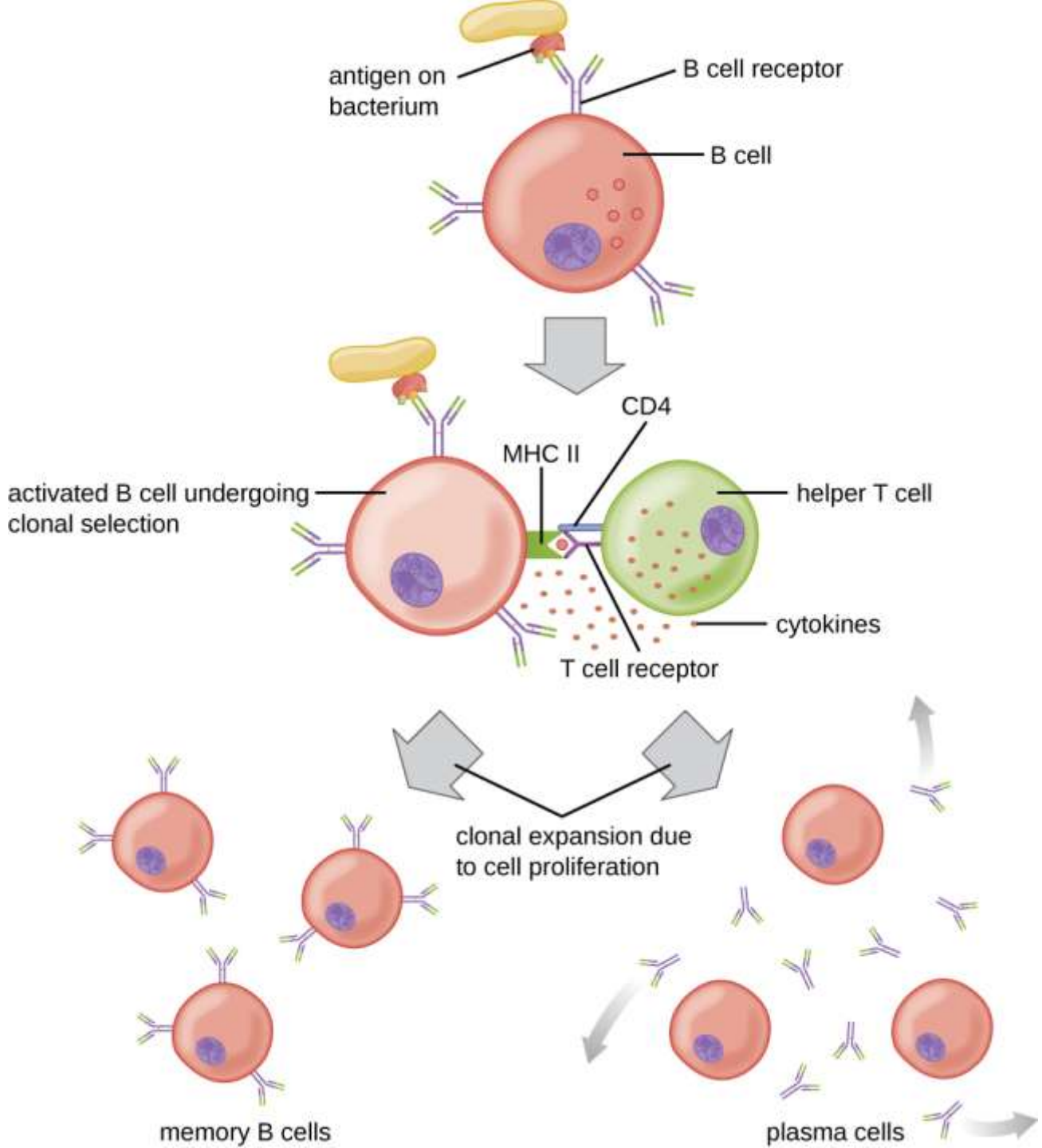
- Once antibody is detected in serum, the **levels rise exponentially** to attain a maximal steady state in approximately 3 weeks, **then decline gradually** with time.

- The **first antibodies** synthesized in the primary immune response **are IgM** and, **then IgG** antibodies arise and eventually predominate.

B Cell Activation

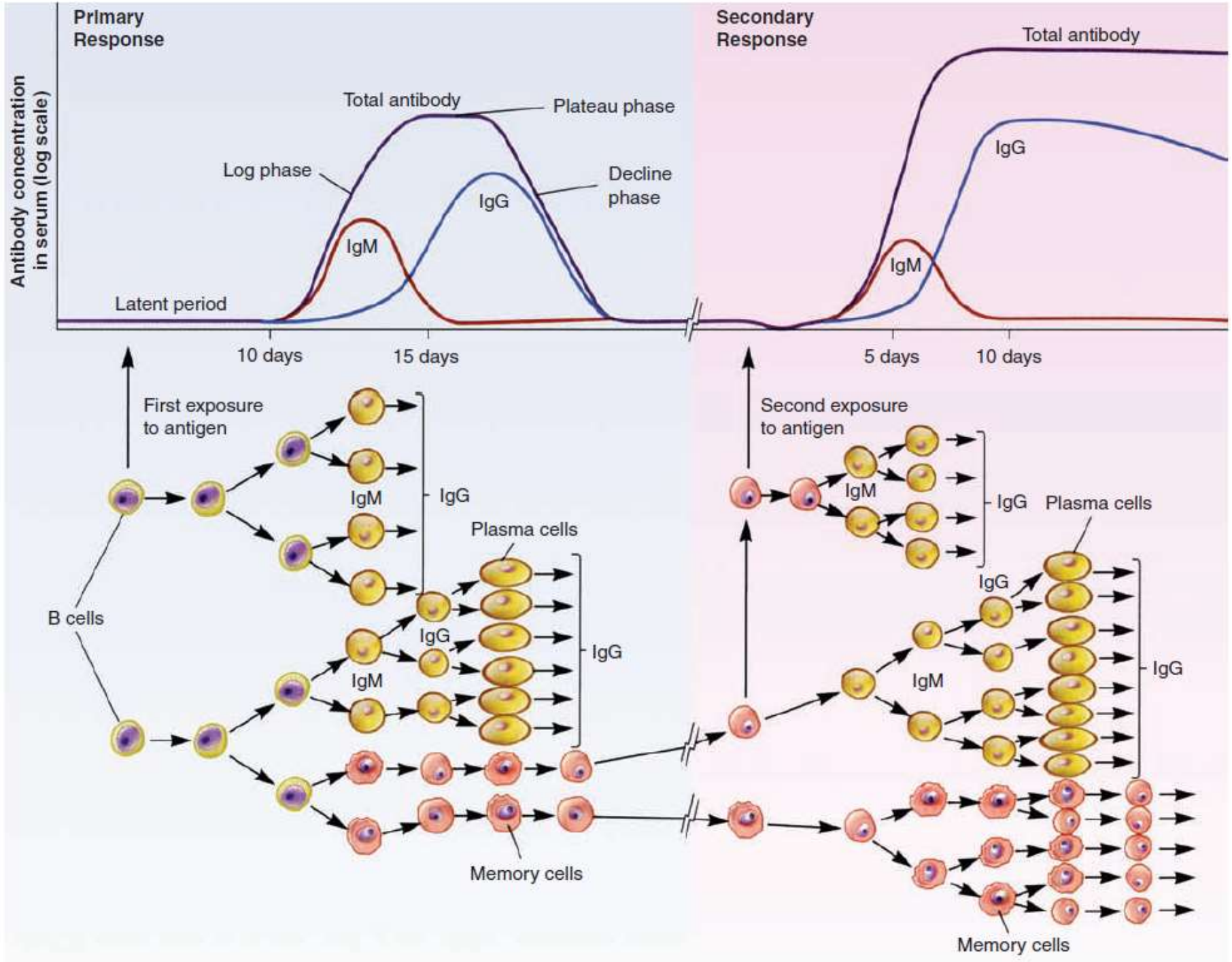
- Resting B cells become activated by antigen **via binding to the BCR and internalization**.
- Once internalized inside the B cell, the protein antigen is processed and **presented with MHC II**. The presented antigen is then recognized by helper T cells specific to the same antigen.
- Once activated by linked recognition, **T-cells produce and secrete cytokines that activate the B cell and cause proliferation** into clonal daughter cells.
- After several rounds of proliferation, additional cytokines provided by the T-cells stimulate the differentiation of activated B cell clones into **memory B cells**, which will quickly respond to subsequent exposures to the same antigen, and **plasma cells** that lose their membrane BCRs and **initially secrete pentameric IgM**

B Cell Activation



- After a subsequent exposure or booster injection of the same antigen, a different sequence called the **secondary response** ensues.

- In the secondary response;
- the **lag time** between the immunization and the appearance of antibody is **shortened**,
- the rate of exponential increase to the maximum steady-state level is **more rapid**,
- and the steady-state level itself is higher, representing a **larger amount of antibody**.
- Another key factor of the secondary response is that the antibodies formed are **predominantly of the IgG class.**


Primary Response
Secondary Response
Antibody concentration in serum (log scale)
Total antibody
Plateau phase
Log phase
Decline phase
IgG
IgM
Latent period
10 days
15 days
5 days
10 days
First exposure to antigen
Second exposure to antigen
B cells
IgM
IgG
Plasma cells
Memory cells

Antibodies/ Class switching

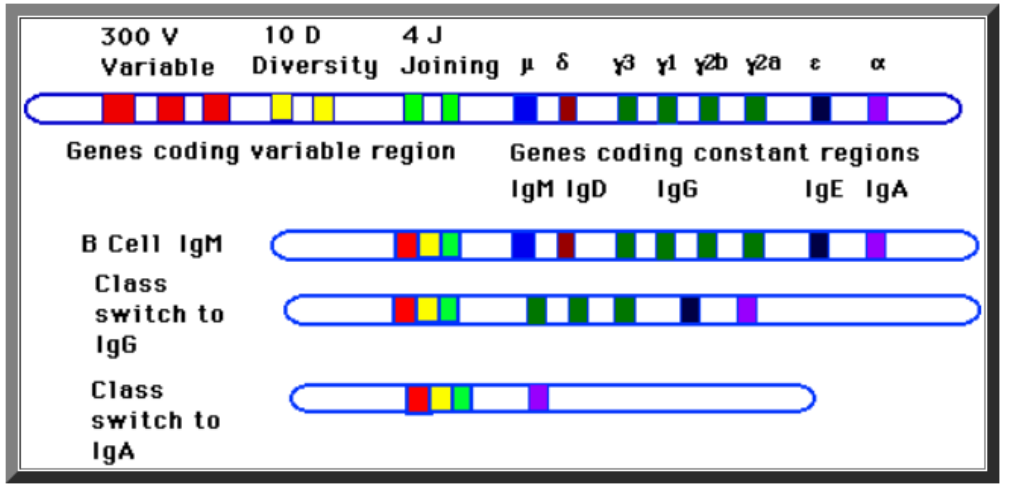
- After initial secretion of IgM, cytokines secreted by T- cells stimulate the plasma cells to **switch from IgM** production to production of **IgG, IgA, or IgE**.

- This process, called **class switching** or isotype switching, allows plasma cells cloned from the same activated B cell to **produce a variety of antibody classes** with the same **antigen** specificity.

- Class switching is accomplished by **genetic rearrangement** of gene segments encoding the constant region, which determines an antibody's class. The **variable region is not changed,** so the new class of antibody retains the original antigen specificity.

Class switch

- DNA rearrangement changing the heavy chain constant gene in memory cells.

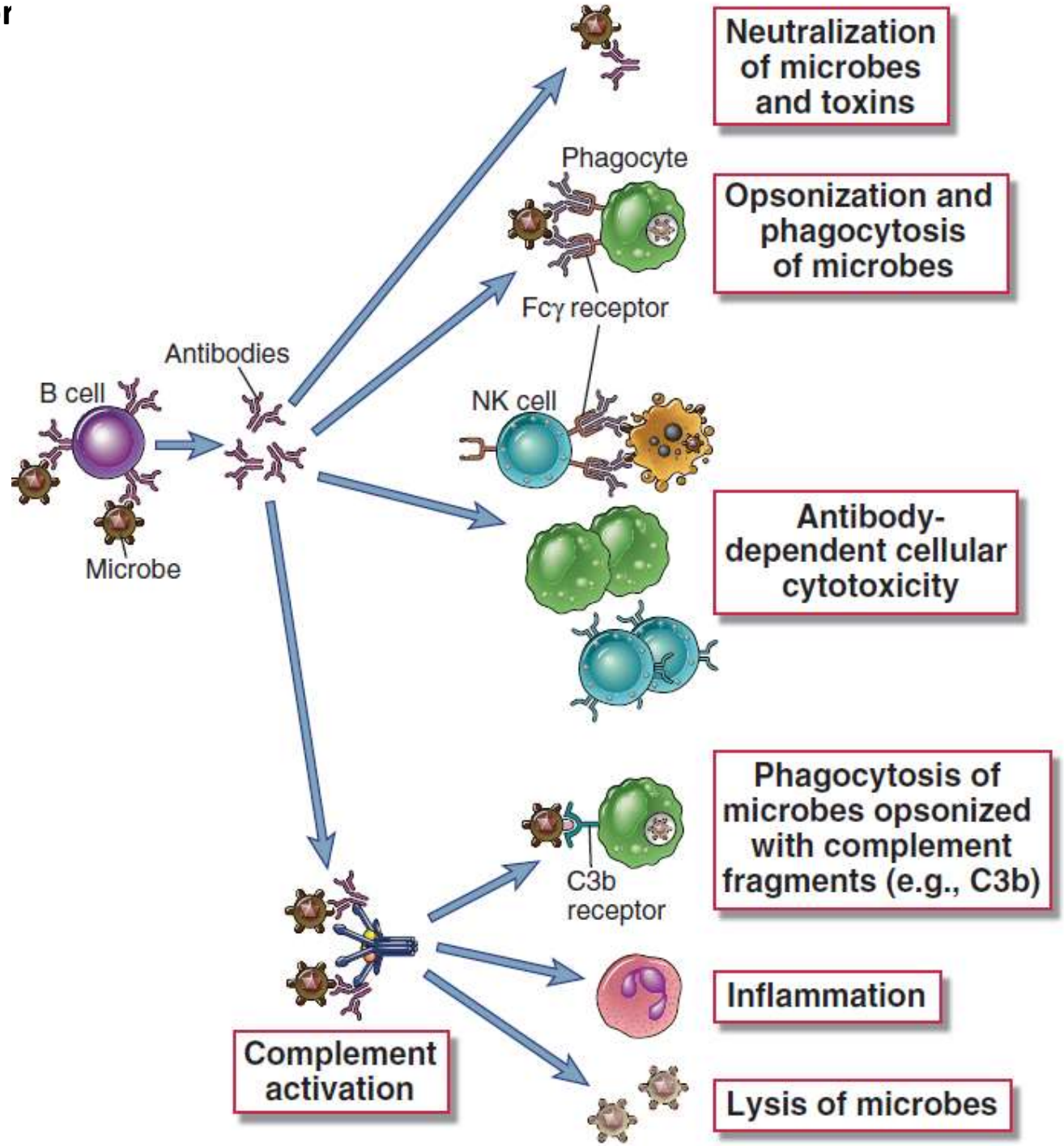


Antibodies and antigens

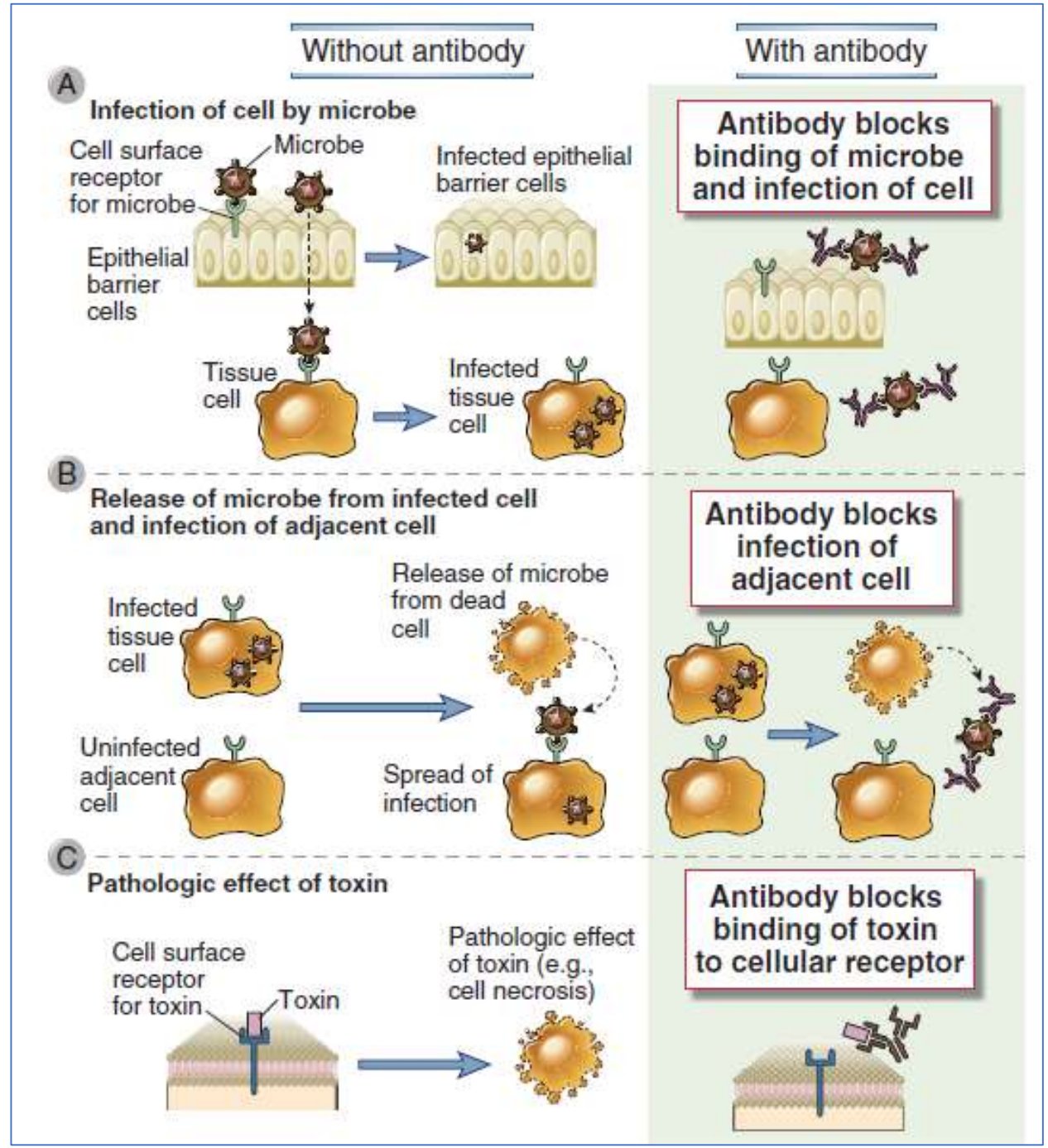
- The **ability** of antibodies in any individual to specifically **bind a large number of different antigens** is a reflection of antibody **diversity**, and the total collection of antibodies with different specificities represents the **antibody repertoire**. The genetic mechanisms that generate such a large antibody repertoire **occur exclusively in lymphocytes**. This diversity is generated by **random recombination of a limited set of inherited germline DNA** sequences to form **functional genes that encode the V regions of heavy and light chains** as well as by the addition of nucleotide sequences during the recombination process.

- **Somatic mutation in antigen-stimulated B lymphocytes** that generates **new V domain structures**, some of which **bind the antigen with greater affinity** than did the original V domains. Those B cells producing higher affinity antibodies preferentially bind to the antigen and, as a result of selection, become the dominant B cells with each subsequent exposure to the antigen. This process is called **affinity maturation**.

Effector mechanisms of humor



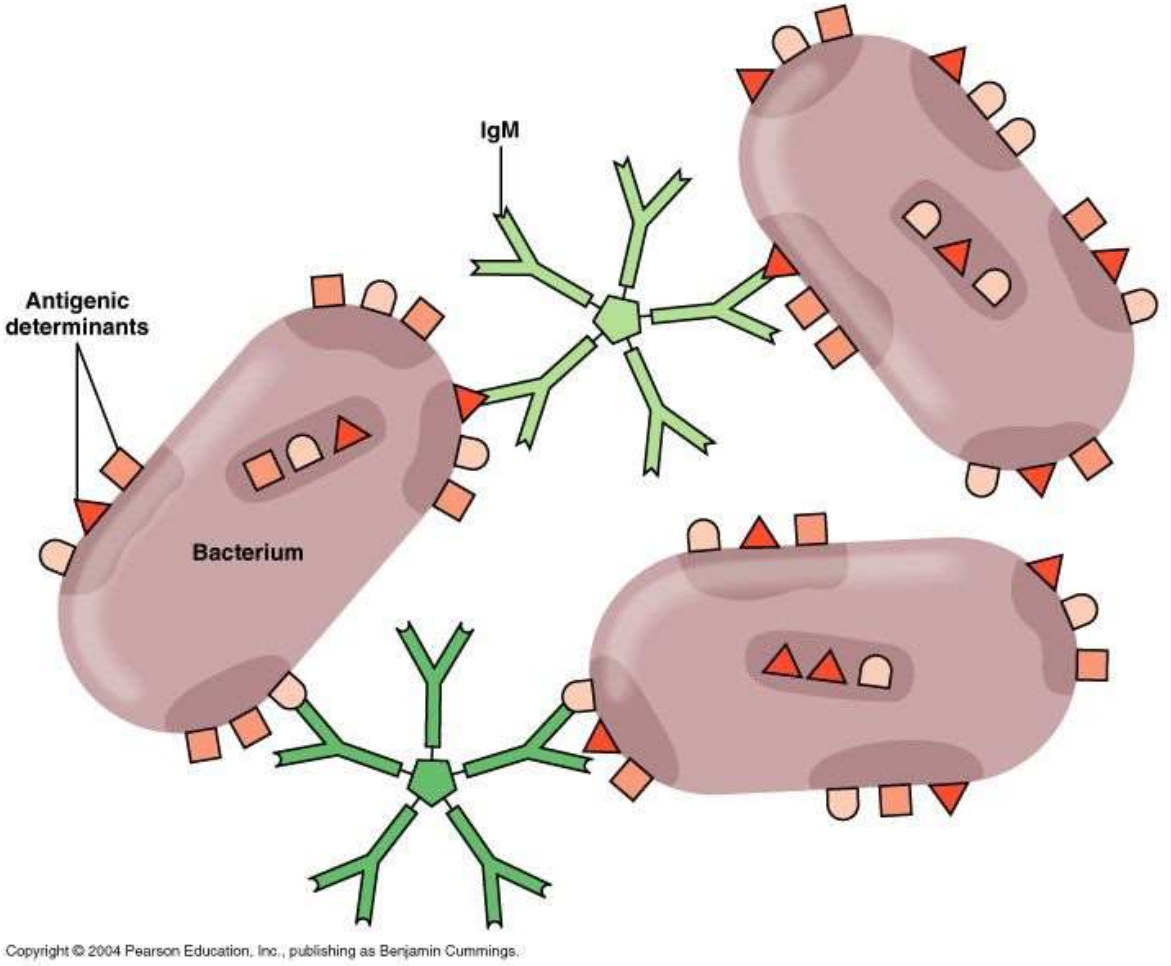
Effector mechanisms of humoral immunity



Antibodies/ *IgM*

- Because of its many specific binding sites, IgM is particularly effective in agglutinating particles carrying antigens against which it is directed.

- IgM is particularly active in bringing about complement-mediated cytolytic damage to foreign antigen-bearing cells.

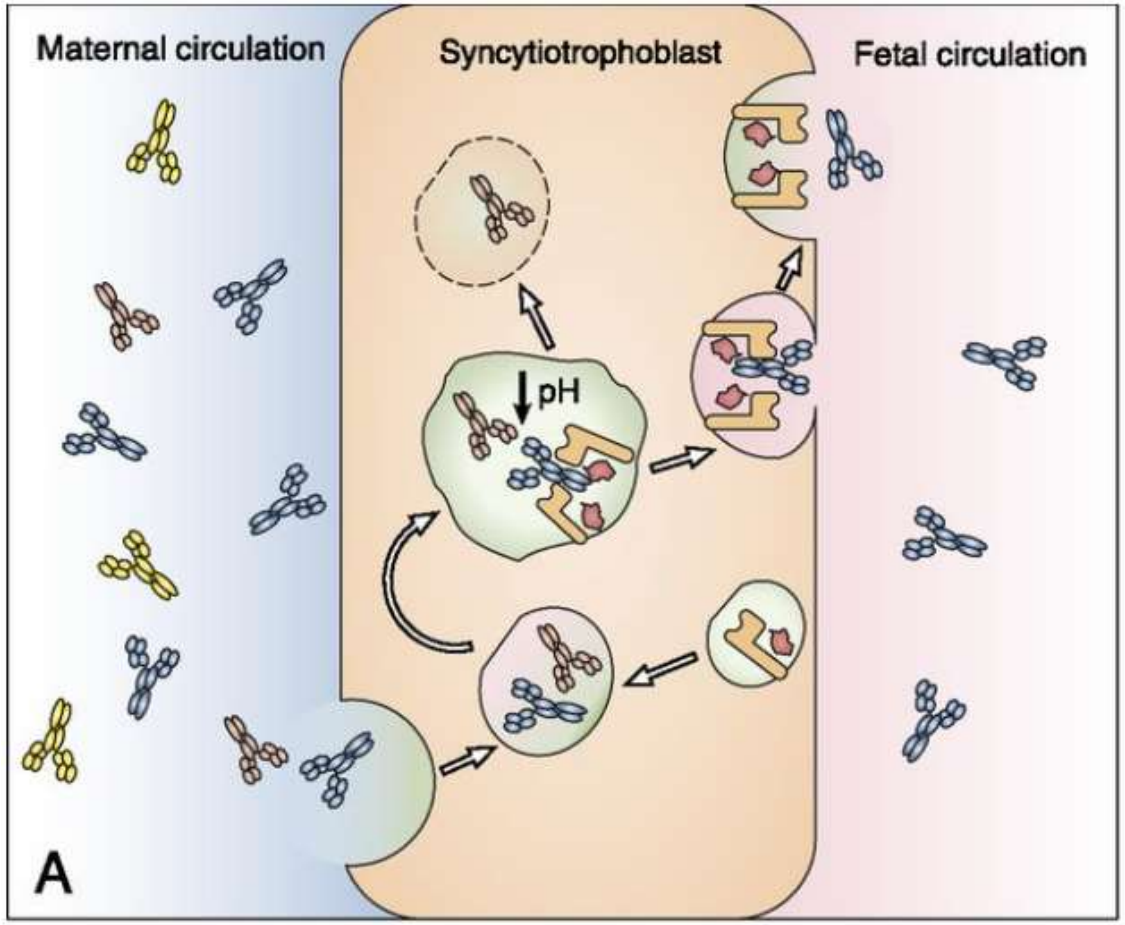


Antibodies/ *IgG*

- Immunoglobulin G (**IgG**) is **the most abundant immunoglobulin in health** and provides the most extensive and **long-lived antibody** response to the various microbial and other antigens.

- IgG antibody is characteristically formed in large amounts **during the secondary response to an antigenic stimulus**, and usually **follows production of IgM** in the course of a viral or bacterial infection.

- IgG is the only immunoglobulin class able to **cross the placental barrier** and, thus, provides passive immune protection to the newborn in the form of maternal antibody.

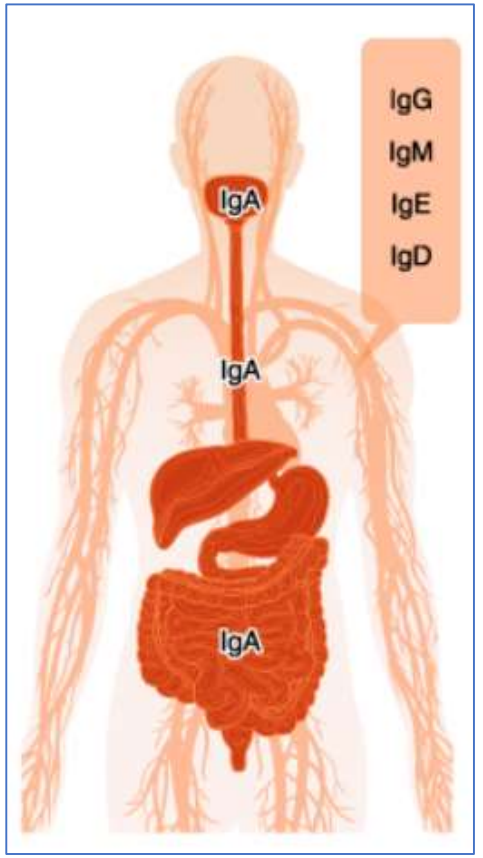


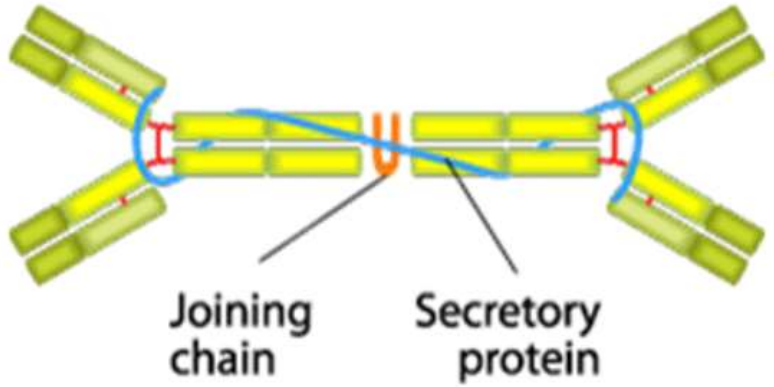
Antibodies/ *IgA*

- Immunoglobulin A (IgA) has a special role as a major determinant of so-called local immunity in **protecting epithelial surfaces** from colonization and infection.

- At the epithelia**, two IgA molecules** combine with another protein, termed the **secretory piece,** which is present on the surface of local epithelial cells. The complex, then termed **secretory IgA** (sIgA),

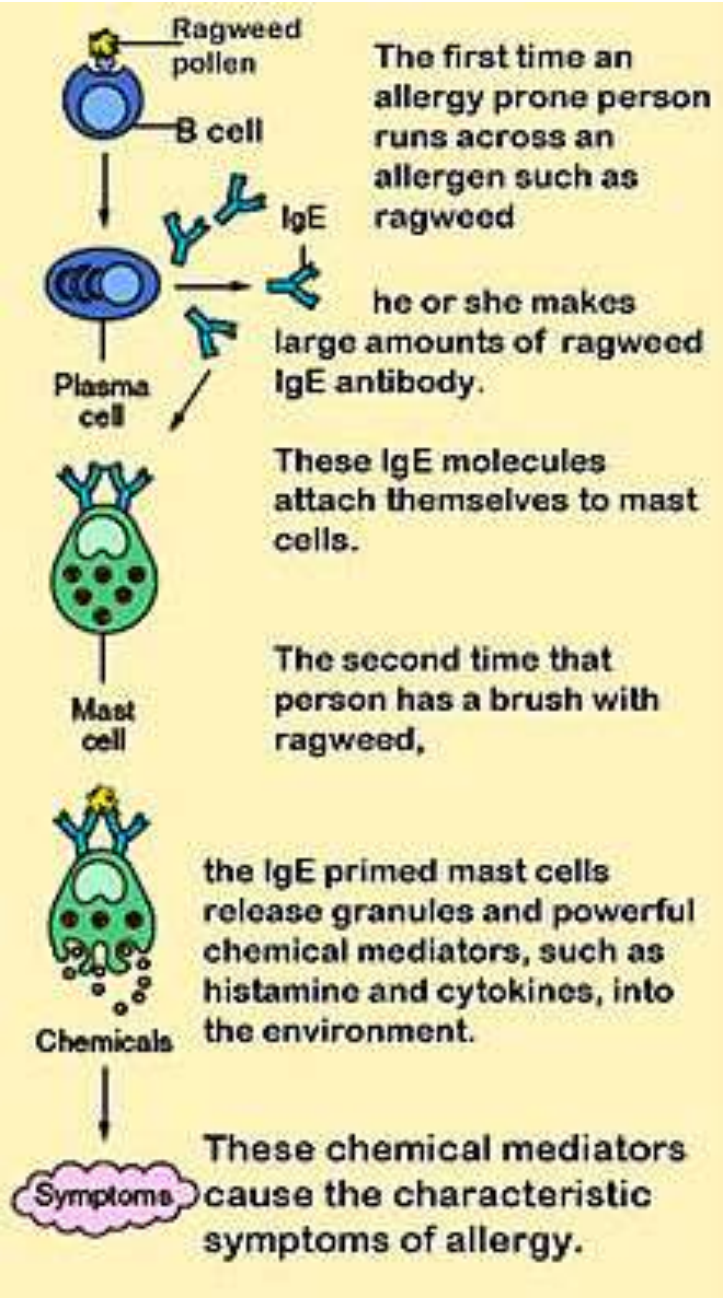
- The **major role of sIgA is to prevent attachment** of antigen-carrying particles to receptors on mucous membrane epithelia





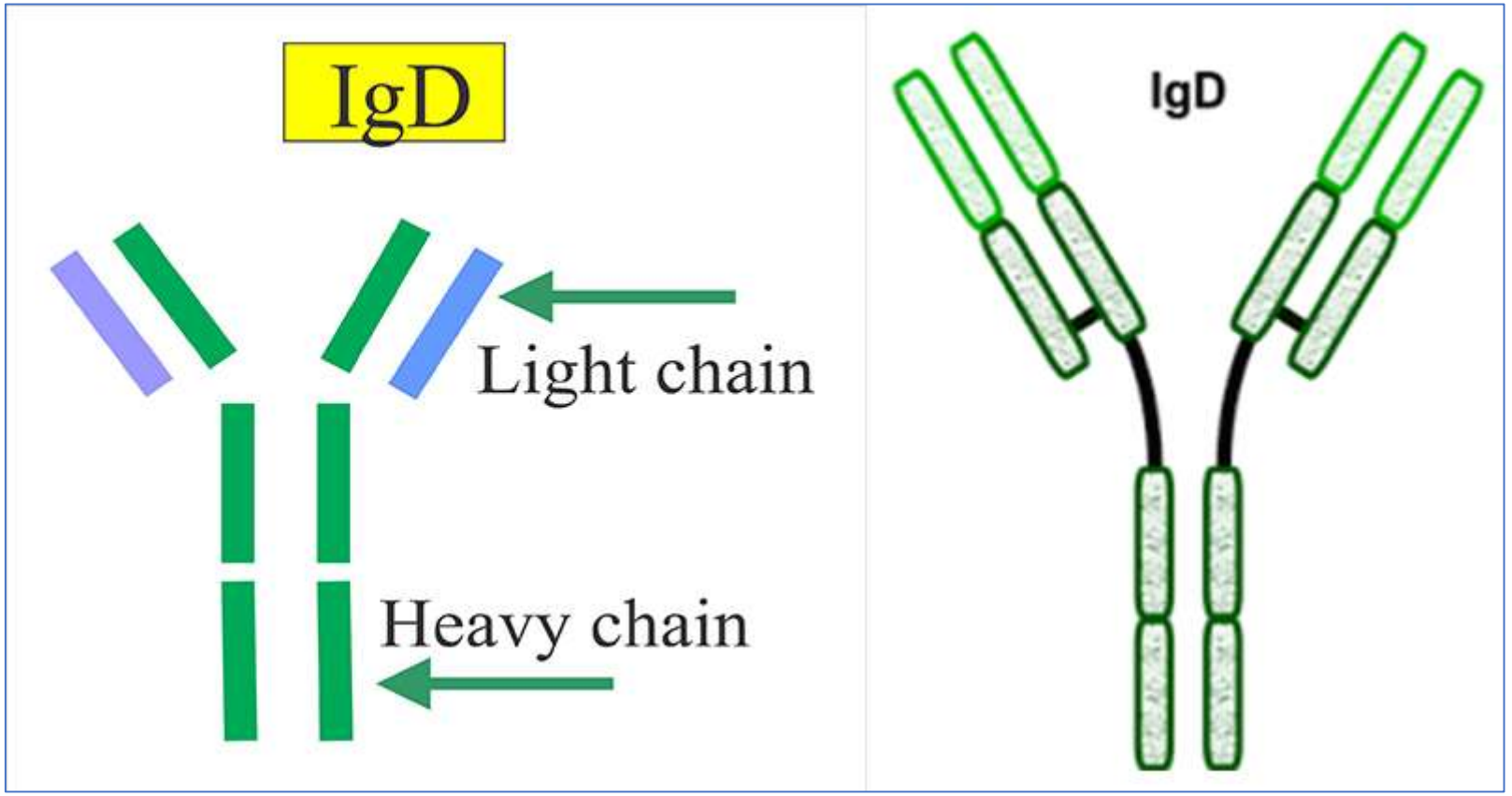
Antibodies/ *IgE*

- IgE not only provides protective immunity against **helminth parasites** but can also mediate the **type I hypersensitivity reactions** that contribute to the pathogenesis of allergic diseases such as **asthma, allergic rhinitis and atopic dermatitis**.



Antibodies/ *IgD*

- **IgD** (**IgD**) is a monomeric antibody isotype that is expressed in the plasma membranes of immature B-lymphocytes. **IgD** is also produced in a secreted form that is found in small amounts in blood serum.
- the function of IgD is to signal the B cells to be activated.



Further reading:

- Cellular and Molecular Immunology. 7th Edition..

Chapter 5. Antibodies and antigens