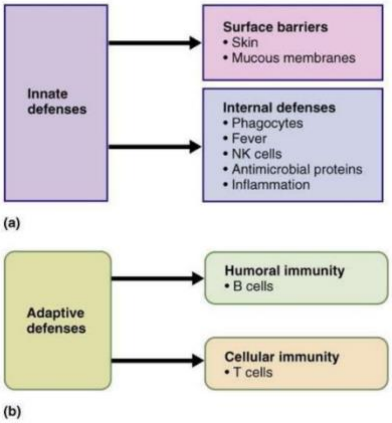
Dr.Ahmad Al-Qawasmi

Biochemistry

Immunoglobulins

❖ Immunoglobulins

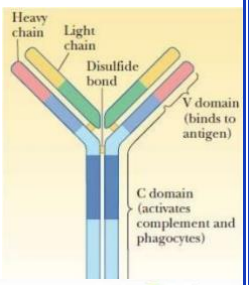
- Types of immunity
  - **Innate:** Non-specific immunity which protects the body from any foreign antigen, and it includes:
    - ✓ Skin and mucous membranes
    - ✓ Phagocytes (such as macrophages) which phagocytose (eat) bacteria once they enter the body
    - ✓ Natural Killer (NK) cells and anti-microbial proteins
    - ✓ Inflammation and fever
  - **Adaptive:** Specific immunity for a specific antigen, it includes:
    - ✓ Humoral immunity using **B cells** (produced by bone marrow)
    - ✓ Cellular immunity using **T cells** (produced by the thymus)
- B and T cells can form **memory cells**



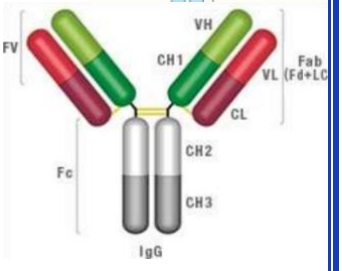
❖ How do B cells work?

- B cells secrete **antibodies**
- Antibodies have three roles:
  - Antibodies bind to pathogens and **induce their phagocytosis** into immune cells
  - Antibodies bind to viruses and microbial toxins **neutralizing** them (preventing their entry to the cell)
  - Antibodies **recruit white blood cells and the complementary proteins system** to lyse and get rid of pathogens
- Newborns don't develop adaptive immunity by themselves, so the fetus and newborns acquire their specific antibodies from their mothers by breast feeding
- When a B cell is activated by antigen, it proliferates & differentiates into antibody-secreting effector cell forming clones of B cells, where each clone secrete changes its DNA and produce a specific antibody
  - Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibody at a rate of about 2000 molecules per second
  - Each individual can produce more than 10^{11} different antibody molecules
    - ✓ This huge **variety** is due to the recombination and mutations in the DNA of B cells causing changes in the sequence of the amino acids and their properties
- Each B cell can produce a large amount of only 1 type of antibodies
  - High affinity antibodies are efficient and persist to perform their function

❖ Structure of antibodies



- Antibodies are **immunoglobulins**
  - Large Y-shaped hetero-tetramers consisting of two identical heavy chains and two identical light chains held together by **disulfide bonds**
  - Within each of the polypeptide Chains there are also intra-chain disulfide bonds
  - They are **glycoproteins**, with oligosaccharides linked to their heavy chains

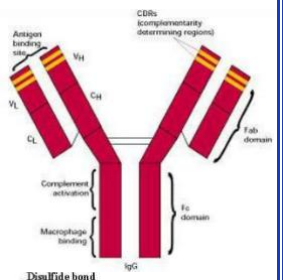


- Each light chain consists of 1 variable domain (V_L) and 1 constant domain (C_L)
- Each heavy chain consists of 1 variable domain (V_H) and 3 constant domains (C_{H1}, C_{H2}, and C_{H3})
  - **V_L pairs with V_H** and **C_L pairs with C_H**

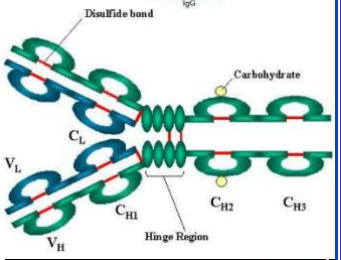
- **Constant regions** are uniform from one antibody to another within the **same isotype**
- **Fc domain:** Formed by the **constant** domains of the heavy chains (mostly C_{H2} and C_{H3}), which are important for binding to immune cells (such as Phagocytes) leading to **phagocytosis** of the antigen or the **activation of the complementary system**, aiding in the antigen clearance

- **Variable regions** are found at the tips of the Y and they bind to the antigen (epitope)
  - Each antibody can bind to **two antigens**
  - The **primary sequences** of the variable regions among different antibodies are quite distinct
  - Each variable domain contains motifs that consist of β-sheets with 3 loops connecting them together

- **Hypervariable regions** also called **Complementarity Determining Regions (CDRs):** They are found within the variable regions of both the heavy and light chains
  - They are the 3 loops in the variable domains, consisting of about 7-12 amino acids in each one that contribute to the **antigen-binding site**
  - They recognize and **bind specifically to antigen with high affinity** (Dissociation constant (K_d) =10^{-12}-10^{-7})
  - As the antibody has a higher affinity, it functions more efficiently and prevent autoimmune diseases



- **Hinge region:** exists where the arms of the antibody molecule form a Y in the constant region of the heavy chain
- It adds some **flexibility** to the molecule, which aids in the high affinity of the antibodies



- **Immunoglobulin fold:** It forms a **Beta-Barrel**
  - It consists of a motif (super secondary structure) that presents in every immunoglobulin
  - Forms a sandwich of two anti- parallel β sheets connected by loops held together by disulfide bonds

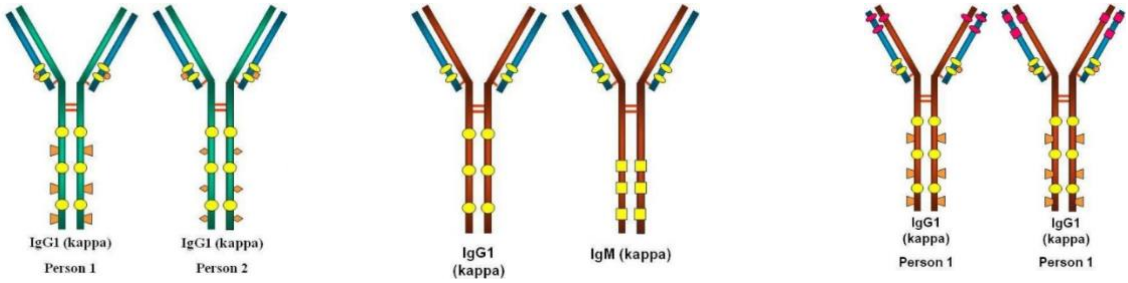
❖ Diversity

- Antigen-antibody binding is mediated by **noncovalent interactions** but also with high-affinity due to the large amount of these bonds
- The enormous diversity of antigen-binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops
- The **overall three-dimensional structure** necessary for antibody function remains constant

- There are two light chains (lambda or kappa)
- There are five heavy chains (alpha, delta, gamma, epsilon or mu)

❖ Idiotype vs. isotypes vs. allotypes

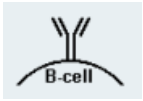
- **Idiotype:** Immunoglobulins with different variable domains of both their light chains and heavy chains (caused by changes and mutations in the variable region)
- **Isotypes:** Immunoglobulins with different constant domains of the heavy chain (caused by changes and mutations in the constant region of the heavy chain)
- **Allotype:** Immunoglobulins of the same class but different among individuals of the same species due to different genetics (polymorphism) between individuals (slight difference in the constant regions)



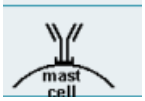
- Immunoglobulin classes (isotypes) are IgA, IgD, IgG, IgE, IgM according to the type of the heavy chain
- **IgM:** Contain Mu heavy chains
  - Expressed on the surface of B-cells
  - The first antibodies produced in significant quantities against an antigen
  - Promotes phagocytosis and activate the complement system that leads to cell killing
  - Appears usually as pentamer

- **IgG:** Contains Gamma chains
  - Monomers
  - Most abundant immunoglobulins in sera (600-1800 mg/dL)
  - Promote phagocytosis and activate the complement system
  - Only kind of antibodies that can cross the placenta

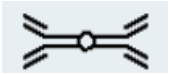
- **IgD:** Contains delta heavy chains
  - Presents on surface of B-cell that have not been exposed to antigens



- **IgE:** Contains epsilon heavy chains
  - A monomer
  - Plays an important role in allergic reactions, and attached to the surface of mast cells



- **IgA:** Contains alpha heavy chains
  - Found mainly in mucosal secretion, and in the breast milk
  - The initial defense in mucous against pathogen agents
  - Appears usually as dimers, and can appear as monomers



❖ Class switching

- **Changing the class** of the antibody but having the same variable region
  - **First**, before binding antigen, B cells contain IgM molecules only
  - Following antigen binding, class switching occurs (IgM switched into IgG)
  - Class switching refers to a DNA rearrangement changing the heavy chain constant gene
  - That causes production of IgG, IgA, and IgE

❖ Hybridoma and monoclonal antibodies

- When an antigen is injected into an animal, the resulting antibodies are polyclonal, meaning they are directed against a number of different epitopes on the same antigen
  - **Polyclonal antibodies:** Antibodies for the same antigen, but produced from different B cells
  - **Monoclonal antibodies:** Antibodies for the same antigen, but produced from same B cells
- **Hybridoma:** **Immortal** monoclonal B cell produced by the fusion of a normal B cell with myeloma (cancer cell)

- Monoclonal antibodies can be used as treatment for many diseases
  - These antibodies are created by introducing and antigen to an animal and then isolating the monoclonal antibodies from it
  - CDRs from the animal antibodies are attached in the appropriate site in the human immunoglobulins then can be used as treatments

- Benefits of monoclonal antibodies
  - Measure the amounts of many individual proteins and molecules (such as plasma proteins, steroid hormones)
  - Determine the nature of infectious agents (such as types of bacteria)
  - Used to direct therapeutic agents to tumor cells
  - Used to accelerate removal of drugs from the circulation when they reach toxic levels

Past papers

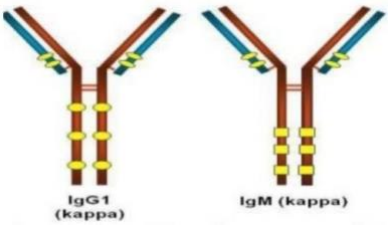
1. In order to immortalize a B cell to produce a monoclonal antibody:

A. B cells undergo class switching
B. Mutations are created
C. B cells with immunoglobulin M are selected
D. B cells are fused with cancer cells
E. B cells are just activated by an antigen

2. Cysteines play an important role in the formation of the quaternary structure of this protein:

A. Carbonic anhydrase
B. Hemoglobin
C. Collagen
D. Immunoglobulin
E. Myoglobin

3. The relation between those 2 antibodies:



A. Idiotype
B. Isotype
C. Allotype
D. Epitope
E. All of the above

4. Newborns of lactating mothers are protected from foreign antigens by:

A. IgA
B. IgE
C. IgM
D. IgG
E. A + D

5. The antigen is bound to which part of the antibody:

A. Fc
B. Fab
C. CDR

6. Which of the following is not true about antibodies:

A. They are glycoproteins
B. They are protomers
C. They consist mainly of Beta sheets stabilized by hydrophobic interactions
D. IgM can bind to 10 epitypes
E. All the above are true

7. Which of the following is not true about antibodies:

A. C_{H2} and C_{H3} domains are the responsible for binding to phagocytes
B. They are a part of the humoral immunity
C. IgE plays an important role in asthma
D. Hinge regions enhances the affinity of an immunoglobulin to the antigen
E. All the above are true