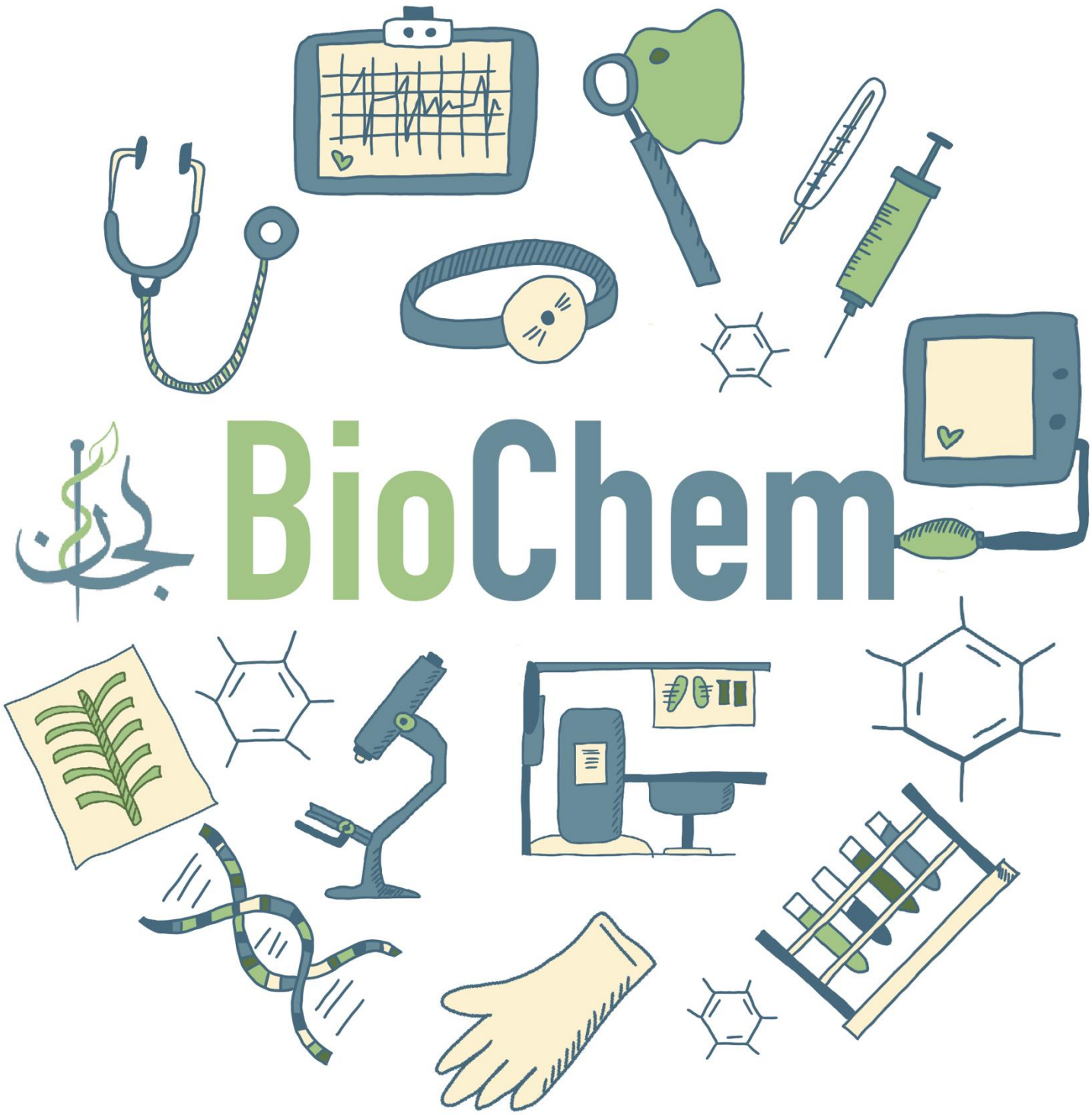


BioChem

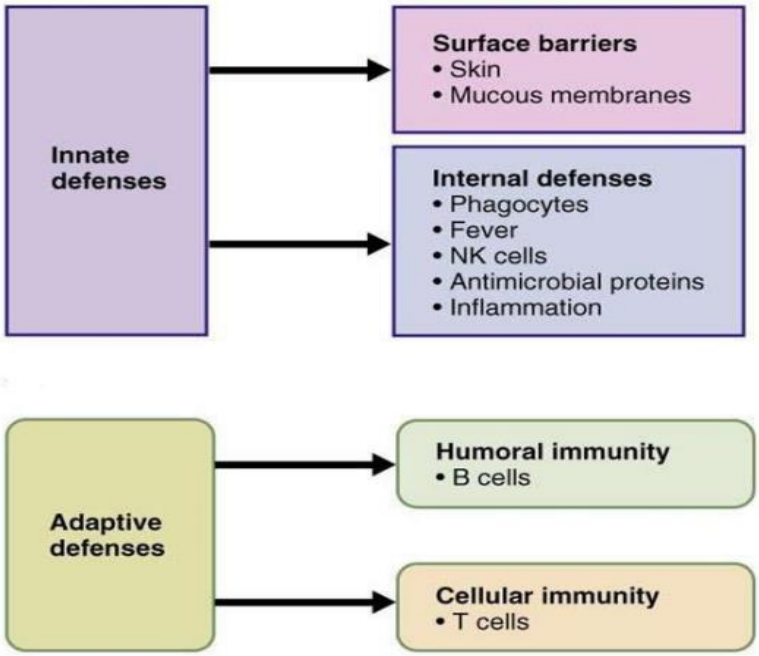
Writer: Abdullah Na’eem

Science: Hani Shehadeh

Grammar:

Doctor: Dr. Diala

Types of immunity



Notes on the **Innate defenses**:

→ The main **function** of immunity is to **protect** the body from **pathogenies** and **causative agents** that result in different types of **diseases**.

→ **Internal defenses** act when pathogenies **enter** the body.

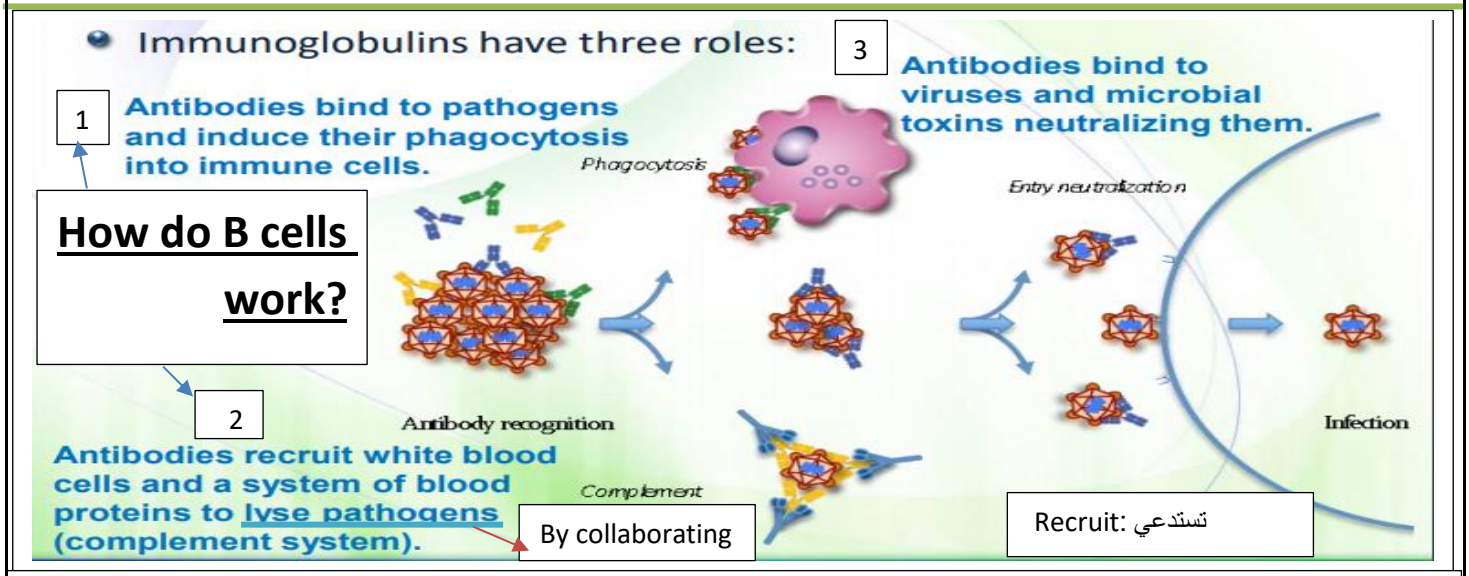
→ **Fever** is considered as a type of **protective reactions**, that's why it's a part of internal defenses

→ **inflammatorial reactions**: reactions caused by **neutralized killer** (NK) **cells** and **Antimicrobial proteins**, where pathogens are **neutralized**.

Notes on the Adaptive defenses:

→ **B cells** (important for **Humoral immunity**): Cells that release **antibodies (immunoglobulins),** which neutralize pathogens

→ **T cells:** have many **subtypes**, and is involved in **cellular immunity,** so these cells **themselves** are involved in the **immunological reactions** rather than the products as in B cells.



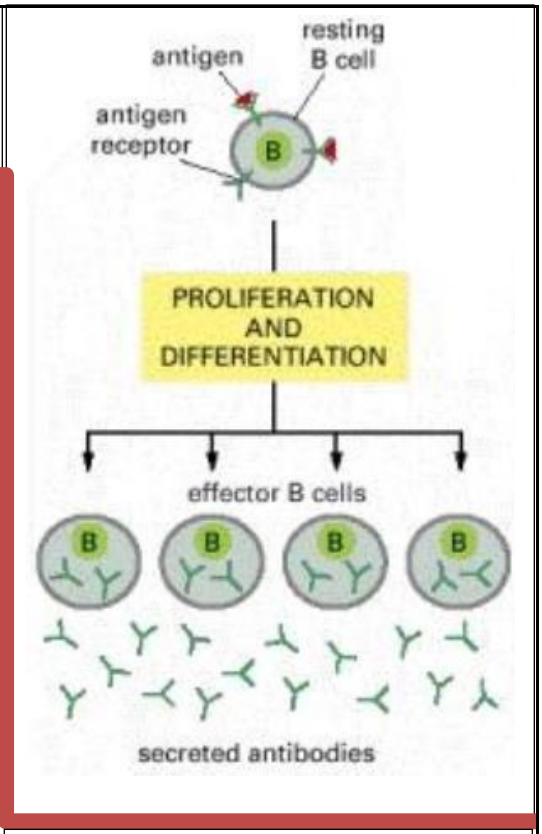
→ If some of pathogens **escape** from these three roles to the **cells**, they will cause **infection.**

→ When B cell is activated by antigen, it proliferates and differentiates into an antibody-secreting effector cell. (B cells in rest state has antigen receptors, once they bound to an antigen, they activate and differentiate into **effector B cells** that produce **antibodies**)

→ Antigens are usually molecules that are exposed on the surface of a bacteria, viruses..etc and defined as foreign bodies from non cells sources

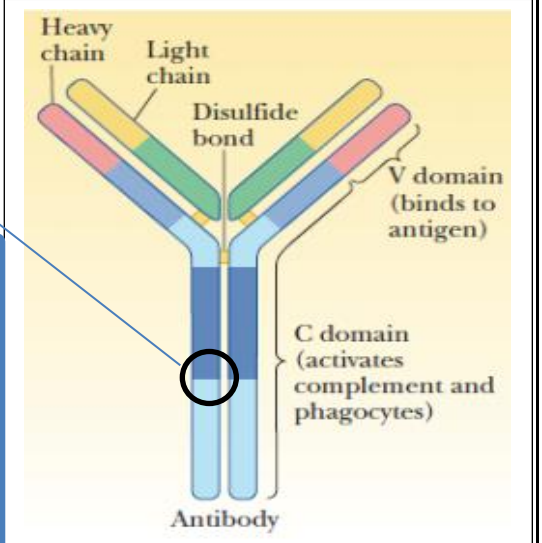
→ Antibodies (globule-proteins) : soluble proteins that aren't a part of the membrane, and can bind to different antigens to get rid of them as a part of the immunological response

→ Such cells make and secrete large amounts of soluble (rather than membranebound) antibody at a rate of about **2000** molecules per second.



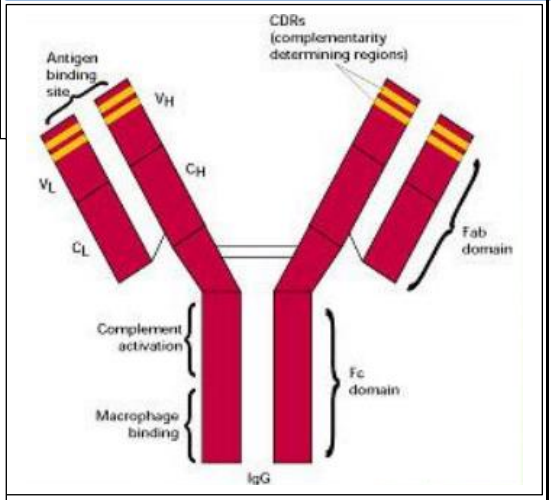
Structure of antibodies

→ Antibodies are Y-shaped molecules consisting of two identical heavy chains and two identical light chains held together by disulfide bonds (which are covalent bonds).
→ The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds
→Within each of the polypeptide chains there are also intra-chain disulfide bonds (bond in the same chain).
→They are glycoproteins, with oligosaccharides linked to their heavy chains.
→ different types of disulfide bridges are present and they're important to hold the quaternary structure together
→ There's an attachment site here for sugars, so antibodies are glycoproteins and these sugars are attached to these sugars of molecules



Antibody regions

→ A light chain consists of one variable (VL) and one constant (CL) domain.
→The heavy chain consists of one variable region (VH) and three constant regions (CH1 , CH2 , and CH3).
→VL and CL pair with VH and CH .
(*)keywords:
L: light chain/ H: heavy chain/ V: Variable/ C: constant



Constant regions

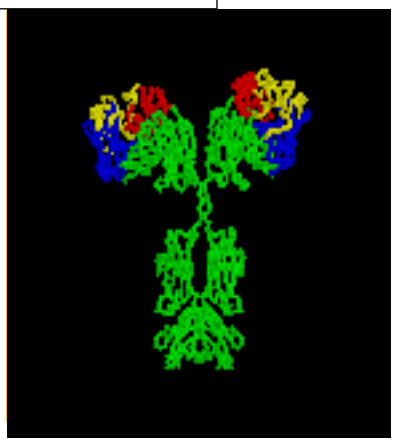
→Constant regions, are uniform from one antibody to another within the same isotype.
→ The Fc domain of antibodies in the heavy chain is divided into two binding regions, one for the complement system and the other for the microphage binding, these are important for binding to phagocytic cells allowing for antigen clearance.

❖ Variable regions

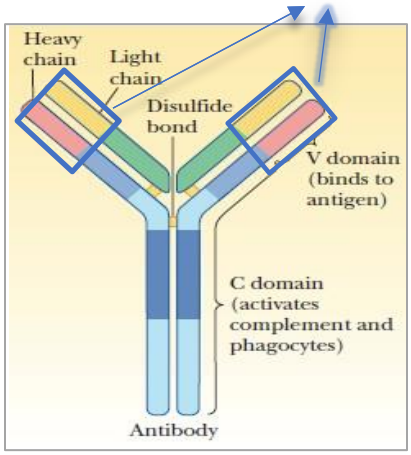
- The variable region is found at the tips of the Y, at the tips of the light chain and heavy chain as well. and is the part of the antibody that binds to part of the antigen (called **epitope**).
- Each antibody can bind to two antigens.
- Since they are variable regions they are important in the diversity of antibody types.
- The primary sequences of the variable regions among different antibodies are quite distinct.

About 7-12 amino acids in each one that contribute to the antigen-binding site, and these amino acids are responsible for the ability of binding to different types of antigens .

Each type of B cell produces only one kind of antibodies and each antibody can bind two types of antigens. (important)

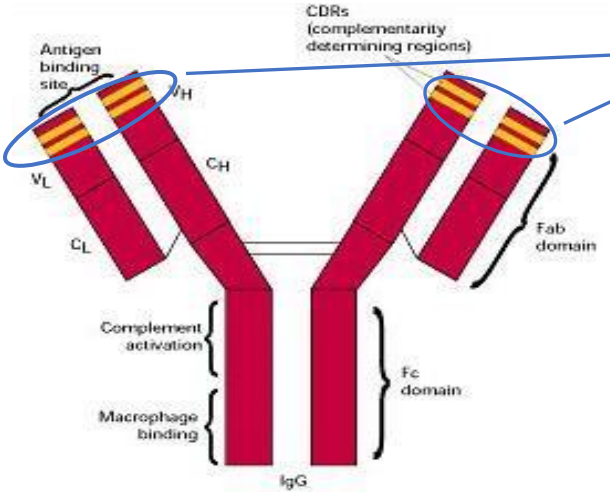


Epitopes



❖ Hypervariable regions

- Within the variable regions of both heavy and light chains there are **Hypervariable" regions**, OR **"Complementarity Determining Regions" (CDRs)**.
- These regions serve to recognize and bind specifically to antigen that is specific and have high affinity so they don't scape or get dissociated from the antibodies causing diseases the cells to which they enter (dissociation constant **(Kd) 10^{-12}-10^{-7}**
  (and that is relatively low so the binding is strong).

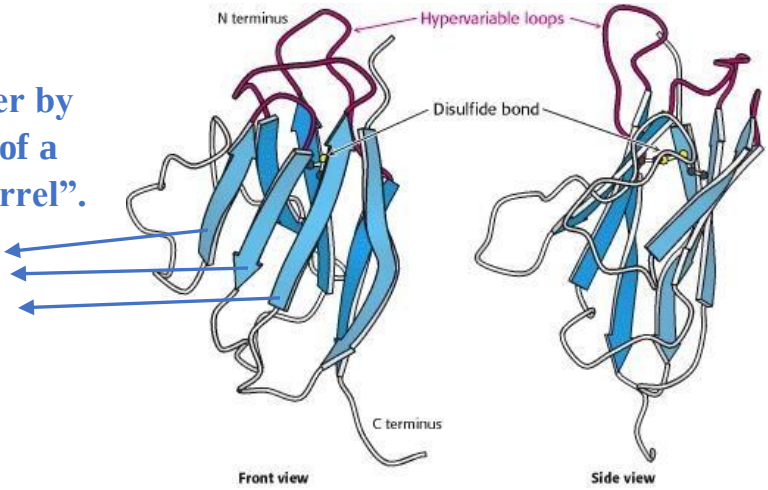


Hypervariable regions (yellow lines)

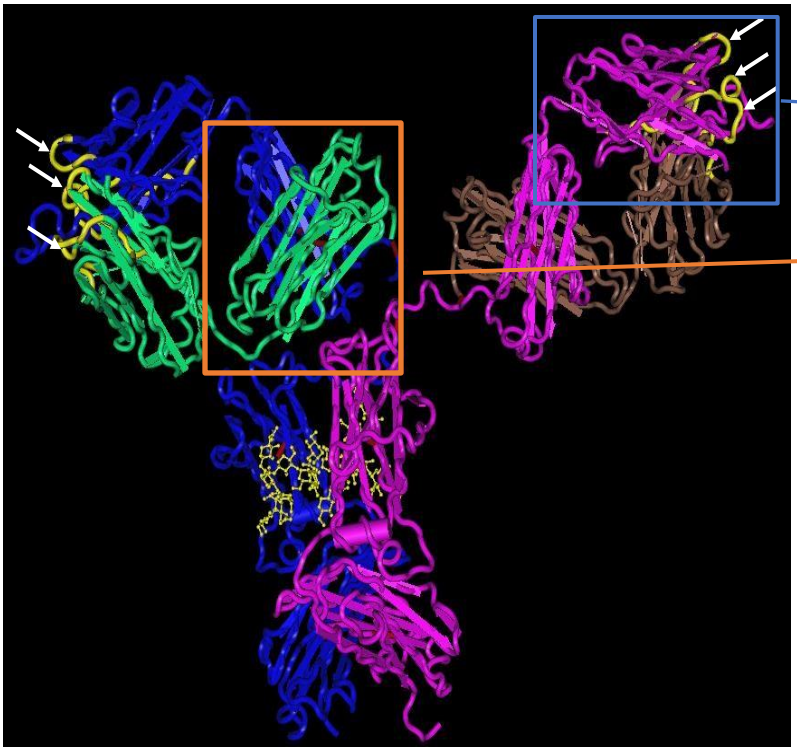
❖ Immunoglobins fold

- Regarding the structure of the hypervariable regions these are actually represent loops exist as a specialized domain (super secondary structure) called **"Immunoglobulin fold",** which is a motif that is present in every immunoglobulin.
- They are presented in every Immunoglobulin.
- The hypervariable regions are specifically in three loops connecting the β sheets to each other.
- Loops generally do not have a very fixed structure so they can move easily that's why they are hypervariable (meaning that they change their location, position and shape)
- **It consists of a sandwich of two anti-parallel β sheets held together by a disulfide bond making a shape of a barrel, hence known as "beta barrel".**

3 β-sheets that go antiparallel to each other and connected via disulfide bonds



- zoom in the structure of the antibody:



Hypervariable regions represented by loops

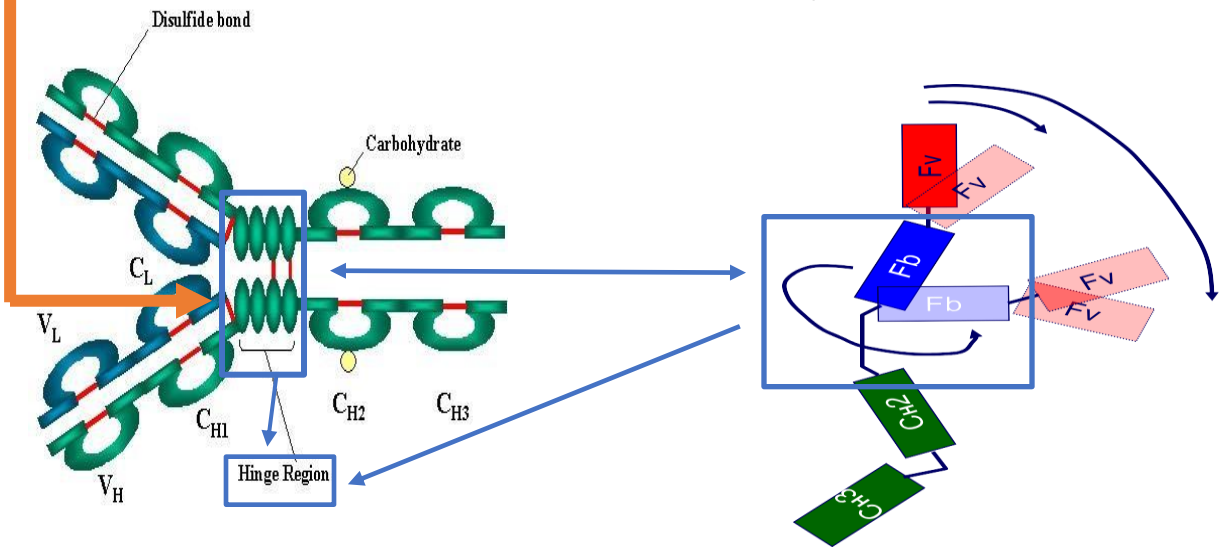
β-sheets represent immunoglobins folds

❖ Diversity

- Antigen-antibody binding is mediated by noncovalent interactions, that is relatively weak, so there must be a sort of specificity so we guarantee this specific antibody bind to the antigen related to it.
  - **how we can create a very large number of antibodies that can be part of the immunological response for very wide variety of microbes, antigens?**
    - The enormous diversity of antigen-binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops, so if they are short or long that would give them different structures, different shapes and that adds to the diversity of the antibodies.
- The overall three-dimensional structure necessary for antibody function remains constant.

❖ Hinger Region

- There is a region in the antibody structure where the arms of the Y-shaped molecule meet the stem of it.
- A hinge region exists where the arms of the antibody molecule forms a Y.
- It adds some flexibility to the molecule as it's made of flexible molecules, so that allows the bend of that molecule easily.



❖ Diversity of antibodies

- Each individual is capable of producing more than 10^{11} different antibody molecules.
- This is done via (here we are going to make more use for the limited number the antibody genes and create a wider and more diverse set of antibodies):

- DNA rearrangement of the different genes
- Imprecise joining of different regions within the genes (just like creating some types of mutations and that would definitely affect the resulting proteins creating a different types of antibodies)
- Addition/deletion of nucleotides during rearrangement and that also change the whole product of that gene.
- Somatic hypermutation in which some nucleotides even if it is just one nucleotide is change within the DNA and this would result in kind of changes in the messenger RNA and then changing in the resulting products

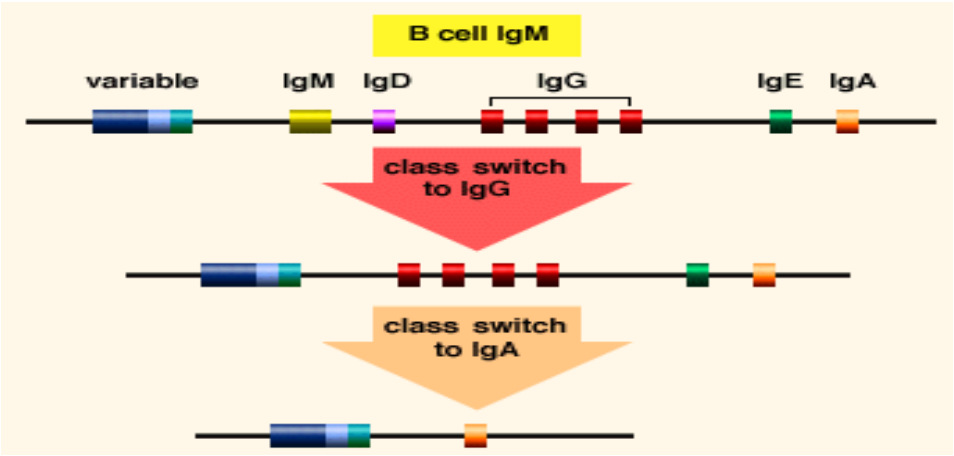
- *Remember: molecular biology last semester*

❖ More diversity

- By having different types of light and heavy chains: there are two "light" chains (lambda or kappa), There are five "heavy" chains (alpha, delta, gamma, epsilon or mu) that make five types (isotopes) of immunoglobulins known as immunoglobulins isotype (IgA, IgD, IgG, IgE, IgM).

❖ Class switching

- B cells that is in a resting state, before binding to any antigen, contain IgM type of antibodies only, (Molecules).
- Then once antigen binds it gets activated and class switching occurs
- DNA rearrangement as a mechanism is involved and it's going to change the heavy chain constant region and this would result a production of other types of antibodies like IgG, IgA, and IgE.



Types of antibodies

Isotype	Structure	Notes
IgM		Contain mu heavy chains Expressed on the surface of B-cells Found primarily in plasma cells The first antibodies produced in significant quantities against an antigen Promote phagocytosis and activate the complement system that leads to cell killing Appear usually as pentamers
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 Present on surface of B-cell that have not been exposed to antigens
IgE		Heavy chains type epsilon A monomer Plays an important role in allergic reactions
IgA		Contain alpha chains Found mainly in mucosal secretion The initial defense in mucosa against pathogen agents Appear usually as dimers

Notes related to the table: here you will find mostly the same information of the table above except that there is clarification for some points:

❖ IgM:

- it's the most important one
- presented in the surface of inactive beta cells
- It's involved in activity of cells to form phagocytosis, activating the complement system that leads to killing of cells as a part of immune response.
- Then as we said before binding of antigens to IgM antibodies results in class switching by DNA rearrangement of the heavy chain's constant region gene

➢ IgG:

- it Contains gamma heavy chains, Present as monomers, they aren't associated with the membrane
- Most abundant immunoglobulin in sera (600-1800mg/dL)
- promote phagocytosis and activate the complement system Only kind of antibodies that can cross the placenta to the blood of the fetus.

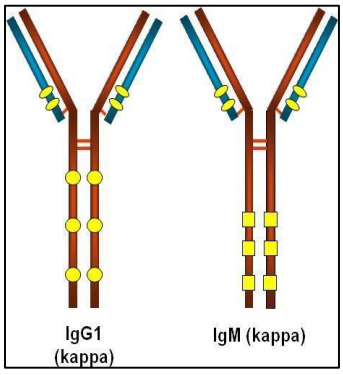
- IgD:
- it Contains delta heavy chains
- Present on surface of B-cells that have not been exposed to antigen, that's why it didn't result by class switching.
- Remember: IgG, IgA, and IgE are resulted from class switching

- IgA:
- it Contain alpha chains
- found mainly in mucosal secretion, the initial defense in mucosa against pathogen agents, (they are important in helping the mucosal coverings/membranes to fight the pathogens they get exposed to)
- Also it Appears usually as dimers

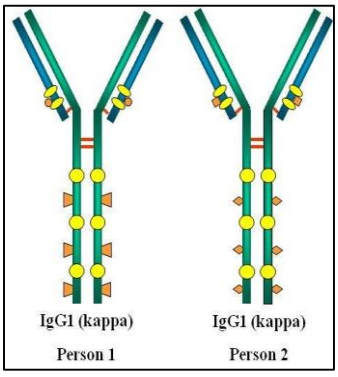
❖ Idiotype vs. isotypes vs. allotypes

❖ We will distinguish between these different terms

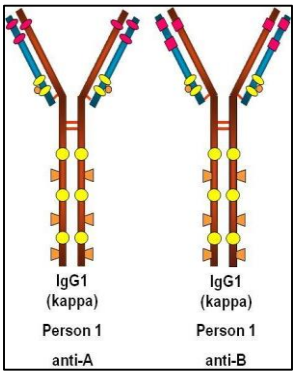
- First, Isotypes, the relationships between IgA, IgG, IgD etc.. these different classes of immunoglobulin depending on the changes and modification or class switching in the constant region of the heavy chains, the relationships is Isotypes
- Second, Immunoglobulin molecules that have different variable domains of both their light (VL) chains and heavy (VH) chains [or one of them] are said to share an idiotype.
- Third, Immunoglobulins belong to the same class but different among individuals of the same species due to different genetics are called: allotypes.



Isotypes



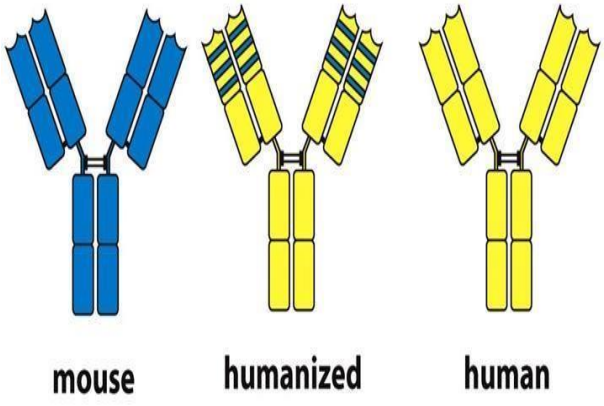
Allotypes

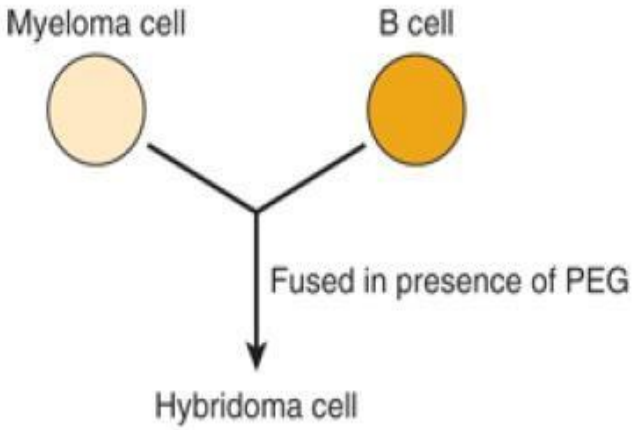


Idiotype

❖ Hybridoma and monoclonal antibodies

- ✓ Here topic we're going to discuss the production of monoclonal antibodies and what they mean
  - ➢ A monoclonal antibody is an antibody that is produced by an immortal B cell, and it's more specific in binding to its antigen in compare to polyclonal antibodies or different types of epitopes " parts of region of the antigen to which they bind ", So we need more specific binding that's why we look on to the better type of antibody for the uses we need. So, It's better to produce monoclonal antibodies than polyclonal antibodies.
  - ➢ **How can we produce monoclonal antibodies?**
  - first, by creating immortal B cells (لا تموت) which we can get from cancer cells, [cancer cells are immortal]
  - and hybridizing of a B cells with myeloma cell which is a blood cancer type, so once they hybrid with each other, the produce cells with the hybrid cell called hybridoma cells which are immortal ones
  - So, this hybridoma (immortal) cells can produce single/specific [monoclonal] antibodies.
- ➢ These monoclonal antibodies were made in mice by injecting their antigens in mice for example or any animal, to induce an immune response and then get these monoclonal antibodies extracted out of the mice , To be able to use these antibodies in human beings, we need them to be humanized for different reasons. then, they can be humanized by attaching the CDRs (because they are the important parts that bind to the antigen) into appropriate sites in a human immunoglobulin molecule
- ➢ now this molecule is a human molecule with these CDRs that are specific for binding to a certain antigen, a specific one, just a single antibody that binds to a specific antigen region to which binding happens





❖ Benefits of monoclonal antibodies

- Monoclonal antibodies have different uses, for example some of these uses can be seen in the medical labs, to measure the amounts (concentrations) of many individual proteins and molecules (e.g. plasma proteins, steroid hormones), present in different ceps and they are going to bind to these molecules and induce certain changes (such as color change), indicating the concentration of these molecules in the patient's sample.
- They determine the nature of infectious agents (e.g. types of bacteria) so we can determine the type of bacteria that infects that person by identifying certain antigen by these monoclonal antibodies in the lab
- They are used to direct therapeutic agents to tumor cells, "cancer treatment, in immunotherapy instead of chemotherapy or radiation, by monoclonal antibodies, for some types of cancers and some patients.
- They are used to accelerate removal of drugs from the circulation when they reach toxic levels.
- (These monoclonal antibodies can bind to these toxic drugs in the blood and damage them and accelerate their removal out of the patient’s blood)