



Globular proteins

Immunoglobulins

Prof. Mamoun Ahram

Dr. Diala Abu Hassan

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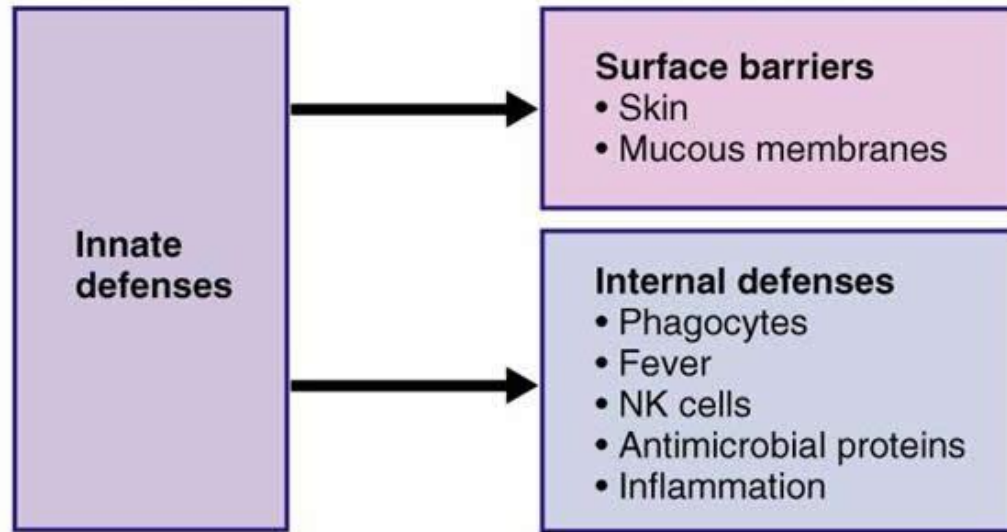
Resources

This lecture

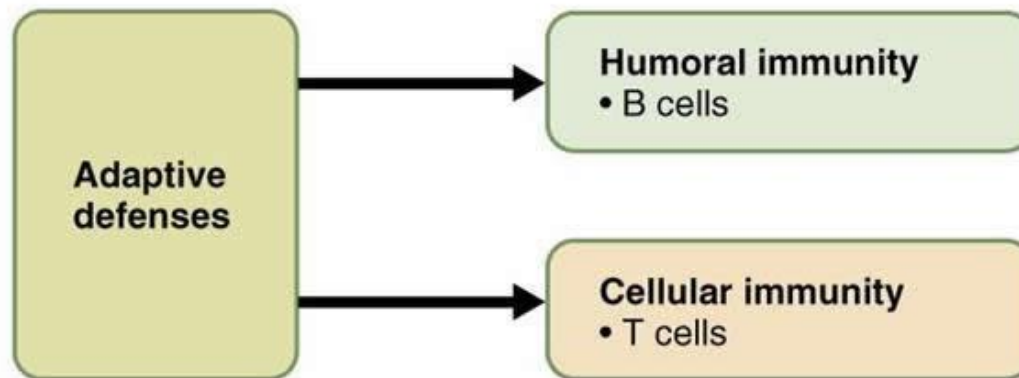
Campbell and Farrell's Biochemistry, Chapters 14 (pp. 421-422)

<http://www.ncbi.nlm.nih.gov/books/NBK26884/#A4469>

Types of immunity



(a)



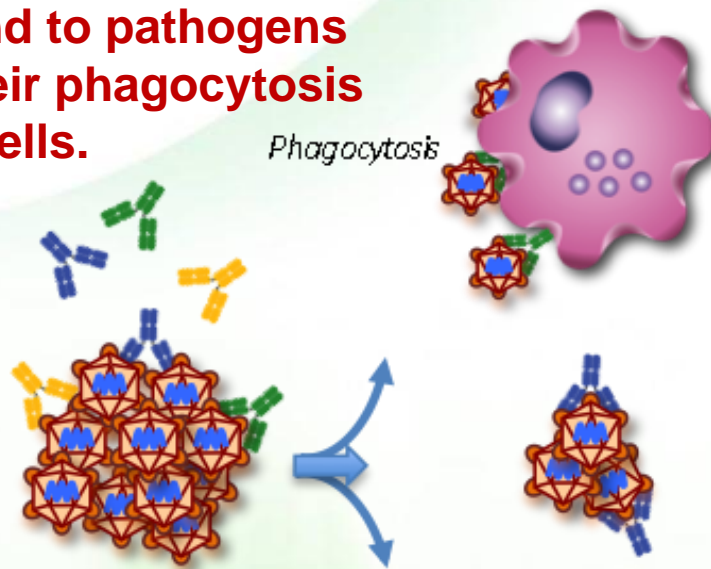
(b)

How do B cells work?

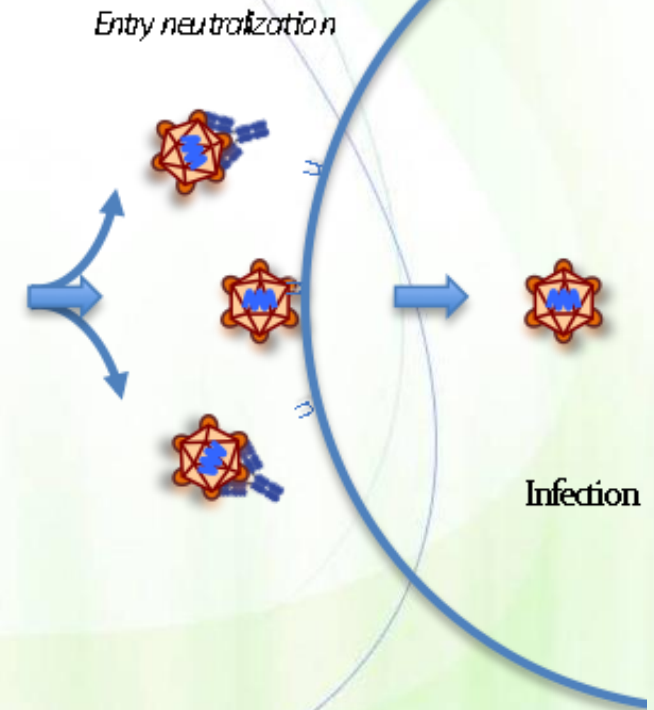


- B cells secrete immunoglobulins (also known as antibodies).
- Immunoglobulins have three roles:

Antibodies bind to pathogens and induce their phagocytosis into immune cells.



Antibodies bind to viruses and microbial toxins neutralizing them.



Antibodies recruit white blood cells and a system of blood proteins to lyse pathogens (complement system).

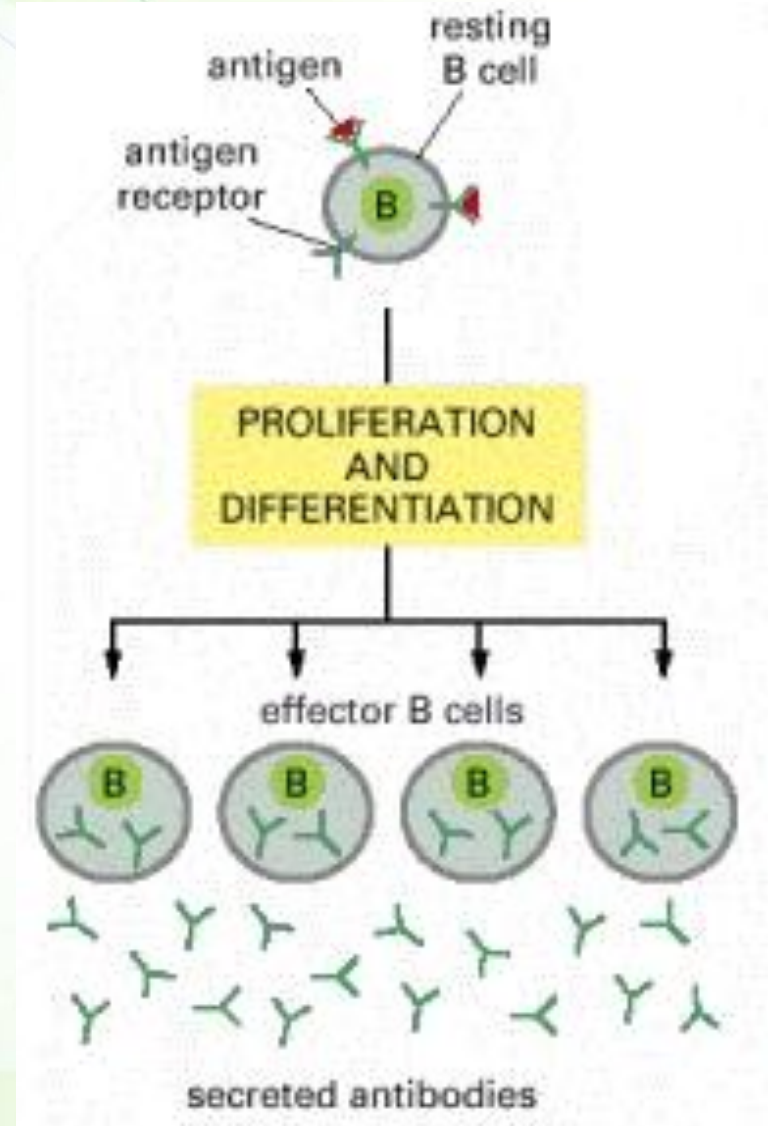
Complement



When B cells recognize an antigen...



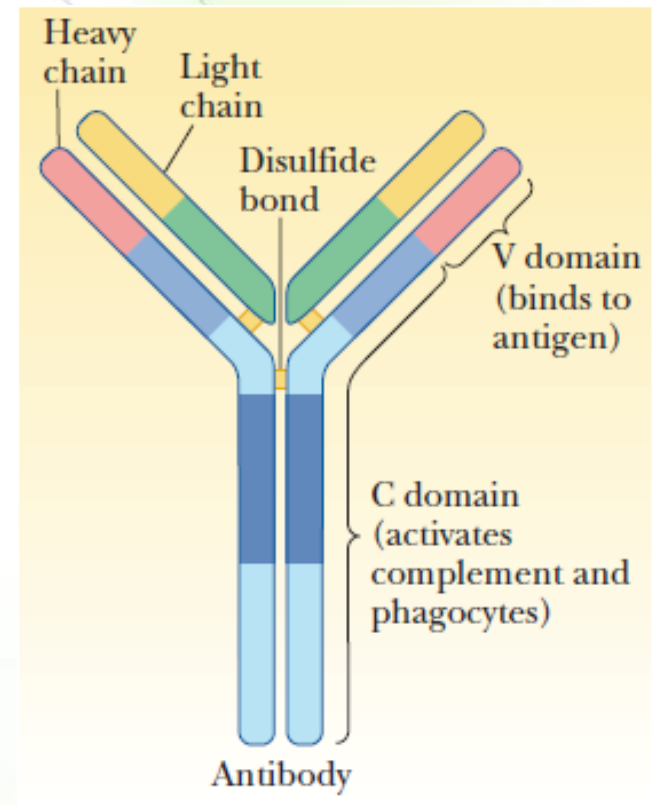
- When B cell is activated by antigen, it proliferates and differentiates into an antibody-secreting effector cell.
- Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibody at a rate of about 2000 molecules per second.



Structure of antibodies



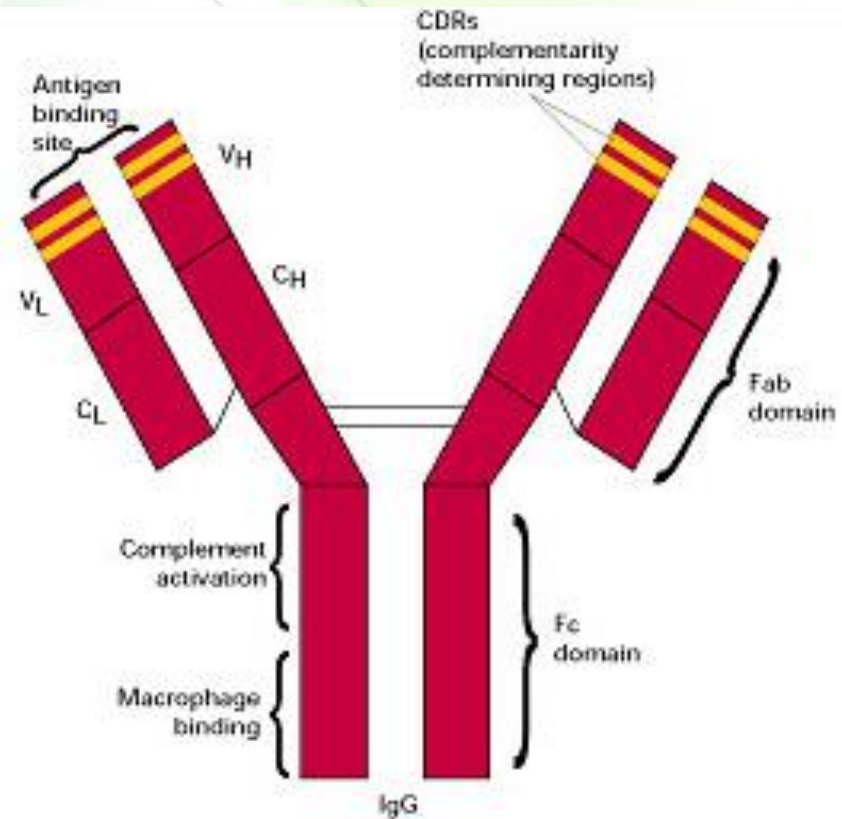
- Antibodies are Y-shaped molecules consisting of two identical heavy chains and two identical light chains held together by disulfide bonds.
- The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds
- Within each of the polypeptide chains there are also intra-chain disulfide bonds.
- They are glycoproteins, with oligosaccharides linked to their heavy chains.



Antibody regions



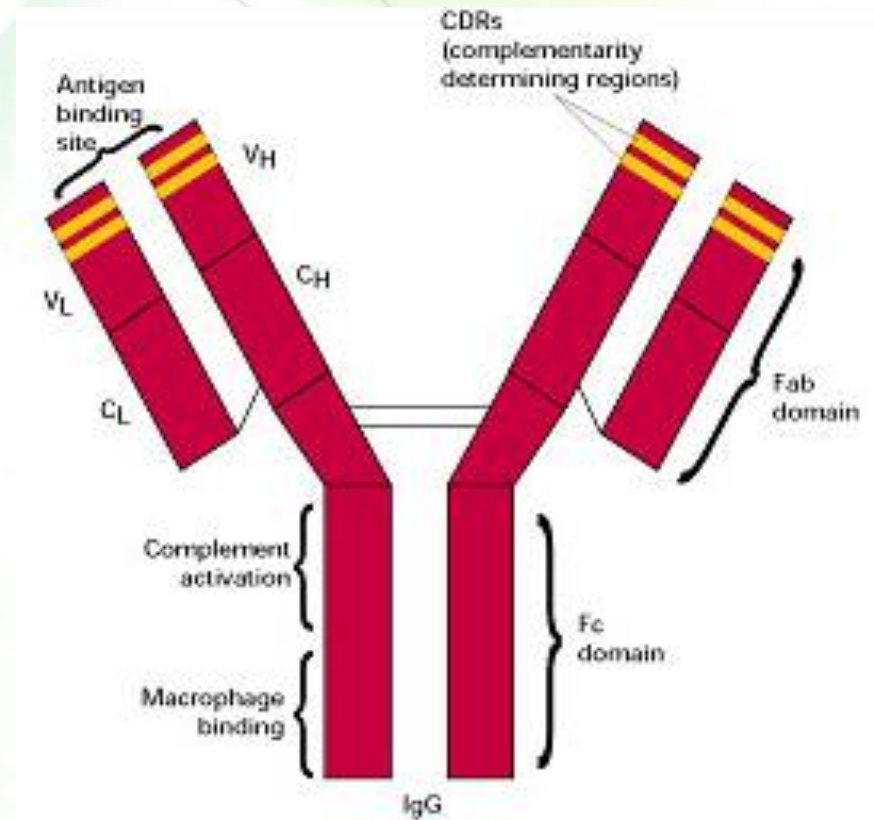
- A light chain consists of one variable (V_L) and one constant (C_L) domain.
- The heavy chain consists of one variable region (V_H) and three constant regions (C_{H1} , C_{H2} , and C_{H3}).
 - V_L and C_L pair with V_H and V_H .



Constant regions



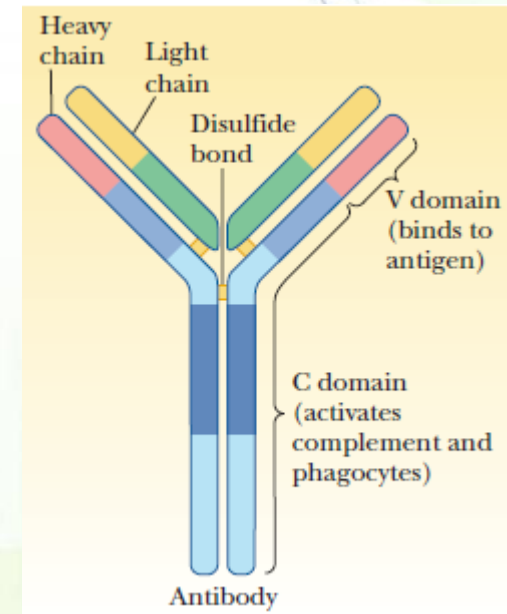
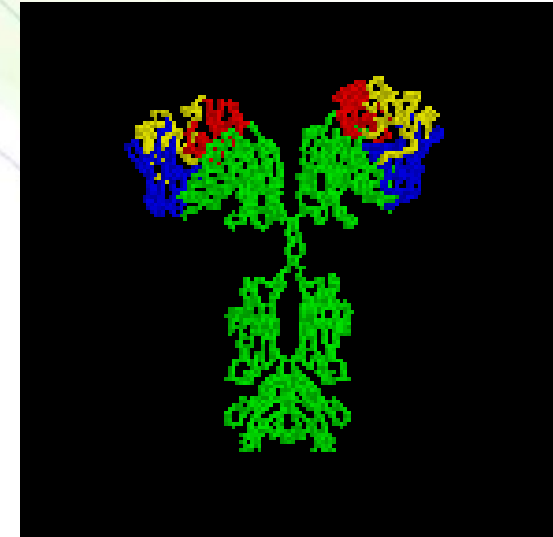
- Constant regions, are uniform from one antibody to another within the same isotype.
- The Fc domain of antibodies are important for binding to phagocytic cells allowing for antigen clearance.



Variable regions



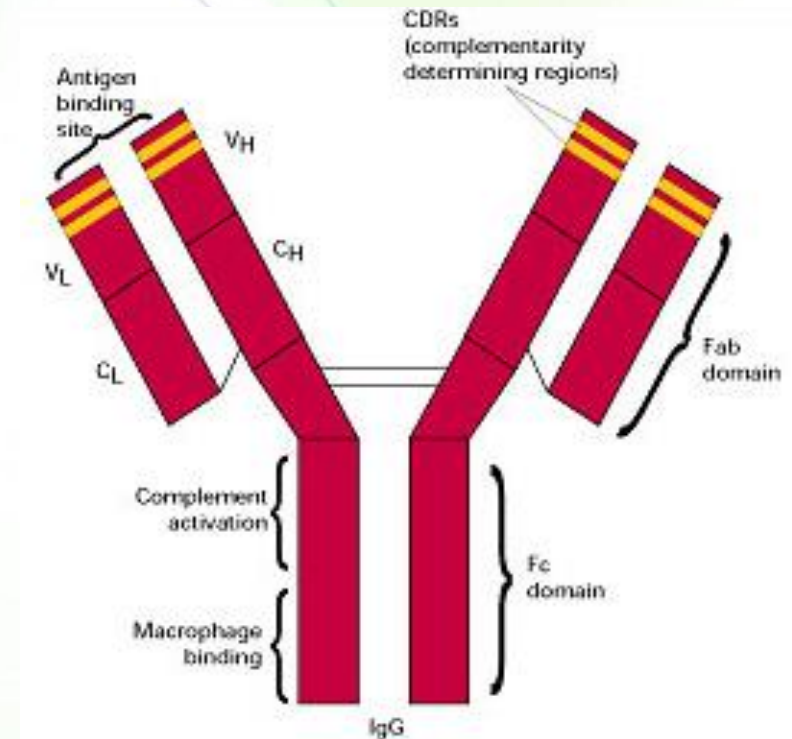
- The variable region is found at the tips of the Y and is the part of the antibody that binds to part of the antigen (called **epitope**).
- Each antibody can bind to two antigens.
- The primary sequences of the variable regions among different antibodies are quite distinct.
 - **About 7-12 amino acids in each one that contribute to the antigen-binding site**
- Each B cell produces only one kind of antibody.



Hypervariable" regions



- Hypervariable" regions, or "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains.
- These regions serve to recognize and bind specifically to antigen with high affinity (dissociation constant (K_d) 10^{-12} - 10^{-7}).

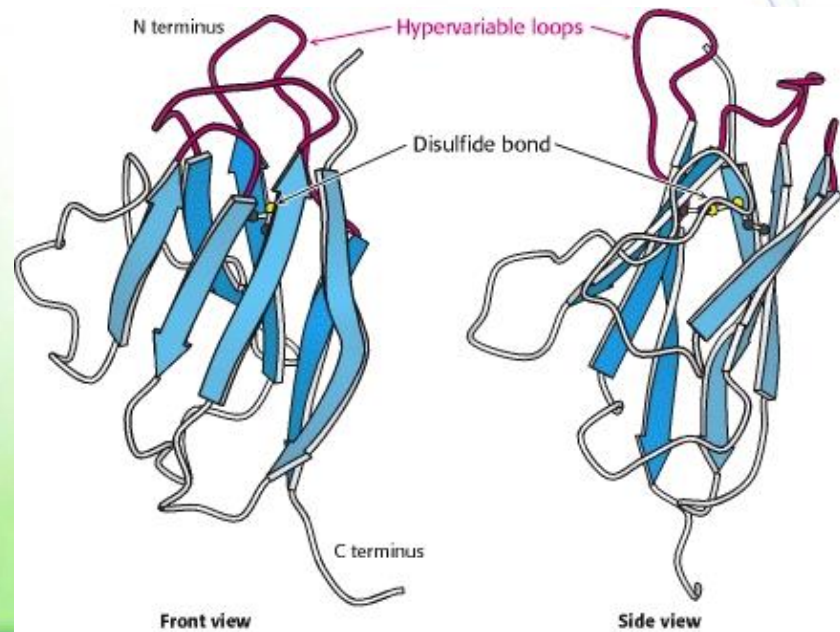


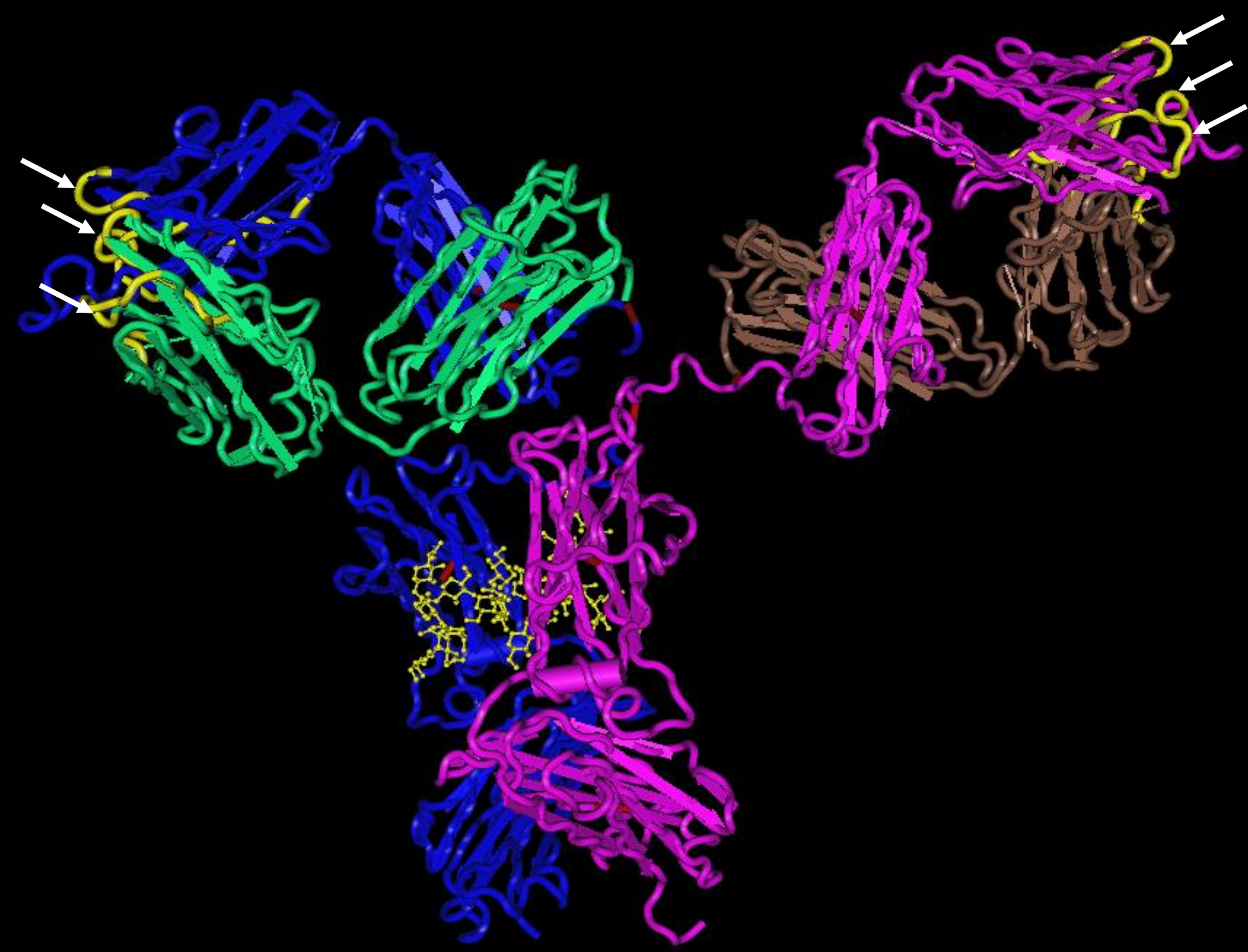
Immunoglobulin fold



- The hypervariable regions exist in a specialized domain called “Immunoglobulin fold”, which is a domain that is present in every immunoglobulin.
- The hypervariable regions are specifically in three loops connecting the β sheets to each other.

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”.



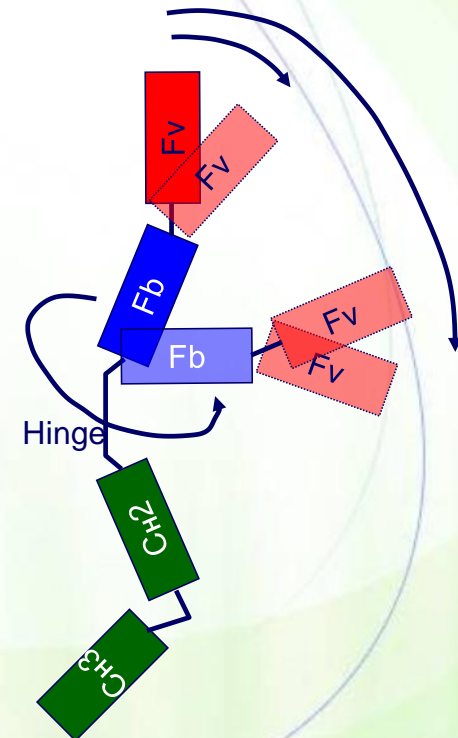


Diversity



- Antigen-antibody binding is mediated by noncovalent interactions.
- 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.

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- The diagram illustrates a protein structure with several key features labeled:
- Disulfide bond:** Indicated by red lines connecting different parts of the protein chain.
 - Carbohydrate:** Represented by yellow circles attached to the protein chain.
 - Hinge Region:** A specific area of the protein structure, highlighted with a bracket and labeled.
 - Labels:** C_L , V_L , C_{H1} , V_H , C_{H2} , and C_{H3} are used to identify specific residues or regions within the protein structure.



Diversity of antibodies



- Each individual is capable of producing more than 10^{11} different antibody molecules.
- This is done via
 - DNA rearrangement
 - Imprecise joining of regions
 - Addition/deletion of nucleotides during rearrangement
 - Somatic hypermutation

Remember: molecular biology last semester

More diversity

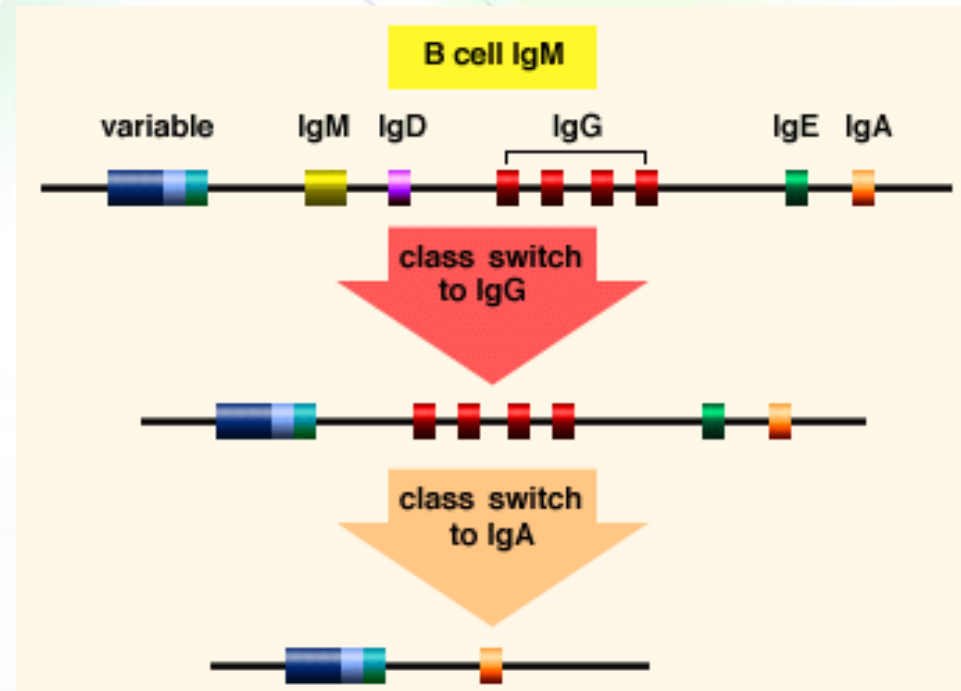


- There are two "light" chains (lambda or kappa),
- There are five "heavy" chains (alpha, delta, gamma, epsilon or mu) that make five types of immunoglobulins known as immunoglobulins isotype (IgA, IgD, IgG, IgE, IgM).

Class switching

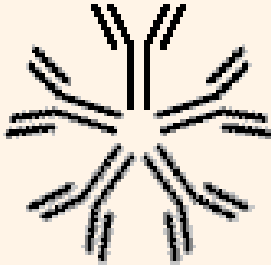
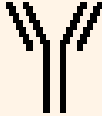
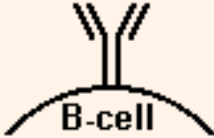
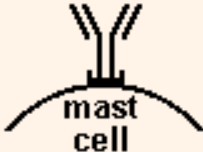
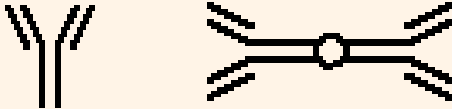


- Before binding antigen, B cells contain IgM molecules only.
- Following antigen binding, class switching occurs.
- Class switching refers to a DNA rearrangement changing the heavy chain constant gene .
- That causes production of IgG, IgA, and IgE.



Types of antibodies

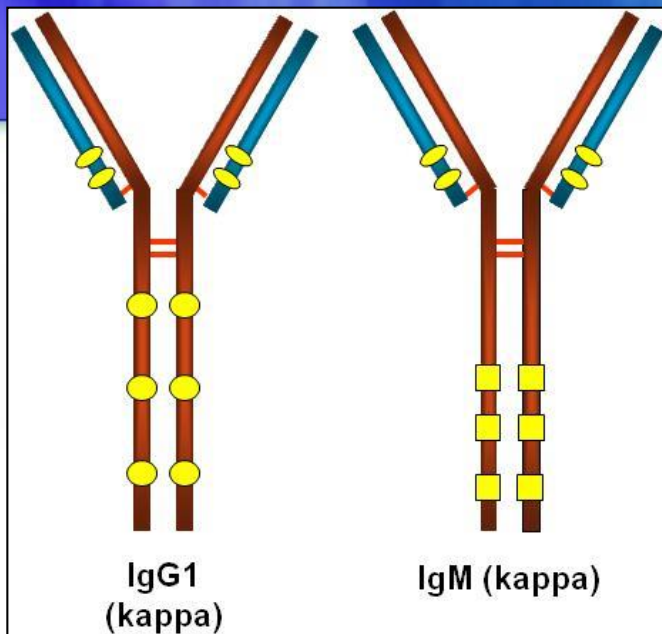


Isotype	Structure	Notes
IgM		<p>Contain mu heavy chains</p> <p>Expressed on the surface of B-cells</p> <p>Found primarily in plasma cells</p> <p>The first antibodies produced in significant quantities against an antigen</p> <p>Promote phagocytosis and activate the complement system that leads to cell killing</p> <p>Appear usually as pentamers</p>
IgG		<p>Contains Gamma chains</p> <p>Monomers</p> <p>Most abundant immunoglobulins in sera (600-1800 mg/dL)</p> <p>Promote phagocytosis and activate the complement system</p> <p>Only kind of antibodies that can cross the placenta</p>
IgD		<p>Contains delta heavy chains</p> <p>Present on surface of B-cell that have not been exposed to antigens</p>
IgE		<p>Heavy chains type epsilon</p> <p>A monomer</p> <p>Plays an important role in allergic reactions</p>
IgA		<p>Contain alpha chains</p> <p>Found mainly in mucosal secretion</p> <p>The initial defense in mucosa against pathogen agents</p> <p>Appear usually as dimers</p>

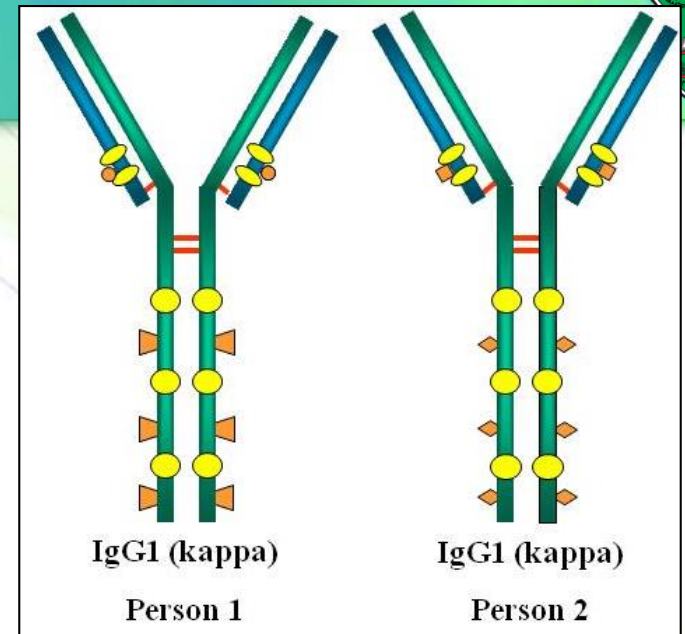
Idiotype vs. isotypes vs. allotypes



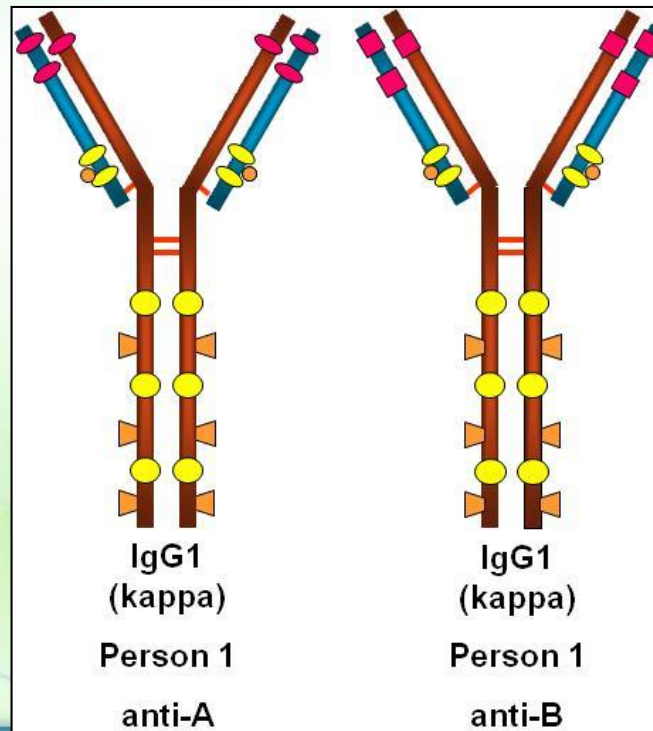
- immunoglobulin molecules that have different variable domains of both their light (VL) chains and heavy (VH) chains and are said to share an **idiotype**.
- The different classes of immunoglobulins are determined by their different C_H regions and called **isotypes**.
- Immunoglobulins of the same class but different among individuals of the same species due to different genetics are called **allotypes**.



isotypes



allotypes

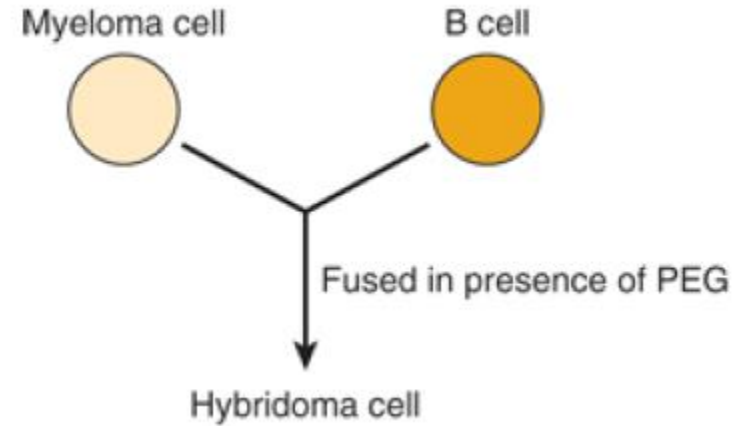


idiotype

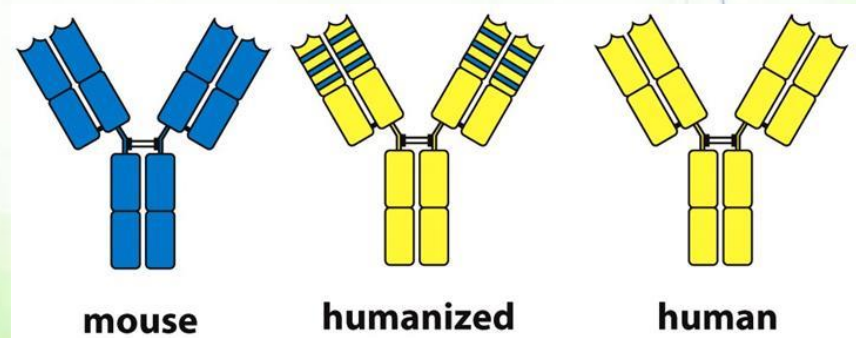
Hybridoma and monoclonal antibodies



- 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 antigen.
- In order to “create” an immortal B cell that produces a single antibody (monoclonal), a B cell hybridizes with a B cancer cell (myeloma).



Monoclonal antibodies made in mice can be humanized by attaching the CDRs onto appropriate sites in a human immunoglobulin molecule.



Benefits of monoclonal antibodies



- Measure the amounts of many individual proteins and molecules (e.g. plasma proteins, steroid hormones).
- Determine the nature of infectious agents (e.g. 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.