Globular proteins

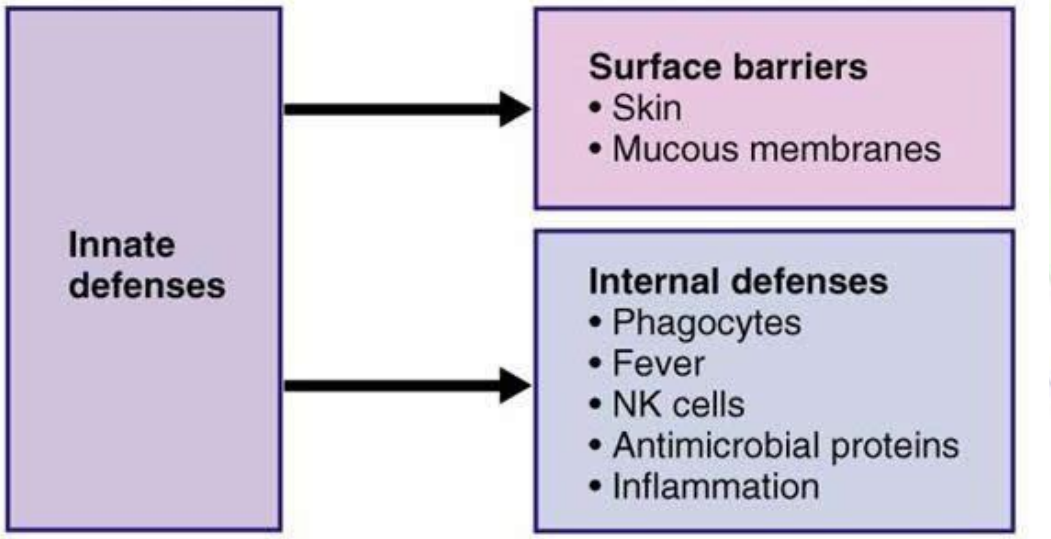
Immunoglobulins

This lecture

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

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

Types of immunity

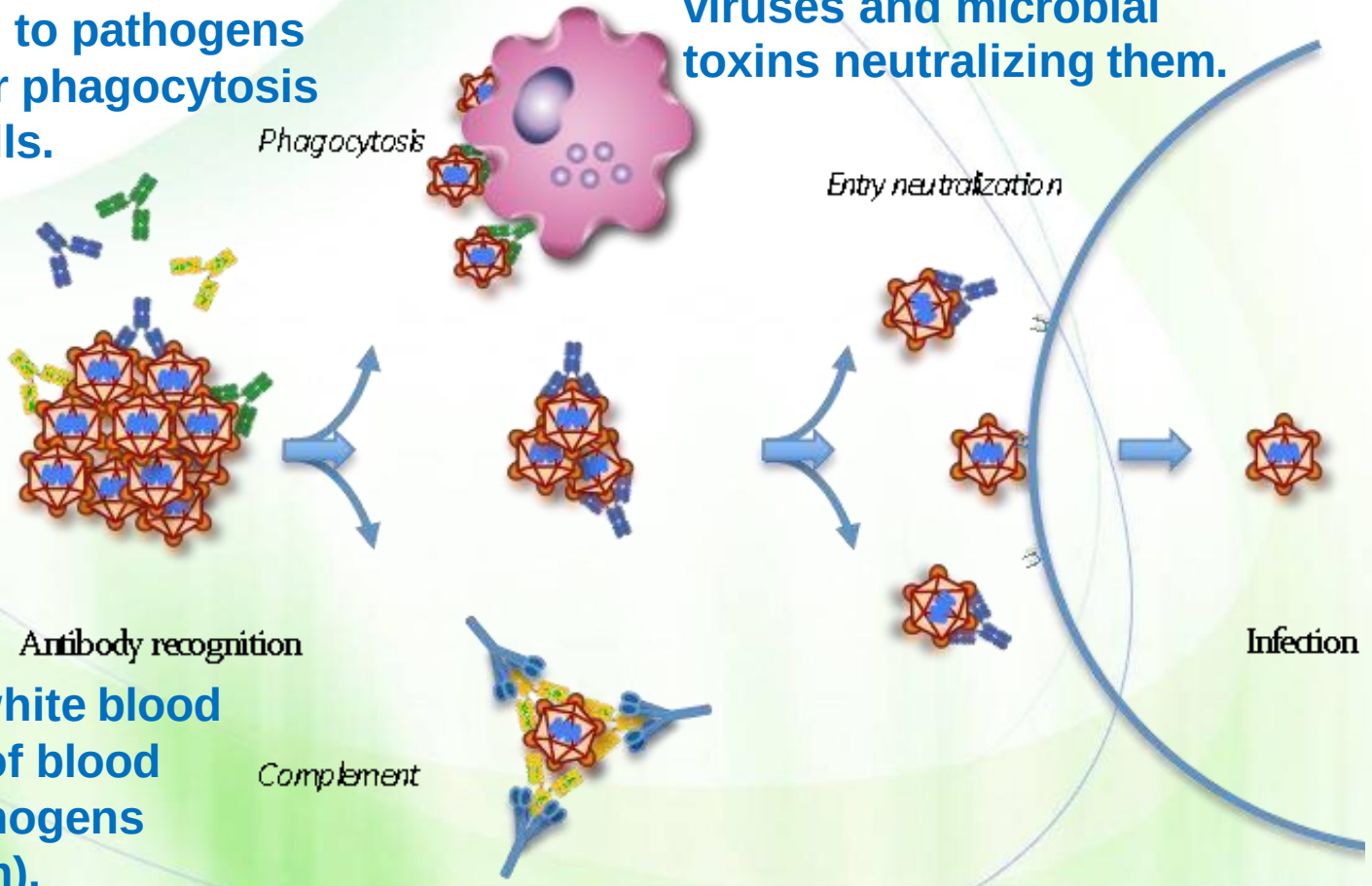


How do B cells work?

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

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

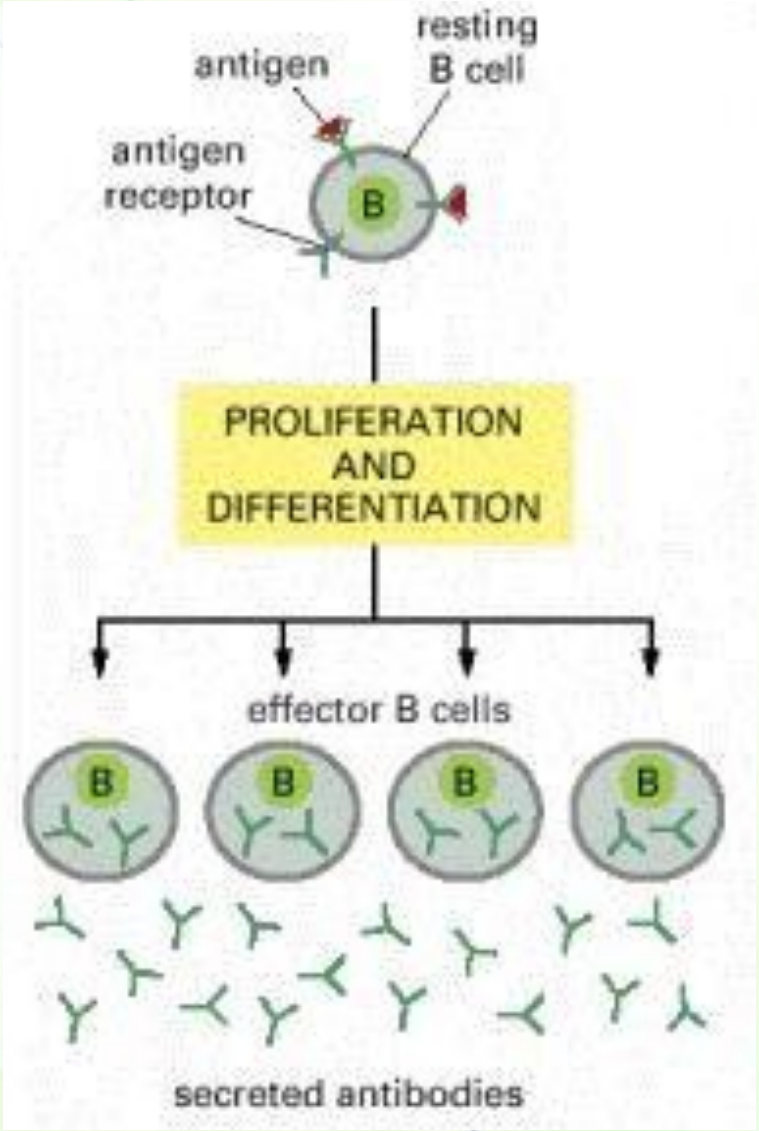
Antibodies bind to viruses and microbial toxins neutralizing them.



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

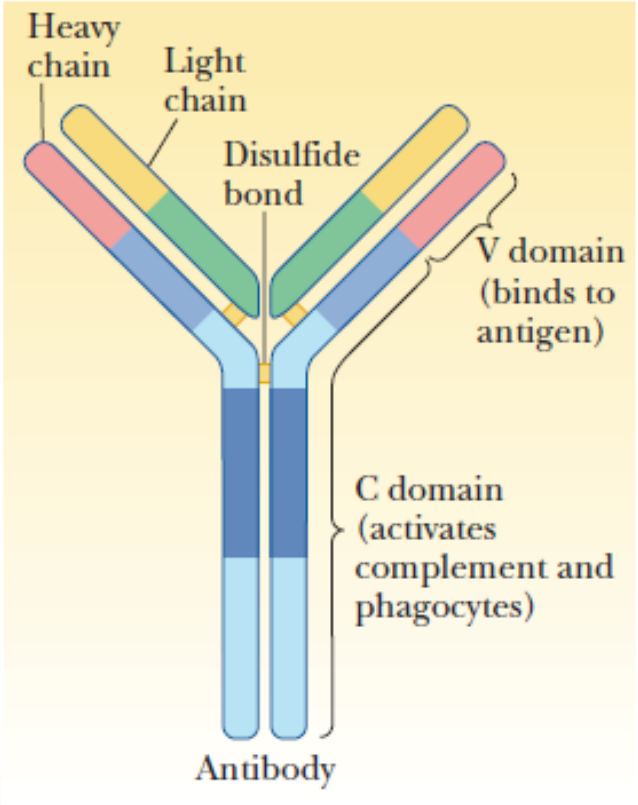
When B cells recognize an antigen…

- 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.



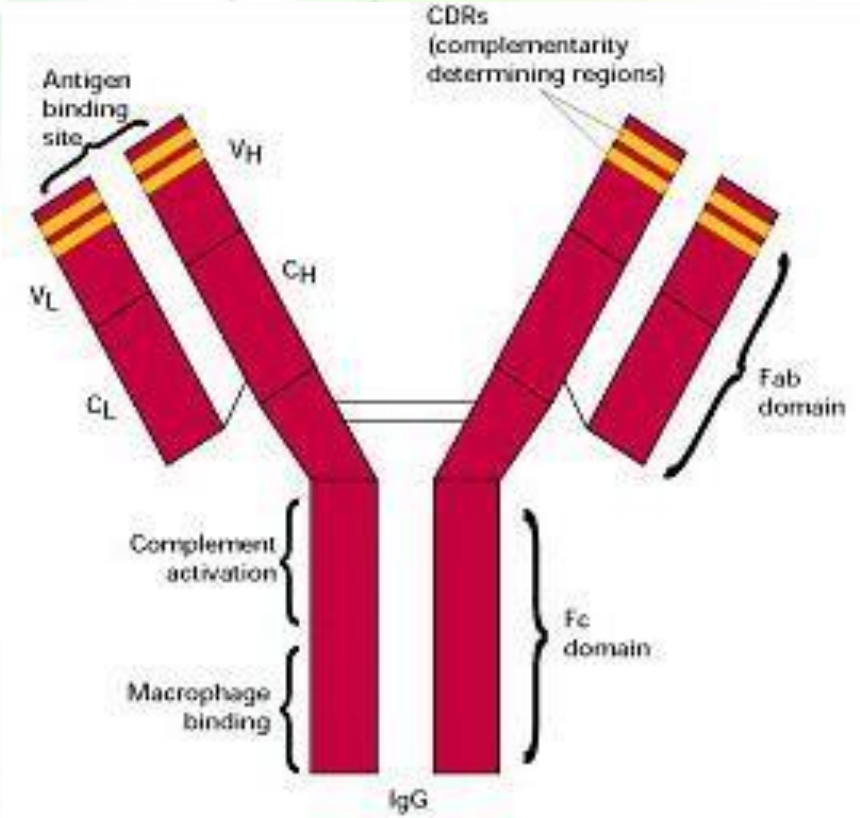
Structure of antibodies

- 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.



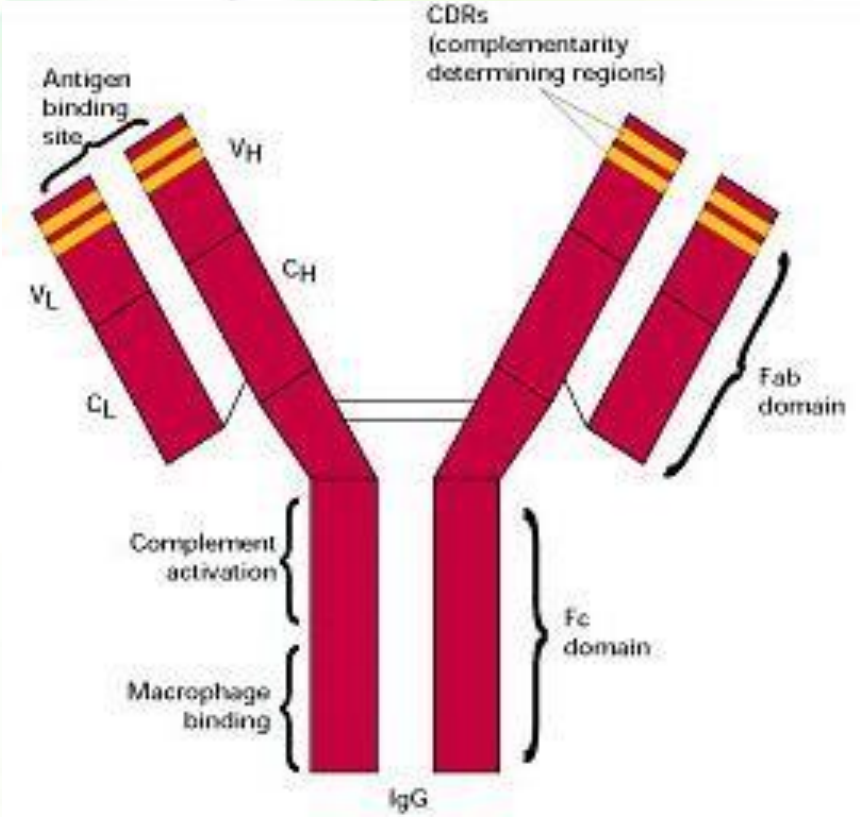
Antibody regions

- 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 C_H .



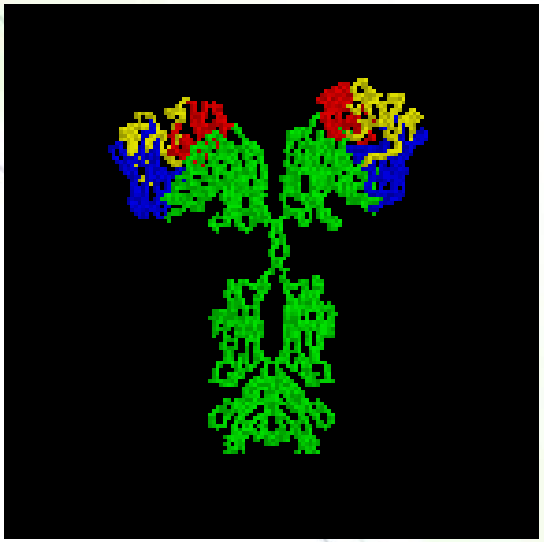
Constant regions

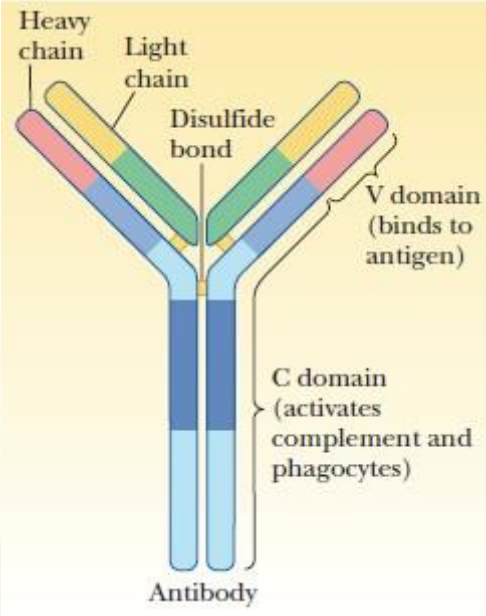
- Constant regions, are uniform from one antibody to another within the same isotype.
- The Fc domain of antibodies are important for binding to phagocytic cells allowing for antigen clearance.



Variable regions

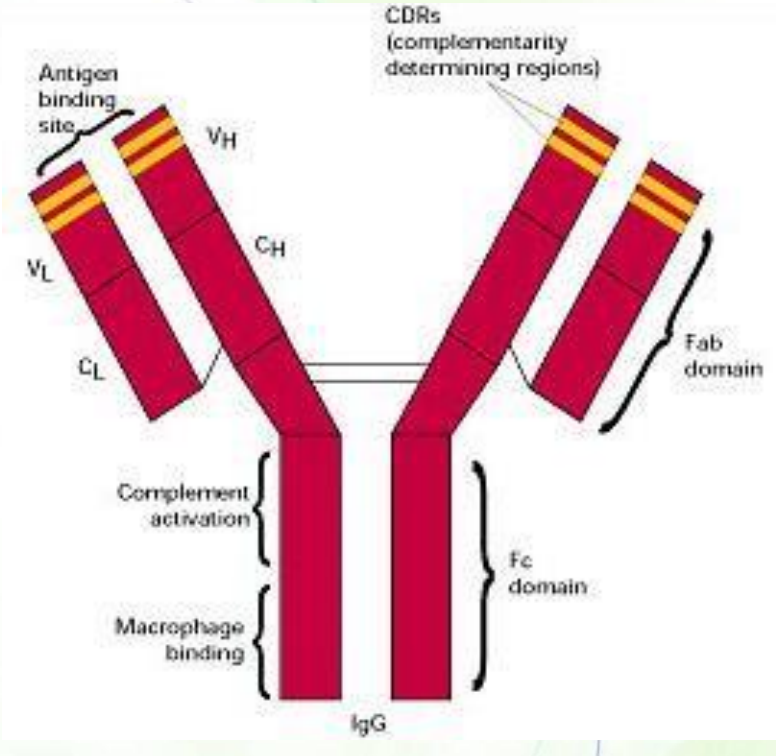
- The variable region is found at the tips of the Y and is the part of the antibody that binds to part of the antigen (called **epitope**).
- Each antibody can bind to two antigens.
- The primary sequences of the variable regions among different antibodies are quite distinct.
  - **About 7-12 amino acids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





Hypervariable" regions

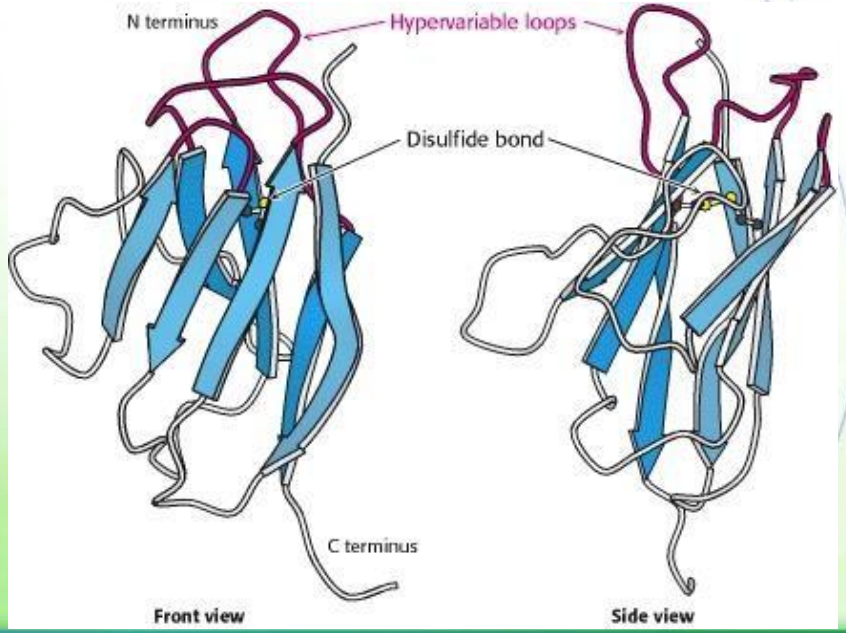
- Hypervariable" regions, or "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains.
- These regions serve to recognize and bind specifically to antigen with high affinity (dissociation constant (Kd) 10^{-12}-10^{-7}).

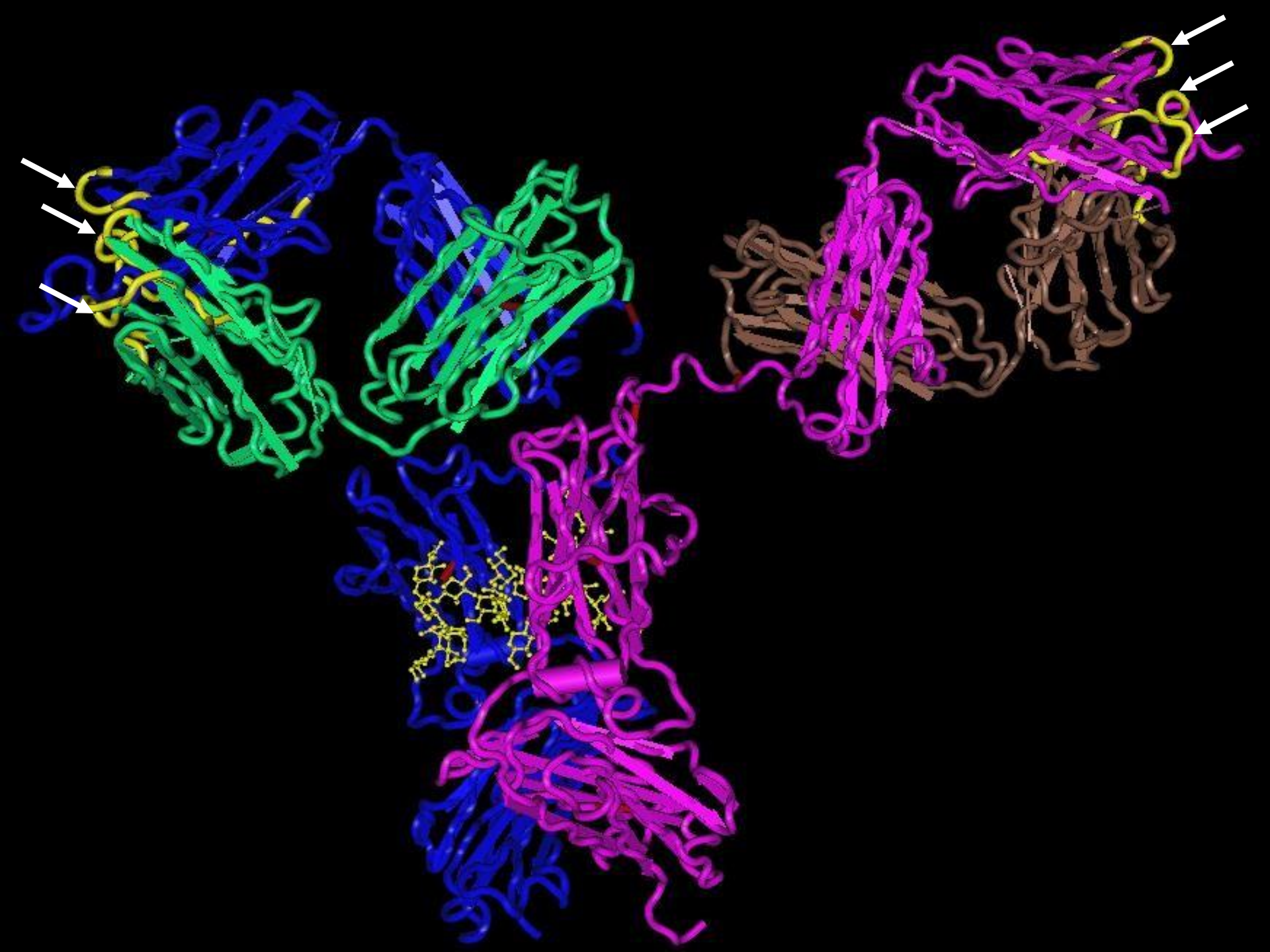


Immunoglobulin fold

- The hypervariable regions exist as a specialized domain called “Immunoglobulin fold”, which is a motif 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”.



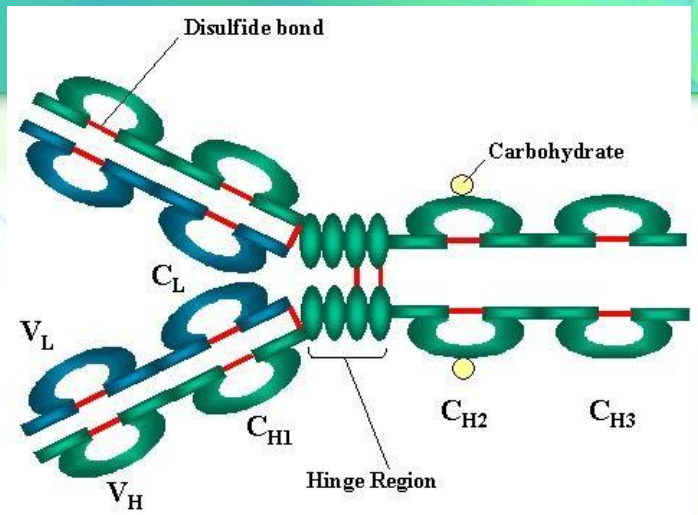


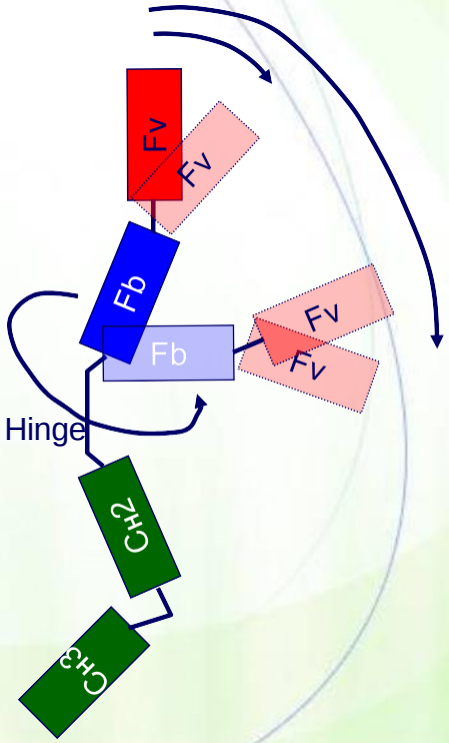
Diversity

- 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.

Hinge region

- A hinge region exists where the arms of the antibody molecule forms a Y.
- It adds some flexibility to the molecule.





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

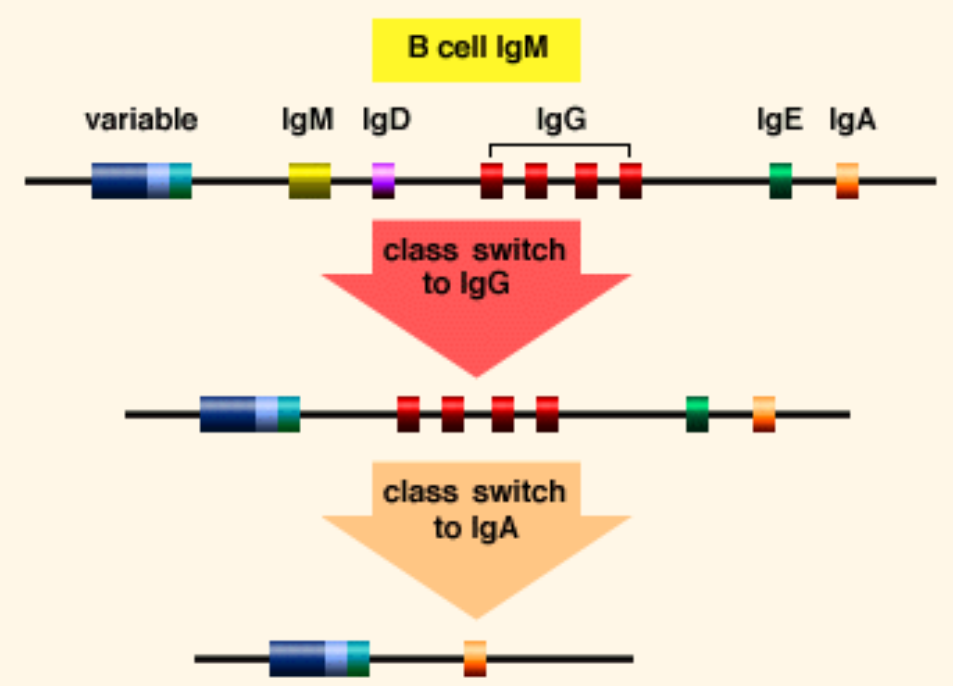
Remember: molecular biology last semester

More diversity

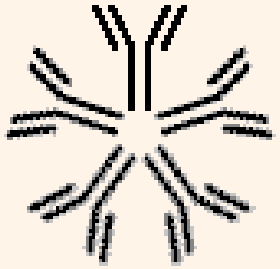
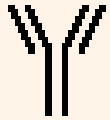
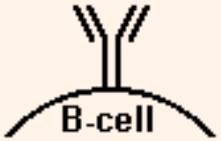
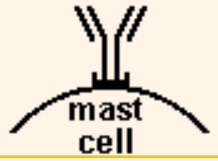
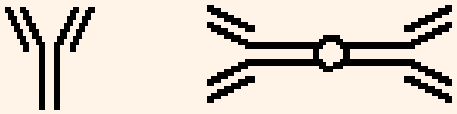
- There are two "light" chains (lambda or kappa),
- There are five "heavy" chains (alpha, delta, gamma, epsilon or mu) that make five types of immunoglobulins known as immunoglobulins isotype (IgA, IgD, IgG, IgE, IgM).

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

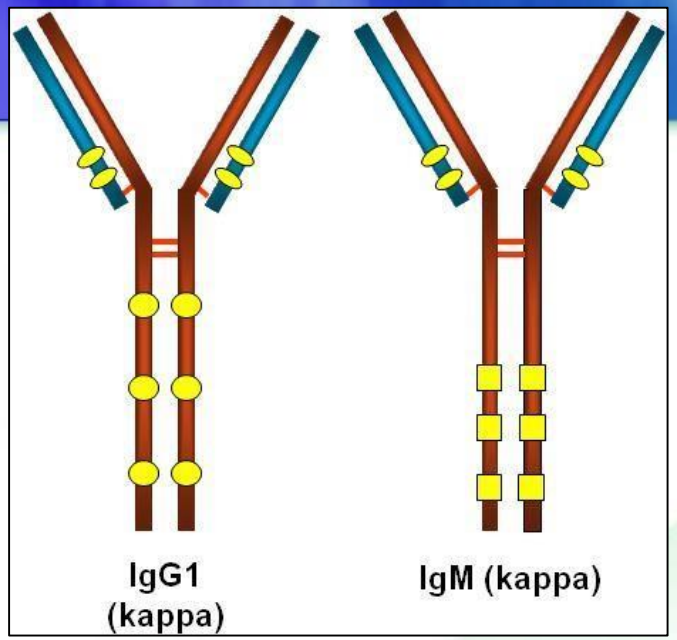
Isotype	Structure	Notes
IgM		Contain mu heavy chains Expressed on the surface of B-cells Found primarily in plasma cells The first antibodies produced in significant quantities against an antigen Promote phagocytosis and activate the complement system that leads to cell killing Appear usually as pentamers
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

Idiotype vs. isotypes vs. allotypes

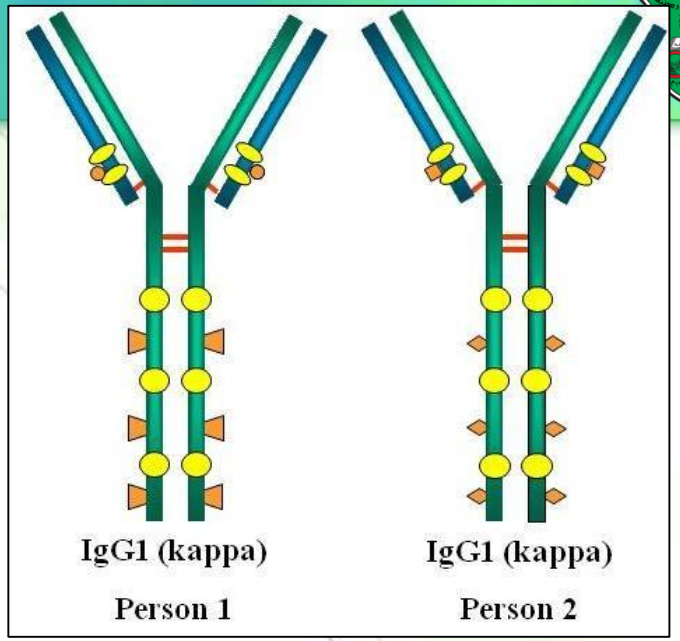
- Immunoglobulin molecules that have different variable domains of both their light (VL) chains and heavy (VH) chains and are said to share an **idiotype.**

- The different classes of immunoglobulins are determined by their different C_H regions and called **isotypes.**

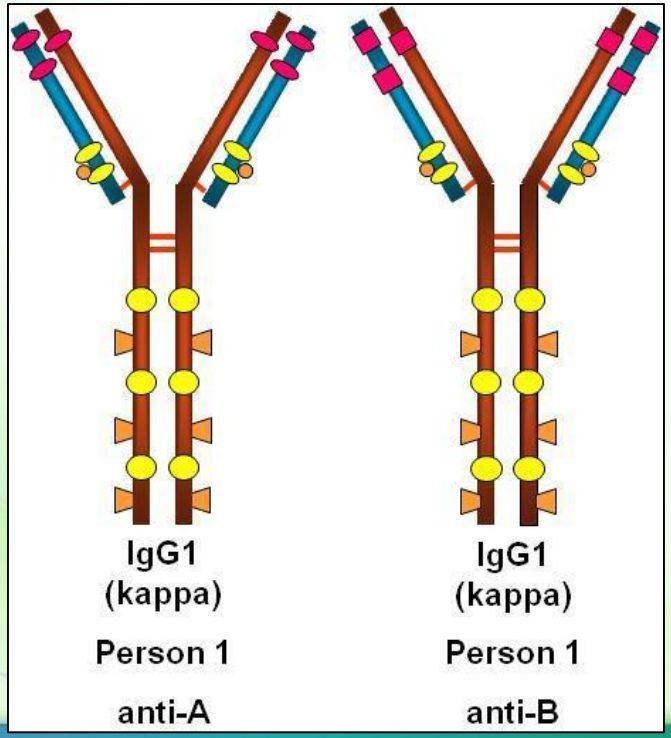
- Immunoglobulins of the same class but different among individuals of the same species due to different genetics are called **allotypes.**



isotypes



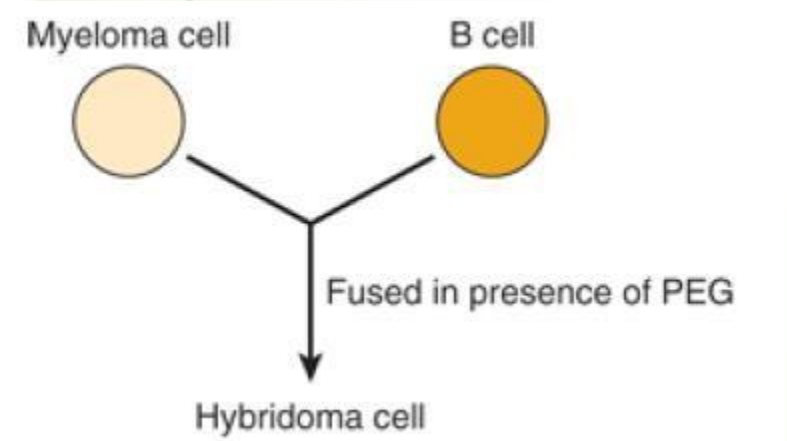
allotypes



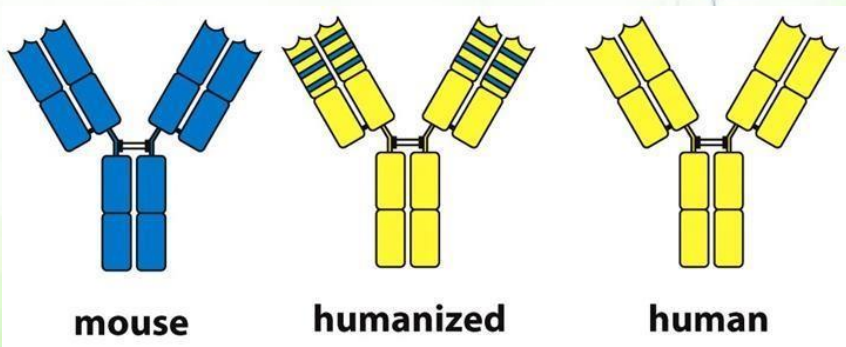
idiotype

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.