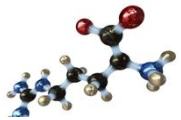
بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ



BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 10

Immunoglobulins

Written by : Lamar Khorma
Rand Alkhateeb

Reviewed by : Shahd Qteifan

Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Globular proteins

Immunoglobulins = Antibodies

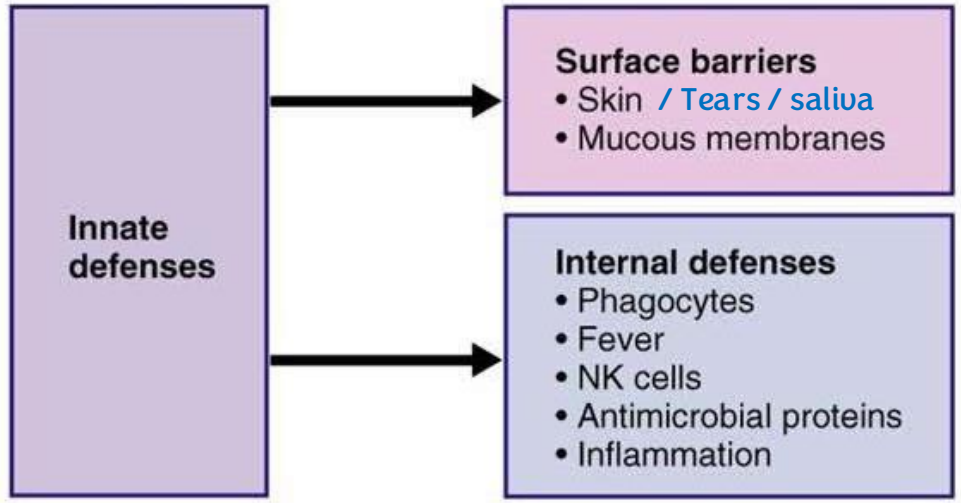
Professor Mamoun Ahram

When we talk about antibodies because they are biotherapeutics which depends on biology, a company cannot simply produce the exact same antibody. It may try to develop an antibody similar to it, but it will have different characteristics.
Actually, antibody-based immunotherapeutics represent both the present and the future. When you hear the term “immuno,” you should immediately think of antibodies.

Types of immunity

Defense against pathogens (like viruses, bacteria, ...) in general

General protection (army)



First-line defense (Physical; chemical) Non- specific

Second-line defense (Non-specific, inflammatory; phagocytic)

When the temperature increases slightly, the effect of the pathogen is reduced. So, this increase in temperature is part of the body's defense mechanism against pathogens.
Also, the inflammation is an alarm system.

Phagocytic cells :
Phago- = eating/engulf
, -cytic = cell
They recognize any foreign body and then eat it by bring it inside the cell, and break it down.

B cells are called B cells because they develop from the bone marrow.
They release immunoglobulin (antibodies) when they are activated.

Third-line defense (Specific)

There are a lot types of them

T cells are called T cells because they develop from the thymus الطُّحال.

There is adaptation depending on the pathogen that enters the body– whether it is a virus, bacteria, a specific type of bacteria, or a parasite–the body develops a system that is specific to that particular bacteria, virus, or parasite.This system changes depending on what enters our system.

How do B cells work?

- When a pathogen enters the body, B cells recognize the pathogen as an (antigen = foreign body).
- B cells produce (Immunoglobulin) antibodies. Antibodies are proteins which have a characteristic Y-shaped structure.

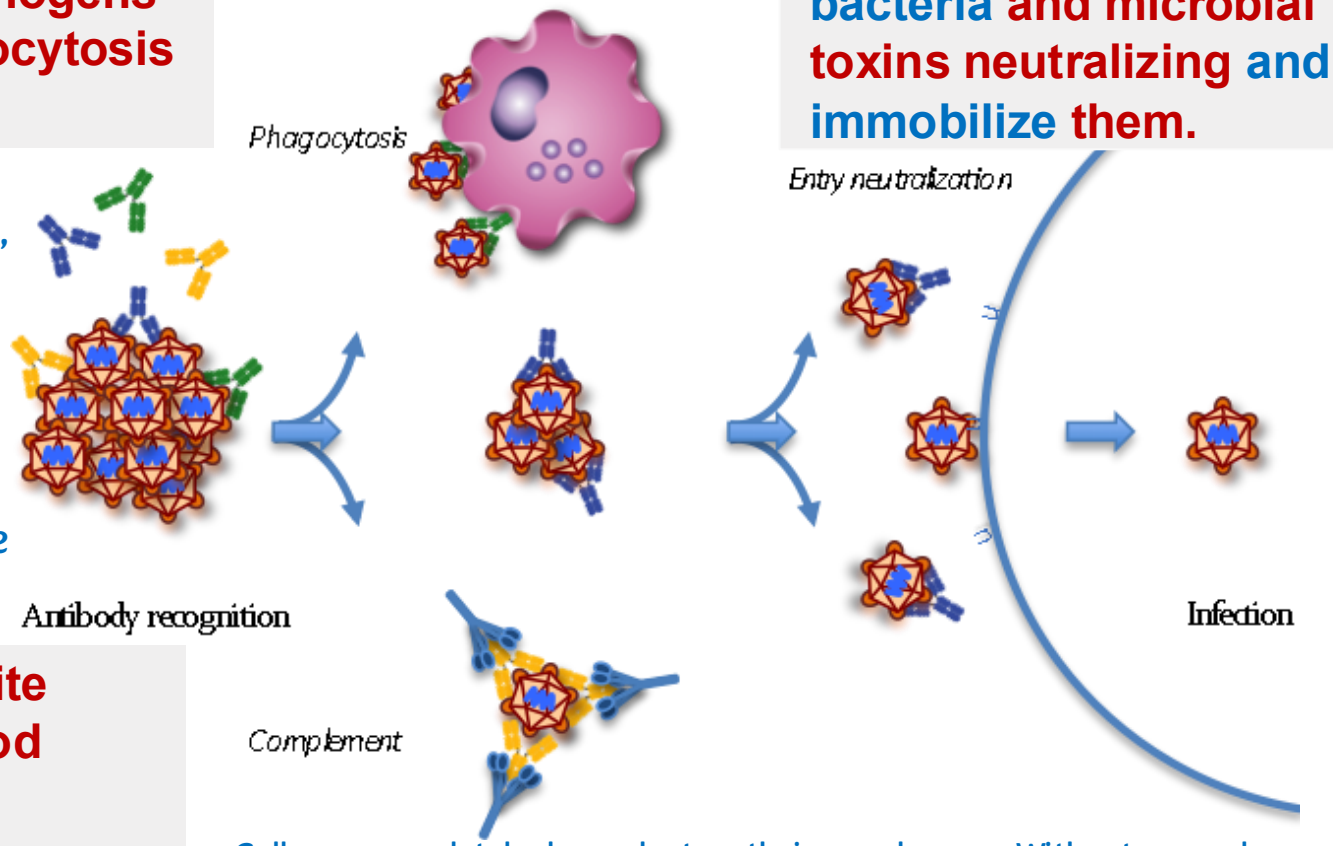
- B cells secrete immunoglobulins (also known as antibodies).
- Immunoglobulins have three roles:

Depends on the type of the B cells, the type of activation, the type of pathogen or the body condition

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

- These antibodies bind to the pathogens. Then, non-specific cells, such as phagocytic cells, come and perform phagocytosis. These cells "eat" the pathogens.
- Through their interaction with the pathogen and with these cells, the antibodies signal the cells that there is something foreign present

Antibodies bind to viruses, bacteria and microbial toxins neutralizing and immobilize them.



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

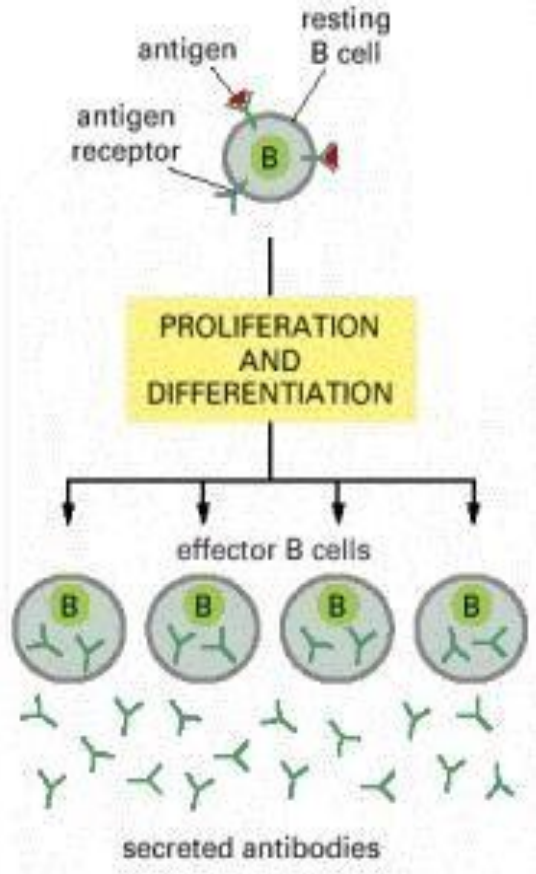
This system consists of a number of proteins which are released by different cells, and they bind to the pathogen and can open up its membrane. يشبه الألغام

Cells are completely dependent on their membranes. Without a membrane, there is no cell, and cells die if their membranes are damaged. These proteins act almost like mines. They are deposited on the pathogen, and when they act on the pathogen, the membrane is disrupted. As a result, the pathogen dies.

When B cells recognize an antigen…

- When a B cell is activated by antigen (a foreign body), it proliferates and differentiates into an antibody-secreting effector cell called plasma cell.
- Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibodies at a rate of about 2000 molecules per second.
- B cells can produce more than 10^{11} different antibody molecules, but each cell produces one type of antibody.
- The antibody has high affinity and specificity to the antigen.

- The B cells are initially dormant and inactive. Each B cell has antibodies on its surface, and this antibody of one B cell is different from the antibody of another B cell.
- When an antigen enters the body, among the millions of B cells present in our body, there may be a few B cells that recognize this antigen. There has to be some sort of interaction between the antibody on the surface of the B cell and the antigen.
- When this interaction occurs, stimulation takes place. It then undergoes proliferation, meaning cell division, and produces many copies of itself. Because this particular B cell has recognized the antigen, it becomes activated and produces different types of cells.
- These cells produce the same antibody and they release it.



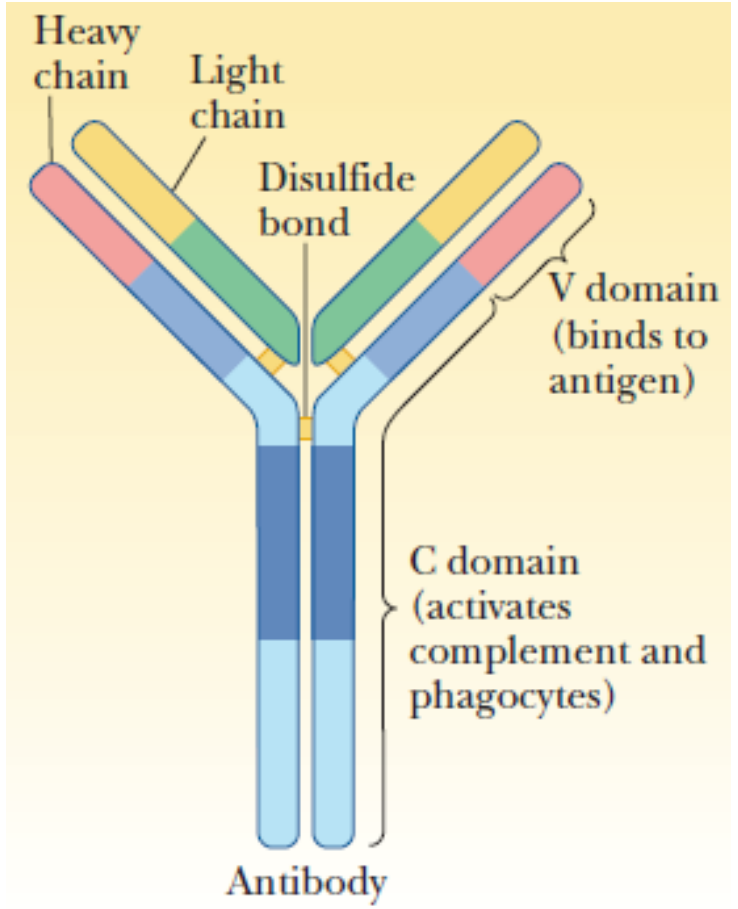
They bind strongly to that antigen and bind to a particular antigen and not to anything else.

This is related to autoimmune diseases. Autoimmune diseases occur when the body starts to attack itself. An antibody may recognize an antigen by mistake—what is sometimes described as "friendly fire." It may recognize something that is present on the body's cells or tissues and start attacking it.

Structure of antibodies

For comparison, the hemoglobin molecule is a heterotetramer. Its four polypeptide chains are linked together through hydrophobic interactions and other non-covalent interactions. Antibodies are also heterotetramers but the polypeptides are linked together covalently (disulfide bonds).

- Antibodies are Y-shaped tetrameric glycoproteins consisting of (four polypeptide chains) two identical heavy chains (50 kDa each) and two identical light chains (25 kDa each). So, the whole immunoglobulin has a size of 150 kDa.
- 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.
- The oligosaccharides are linked to the heavy chains. So it's a glycoprotein



Constant region is shared among different antibodies produced by different cells but Variable region is different from one antibody to another and from one B cell to another.

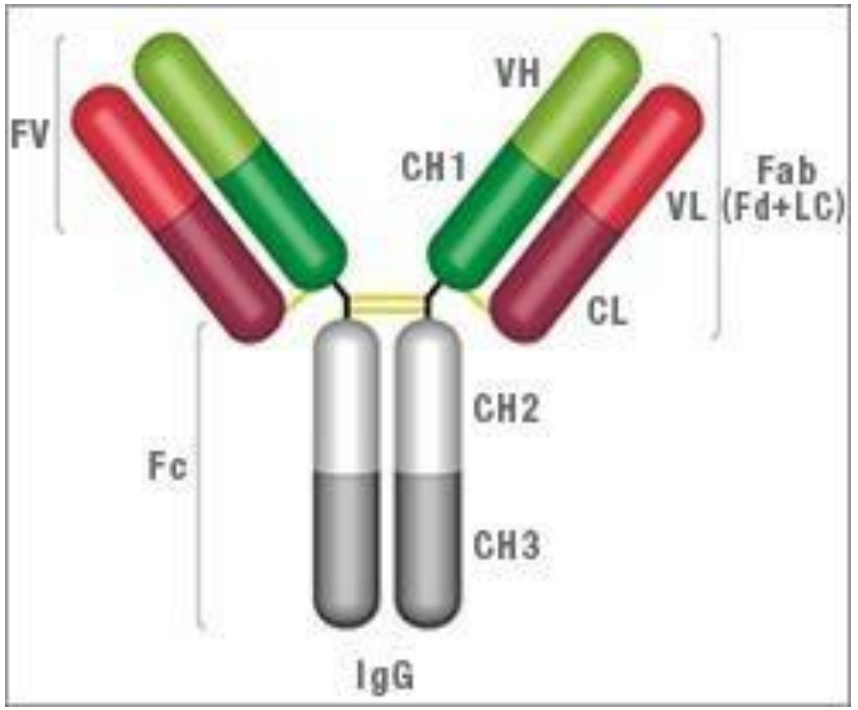
Each B cell produces one type of antibody. The B cell produces many copies of that same antibody.

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 or domains (V_H) and three constant regions/domains (C_{H1}, C_{H2}, and C_{H3}).
  - V_L and C_L pair with V_H and C_{H1}, respectively.
- Constant regions are uniform from one antibody to another within the same isotype.
- The Fc domain of antibodies is important for binding to the surface of phagocytic cells, allowing for antigen clearance.

The same variable region is present in the other light chain, and the constant region is also the same.

The second heavy is an identical copy of the first heavy chain, with the same variable region and the same constant regions.



Super Secondary Structure

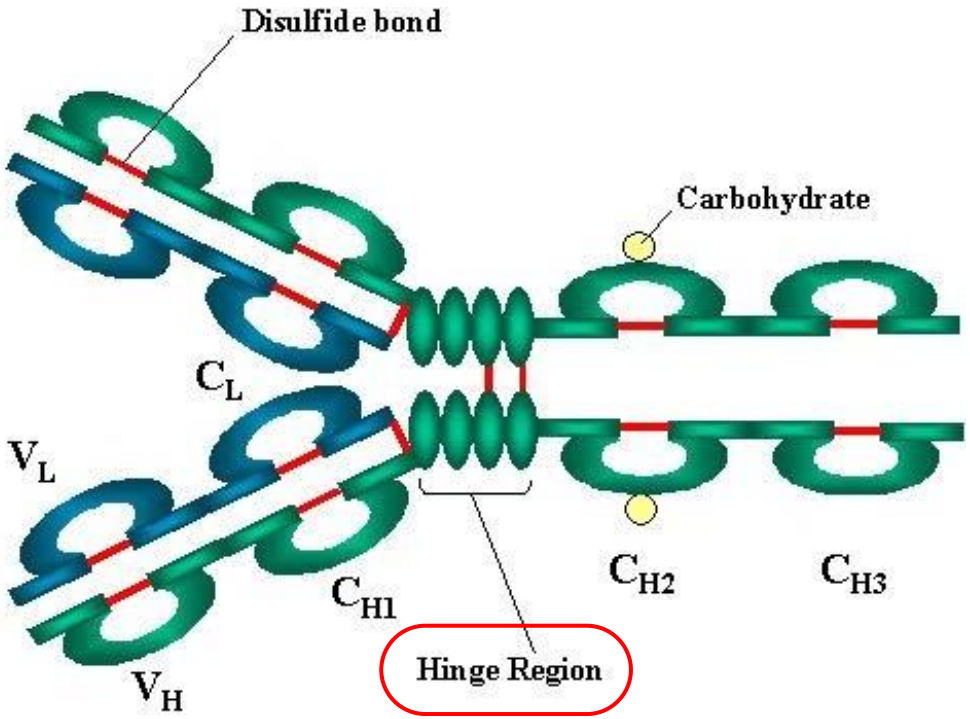
The antibodies bind to the surface of phagocytic cells, which are non-specific cells. These cells can then interact and bind with the pathogen or antigen and engulf it.
So, the interaction between the antibody and the phagocytic cells occurs through the Fc domain.

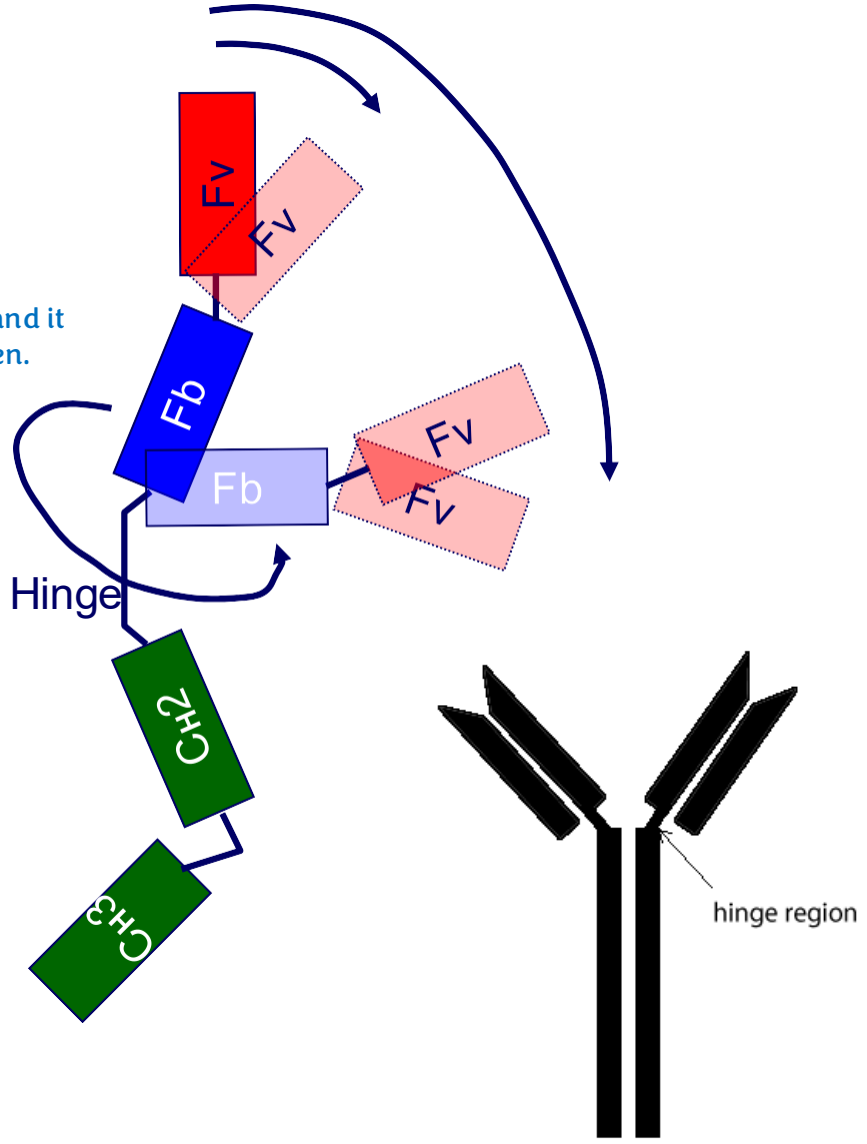
Hinge region

It is a region between the constant domains, specifically between constant region 1 and constant region 2.

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

So, the antibody can change its structure and it can bind to the antigen.





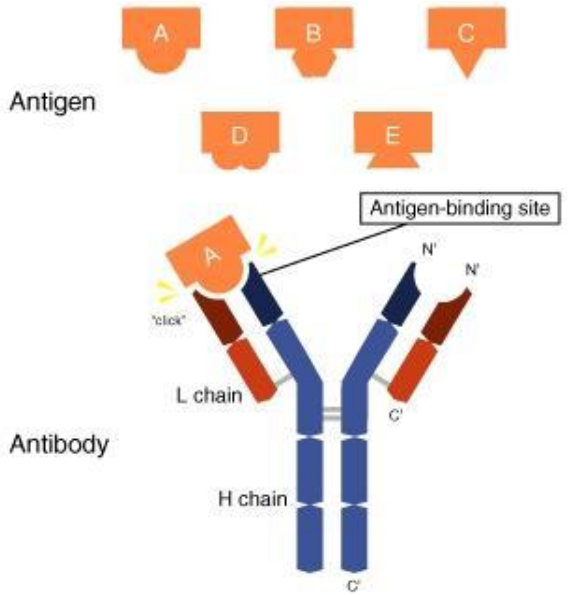
- Each antibody can bind to two antigens of the same type. The two antigen-binding sites are identical, so they recognize the same antigen.

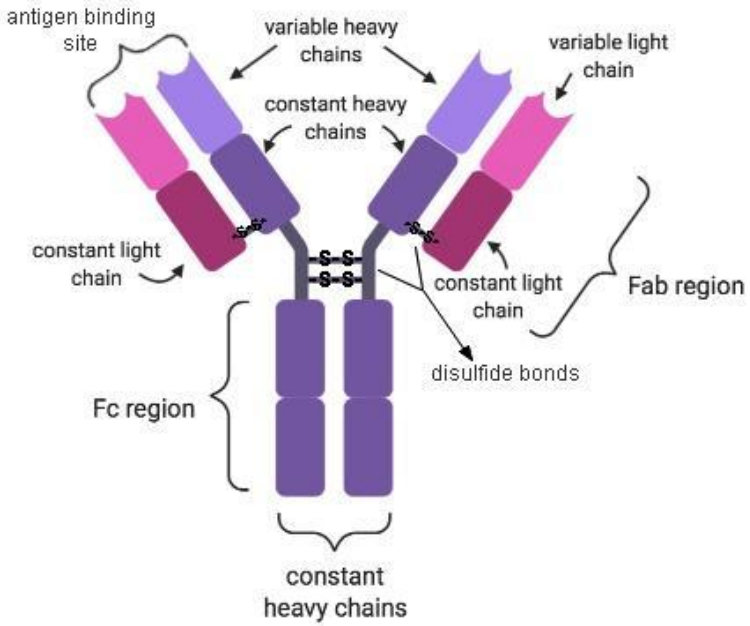
- For example, we have one bacterial cell with a certain protein on its surface, and another bacterial cell with the same protein. The antibody will bind to this protein through the variable region then bind to the same type of antigen on the other bacterial cell because the two antigen-binding regions are identical so the antibody binds to the same antigen at the same site.

- Another example with the same concept, we have one bacterial cell with hemoglobin and another bacterial cell with hemoglobin. Not just any part of the hemoglobin is recognized; it is a specific site. If the antibody binds to a particular amino acid or site on the hemoglobin, it will bind to the same amino acid or the same site on the hemoglobin of the other antigen. This is because we said that antibody binding is highly specific.

- Also, there is a need of two variable regions to bind to one antigen (the variable region of the heavy chain with the variable region of the light chain in order to bind to the antigen).

Two additional images for clarification





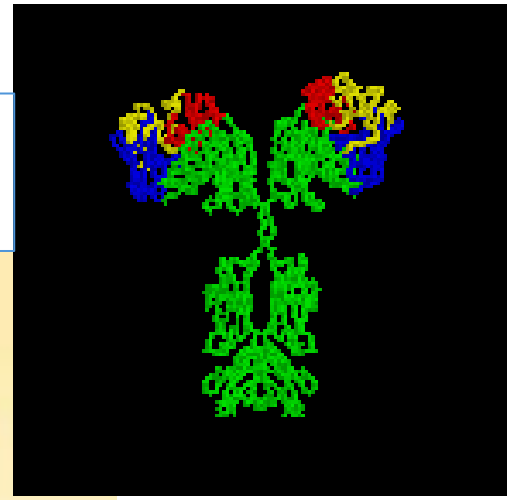
Variable regions

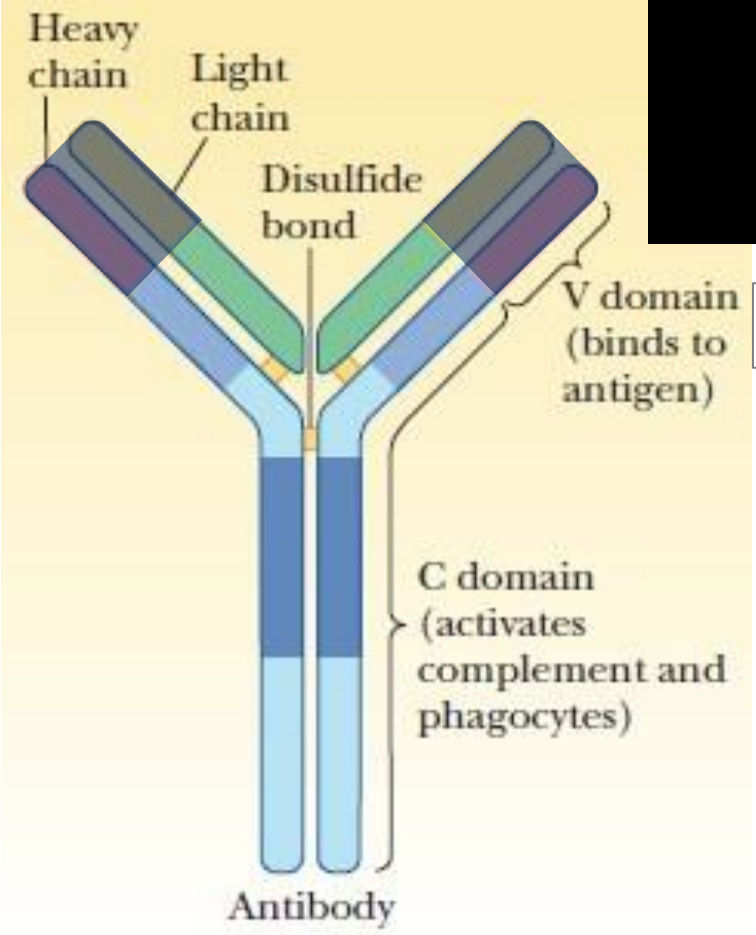
Recognition of the antigen

- 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 epitope is a part of the antigen

Can bind to proteins on top of the cells and also the secreted proteins, by bacteria for example





V stands for Variable

- The primary sequences of the variable regions among different antibodies are quite distinct.

They could bind different antigens or at the same antigen, but to different regions with different amino acids sequences of the antibodies

- Each B cell produces only one kind of antibody.

- No two variable regions in different humans are identical.

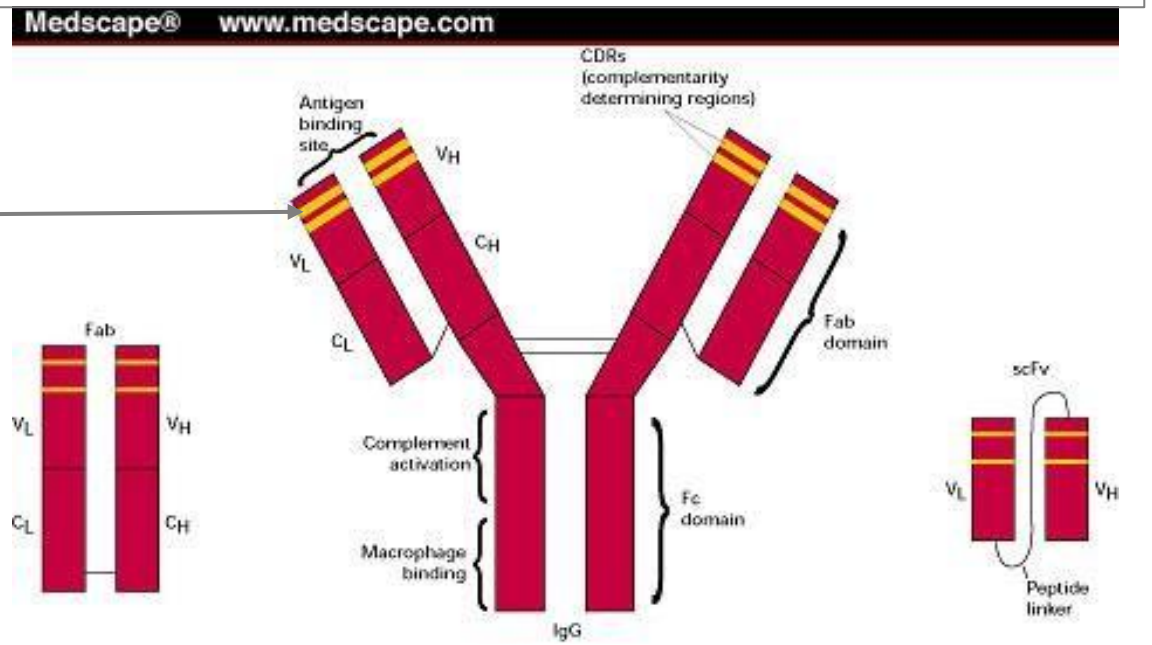
Two patients might get exposed to the same kind of bacteria, but they will generate different antibodies

- Relatively invariable regions and other hypervariable regions.
- L chains have 3 hypervariable regions (in V_L) and H chains have four (in V_H).

Hypervariable" regions

– Hypervariable region : on the antibody (does the organization)
– Epitope : on the antigen (the piece being recognized)

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



The dissociation constant (K_D) is used to measure the rate at which the antibody dissociates from its target. K_D is inversely proportional to affinity, so the lower the K_D value (the lower the concentration), the higher the affinity of the antibody. Recall from Dr. Faisal's lectures in physiology

Immunoglobulin fold

A type of a super secondary structure

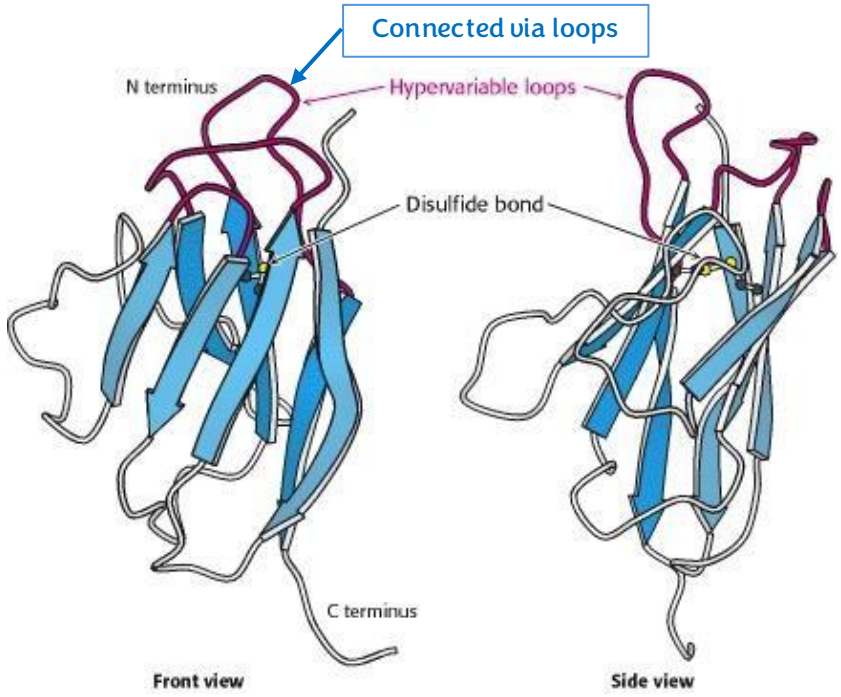
- The hypervariable regions exist in a specialized domain called “Immunoglobulin fold”, which is a domain that is present in every immunoglobulin.
- The hypervariable regions are specifically in three loops connecting the antiparallel β sheets to each other.

The loops are represent the hypervariable region

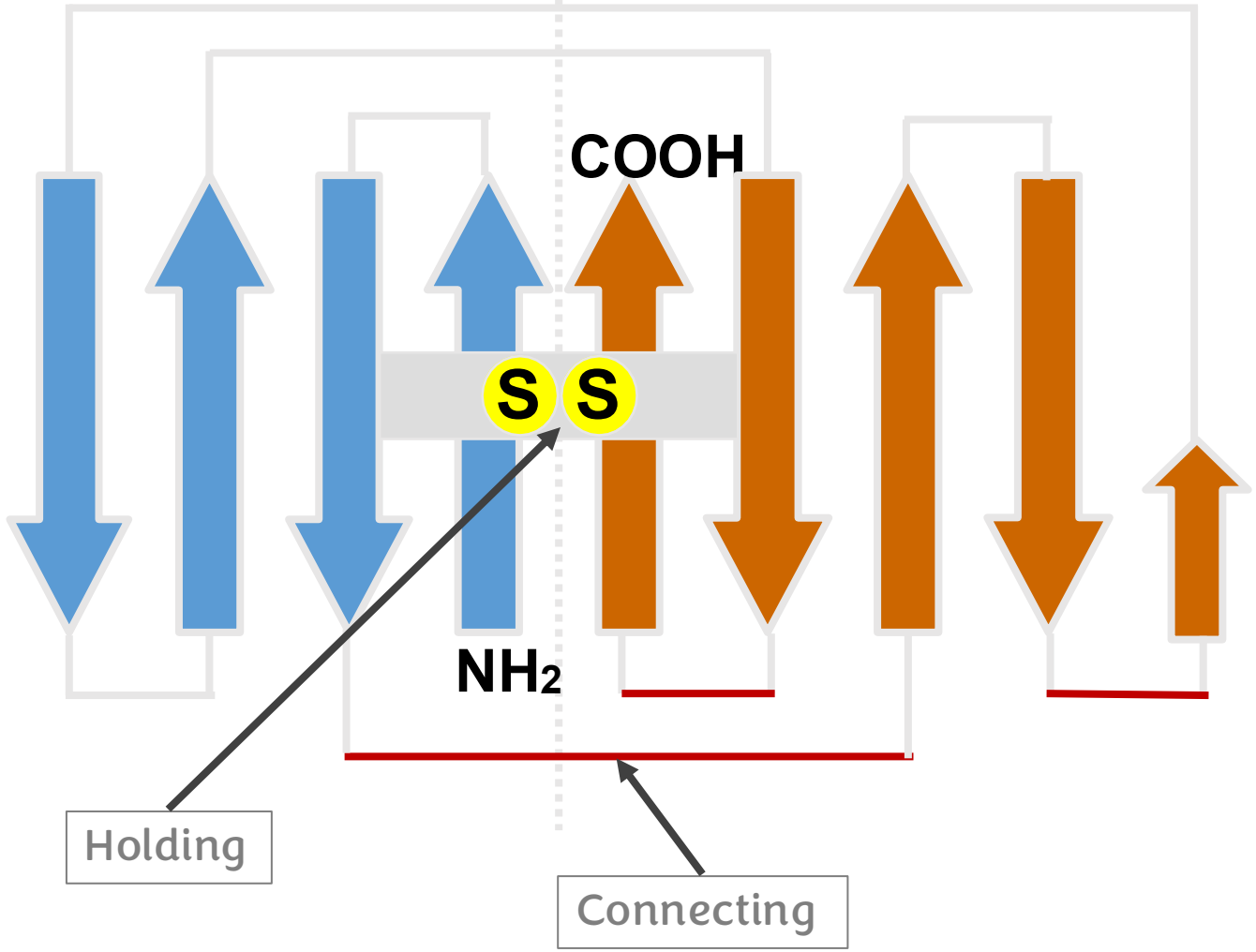
- About 7-12 amino acids in each one that contribute to the antigen-binding site.

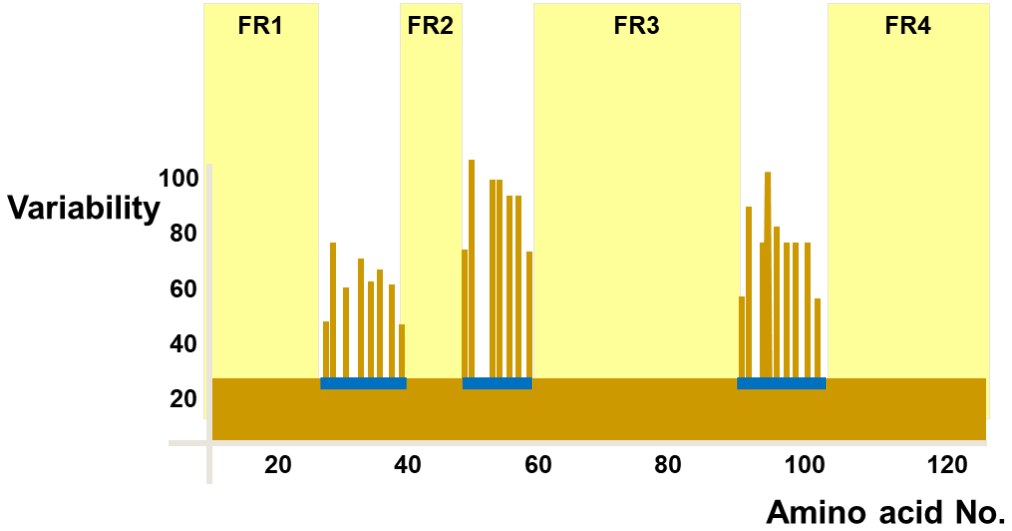
Different sequences of different loops at the antibodies

It consists of a sandwich of two anti-parallel β sheets of 4 β strands each **held** together by a disulfide bond making a shape of a barrel, hence known as “beta barrel”.

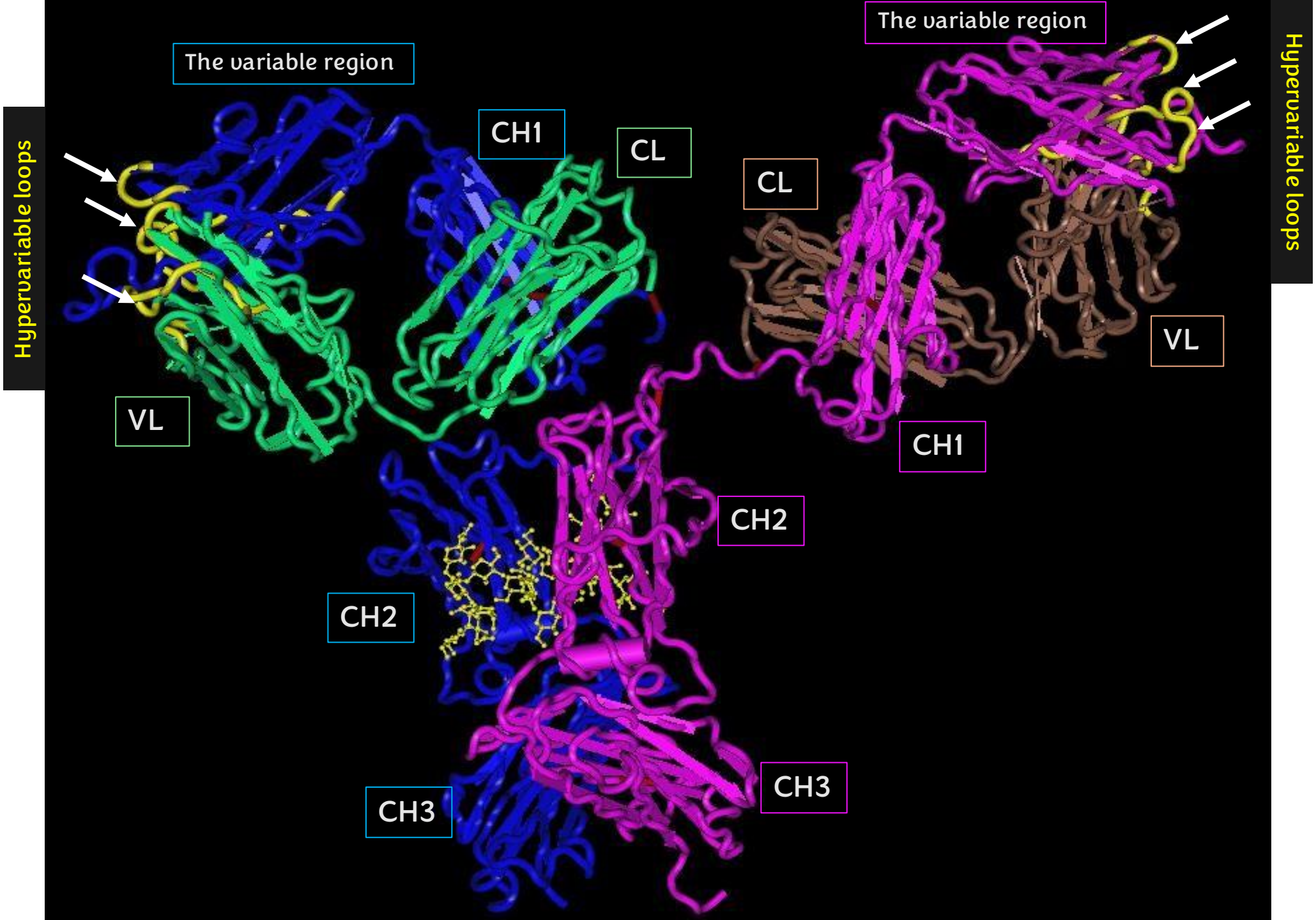


Hypervariable loops





Highly variated

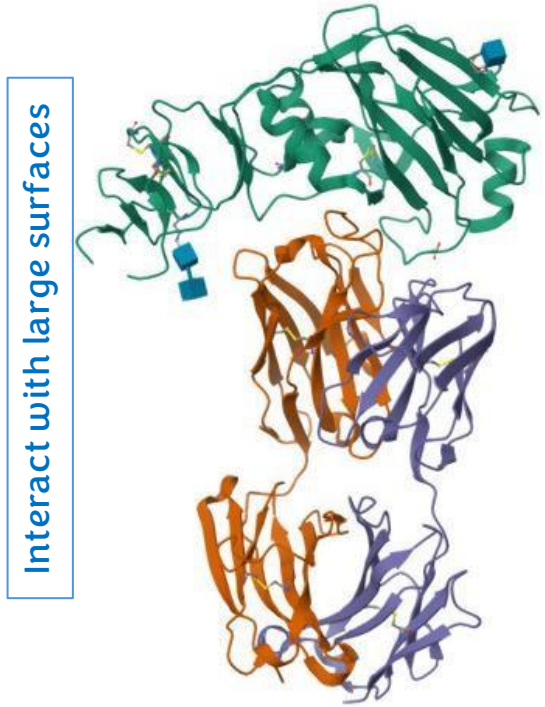

The variable region
CH1
CL
Hypervariable loops
VL
The variable region
CL
Hypervariable loops
VL
CH1
CH2
CH2
CH3
CH3

Antigen-antibody interaction

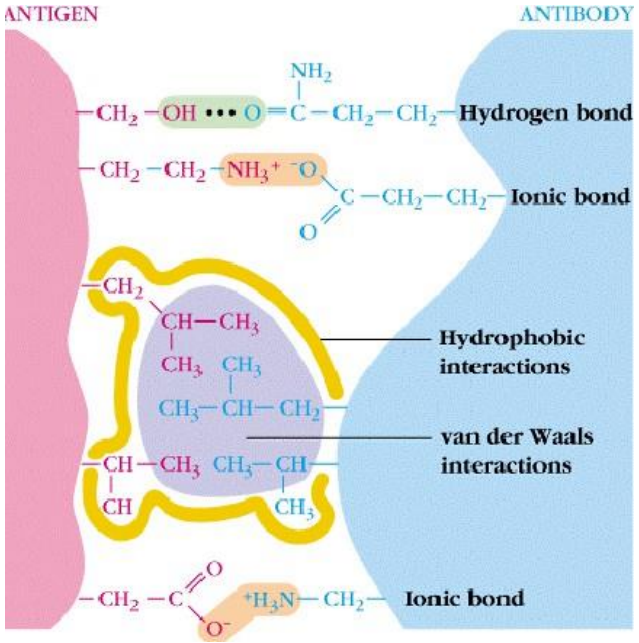
Antigen-antibody binding is mediated by noncovalent interactions between the complementary surfaces.

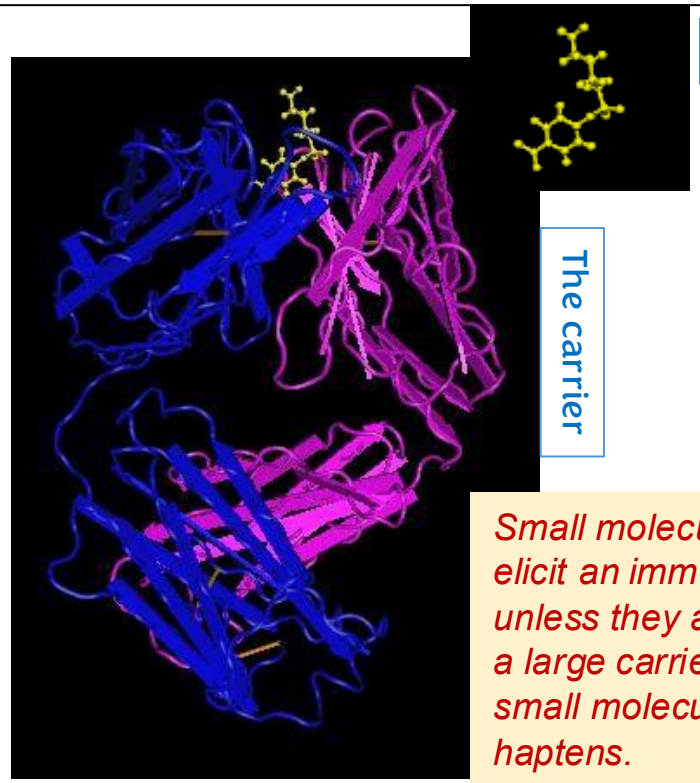
Large antigens interact with all of the CDRs of an antibody.

Small antigens interact with only one or a few CDRs that form a pocket or groove in the antibody molecule.



Human antibody complexed with influenza hemagglutinin





Small molecules cannot elicit an immune response unless they are attached to a large carrier. These small molecules are called haptens.

Hapten: 5-(para-nitrophenyl phosphonate)-pentanoic acid

How is diversity of antibodies created?

- The enormous diversity of the amino acid sequence of the antigen-binding sites that are generated by
  - Erroneous combinations (genetic recombination) of the many components of the genes.

    Deletion, substitution, etc
  - The introduction of genetic mutations during B cell differentiation resulting in changes of the lengths and amino acid sequences of the hypervariable loops.
- The combinations of different heavy and light chains.

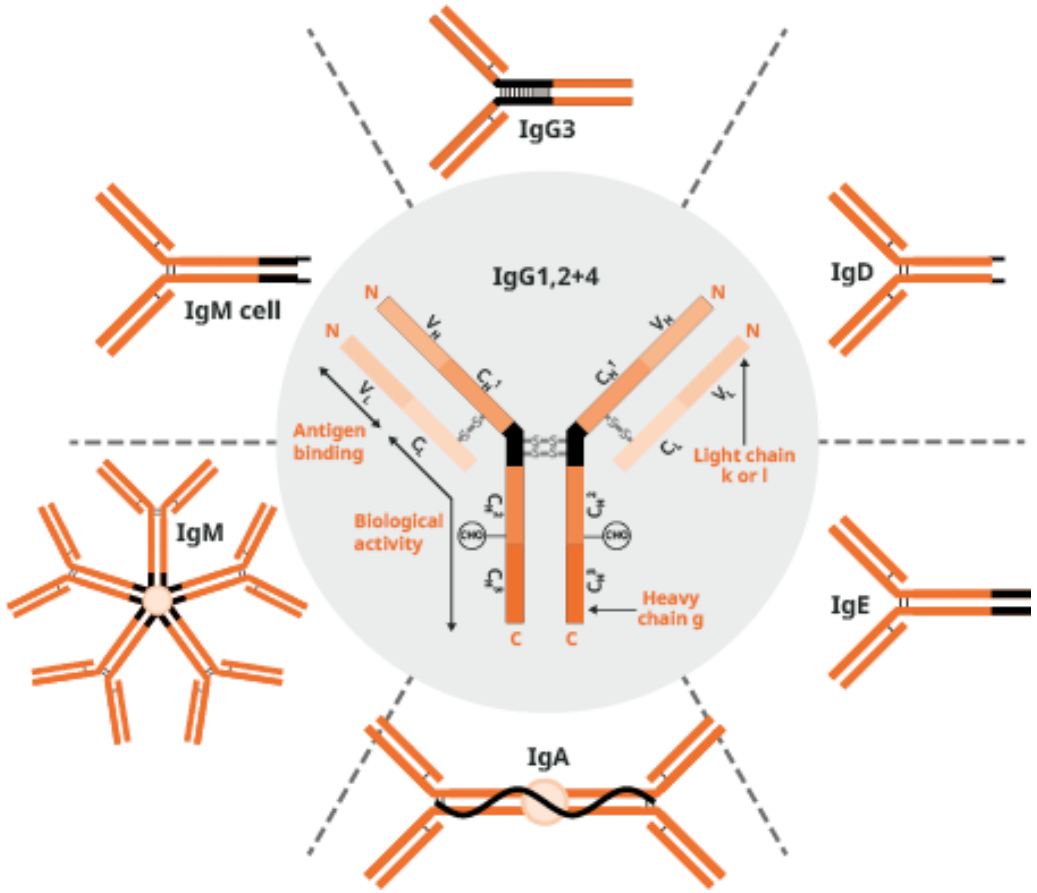
  The structure of the original antibody differs from the final product (antibody) secreted by the differentiated B-cells
- The overall three-dimensional structure of the antigen-binding sites.

  Different types of antibodies from different types of B-cells , yet from the same origin

https://www.youtube.com/watch?v=Na-Zc-xWCLE

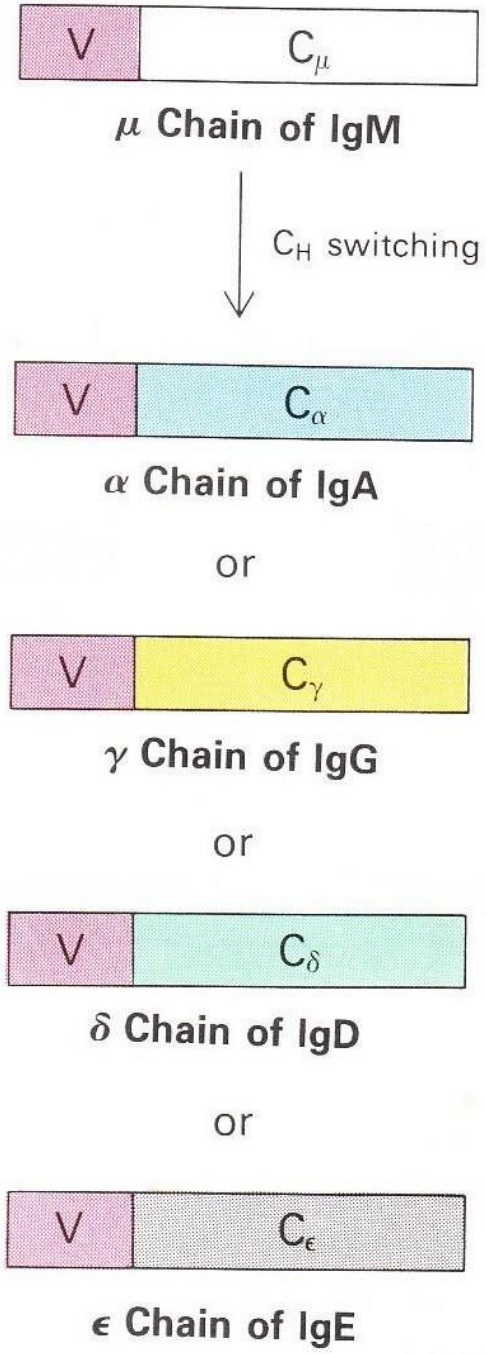
More diversity: immunoglobulin classes

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

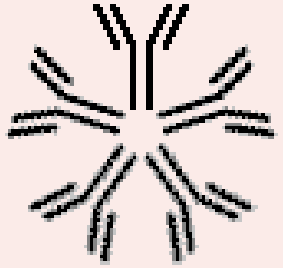
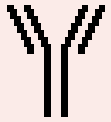
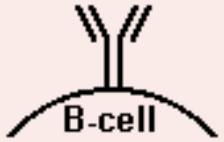
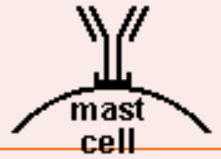
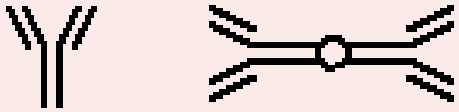


Class switching

- Before binding an antigen, B cells contain IgM molecules only. Naïve
- Following antigen binding, class switching occurs. At maturation
- Class switching refers to a DNA rearrangement changing the heavy chain constant gene.
- The variable regions stays the same.
- That causes production of IgG, IgD, IgA, and IgE.



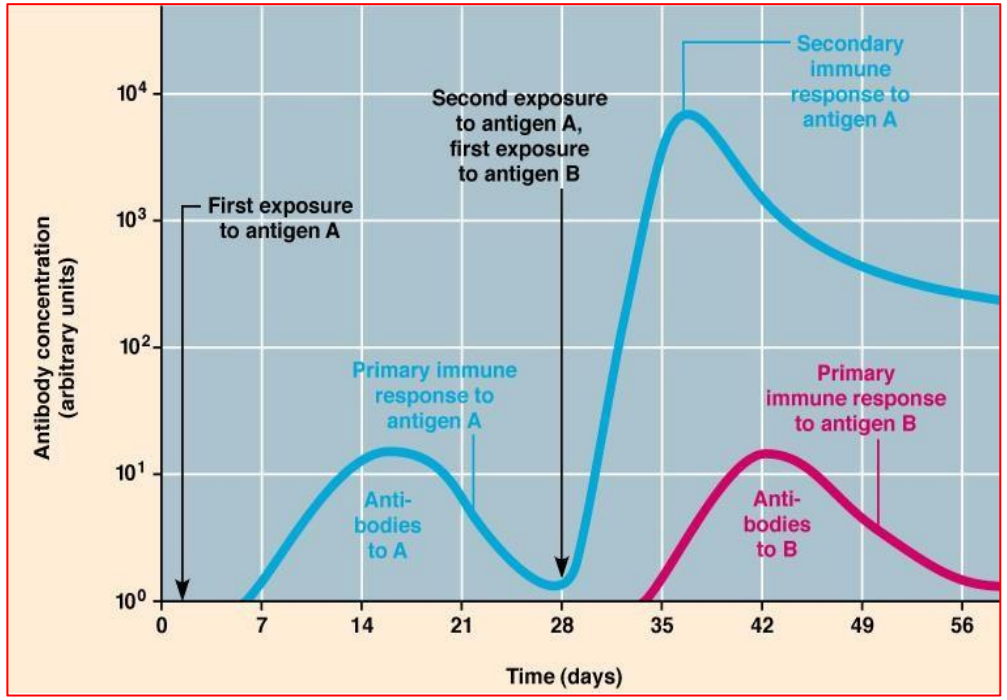
Types of antibodies

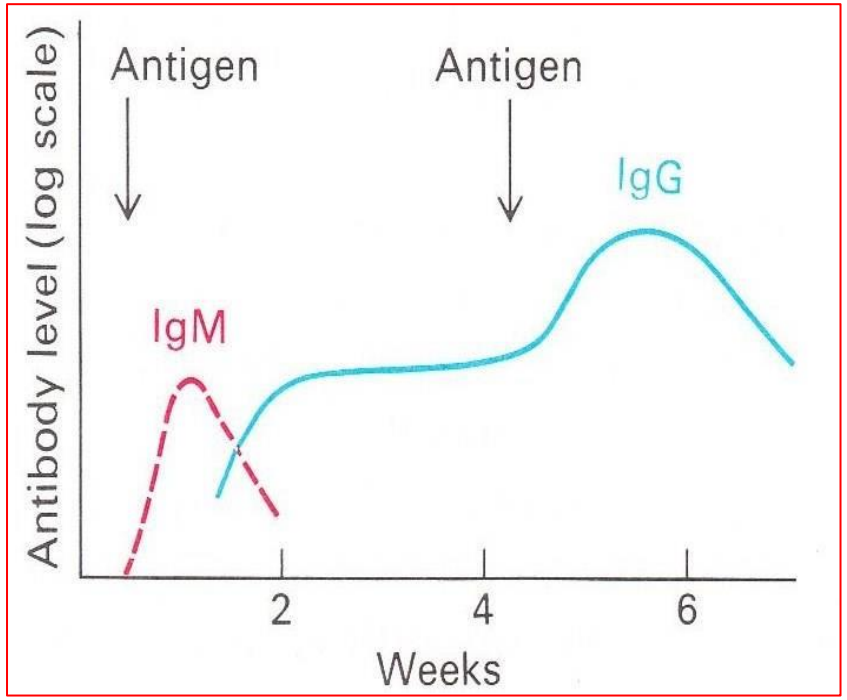
Isotype	Structure	Notes
IgM		Contains Mu heavy chains Expressed on the surface of B-cells (as a monomer only) The first antibodies produced in significant quantities against an antigen Appears usually as a pentamer when released, but can exist as a monomer and hexamer This one can recognize & bind to 10 antigens
IgG		Contains Gamma chains Monomers Most abundant immunoglobulins in sera Produced as a second immune response Only kind of antibodies that can cross the placenta
IgD		Contains Delta heavy chains Presents on surface of B-cell and can initiate immune responses No function known yet
IgE		Heavy chains type Epsilon A monomer Plays an important role in allergic reactions
IgA		Contains Alpha chains Found mainly in mucosal secretion The initial defense in mucous against pathogen agents Appears usually as dimers, but can appear as monomer and trimer

Immunological Memory

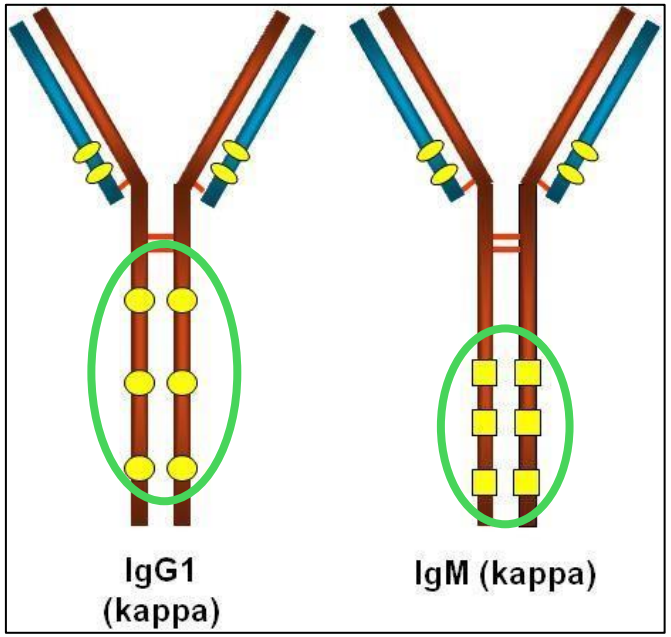
- IgM for new pathogens
- IgG for known pathogens

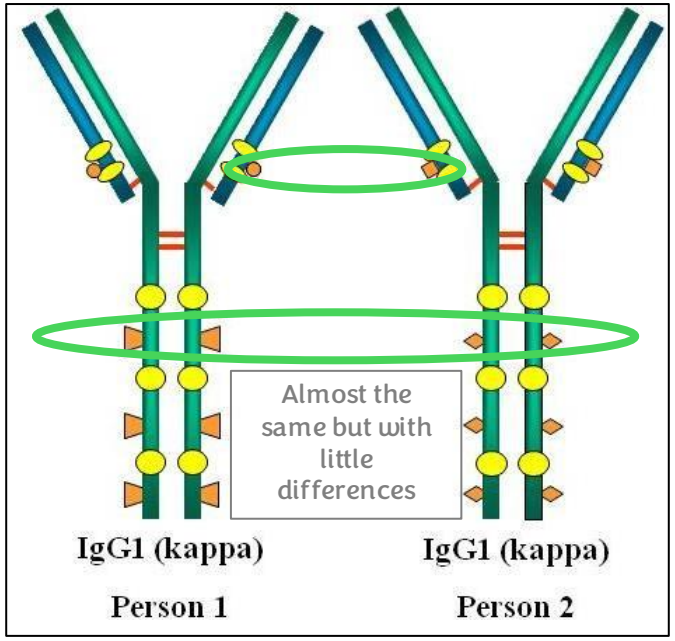
Study these diagrams



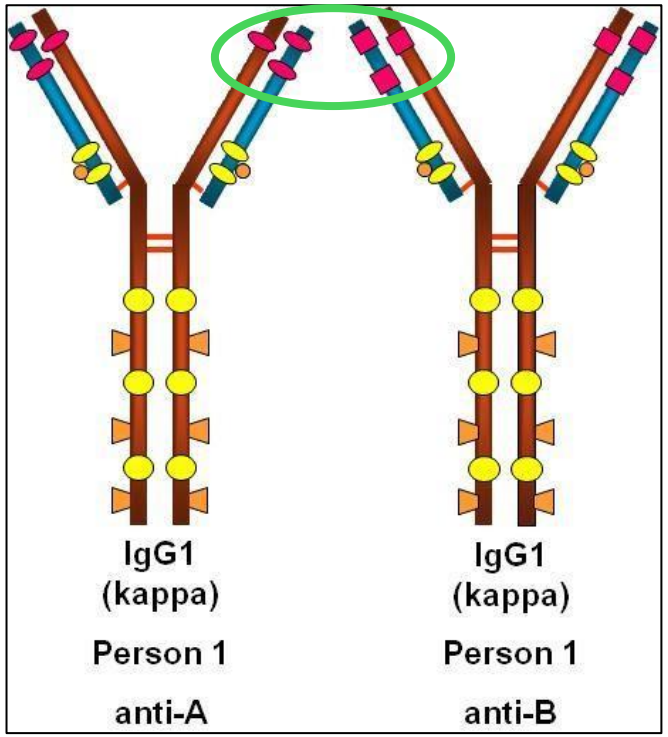


Isotypes:
Immunoglobulins of different classes based on their different constant regions.





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



Idiotype:
immunoglobulin molecules that have the same constant regions, but different variable domains within their light (VL) chains and heavy (VH) chains

Hybridoma and monoclonal antibodies

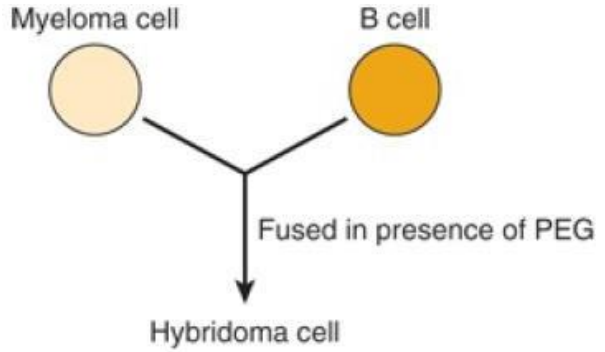
- When an antigen is injected into an animal, the resulting antibodies are polyclonal, meaning they are directed against a number of different epitopes on the antigen.

But remember that Each B cell produces only one kind of antibody

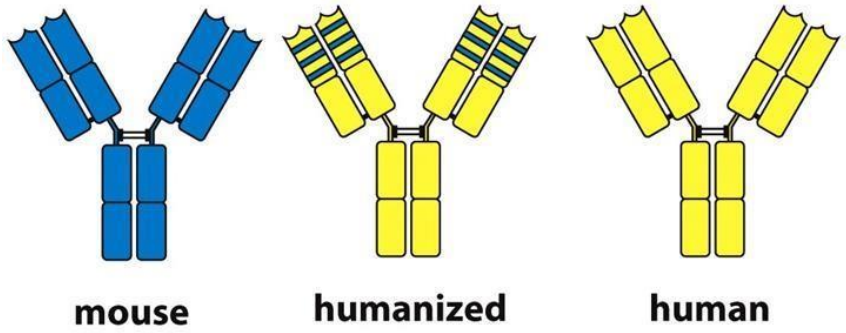
- In order to "create" an immortal B cell that produces a single antibody (monoclonal), a B cell hybridizes with a B cancer cell (myeloma).

Hybridoma cell : mature (plasma) B-cell + cancer cell ~ endless antibodies and yet all of them have the same results

https://www.youtube.com/watch?v=CNPwxbeP7B8
https://www.youtube.com/watch?v=U76LI3OuBsU



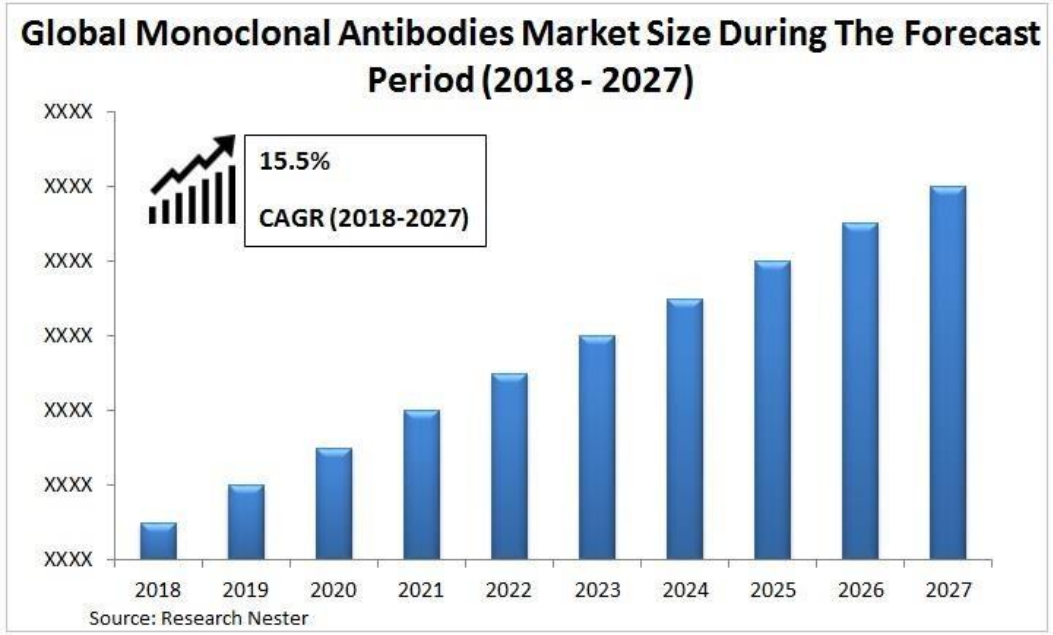
Monoclonal antibodies made in mice can be humanized by attaching the CDRs onto appropriate sites in a human immunoglobulin molecule.



Constant region from the human antibody & the variable region from the mouse's antibody

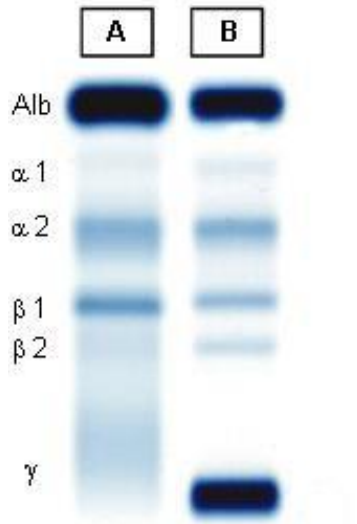
Benefits of monoclonal antibodies

- 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 the removal of drugs from circulation when they reach toxic levels.

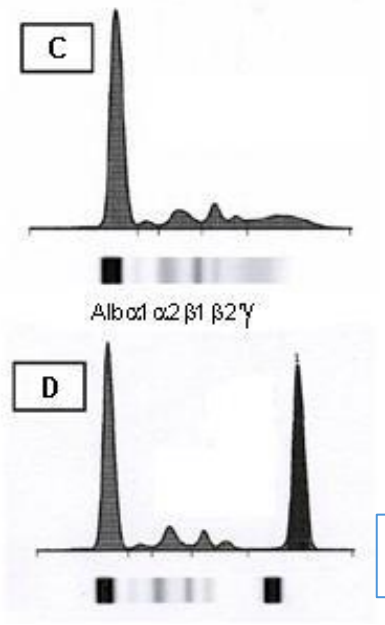


Diseases

- Multiple myeloma: a neoplastic condition where plasma cells grow out of control in the bone marrow and overproduce abnormal monoclonal proteins (M proteins) instead of useful antibodies.
- Decreased production may be restricted to a single class or may involve underproduction of all classes (ex. agammaglobulinemia)



Overproduction of gamma globulin



Multiple myeloma

Quiz!

https://share.gemini.google/80u9b5oZH2QI

For any feedback, scan the code or click on it

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
https://youtu.be/mJlToyu8YOE?si=Vaok2srqGqzI5DD7

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)

"اللهم لا سهل إلا ما جعلته سهلاً، وأنت تجعل الحزن إذا شئت سهلاً ..ويسّر لي أمري وافتح عليّ فتوح العارفين".
سمِّ الله، وابدأ بقلب مطمئن.