بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture 22

Immunoglobulins

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا لنصرة دينك

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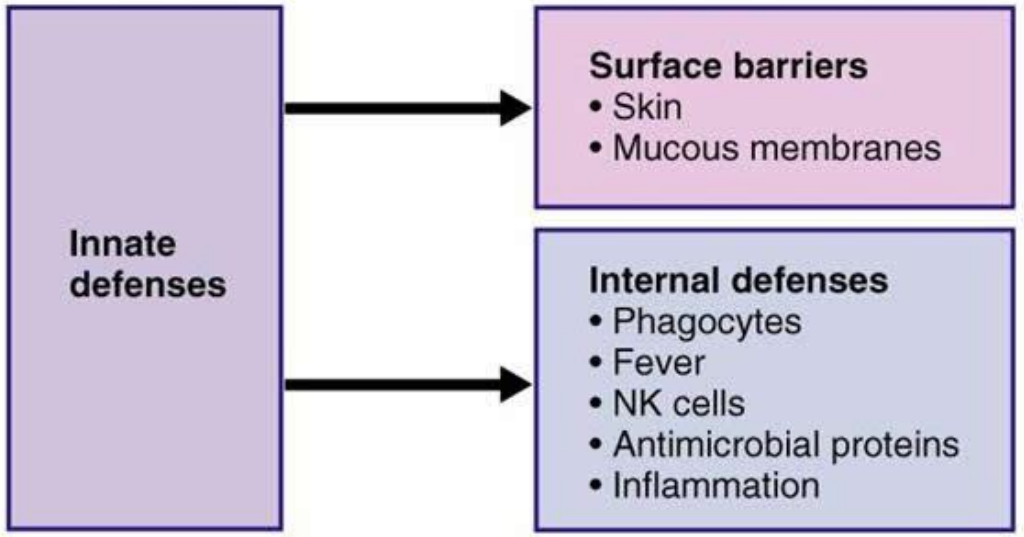
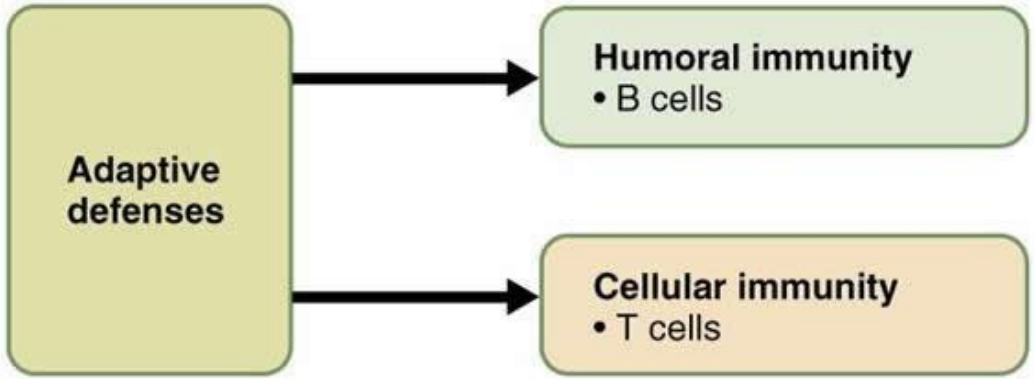
Types of immunity



(a)



(b)

Produce antibodies (immunoglobulins)

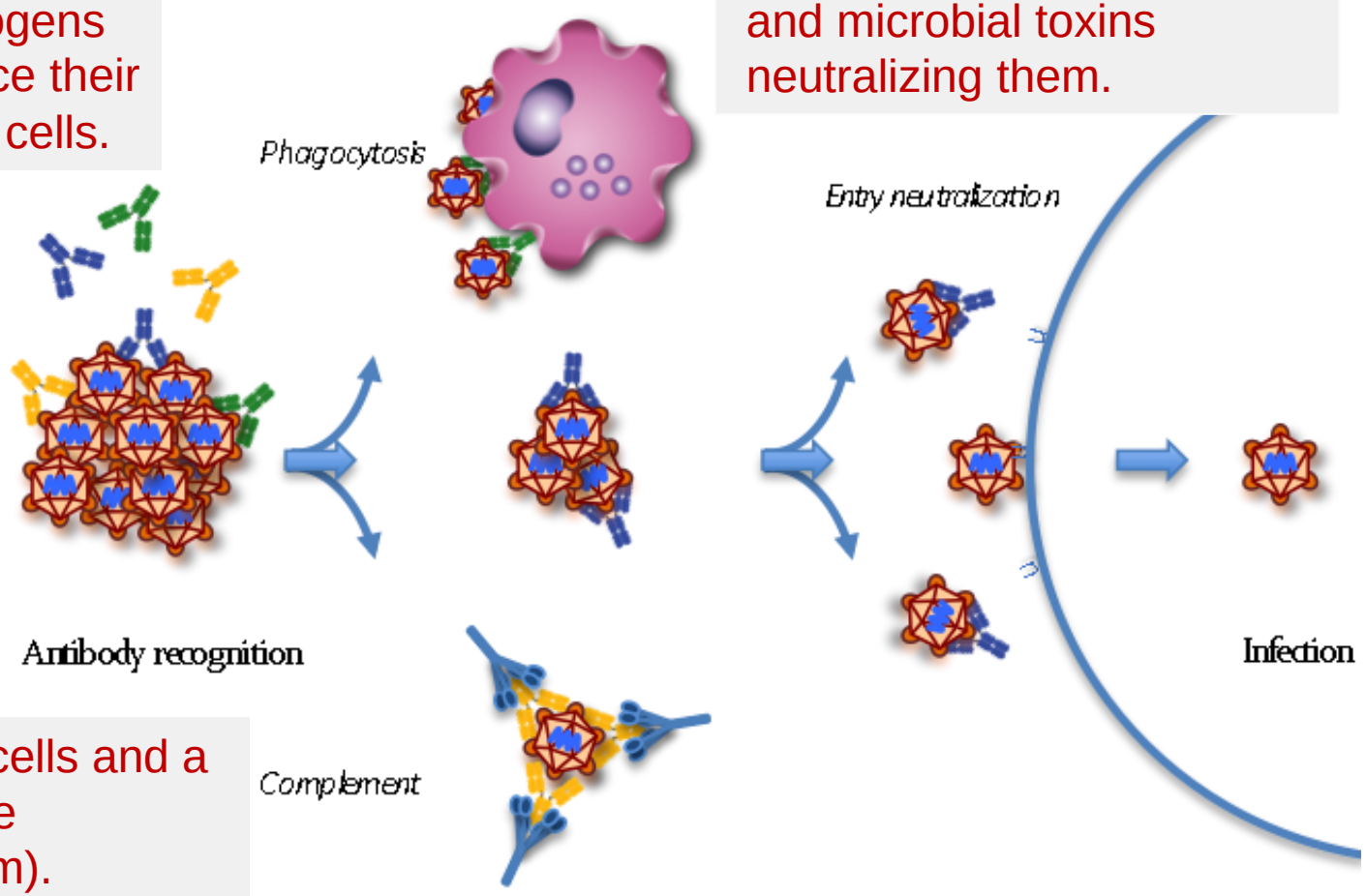
Immunoglobulins are an example of globular proteins, and they are immune proteins (fight antigens).

Cell based immunity

How do B cells work?

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

1- Antibodies bind to pathogens (and foreign cells) 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).

*Professor said that he won't test us in understanding these mechanisms . However, we think that it is beneficial to know them at least.

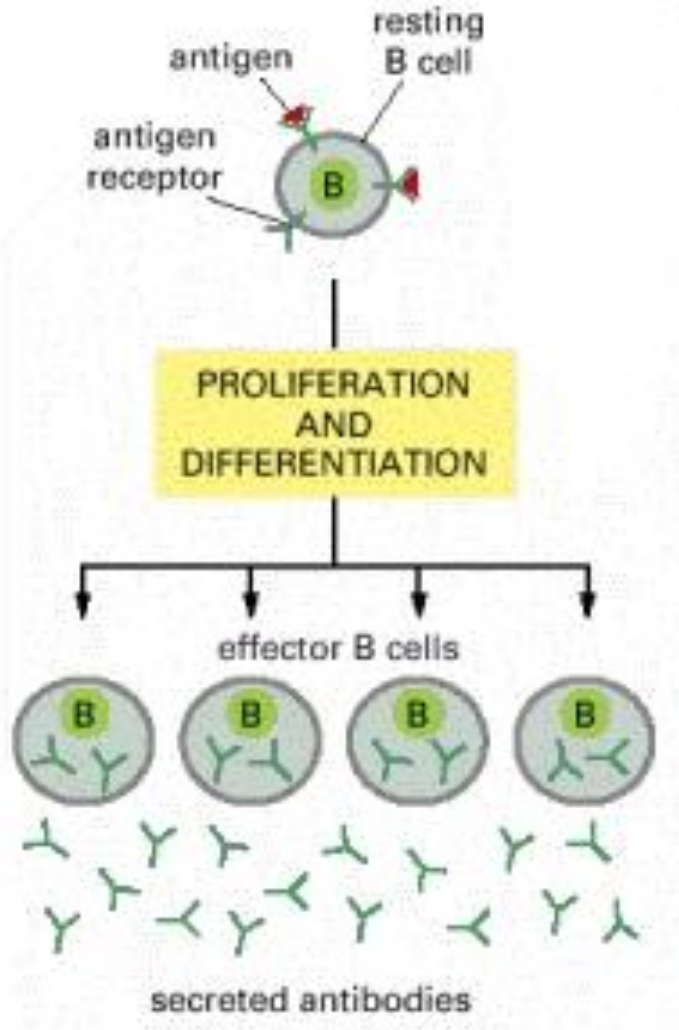
When B cells recognize an antigen…

- When foreign particles (i.e. antigens) bind to their receptors on the surface of B-cells, B-cells get activated.

- When a 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.
- Each individual can produce more than 10^{11} different antibody molecules.

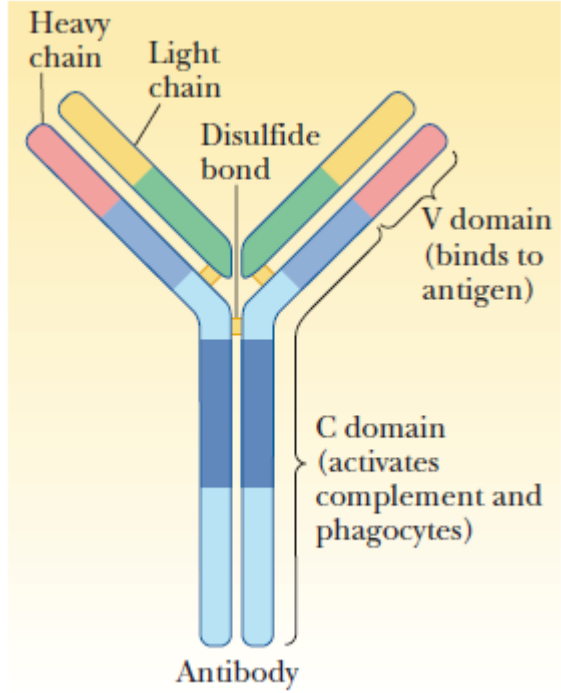
During proliferation, the genetic material of B-cells coding for antibody sites responsible for recognizing antigens undergoes changes. These changes occur through two mechanisms mentioned later on slide 12.

B-cells undergo a process of gene and protein modification, resulting in the production of the most effective and potent antibodies, in a process called the election mechanism (as illustrated in slide 13). Subsequently, these B-cells are transformed into plasma cells, which secrete a substantial quantity of the elected antibody.



Structure of antibodies

- Antibodies are Y-shaped molecules consisting of two identical heavy chains and two identical light chains held together by disulfide bonds. So, they have a quaternary structure (multiple chains).
- 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. i.e., between residues of the same chain
- They are glycoproteins, with oligosaccharides linked to their heavy chains.

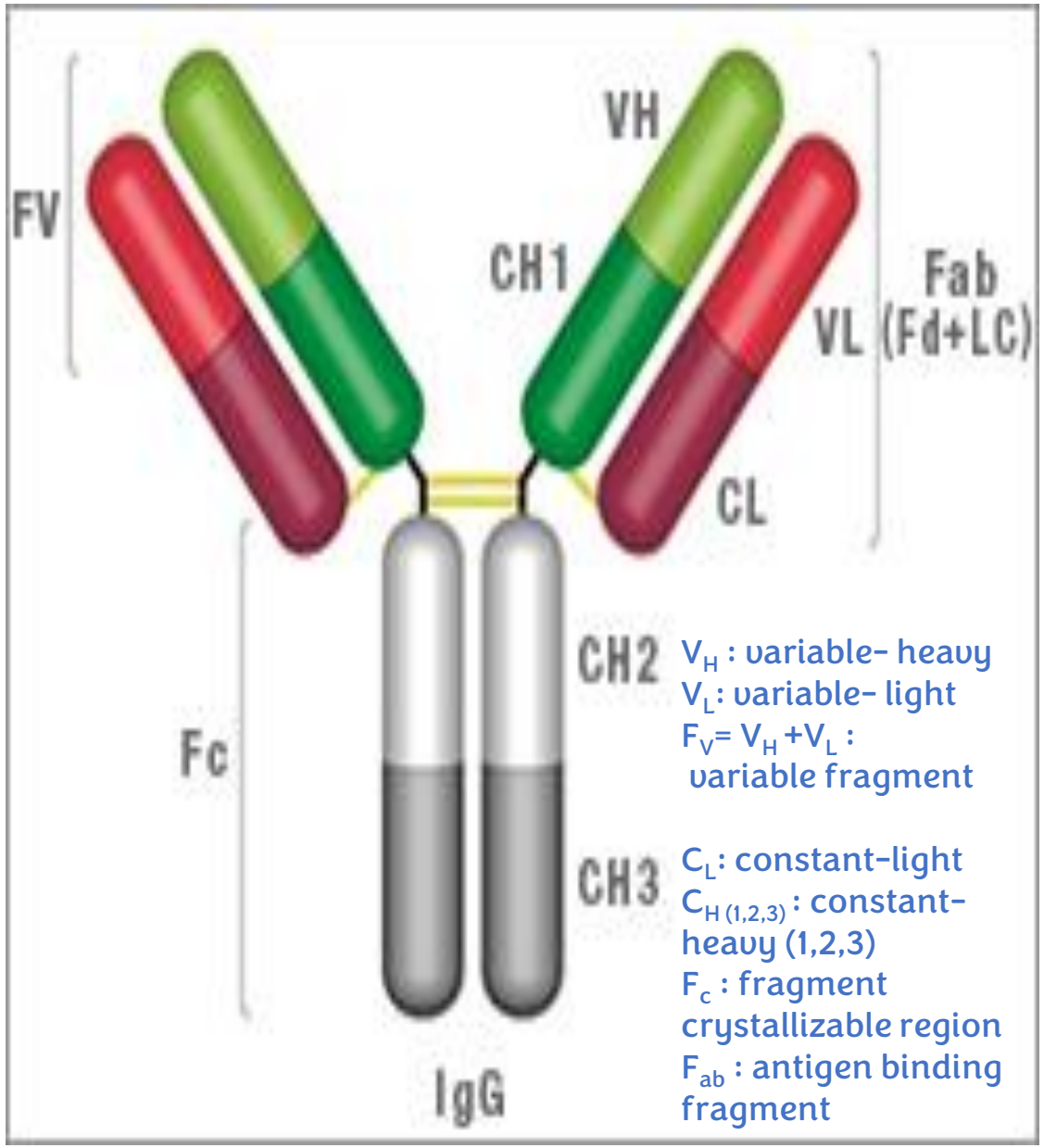


Whether the sulfide bond is intra-chain or between different chains it is between cystine residues.

Antibody regions

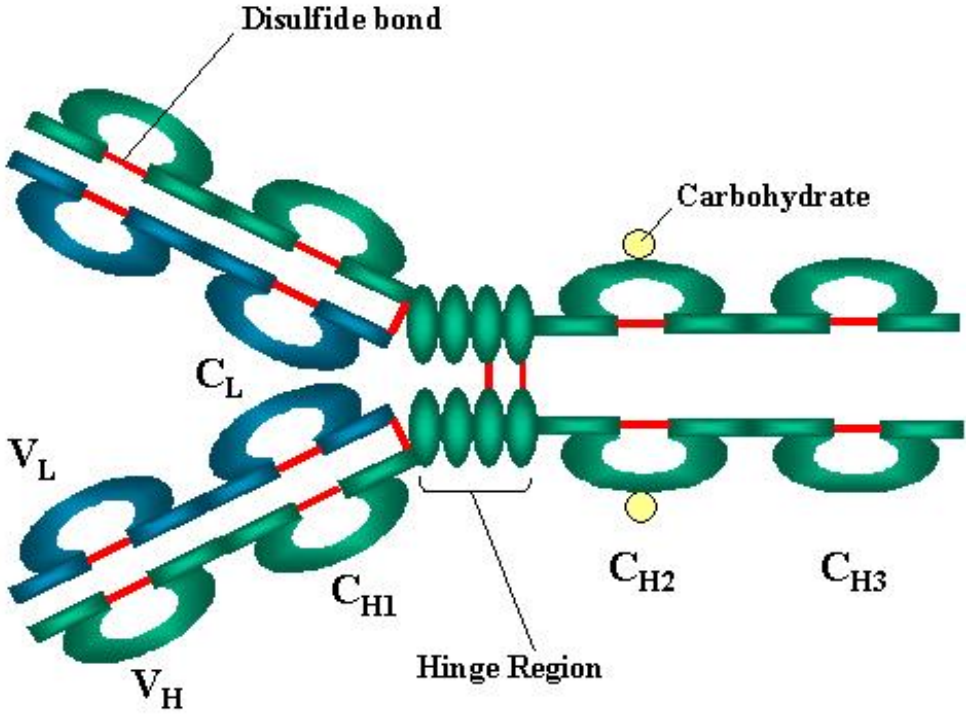
Variable regions of both heavy and light chains are responsible for binding to antigens.

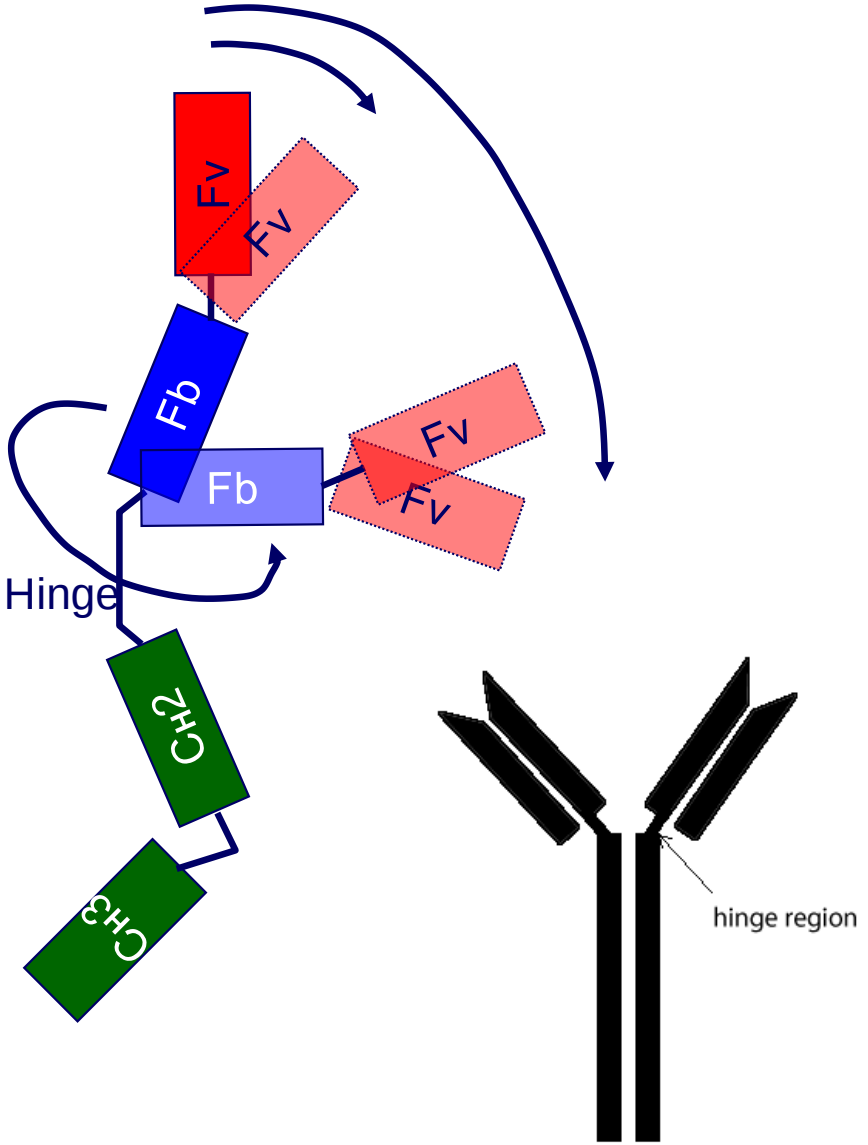
- A light chain consists of one variable (V_L) and one constant (C_L) domain.
- The heavy chain consists of one variable region (V_H) and three constant regions (C_{H1}, C_{H2}, and C_{H3}).
  V_L and C_L pair with V_H and C_H, respectively.
- Constant regions, are uniform from one antibody to another within the same isotype. (Explained in slide 19)
- The F_c domain of antibodies is important for binding to phagocytic cells allowing for antigen clearance.



Hinge region

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

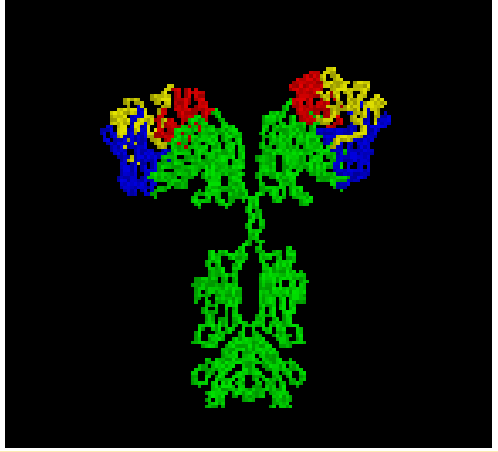




Fb = C_L +C_{H1}

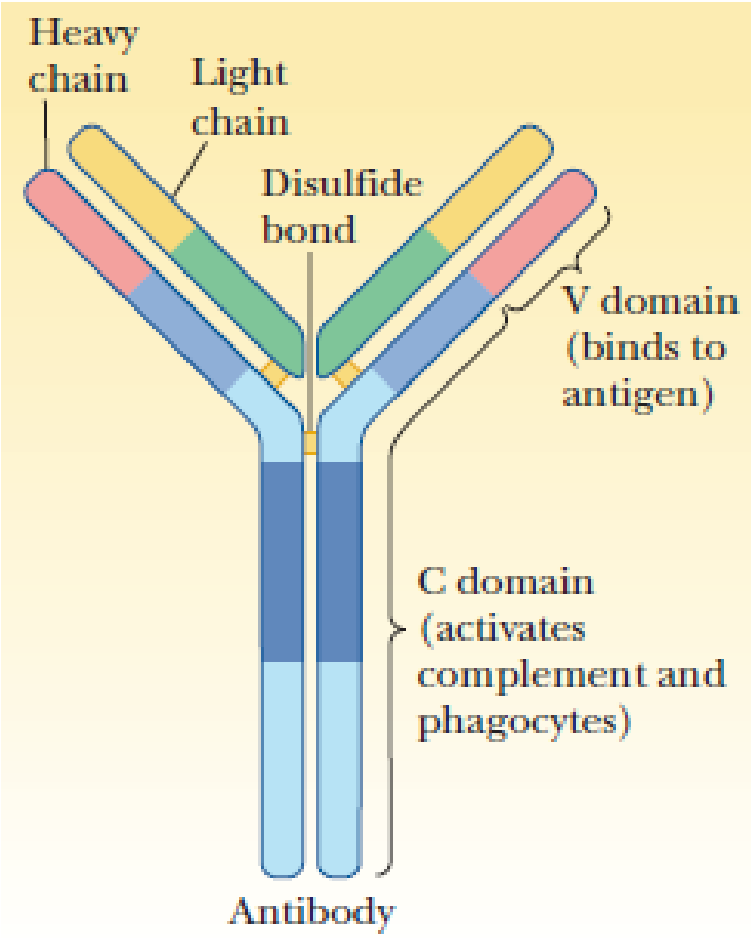
Variable regions

- 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.



Interesting Math:
There are billions of antigens, however these sequences are enough to produce billions of antibodies specific to these antigens.

Here is the calculation:
$(20^7$ to $20^{12})$
$= (1.28\times10^9$ to $4.096\times10^{15})$
different possible combinations of amino acids, leading to the sufficient diversity that can deal with the huge number of possible antigens.



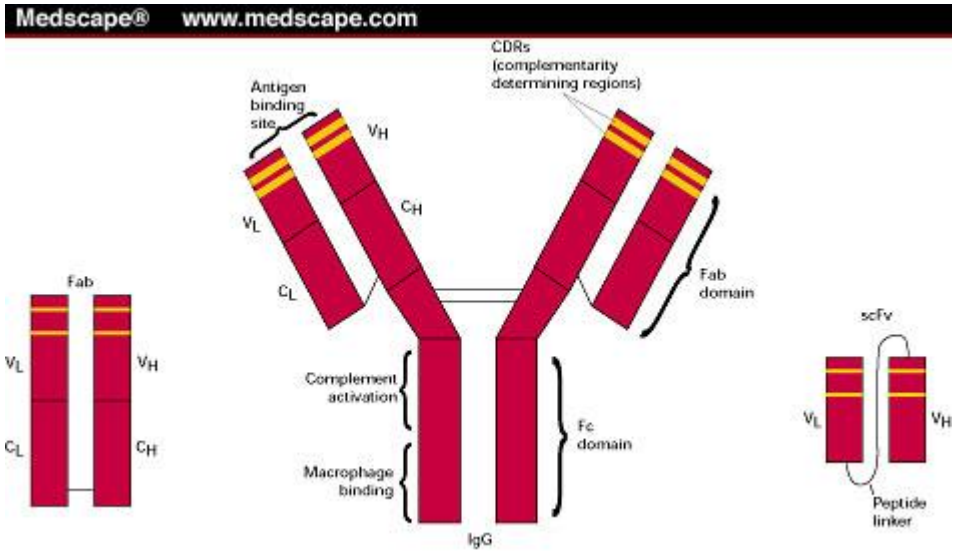
Hypervariable regions

- 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}).

These regions are responsible for binding to antigens through interactions that are highly specific, with each antibody capable of binding to only a single antigen type. Additionally, CDRs have a low K_d indicating their strong affinity for antigens.

The region is named hypervariable because it is responsible for most of the diversity in antibodies, which is caused by the variability of amino acid sequences in this region.

They are named CDRs because these sequences result in a structure that is complementary to the shape of the specific antigen bound by the Immunoglobulin.

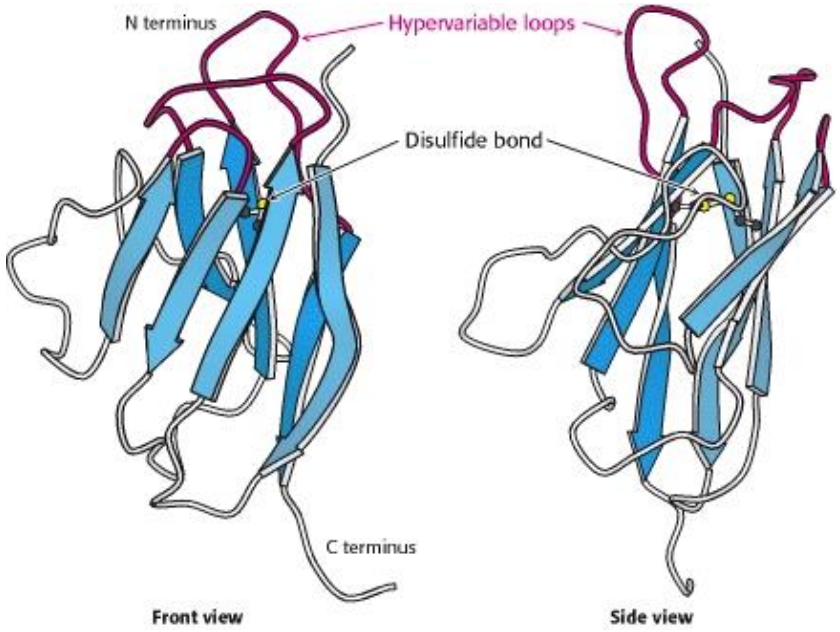


The dissociation constant (K_D) is used to measure the rate at which the antibody dissociates from its target. K_D is inversely proportional to affinity, so the lower the K_D value (the lower the concentration), the higher the affinity of the antibody.

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".



Color code :

pink & blue :2 identical heavy chains

brown & green : 2 identical light chains

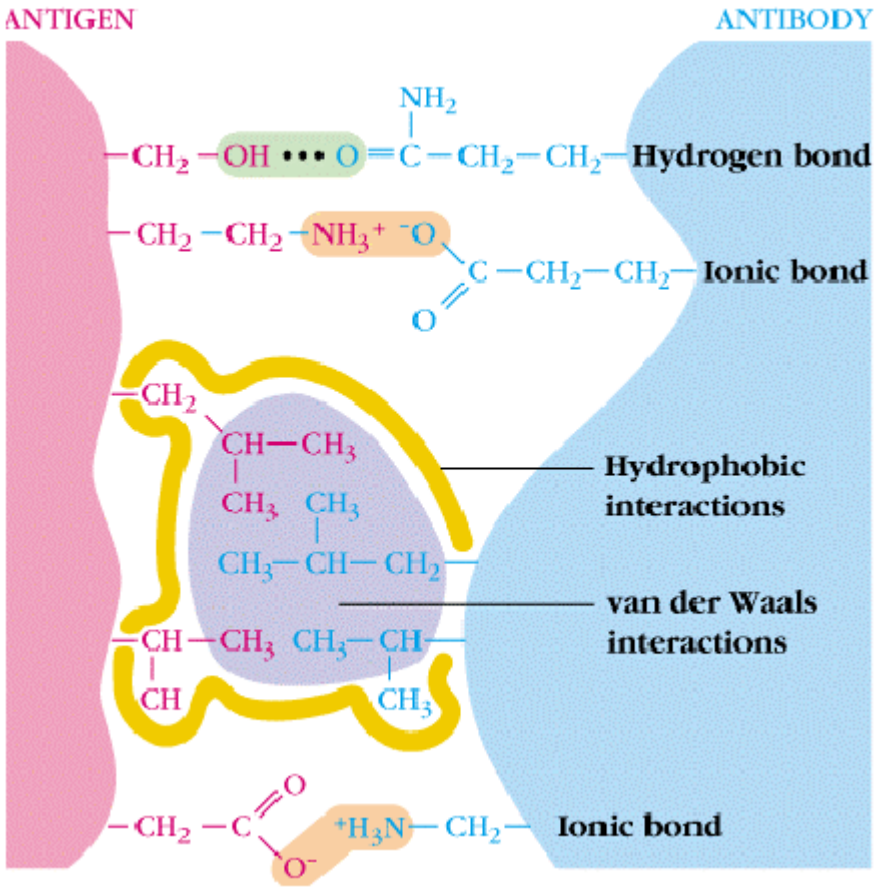
Yellow loops (and pointed to by the arrows): CDRs

Note that these are sugars binding to the constant region of the heavy chains.

For explanation, look over the next slide.

Diversity

- Antigen-antibody binding is mediated by non-covalent interactions.
- The enormous diversity of antigen-binding sites can be generated by
  - 1 - Creating genetic mutations resulting in changes of the lengths and amino acid sequences of the hyper-variable loops.
  - 2- Genetic recombination of different components of the genes.
- The overall three-dimensional structure necessary for antibody function remains constant.



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

The question arises: how can the limited number of B-cells recognize the billions of antigens present in nature?

During the maturation of B-cells, there are some specific processes that causes different antibodies to be produced:

1- For the cells to replicate, the DNA must duplicate. During that process, random mutations can occur, producing different types of B-cells (each produces one type of antibody as previously mentioned). Each type differs from the others in the hyper-variable region (in the 7-12 A.As).

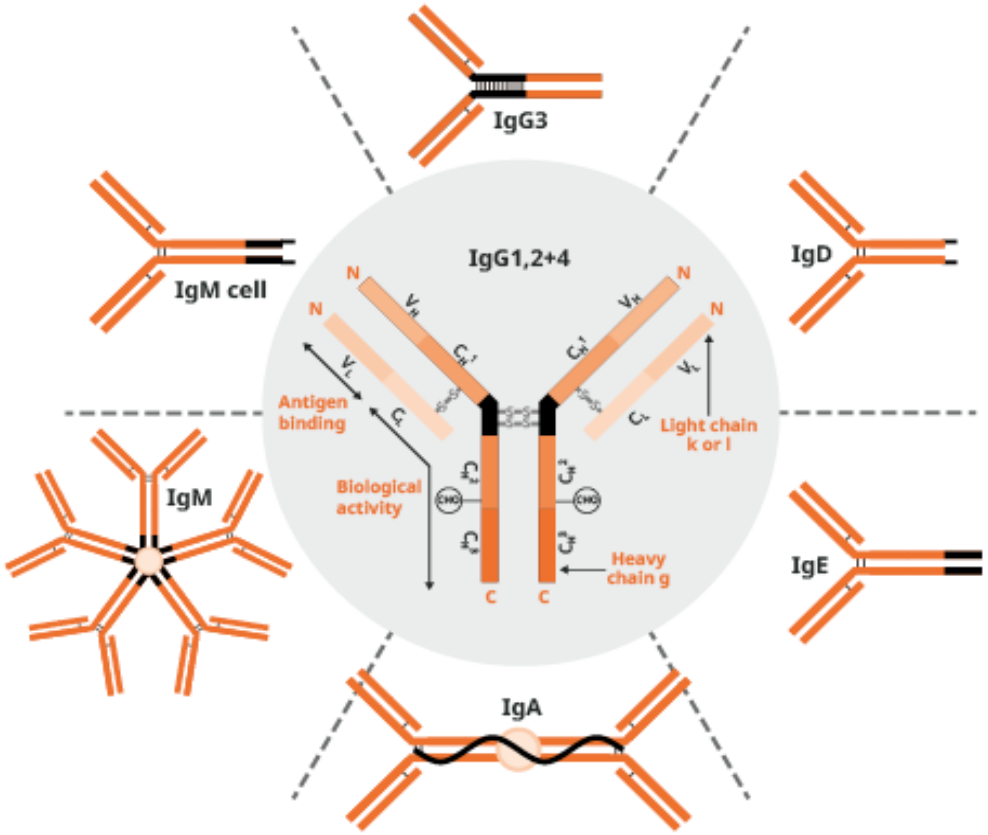
The alteration of certain amino acids can either result in the formation of strong interactions or weaker interactions with antigens. The weak interacting antibodies would be eliminated, while the strong interacting would survive and proliferate.

2- Cells occasionally produce various combinations of different regions of DNA, resulting in the production of distinct antibodies.

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).



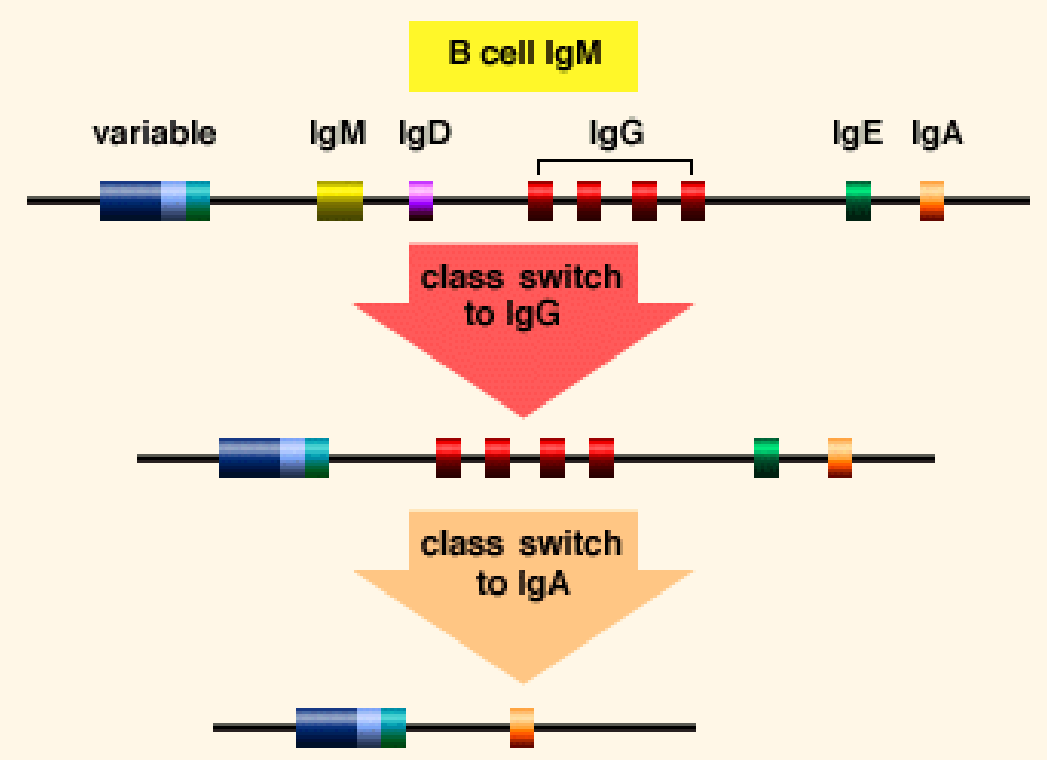
- IgA ; immunoglobulin **A** (Ig with 2 **alpha** heavy chains)
- IgD ; immunoglobulin **D** (Ig with 2 **delta** heavy chains)
- IgG ; immunoglobulin **G** (Ig with 2 **gamma** heavy chains)
- IgE ; immunoglobulin **E** (Ig with 2 **epsilon** heavy chains)
- IgM ; immunoglobulin **M** (Ig with 2 **mu** heavy chains)

Change of the constant region of the heavy chain

Class switching

- Before binding to an 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.

ALERT!! see next slide first.



In a B cell, the variable region remains unchanged. Initially, the B-cell only contains IgM molecules. Upon antigen identification and binding, DNA rearrangements occur, resulting in the deletion and replacement of IgM genes with IgG genes. Additionally, a class switch from IgG to IgA can also occur.

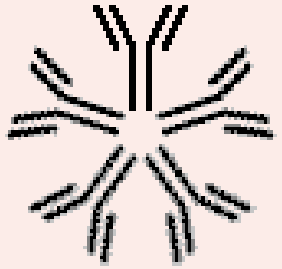
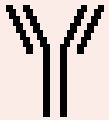
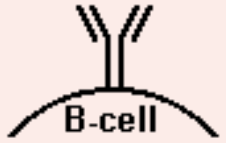
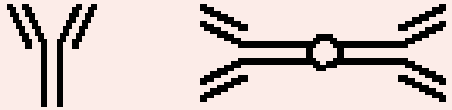
Explaining the previous slide

- 1-Resting B-cells, which have never encountered an antigen, express IgM on their surface. Upon exposure to an antigen, some of these B-cells will recognize it through their variable regions.
- 2-After the recognition, B-cells initiate division and proliferation, resulting in the conversion of IgM into IgG. This process preserves the variable region while altering the constant region of the heavy chains.
- 3- Occasionally IgG will be converted into IgA.

Isotypes of antibodies

according to the types of heavy chains

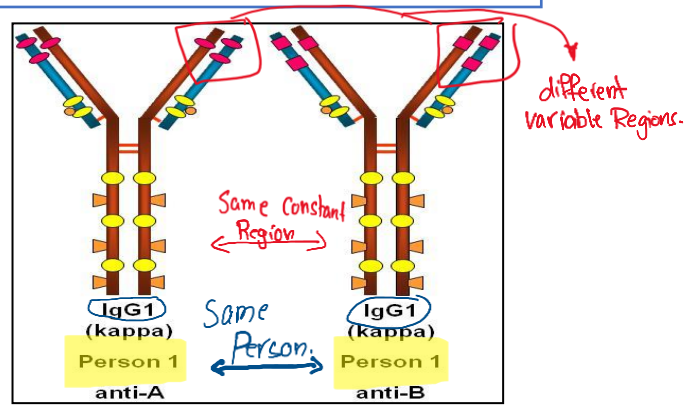
All underlined and blue text is complementary

Isotype	Structure	Notes
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 pentamers with 10 arms to maximize the probability of antigen binding as efficiently as possible.
IgG		Contains Gamma chains Monomers Most abundant immunoglobulins in sera (600-1800 mg/dL) (in blood serum) Promote phagocytosis and activate the complement system Only kind of antibodies that can cross the placenta
IgD		Nothing much known about it Contains delta heavy chains Presents on surface of B-cell that have not yet been exposed to antigens
IgE		Heavy chains type epsilon A monomer Plays an important role in allergic reactions, as it recognizes allergens. Present on the surface of mast cells, which release histamine.
IgA		Contains alpha chains Found mainly in mucosal secretions The initial defense in mucous against pathogen agents Appears usually as dimers

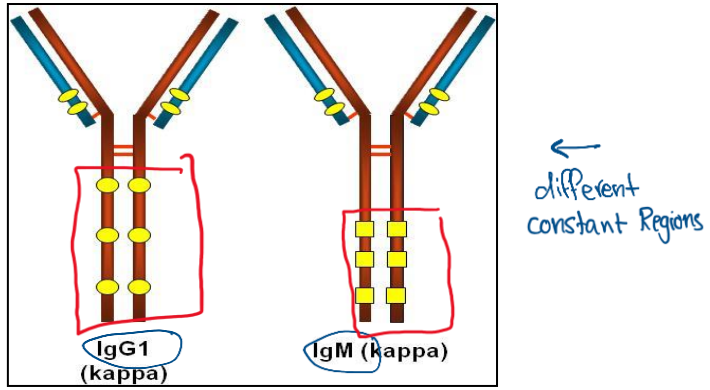
Since the fetus's and baby's immune systems are still developing, they rely on the antibodies present in their mother's blood and milk. These antibodies, specifically IgG, provide the fetus and baby with protection against potential antigens they may encounter during their early development.

Idiotype Vs. isotypes Vs. allotypes

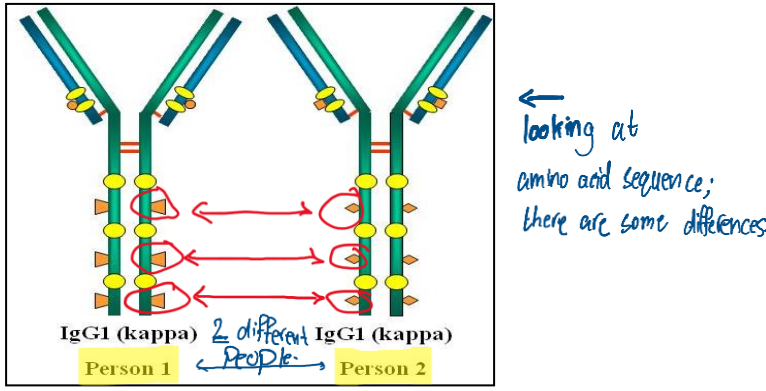
- immunoglobulin molecules that have different variable domains of both their light (V_L) chains and heavy (V_H) chains and are said to share an idiotype.
- They recognize different epitopes and therefore different antigens.



- The different classes of immunoglobulins which are determined by their different C_H regions are called isotypes.
- IgG, IgA, etc.
- The variable regions may be the same or different.

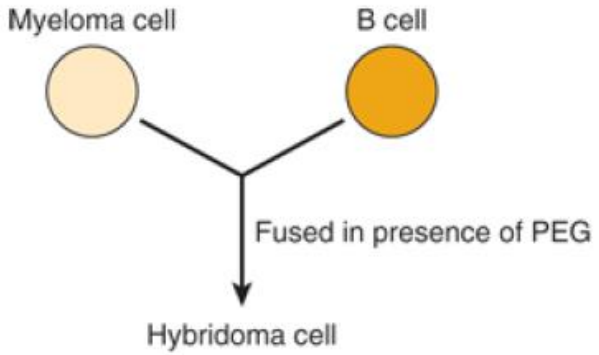


- Immunoglobulins of the same class but different among individuals of the same species due to different genetics are called allotypes.
- There might be slight variations in the amino acid sequence of C_H chains, but these variations do not result in the formation of new isotypes.



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



https://www.youtube.com/watch?v=CNPwxbeP7B8
https://www.youtube.com/watch?v=U76Ll3OuBsU

Definitions:

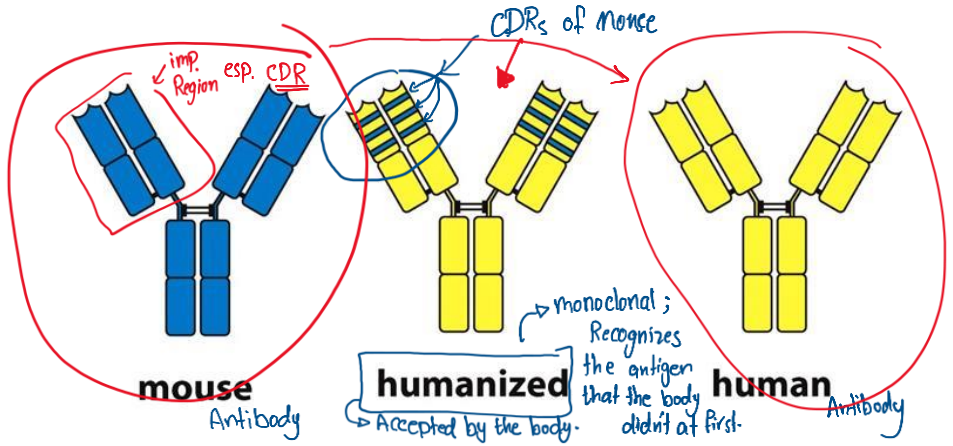
Polyclonal antibodies: A term referring to a collection of antibodies produced from different B-cells, each recognizing a different epitope on the same antigen and binding to it with a different affinity.

A monoclonal antibody: An antibody produced from a single B-cell that specifically recognizes and bind to a single epitope on an antigen. Giving it the unique property of binding to the same antigen with the same affinity all the time.

Hybridoma cells: The immortal B-cells that can produce the same type of antibody.

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

*CDRs are the most important regions in an antibody.



Detailed explanation (not for memorization):
Scientists create mouse antibodies to cure human diseases, but when used in humans, the immune system perceives them as foreign and can halt the treatment. To address this, they humanize the mouse antibodies. This involves replacing most of the mouse antibody's protein structure with the corresponding human antibody structure, except for the region (CDRs) that interacts with the target antigen epitopes. The resulting humanized antibody reduces the likelihood of rejection by the human immune system, enhancing its safety and efficacy in treating human diseases.

Benefits of monoclonal antibodies

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

  Medical labs may use an antibody that specifically binds to a molecule we want to measure the levels of. The results must be reproducible over different tests as monoclonal antibodies are used.

- Determine the nature of infectious agents (e.g. types of bacteria).

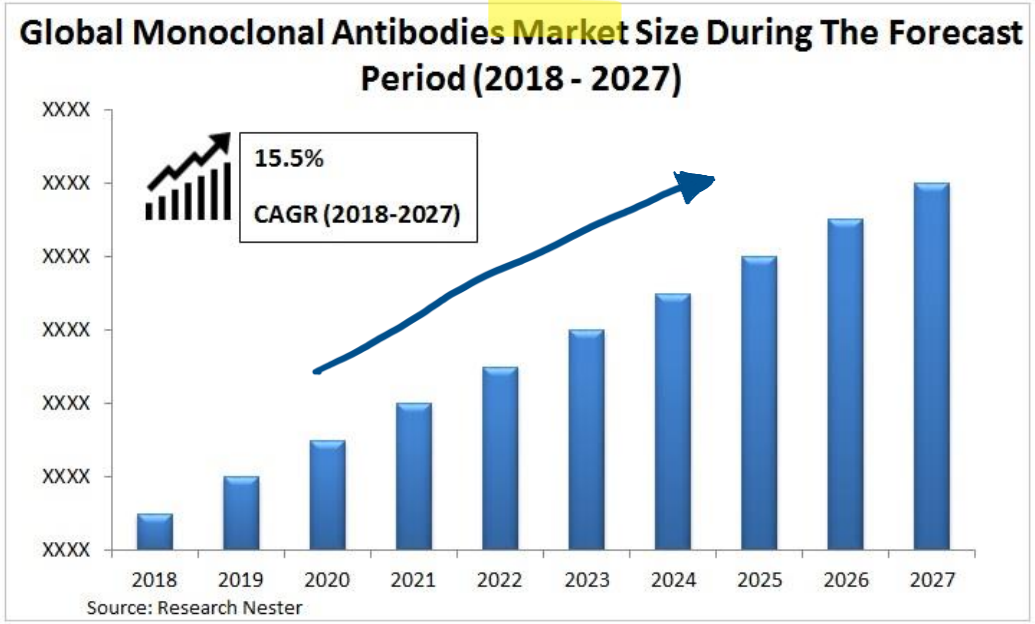
  Antibodies can only bind to a specific epitope on a particular antigen produced by a specific infectious agent, such as bacteria or viruses.

- Used to direct therapeutic agents to tumor cells.

  Recently, scientists started utilizing monoclonal antibodies to treat diseases and cancer, utilizing their ability of specific and strong binding to epitopes.

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

  Antibodies attach to chemicals found in drugs, preventing them from reaching the central nervous system. They also aid in the elimination of drugs from the body.



↑ shows Market growth of monoclonal antibodies; Meaning that they're Beneficial.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	1. #13, there are numerous different types of B-cells, which cannot be bound by a number. 2. General rephrasing and auditing	“Producing 10 types of B-cells”	“Producing different types of B-cells”
V2 → V3			

Additional Resources Used:

1. class switching and B-cell maturation .
2- monoclonal Antibodies and hybridoma.
3- immunoglobulins general overview

رسالة من الفريق العلمي:

كان نبينا صلى الله عليه واله وسلم إذا لقي العدو يقول:
(اللهم منزل الكتاب، ومجري السحاب، وهازم الأحزاب اهزمهم، وانصرنا عليهم).