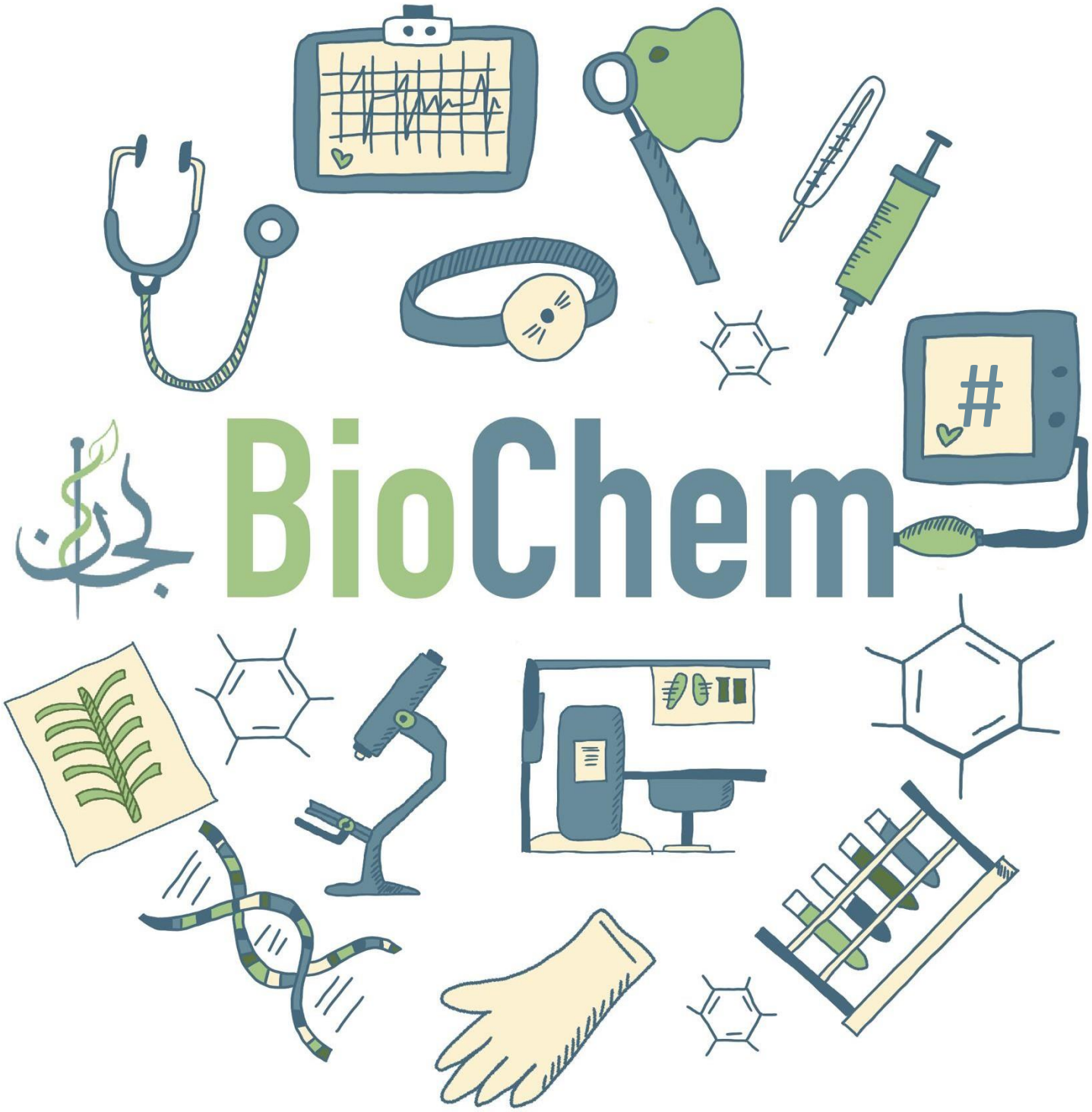

BioChem

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Globular proteins: **Immunoglobulins**

Types of immunity:

A) Innate defenses.

non-specific for a certain pathogen immunity includes surface barriers like skin, internal defenses like phagocytic cells that eat every foreign antigen.

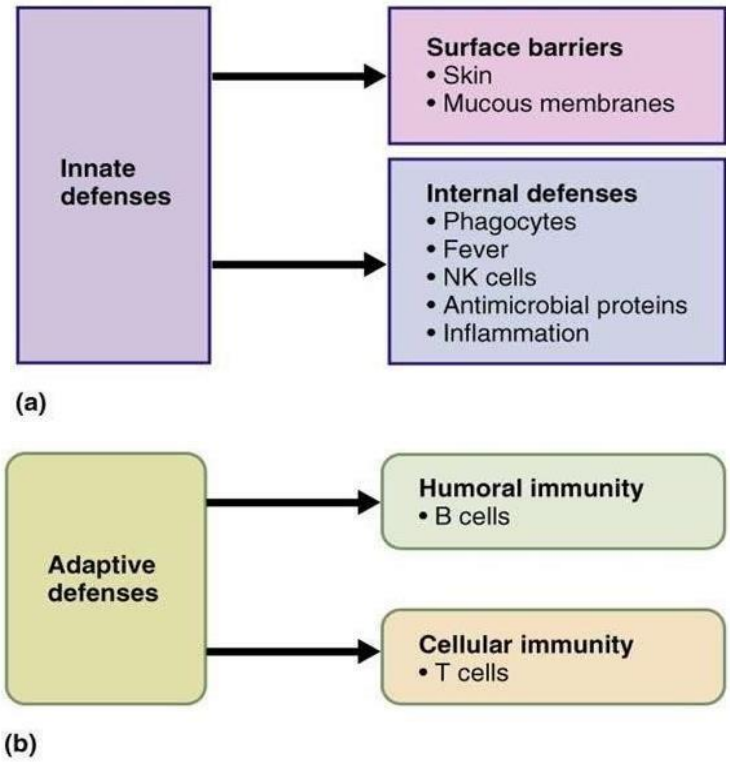
B) Adaptive defenses.

more specific branches of immunity, they attack specific antigens like bacterial proteins, or bacteria viruses, for example the immune system would respond to E.coli bacteria differently than responding to salmonella bacteria, and the response is different from one individual to another.

This immunity is mediated by two types of cells:

1.B cells formed in the bone marrow and produce antibodies.

2.T cells.

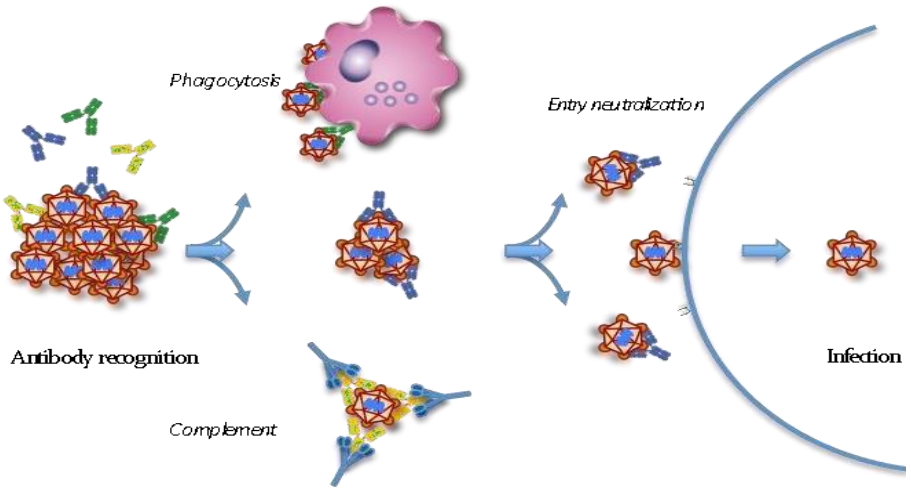


How do B cells work?

B cells represent a branch of adaptive immunity, once activated they secrete molecules called immunoglobulins also known as antibodies.

The three basic primary roles of Immunoglobulins are:

1.phagocytosis. 2.antibody recognition. 3.neutralization.



1) Phagocytosis: immune cells eating antigens

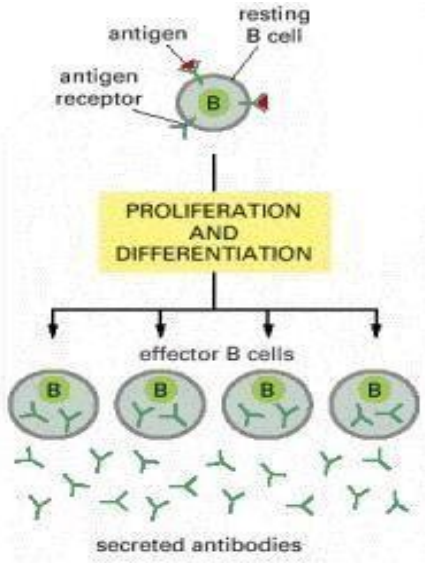
Antibodies bind to pathogens and induce their phagocytosis by immune cells.

in which antibodies guide phagocytic cells like macrophages to where the antigens exist, allowing these cells to eat them leading to antigen degradation.

2) Antibody recognition
Antibodies recruit white blood cells (which are non-specific immune cells) and a system of blood proteins to lyse pathogens, this process is known as complement system activation, the system in turn activates immune cells to release certain proteins that bind to the bacterial cells membrane poking a hole in it and causing lysis or disruption of the plasma membrane so they will eventually die.

3) Neutralization simplest mechanism of action of antibodies
Antibodies bind to pathogens like viruses, bacteria, and molecules like microbial toxins neutralizing them and preventing them to infect other cells.

What happens when B cells recognize an antigen?

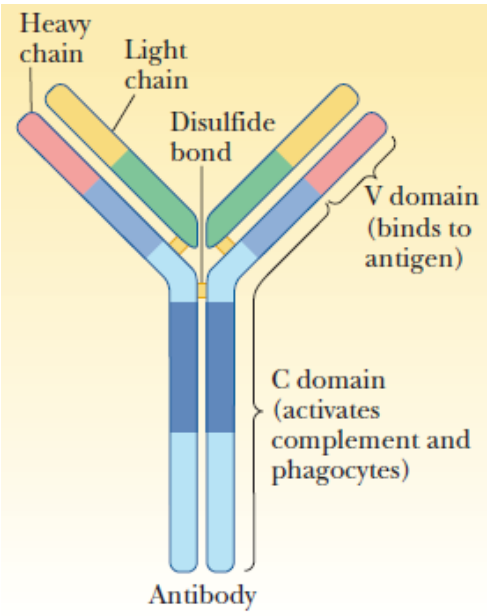


Each B cell in our immune system has an antibody on its surface that functions as antigen receptor.
B cells that have not yet interacted with an antigen are called naïve.

antigen binding activates B cells causing them to proliferate, grow, and divide forming clones of B cells, some of these cells produce antibodies with strong antigen-affinity and as a result they continue to divide, and the others that produce antibodies with less affinity than the parental B cell die.

B cells differentiate and undergo changes in the gene that produces immunoglobulins into effector B cell—an antibody secreting cell, changes vary from one B cell to another producing different types of antibodies 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.
- Heterotetramers composed of four chains:

two identical heavy chains and two identical V-shaped light chains held together by disulfide bonds.
the heavy chains are structurally different than the light chains.

- The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds

-the heavy chains are connected to each other via a disulfide bond, and each light chain is connected to a heavy chain by a disulfide bond.

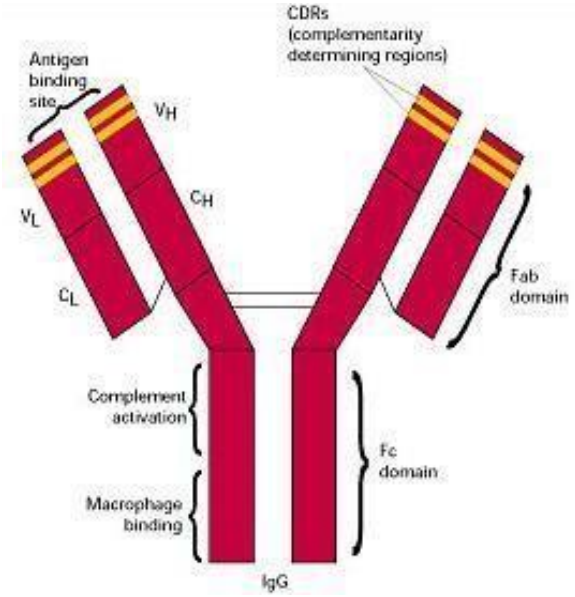
-Within each of the polypeptide chains there are also intra-chain disulfide bonds stabilizing the structure of the molecule (chain).

- They are glycoproteins, with oligosaccharides linked to their heavy chains(only).

Antibody regions

1.variable region. 2.constant region. 3.Fc region. 4.hinge region.

antibodies consist of two domains, the first one is the constant domain and the other is the variable domain.



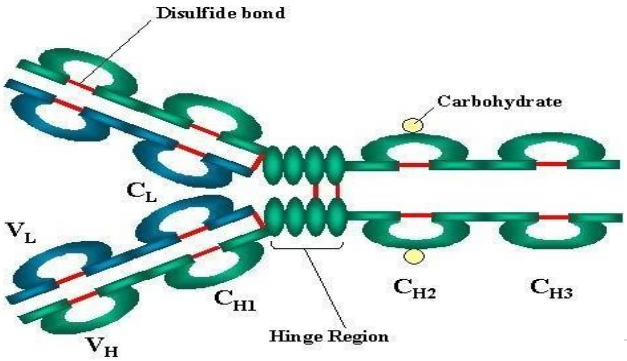
- A light chain consists of two domains: one variable (VL) and one constant (CL) domain.
- Each heavy chain consists of one variable region (VH) and three constant regions (CH1, CH2, CH3).
- VL and CL pair with VH and CH, respectively.

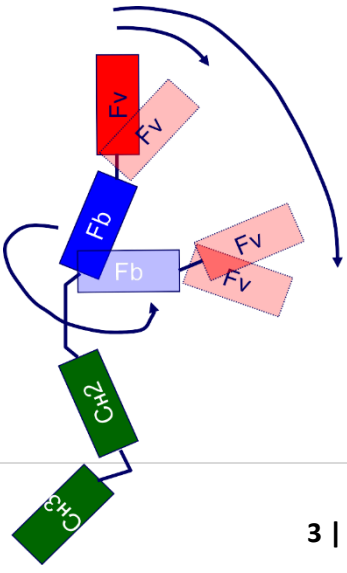
the variable domain of the light chain interacts with the variable domain of the heavy chain and the constant domain of the light chain interacts with one of the constant domains of the heavy chain.

- Constant regions are uniform from one antibody to another within the same idiotype (same class) meaning antibodies could have the same constant domain but differ in the variable domain.

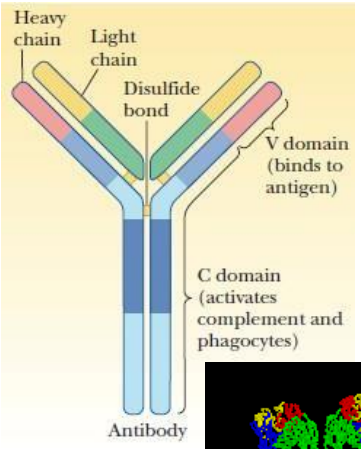
- The Fc domain of antibodies are important for binding to phagocytic cells allowing for antigen clearance.

Right in the middle of the antibodies' structure, present at the constant region of the heavy chains only is the **Hinge region** which exists where the arms of the antibody molecule form a Y, as it provides flexibility to the molecule so when it binds to an antigen it can orient itself in a proper angle and bend for a better interaction with a higher affinity.





Variable regions **the most important part of an antibody



The variable region is found at the tips of the Y-shape of the immunoglobulin.
it recognizes the "epitope" of bacteria which is the antigen binding site.

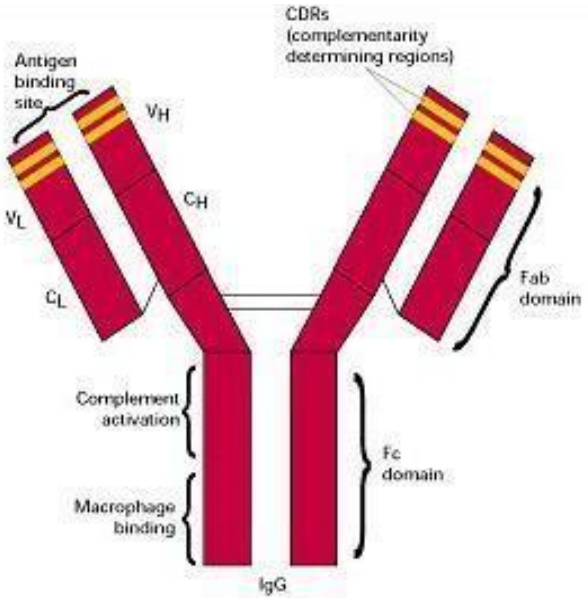
- each immunoglobulin can bind to two antigens of the same type; the variable regions of both the heavy and light chains on one side of the antibody can bind to an antigen at a particular position on the antigen (the epitope), this also applies to VL and VH on the other side binding to the same exact type of another antigen.
- The variable region is different among different antibodies, the primary sequences of the variable regions among different antibodies are quite distinct, in which they differ in structure and length of (7-12) amino acid sequence.
  this determines which antigen an immunoglobulin would bind to.
- Each B cell clone produces only one kind of an antibody, having the same exact type of a variable region different from antibodies of other clones.

Hypervariable regions

"Hypervariable" regions, also known as "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains right at the tip of the immunoglobulin.

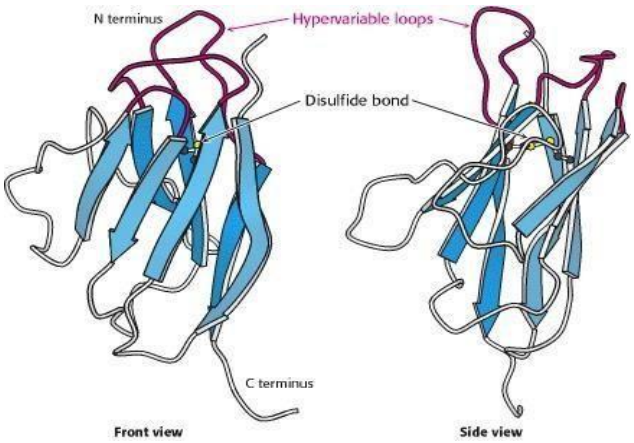
-These regions recognize and bind specifically to antigens with high affinity (dissociation constant (Kd) 10^{-12}- 10^{-7}).

-The differences in the amino acid sequence among antibodies are in the hypervariable region, these differences provide both diversity and affinity in terms of specificity in binding to antigens.
so, once the hypervariable regions bind to the antigen, the binding is usually strong which means high affinity.



further analysis:
hyper → very.
variable → diverse.
hypervariable → very diverse.
so, at the hypervariable regions you'd find very diverse differences in amino acid sequence.

Immunoglobulin fold (beta strands + loops)
further zooming at the structure of the hypervariable region lies the immunoglobulin fold.

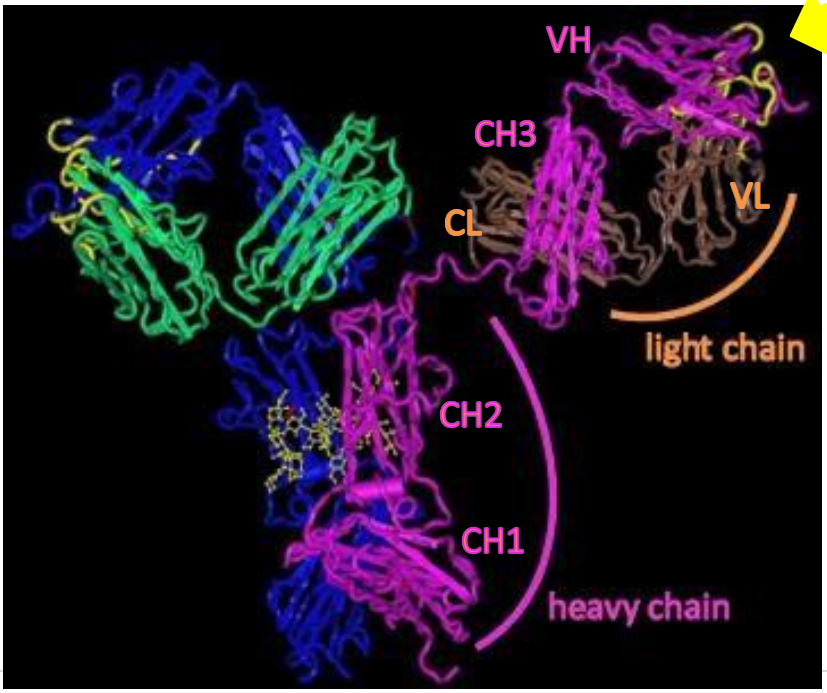


- The hypervariable regions exist in a specialized domain that's present in every immunoglobulin called "Immunoglobulin fold".
- The hypervariable regions exist in three loops connecting the β sheets to each other.
  each loop is a part of a larger structure that is full of β strands.
  these loops recognize and interact with antigens.
- It's like a sandwich of two halves of anti-parallel β sheets held together by a disulfide bond, four are present in the front and four in the back.

the orientation of loops with beta strands forms a barrel-shaped structure, hence the name "beta barrel".
zooming sequence: variable region → hypervariable region → immunoglobulin fold → loops
the very specific part where 7-12 amino acids **variety exists, and the part that recognizes the epitope and binds to the antigen is the loop.

in the picture below u can see the different domains of heavy and light chains in an immunoglobulin.
the structure on the right colored in pink, starting from the bottom to the top respectively: constant domain1, followed by constant domain2, then constant domain3 of the heavy chain, ending with the variable region that includes the immunoglobulin fold where the hypervariable region extends to the outside (yellow-colored loops.)
the light chain is **brown**-colored, with one constant region and one variable region that has the beta barrel structure and the extending loops in yellow.

loops of VH AND VL



the blue and pink structures are heavy chains with identical structures and 3 constant regions + 1 variable region

the brown and green structures are light chains with identical structures with 1 constant region + 1 variable region

Diversity

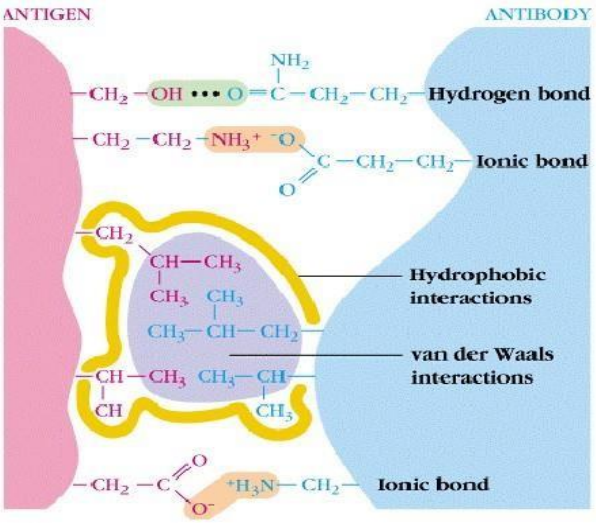
antigen-antibody binding is mediated by non-covalent interactions such as:

1.hydrogen bonds.

2.electrostatic interactions.

3.hydrophobic interactions.

4.van der waals interactions.



The enormous diversity of antigen-binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops, so again binding affinity is determined by the amino acid sequence within the loop as well as the length of the loops.

- Conclusion: depending on the antibody it recognizes either the overall 3D structure of the antigen or its amino acid sequence.

Diversity of antibodies

The number of antigens exceeds the number of B cells in our body. So how can antibodies recognize all the different antigens?

Antibodies can do that because each individual is capable of producing more than 10^{11} **different** antibody molecules with different sequences.

This is done during activated B cells differentiation via:

1. DNA rearrangement for the different exons that code for the variable region and the joining region of antibodies.
2. Imprecise joining of regions during the DNA rearrangement.
3. Addition/deletion of nucleotides during rearrangement.
4. Somatic hypermutations:
   as B cells proliferate and synthesize DNA, they introduce mutations in certain regions especially in the hypervariable region.
5. Alternative splicing of the RNA molecule.

these genetic mutations produce different clones of B cells, but one or two clones would have high affinity interaction with a specific antigen.

- Another level of diversity that can be created in immunoglobulins is in the constant region ofboth the light chain and the heavy chain:

two genes can code for the **light chains** known as “lambda” or “kappa”

there are also **five heavy** chains that make five different types of immunoglobulins known as Immunoglobulin isotype in which each constant region is produced from certain exons in the heavy chain gene.

1.IgA	2.IgD	3.IgG	4.IgE	5.IgM
A→ alpha exon	D→ delta	G→ gamma	E→ epsilon	M→ mu

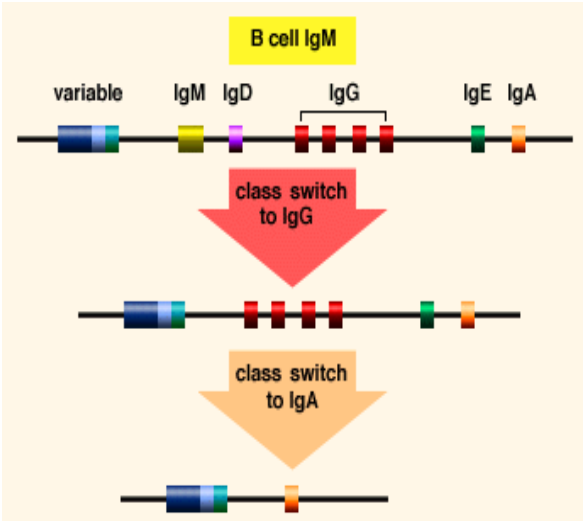
Ig stands for **I**mmuno**g**lobulin

Class Switching occurs in the heavy chain

naïve B cells usually have an immunoglobulin on their surface (antigen receptor), specifically IgM molecules. (That means before antigen binding, B cells contain IgM molecules **only)**.

-when these B cell recognize an antigen and bind to it, it gets activated and class switching occurs.

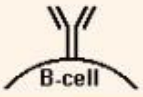
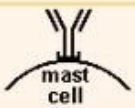
-class switching is DNA rearrangement in which the exons that code for the constant region gene of the heavy chain get rearranged, the variable region would move closer to other class exons.
this causes the production of IgG, IgA, and IgE.



Class switching from IgM to IgG to IgA

The constant region of the antibody heavy chain is changed, but the variable VH stays the same and since the variable region does not change, the antibody retains specificity and affinity for the same antigen but can interact with different effector molecules.
the constant region plays an important role in the type of immune cells the antibody can bind to.

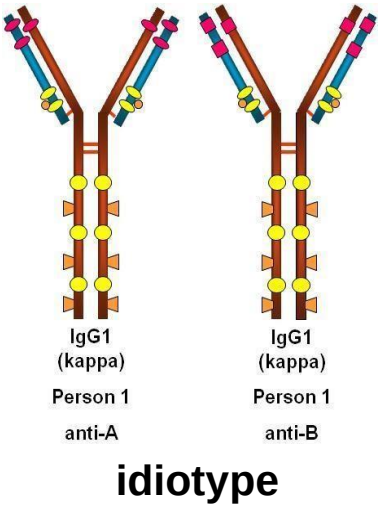
Types of Antibodies upon class switching as each has its own function

Isotype	Structure	Notes
IgM		Contain mu heavy chains Expressed on the surface of B-cells, right before recognizing the antigen. Once the IgM on the cell surface interacts with the antigen, the B cell becomes activated, starts to proliferate and differentiate further and undergoes class switching. 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 so this antibody can bind to 10 different antigen molecules
IgG		Contains Gamma chains, produced from the gamma exon. 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 Presents 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, as it binds to the cell surface of immune cells (mast cells).
IgA		Contains alpha chains Found mainly in mucosal secretion The initial defense in mucos against pathogen agents Appears usually as dimers

***IgM and IgD are always on the cell surface and not secreted*

Idiotype vs. isotypes vs. allotypes

antibodies can be Idiotype, isotypes, or allotypes to each other.



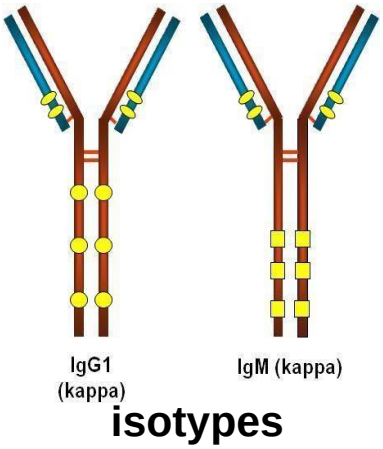
- idiotypes→ same constant region, different variable region.

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

these immunoglobulins have different amino acid sequence at the hypervariable region, they can be produced against the same antigen, and one would have higher affinity than the other, or they can bind to different antigens.

- isotypes→ same variable region, <u>different constant region</u>.

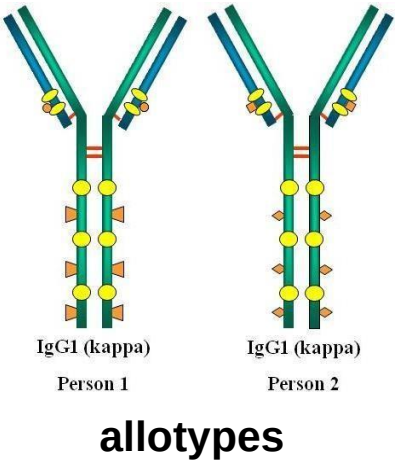
The <u>different classes</u> of immunoglobulins are determined by their different CH regions and called **isotypes**.



this happens in the case of class switching, once antigen-interaction occurs B cell proliferate and produce IgM antibodies at first, then class switching takes place, in which the variable region would be close to the Gamma exon instead of being close to the Mu exon, producing an IgG antibody that has the same variable region as IgM antibody.

**isotypes recognize the same epitope

- Immunoglobulins of the same class—same type of constant region, but different in the primary structure of immunoglobulins among individuals of the same species due to different genetics are called **allotypes**.



allo→ comes from alleles which are different forms of the same gene.

little differences in DNA sequences among individuals that result from single-nucleotide polymorphisms, so even though individuals would have the same type of constant region but there are little differences that can differentiate one person from another.

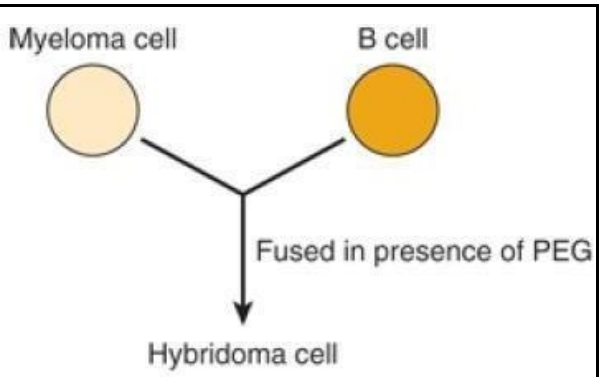
Hybridoma and monoclonal antibodies

-monoclonal antibodies are important in diagnosing diseases and treating patients.

-the concept is producing the same exact antibody (monoclonal) from an immortal B cell.

Formation of hybridoma

hybridoma is a hybrid cell produced from fusion of two cells, one is a B cell and the other is a cancer cell (myeloma cell).

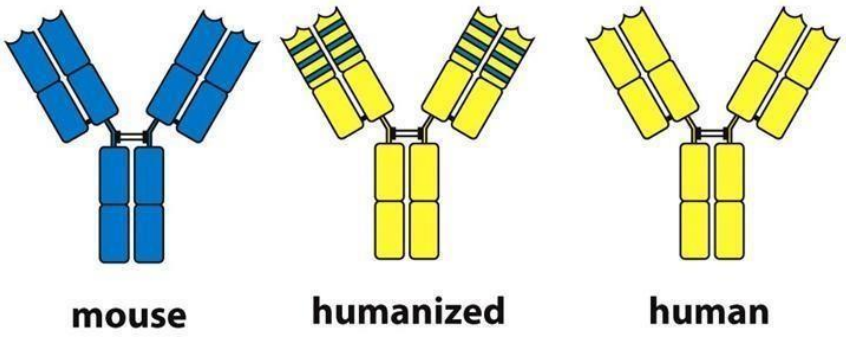


the hybridoma cell is immortal because it behaves like a cancer cell that keeps on dividing, and it behaves like a B cell producing the same exact type of antibodies.
these antibodies are known as monoclonal antibodies.
why cancer cells? because normal cells in our body die eventually, and cancer cells don't

polyclonal antibodies: produced from different B cells
when an antigen enters an individual's body, different B cells can recognize the antigen, and each one of them would be activated to produce antibodies; antibodies produced from the same B cell are identical, however antibodies produced from different B cells are different in structure and sequences.
these antibodies are said to be polyclonal, meaning they are directed against a number of different epitopes on the antigen.

it's not possible to produce monoclonal antibodies against a specific antigen in humans as its unethical to inject an antigen in a human and collect antibodies.
and in order to have a single type of an antibody a hybridoma is created, in which scientists have created humanized antibodies, by injecting an antigen into an animal (like a mouse)—the mouse would produce antibodies.

they take the gene that produces the antibody, and with genetic engineering technologies the constant region would be a human and the produced immunoglobulin has all the sequences and domains of human immunoglobulin, but the hypervariable region (CDRs) that recognizes a certain antigen would be from the mouse (it would be attached to specific regions of the immunoglobulin), thus creating a humanized immunoglobulin.



humanized immunoglobulins can be injected in humans as it's considered normal, not an antigen unlike injecting a complete mouse immunoglobulin that induces an immune response once injected because it is an antigen by itself.

Benefits of monoclonal antibodies

1)Measure the amounts of many individual proteins and molecules, in clinical laboratories monoclonal antibodies are used to measure the amount of plasma proteins, steroid hormones and the same exact results are obtained consistently and reproducibly because the same antibody would bind to the same antigen at the same site with the same affinity every time.
2)Determine the nature of infectious agents (e.g. types of bacteria).
by using an antibody against a known antigen, for example if you use an antibody against E.coli in a sample that has a bacterium of an unknown type and the bacteria specifically binds to the antibody, u can confirm that E.coli is present in the examined sample.

3) Used therapeutically, immunoglobulins are used to target tumor cells, or recognize cytokines or inflammatory proteins.

4) Used to accelerate drug removal from the circulation when they reach toxic levels.

Self-Assessment questions

Q1) the main purpose of the hinge region of antibodies is:
A. antibody clearance.
B. binding phagocytic cell.
C. allowing better binding to the antigen.
D. site of sugar binding.
E. binding to antigenic epitopes.

Q2) Monoclonal antibodies:
A. are less specific than polyclonal ones.
B. are produced by myeloma cells.
C. can be humanized by attaching the CDRs to specific regions of it.
D. b and c are correct.
E. none of the above.

Q3) After binding to its specific antigen, a B lymphocyte (cell) may switch its:
A. immunoglobulin light-chain isotype.
B. immunoglobulin heavy-chain class.
C. variable region of the immunoglobulin heavy chain.
D. constant region of the immunoglobulin light chain.

Q4) what is wrong regarding immunoglobulins:
A. have motifs to help bind to other molecules.
B. can be used to detect antigens.
C. have disulfide bonds.
D. lipoproteins.
E. have quaternary structure.

Q5) Immunoglobulin structure has the following feature:
A. Noncovalent interactions connect the heavy chains to light chains.
B. All immunoglobulin types are found as dimers.
C. Differences in the constant regions make isotypes.
D. The hypervariable regions are present on the tips of the heavy chains but not the light chains.
E. The carbohydrate moiety is linked to the Fc domain of the light chains.

Q6) Most effective antibody is
A. IgM
B. IgE
C. IgG
D. IgA

Q7) IgM is a
A. pentamer with 10 antigen binding sites
B. monomer with 2 antigen binding sites
C. dimer with 4 antigen binding sites
D. tetramer with 8 antigen binding sites

Q8) The immunoglobulin fold is
A. found only in IgG molecules.
B. composed of two antiparallel b-strands folded into a globular domain.
C. a b-barrel composed of a three-loops and a four-stranded antiparallel b-sheet.
D. found six times in the IgG molecule.
E. The third and fourth choices are both correct.

Q9) Antigenic determinants bind to which portions of an antibody?
A. variable regions.
B. constant regions.
C. only light chains.
D. only light chains.
E. the effector region.

Q10) Monoclonal antibodies produced in the laboratory
A. lack the constant regions of IgG.
B. cannot be used for disease diagnosis yet.
C. derive from human cancer patients.
D. can be selected to bind to almost any known molecule.

Question	1	2	3	4	5	6	7	8	9	10
Answer	C	C	B	D	C	A	A	C	A	D

THANK YOU