Doctor 022

Biochemistry

Sheet no.

19

Immunoglobulins

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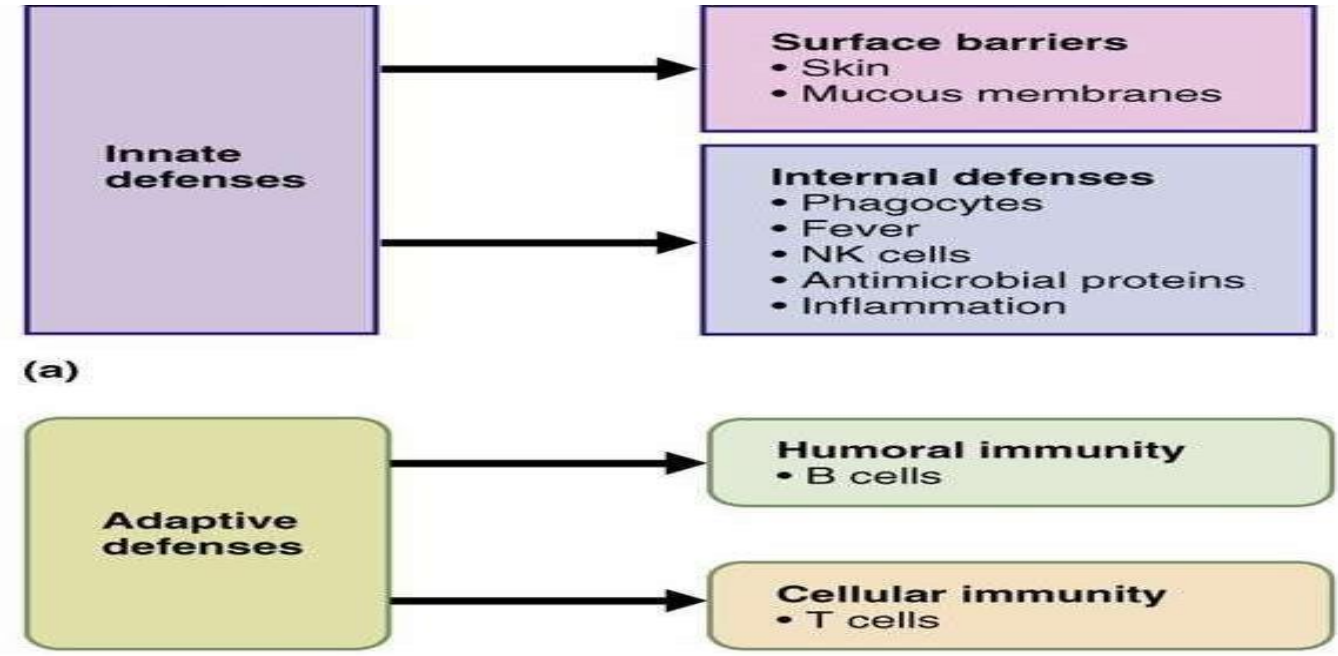
Immunoglobulin(antibody)

We have 2 types of the "immune system":



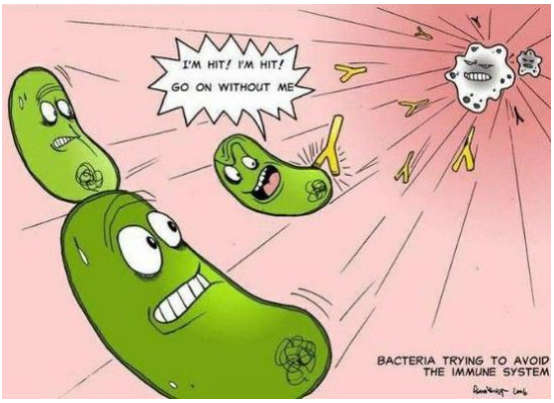
Innate (nonspecific)

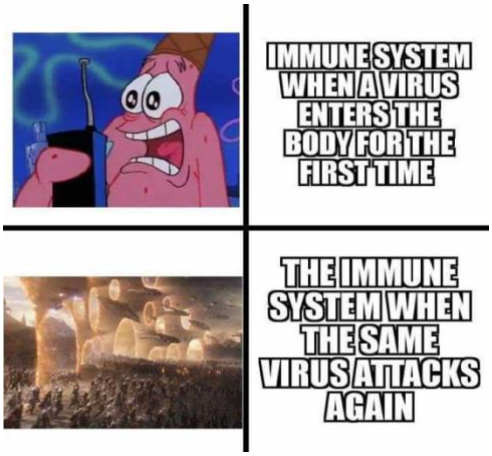
- When any antigen enters or try to enter the body and the body resists it .
- (we won't talk about the skin and mucous membrane and how they physically prevent the invade) we will talk about human cells, which are natural killer cells, macrophages and different types of immune cells
- Internal Defense includes the case when an antigen enters the body, how is the body going to deal with it?! For example, when a bacteria enters the body, they eat it by phagocytosis (cell eating) whatever the kind of bacteria is, that's how nonspecific immune system works.

- **Also, fever is part of the inflammatory response that indicates that the body is dealing with something (not necessarily an invade, it may be a fracture..)**

Adaptive

- **adapts with the virus or the bacteria that enters the cell, this type of immune defense develops over a longer period of time.**
- **there are two types of cells: B cells and T cells, they are specific cells for specific antigens**
- **Not any B cell can attack the bacteria , a specific B cell attack it according to the antigen or the bacteria that enters the cell, so that the B cell that recognizes the antigen will be activated so it will attack the bacteria , a part of B cells will transform to memory cells, therefore if the bacteria enters the cell again it will be easy for the B cell to recognize the bacteria so the reaction will be fast , and not any T cell can attack the virus , the same as B cell.**
- **The name of B cell is related to the source of this cell that is (Bone marrow) and T cell from (thymus).**





How Do B cells work?

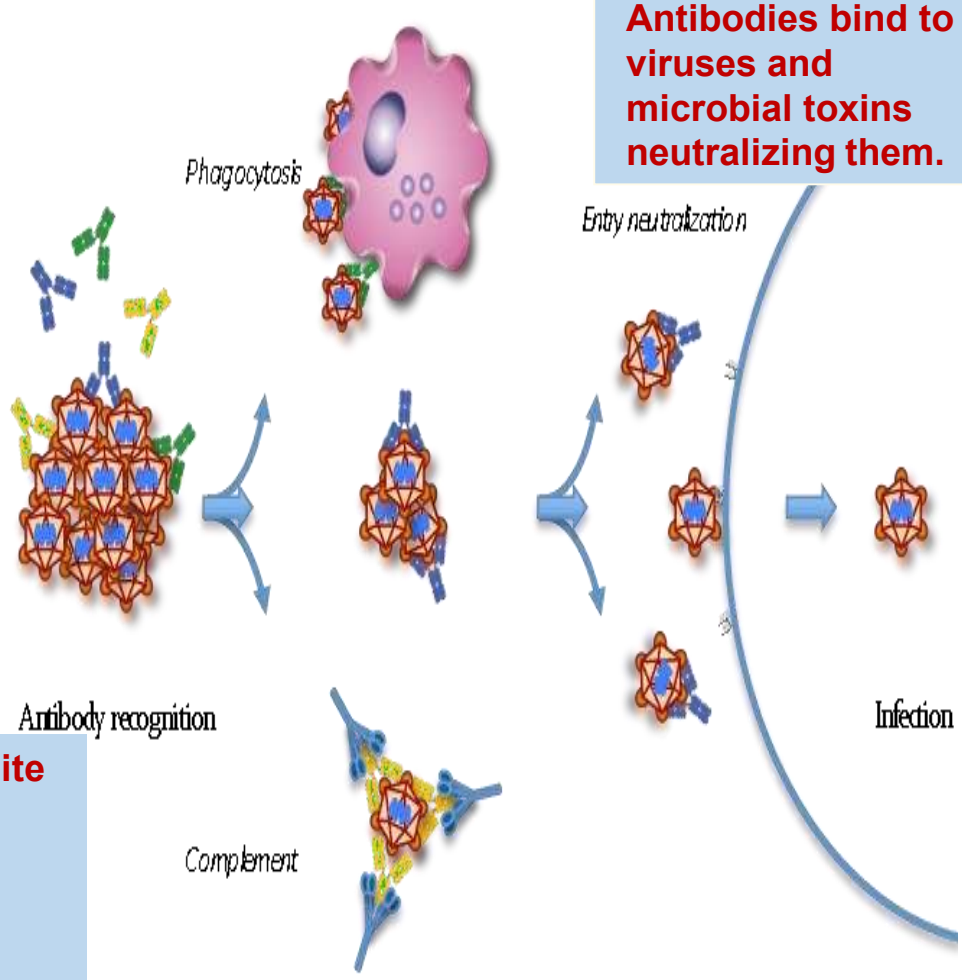
- B cells secrete immunoglobulins after activation (also known as antibodies), **these antibodies are specific proteins that attack or bind to the antigen and neutralize it, therefore the bacteria won't be able to attack or enter the cell. Another thing, these antibodies may help cells like (macrophages, neutrophils) to be able to phagocyte the bacteria or the antigen.**

Immunoglobulins have three roles (functions):

The 2 roles on the left are done by directing other cells to accomplish the degradation, but the role on the right is done by the antibodies themselves.

Antibodies bind to pathogens and induce their phagocytosis

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*)

[The mechanism for this process is that antibodies can stimulate complement system (specific proteins that are

secreted from B cells), that makes holes on the bacterial membrane, thus they help to fight and remove the antigen.] (we won't talk about this in details).

- **There is no strong immune system in newborns because they haven't recognized any antigen so far, so they take the antibodies from their mothers while they are fetuses (through the placenta), then they take the antibodies from the mother's milk, that's why babies who are breastfed have stronger immunity than those who aren't breastfed .**
- **It takes few months for the infant to have and develop their own immune system (when the antigens start to enter the body).**

When B cells recognize an antigen…

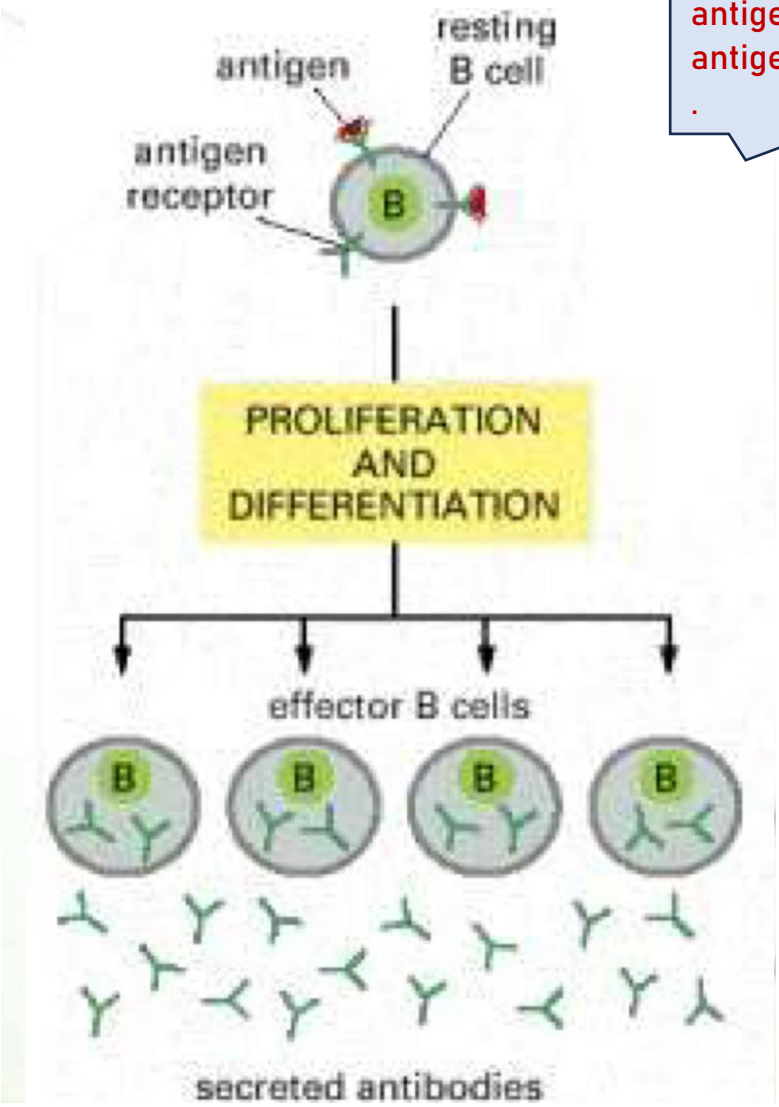
After development of the B cells, each B cell makes only one antibody on its surface. This B cell can be cloned (by producing a single antibody type).

For infants, their bodies store B cells. For example, they have million dormant B cells (inactive ,sleeping). When an antigen enters the body and bind to its receptor on the resting B cell (as indicated in the figure below), let's say ten out of million cells recognize the antigen, so these cells will be stimulated and start to grow, divide and proliferate. The rest cells (that haven't recognized the antigen) are still dormant.

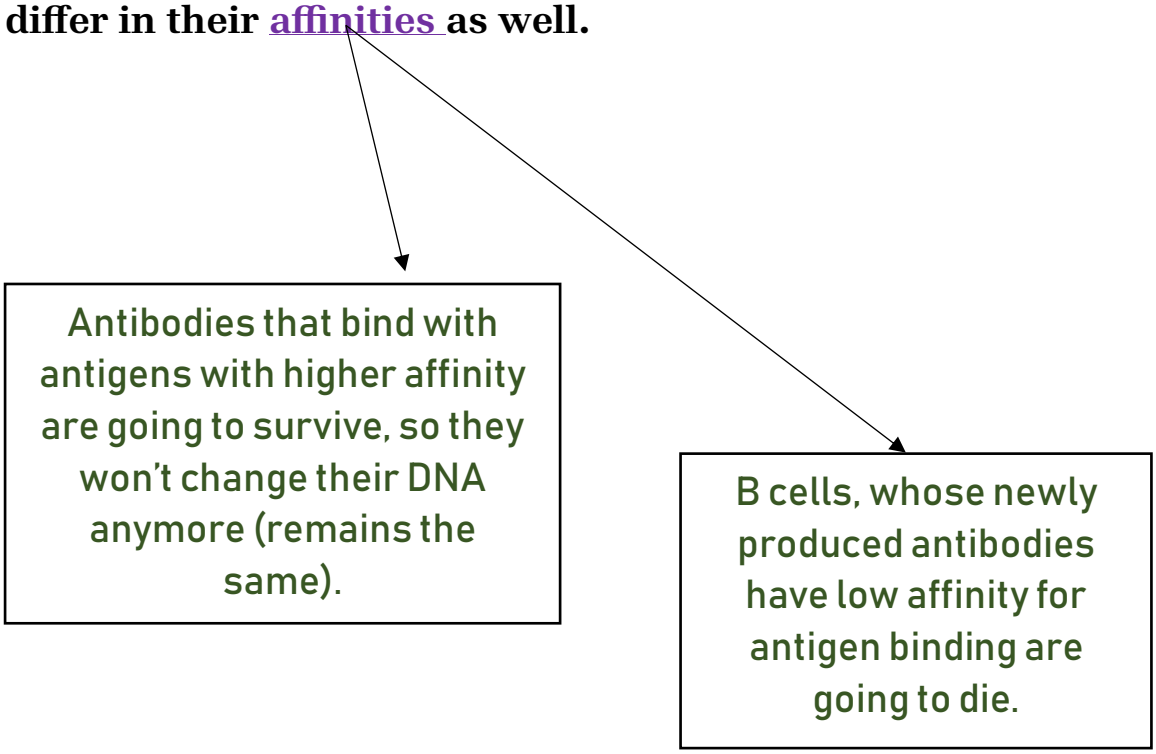
- **When a B cell is activated by antigen, it proliferates and differentiates into an antibody-secreting effector cell .**
  Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibody at a rate of about 2000 molecules per second, then these antibodies will start functioning by the 3 pathways that were mentioned before.

- **Each individual can produce more than 10^{11} different antibody molecules.** Surely, these antibodies haven't been coded from different 10^{11} genes, there are some mechanisms we will study later on to use 1 gene for the production of many antibodies.

Antibodies bind with antigens NOT with antigens' receptors .



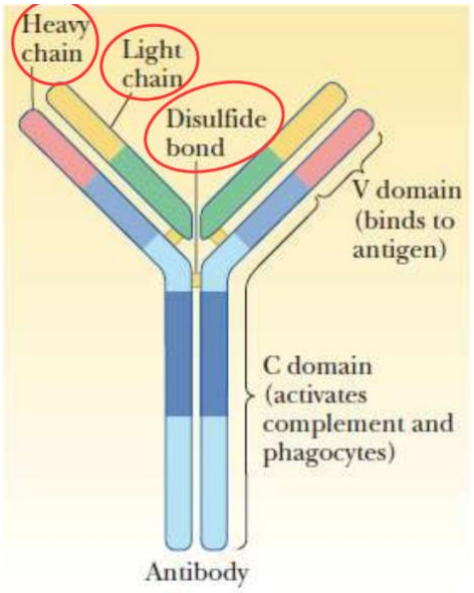
- **DNA synthesis is required during growth and division of cells (that have been stimulated). Each one of the 10 stimulated cells will start to change its DNA. Each will differentiate into (for example) 10 different antibody-secreting effector cells, with different antibodies for each .So, now we have 100 different types of B cells (100 different antibodies) and these antibodies differ in their affinities as well.**



- **So, after proliferation and divison, only B cells with high affinity antibodies are going to survive .**

- **Eventually, active B cells will release a single type (not molecule) of antibodies. And, as we know, antibodies will neutralize antigens, help in phagocytosis by binding to the surface of phagocytic cells or they will activate the complement system by which antigens are killed.**

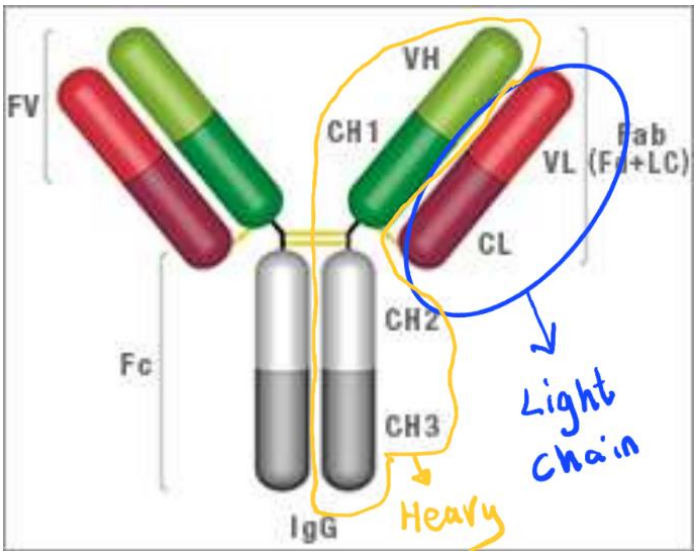
Structure of antibodies



- Antibodies are Y-shaped molecules consisting of two identical heavy chains and two identical light **(so it is a hetro-tetramer as hemoglobin)** 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

C stands for constant , V for variable , H for heavy , and L for light



- 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_{H1}, respectively.**

The variable regions in both light & heavy chains form an antigen binding site, the other 2 variable regions also form an antigen binding site. Thus, we have 2 antigen binding sites that both recognize only one 'type' of antigens . Variable regions identify antigens (that's why they're 'variable', to recognize different

antigens and interact with them) .(NOTE from the picture above : variable regions of light chains are identical and variable regions of heavy chains are also identical , but the variable region in the light chain is different from that in heavy), while constant regions help in stimulation of the complement system or other immune cells such as : (macrophages & natural kill cells, which will do phagocytosis). (NOTE : Don't worry about different locations of light chain between last 2 illustrations). Each variable region binds to a single antigen.

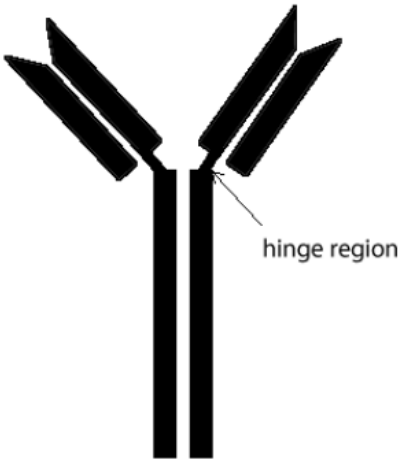
- Constant regions, are uniform from one antibody to another within the same isotype , they also helps the antibody's attachment to cell surfaces (example the antibody on the B-Cell's surface)

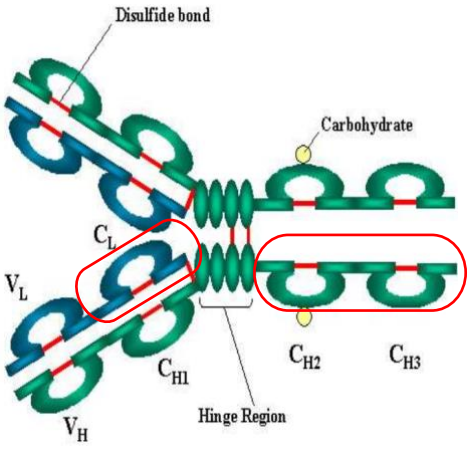
- The Fc domain of antibodies is important for binding to phagocytic cells allowing for antigen clearance.

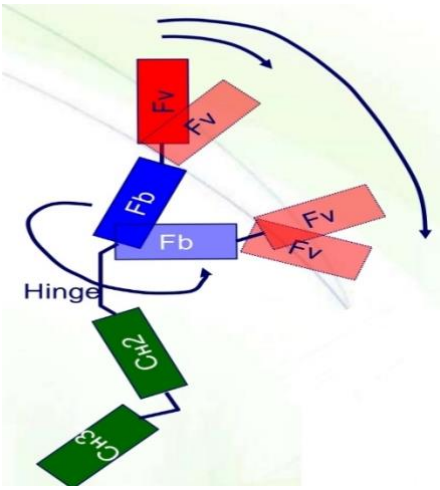
Hinge region

Antibodies should have a kind of flexibility.. why? Because the distance between variable regions may have to change so that they can bind effectively with higher affinity with antigens. And for that purpose we have the hinge domain (زي الزمبرك).

- A hinge region exists where the arms of the antibody molecule forms a Y, it links CH with CL.
- It adds some flexibility to the molecule.







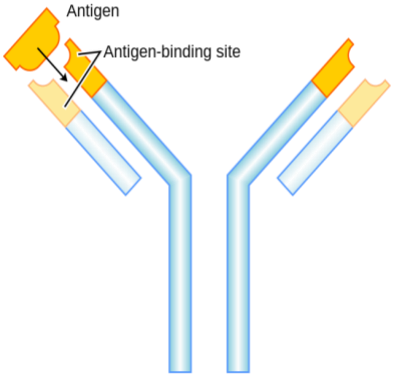
Variable regions

- **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) , the antibody recognizes the antigen by interacting with an epitope.**

** Epitope : is part of the antigen (not antibody), and it is the site or the part that binds to the variable region of the antibodies.

Each antigen contains many epitopes

- Each antibody can bind to two antigens.
- The primary sequences of the variable regions among different antibodies are quite distinct.



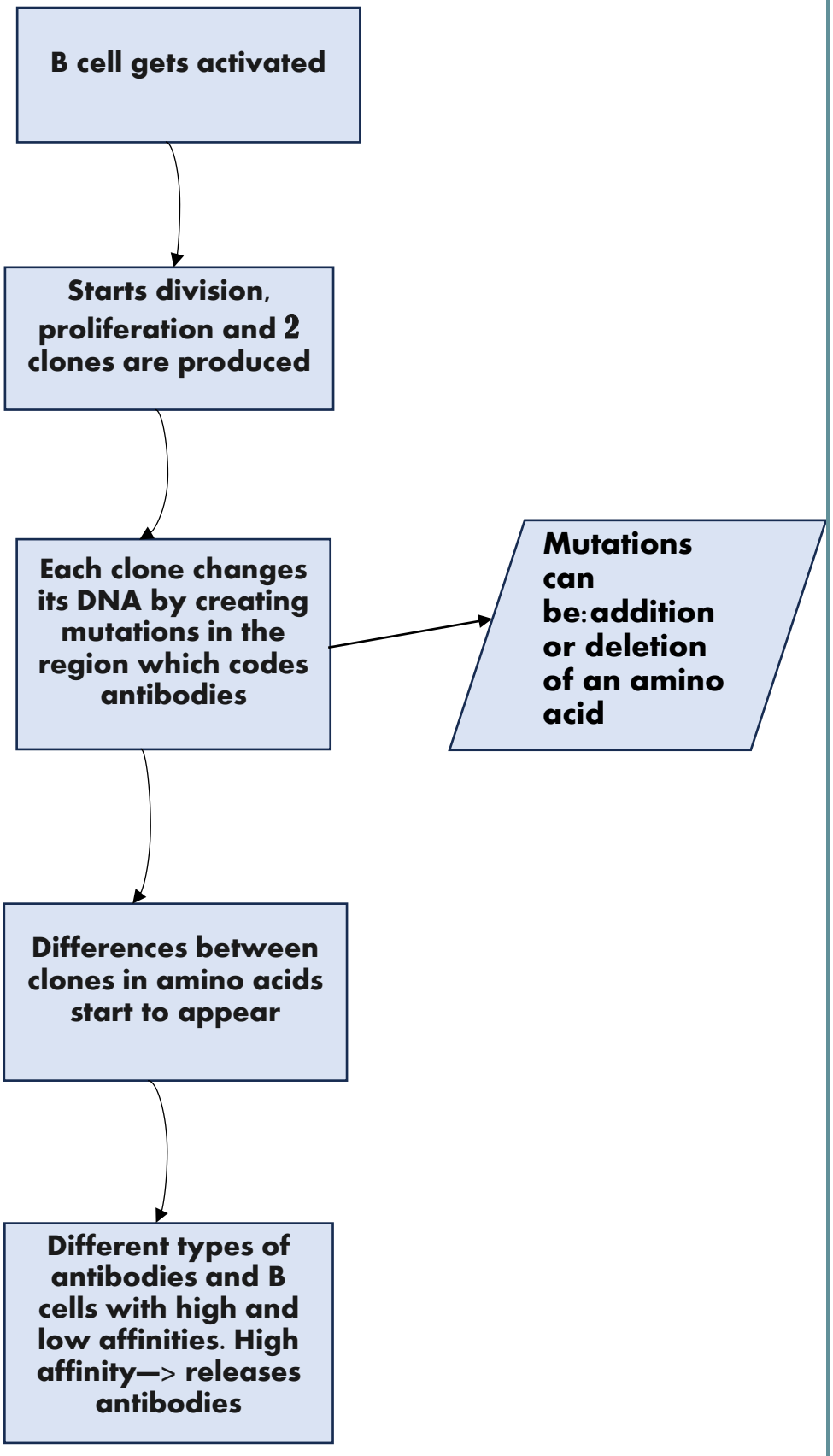
Specifically the region that recognizes the antigen isn't all of the variable region but a small part of it, which consists of about 7-12 amino acids – exists especially in secondary structures such as : loops (remember : loops connect secondary structures , and we have 'secondary structure immunoglobulin folds' which are -beta strands,loops - ; -beta strands, loops-...) which is a complex motif.

For further illustration

So the loop recognizes the epitope.

- **Each B cell produces only one kind of antibody.**

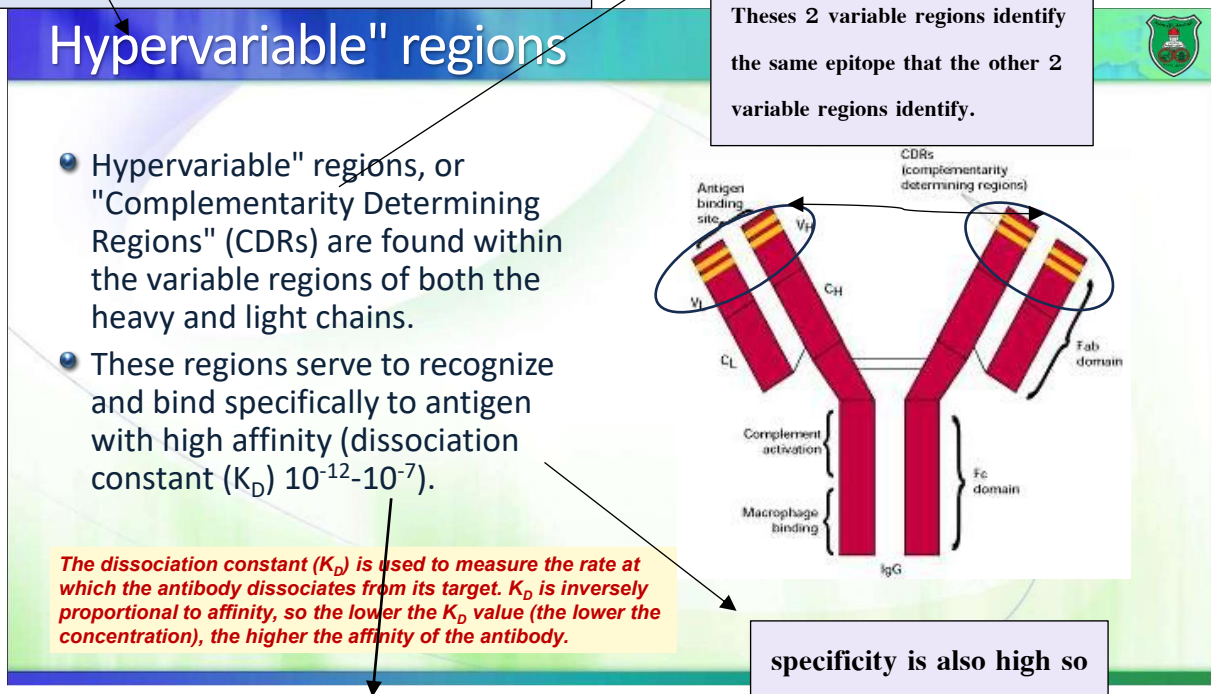
The explanation of this point is similar to what was mentioned in page 6+7, so let's simplify it using the following cascade..!


B cell gets activated
Starts division, proliferation and 2 clones are produced
Each clone changes its DNA by creating mutations in the region which codes antibodies
Mutations can be:addition or deletion of an amino acid
Differences between clones in amino acids start to appear
Different types of antibodies and B cells with high and low affinities. High affinity—> releases antibodies

The name indicates that they aren't only variable, they are excessively variable in their amino acids' sequence (in specific regions). These 'regions' are the loops

They are complementary between antibody and antigen.

Theses 2 variable regions identify the same epitope that the other 2 variable regions identify.



means that 1 antibody and 1 antigen are free

specificity is also high so antibodies bind with specific antigens

-Antibodies produced by B cells have high affinity for antigens, the specificity also is high. So an antibody binds with a single specific and only specific antigen.

-If an antibody binds with an antigen with a similar shape(not the exact antigen), it can lead to an autoimmune disease.

-The affinity is measured by the 'dissociation constant (Kd)' which is defined as the ratio of the concentrations of the free molecules (antibody and antigen) to the concentration of the bound complex (antibody-antigen).

-A low ratio indicates high affinity between the antibody and antigen and vice versa.

Extra:

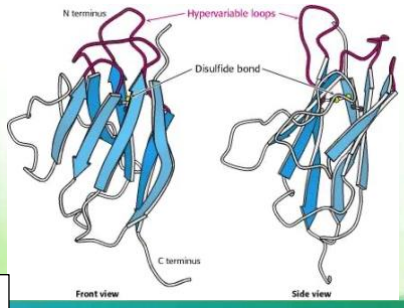
- **The Pfizer vaccine targets only one epitope, while the Chinese vaccine relies on the inactivated virus and targets more than one epitope.**
- **Pfizer's vaccine is considered more effective.**

The hyper-variable region is within the loop, which is a part of the immunoglobulin fold.

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".



بتشبه البرميل

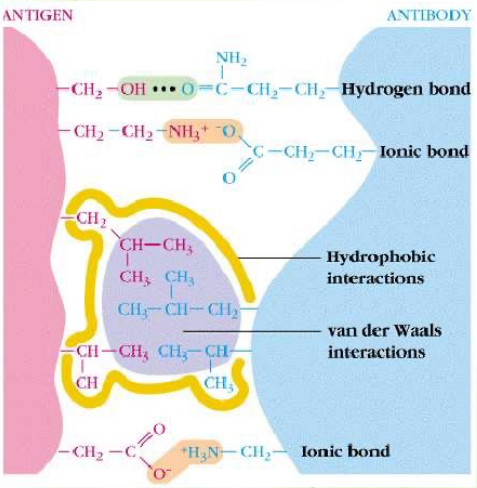
- **The immunoglobulin fold is a complex motif in the variable region.**
- **It is composed of anti-parallel beta sheets connected by loops, and this pattern repeats in the structure.**
- **The loops within the immunoglobulin fold are known as Complementarity Determining Regions (CDRs), and they are responsible for interacting with antigens.**
- **The variations in amino acids (around 7-12) between different antibodies predominantly occur within these loops. Mutations, growth, and synthesis of DNA also happen within these loops.**

This is the variable region(immunoglobulin fold) especially the loops (pointed with arrows)

- Antibodies are glycoproteins since they are glycosylated. Glycosylation occurs on the constant region of heavy chains.
- The 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.
- These interactions are non-covalent, meaning they are usually weak. However, the strength of binding is high because the dissociation constant is very low and the affinity is high.
- The binding process involves multiple non-covalent bonds.
- Any change in amino acids within loops will affect the non-covalent interactions.

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.



- **Let's consider that we have 1 million B cells, each with a different type, in a dormant (inactive) state. The question is, how many antigens exist in nature? There are approximately 10^11 antigens. B cells possess sufficient diversity to recognize all these antigens present in nature.**

- **However, it's challenging for B cells to produce an exact match for each antigen. For instance, when we produce 1 million antibodies , only some of them will bind effectively, and the rest will undergo a process of evolution.**

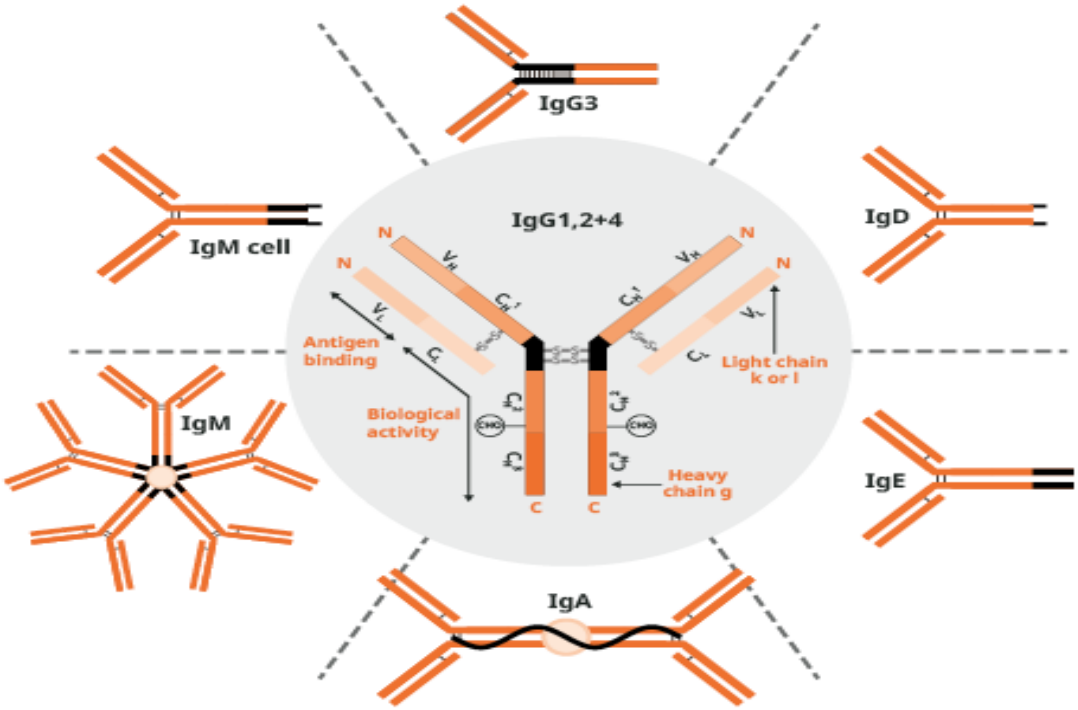
- **Some B cells may bind with low affinity, resulting in a suboptimal product. In such cases, the body disposes of these ineffective B cells.**

More diversity

- **We have lots of (B) cells with immunoglobulins attached, so there are many differences between their variable regions. Also, differences exist among the constant regions but with less variation compared to variable ones.**
- **we have only five different constant regions for the heavy chains and two different constant regions for the light chains. Each one of them has it's own function.**

- **Different heavy chains come from different genes.**

There are 5 "heavy" chains (alpha, delta, gamma, epsilon or mu) that make five types of immunoglobulins known as immunoglobulins isotype (IgA, IgD, IgG, IgE, IgM).
But light chain are only (lambda or kappa).
A combination between heavy and light chains may occur but it is not a random process. Note that in a single immunoglobulin, heavy chains must be the same type, also light chains.



The story goes like this:

An antigen enters the body, dormant B cells have (IgM) on their surfaces. IgM recognizes the antigen and they interact with each other. Thus, B cells are stimulated , proliferate, divide, synthesize and make mutations in DNA. Different clones of B cells (different amino acids, different lengths of loops, different affinities) are produced. B cells with a high affinity IgM start to release it. The advantage of IgM is its ability to form a pentamer (5 different IgMs get connected to each other—> forming a pentamer). This pentamer can bind to 10 different antigenic molecules. It's a quick way of neutralization (IgM's release is followed by its binding with 10 antigens to neutralize them).

Class switching (5 different classes of heavy chain) :

1. **Before binding antigen, B cells contain IgM molecules. only.**

2. **Following antigen binding, class switching occurs.**

The IgM is released, after that a manipulation of the DNA occurs. Instead of the variable region interacting with IgM and producing IgM with a variable region, a switch will happen that the part of DNA which makes the variable region will 'jump' and attach to the constant region which produces IgG. So first of all IgM comes out, then IgG....

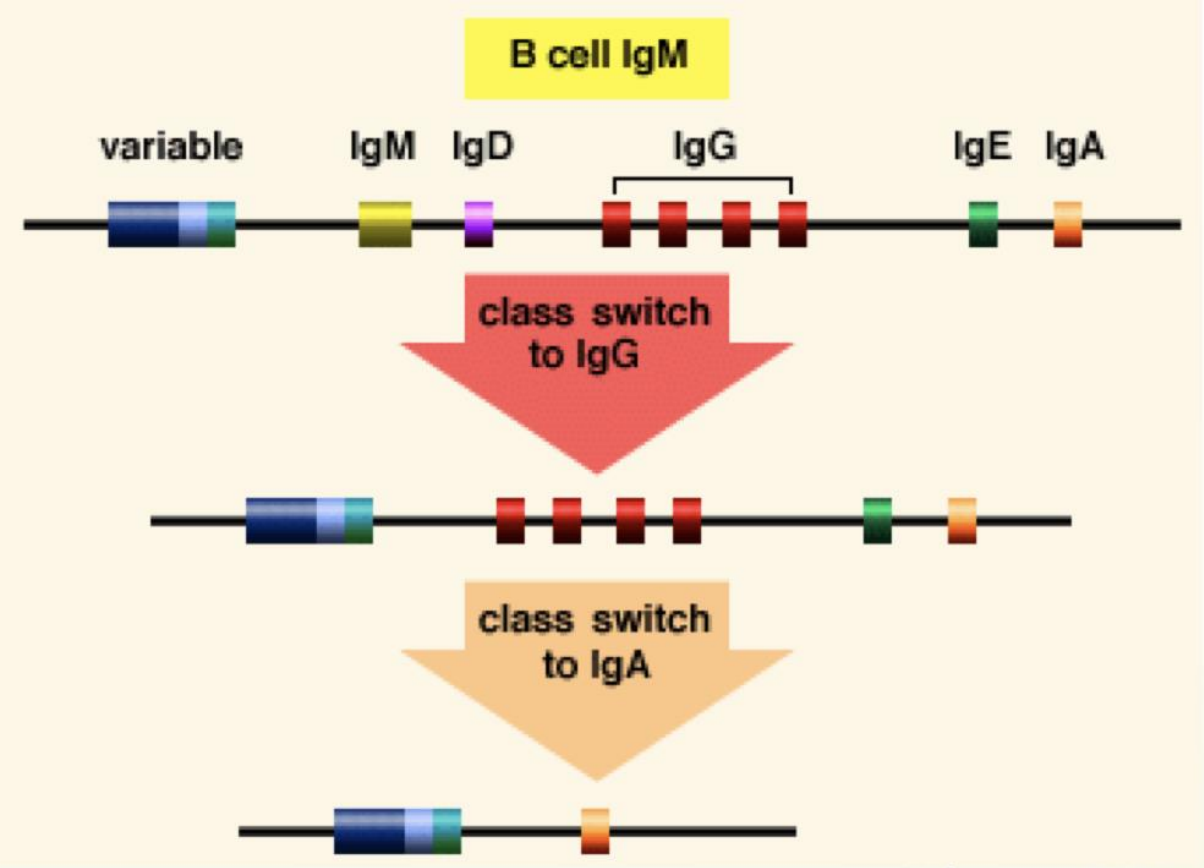
**** Note that the constant region is different whilst the variable region is the same.**

3. **Class switching refers to a DNA rearrangement changing the heavy chain constant gene.**

When a class switching occurs, that cell will be specified to produce IgG and no IgM production anymore

4. That causes production of IgG, IgA, and IgE.

- ✓ The whole state happens when the B cell becomes mature so it makes IgG because IgG is more efficient (in phagocytosis, complement system,etc) than IgM and when it starts to produce IgG it can't go back again and produce IgM as we mentioned earlier.



﴿ أَوَلَمْ يَعْلَمُوا أَنَّ اللَّهَ يَبْسُطُ الرِّزْقَ لِمَن يَشَاءُ وَيَقْدِرُ ۚ إِنَّ فِي ذَٰلِكَ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ﴾

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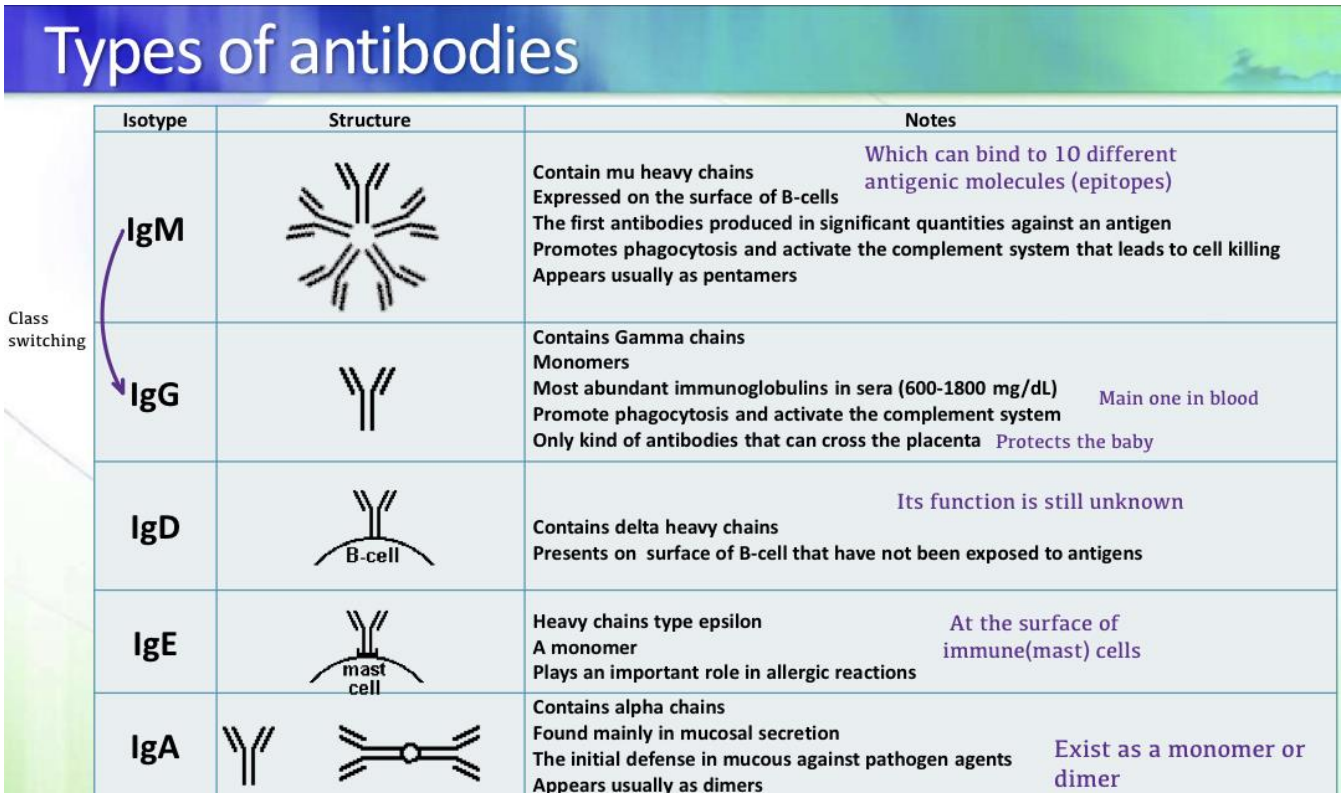
Types of antibodies:

So, we have 5 different classes of antibodies according to the constant region of the heavy chain.



There are some gentic polymorphisms (اختلافات جينية) between people in the (constant regions). So if an IgG form one person enters your body, it's going to deal with it as a foreign body. And there are different types of morphisms (different relationships) as follows:

*Idiotypes vs isotypes vs allotypes

1. Allotypes **(the relationship between one's IgG and another's IgG for example)**

Immunoglobulins of the same class but different among individuals of the same species due to different genetics are called allotypes (differences in constant regions)

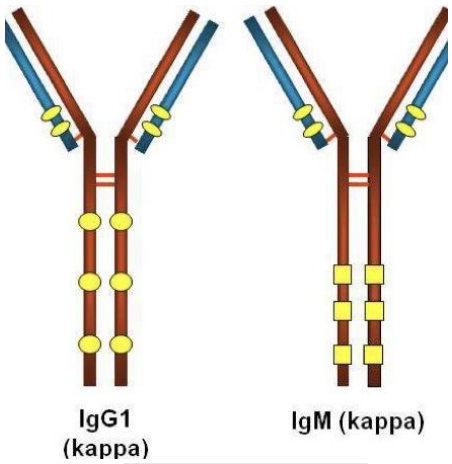
2.Isotypes (the relationship between types of antibodies' IgG,IgA..' within an individual)

The different classes of immunoglobulins are determined by their different CH regions and called isotypes(IgA,IgM,IgE etc.) for the same person.

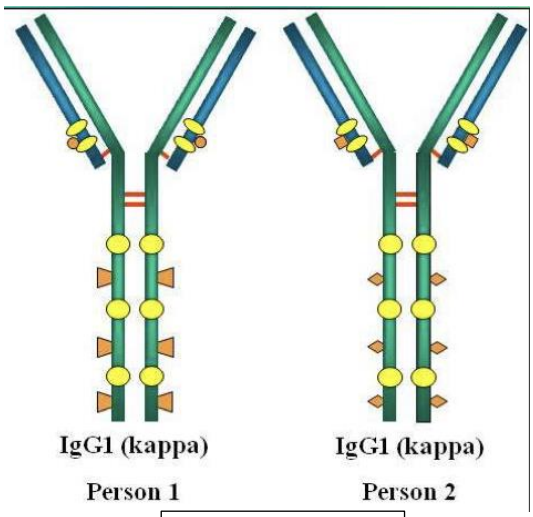
Differences are in constant regions of heavy chains (Fc).

3.Idiotypes (relationship between variable regions in one IgG and another IgG)

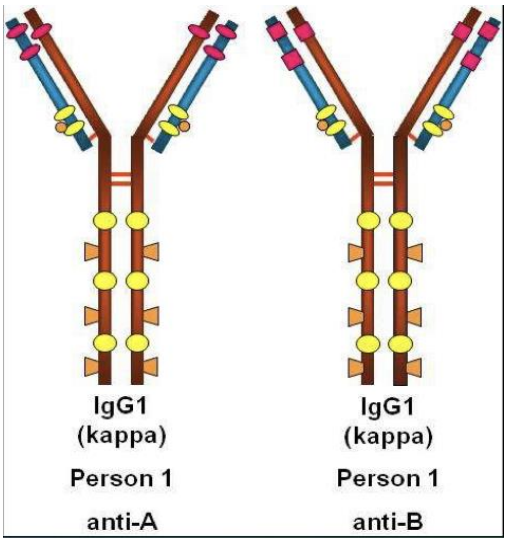
Immunoglobulin molecules that have different variable domains of both their light (VL) chains and heavy (VH) chains. Constant regions are the same.



Isotypes



Allotypes



Idiotypes

Hybridoma and Monoclonal Antibodies:

Recent developments in regards to immunology which aren't limited to understanding, but rather utilizing antibodies in different manners have showed great discoveries:

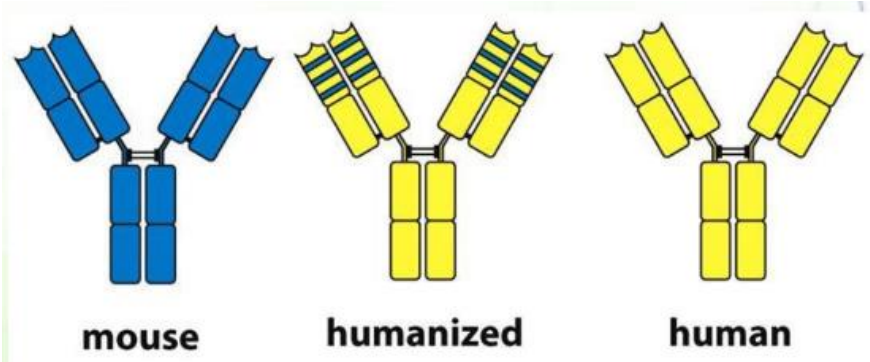
Let's say we inject an antigen into a mouse, the mouse's immune system recognizes that it's foreign, prompting it to produce antibodies against this antigen. Now let's repeat that process with another mouse, the second mouse has a high probability of producing different antibodies to that of the first's; both would recognize different epitopes of the same antigen.The process is 'random'. Moreover, if we use both kinds of those antibodies in an experiment under the same conditions, they both would give different results. These two antibodies produced against the same antigen would be considered Polyclonal antibodies; because they come from different B Cells. For scientists to gain more similar results, they'd have to isolate one singular B Cell for it to produce One type of antibody. Gathered together, antibodies from one singular B Cell are called Monoclonal antibodies.

Monoclonal antibodies prove to be better than Polyclonal antibodies in regards to reproducibility 'having the same outcomes every single time a procedure is performed' in treatments that result of diseases or experiments. However, one issue occurs in producing them which is that B Cells are short-lived 'mortal'.In order to "create" an immortal B cell that produces a single antibody (monoclonal), a B cell hybridizes with a B cancer cell (myeloma), the DNAs become mixed, causing for the fused cell to become a Hybridoma cell :

Hybridoma cells behave like cancer cells; in the matter that they keep growing and are immortal, and also behave like B Cells; in regards to producing antibodies of one kind (Monoclonal antibodies). It's like hybrid cars behaving like electrical cars when using electricity to run, and behaving like normal gasoline cars when using gasoline.

It's considered unethical to attempt to create B Cells by injecting antigens into Humans; because we'd be unsure whether or not the antigen might hurt the person, so we do this on Animals. But, if we inject the antibodies we take from animal B Cells/Hybridomas into humans directly without any further modifications, they'd be recognized as foreign by the Human body, and become destroyed by the immune system.

To make these monoclonal antibodies seem like Human, we use Genetic Engineering to humanize them by taking the most valuable part in the mouse's antibody that recognizes foreign antigens (the CDR), and attach it to normal human immunoglobulin molecules at appropriate sites. Making it not foreign and acceptable by the human body's immune system.



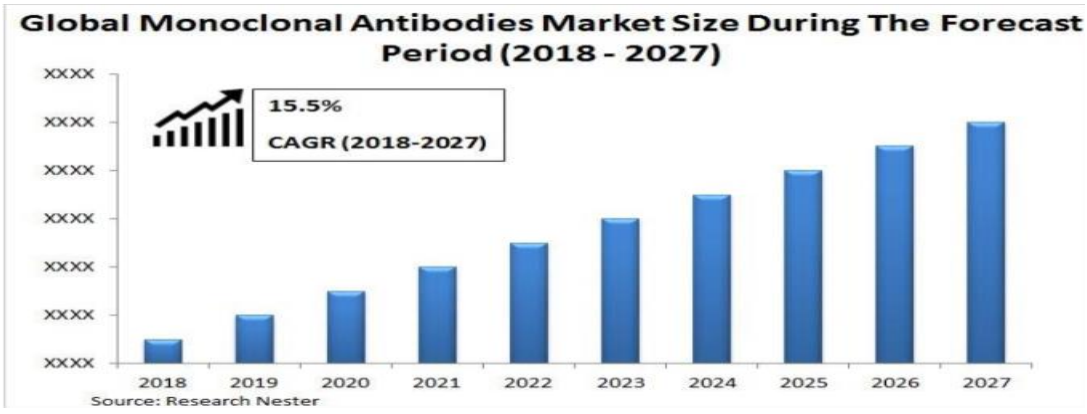
Benefits of Monoclonal Antibodies:

In the last 10 years, scientists where able to use antibodies produced by immune systems of creatures to treat diseases, examples would be Treatment of cancer cells, of inflammatory diseases (like Chron's disease) in GI, and Arthritis. In treating these diseases, they use Monoclonal antibodies that target certain proteins such as Cytokines, Growth Factors, Hormones and nuetralize them. Which would go on to give similar results to different people using them.

Specific benefits are listed below:

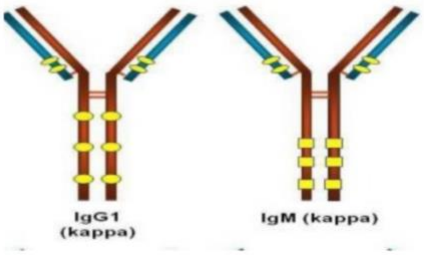
- **Measure the amounts of many individual proteins and molecules (e.g plasma proteins, steroid hormones).**
- **Determine the nature of infections agents (e.g. testing different types of bacteria or viruses like covid-19)**
- **Used to direct therapeutic agents to tumor cells.**
- **Used to accelerate the removal of drugs from circulation when they reach toxic levels.**

The market for monoclonal antibodies has been on the rise in markets lately, because they're effective, have few side-effects, and much simpler in regards to treating different ailments rather than normal medication.



Some past years' questions to test your study:

The relation between these two antibodies:



A. Idiotypes
B. Isotypes
C. Allotypes
D. A+C
E. There is no relationship

. Newborns of lactating mothers are protected from antigens through the action of:

A. IgA
B. IgD
C. IgM
D. IgG
E. both A and D

The antigen binds specifically to which part of the antibody?

A. Fc domain
B. Fab domain
C. CDR
D. Hinge region

- The main purpose of the hinge region of antibodies is:

a. Antibody clearance
b. Binding phagocytic cells
c. Allowing better binding to antigen
d. Site of sugar binding
e. Binding to antigenic epitopes

In order to immortalize a B cell to produce a monoclonal antibody:

a. Mutations are created
b. B cells are just activated by an antigen
c. B cells with immunoglobulin M are selected
d. B cells undergo class switching
e. B cells are fused with cancer cells

Answers : B, E, C, C,E

The end of sheet 19

V2

The edits were made upon Dr.Diala's lecture. Some notes were fixed to indicate a simpler meaning, and some were added to further clarify a specific definition.Thus, you can still stick to the previous one as no peculiar scientific mistakes were reported.

Al-Razi Node