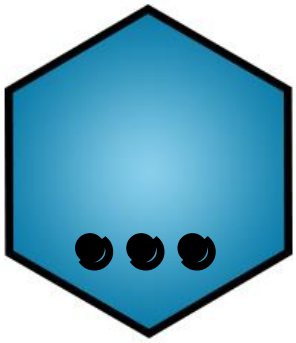



**Faculty of medicine – JU2018**

◉ Sheet ○ Slides


**DONE BY**

رغد ظاهر & كرام سلامة

**CONTRIBUTED IN THE SCIENTIFIC CORRECTION**

كوفحي دينا

**CONTRIBUTED IN THE GRAMMATICAL CORRECTION**

كوفحي دينا

**DOCTOR**

Dr.Mamoun Ahram

# Immunoglobulins

*** A quick revision:

Proteins have two types:

1) Fibrous proteins: which have 3 subtypes:
   a- Collagens
   b- Elastin
   c- Keratins
2) Globular proteins: which have 3 subtypes:
   a- Myoglobin
   b- Hemoglobin
   **c- Immunoglobulins (which is going to be our topic for this sheet)**

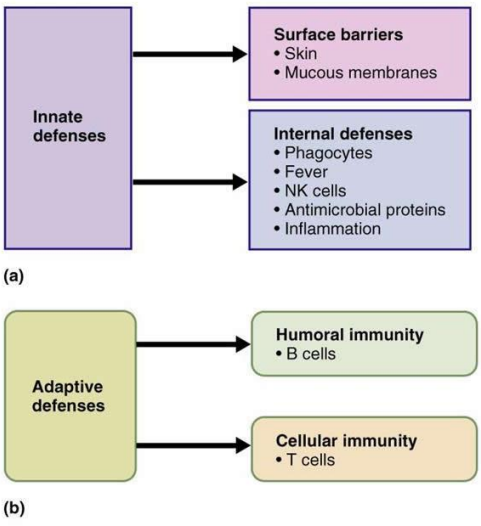
---

00:00:00

- Immunoglobulins are also known as antibodies.
- They are involved in immune responses, protecting the body from antigens and foreign bodies.

The immune system in the human body has two phases for the immune response using two mechanisms:

1) Innate Defense: A nonspecific mechanism of protecting the body from foreign antigens.
2) Adaptive Defense: A more specific mechanism in terms of fighting the antigens (they target antigens specifically).



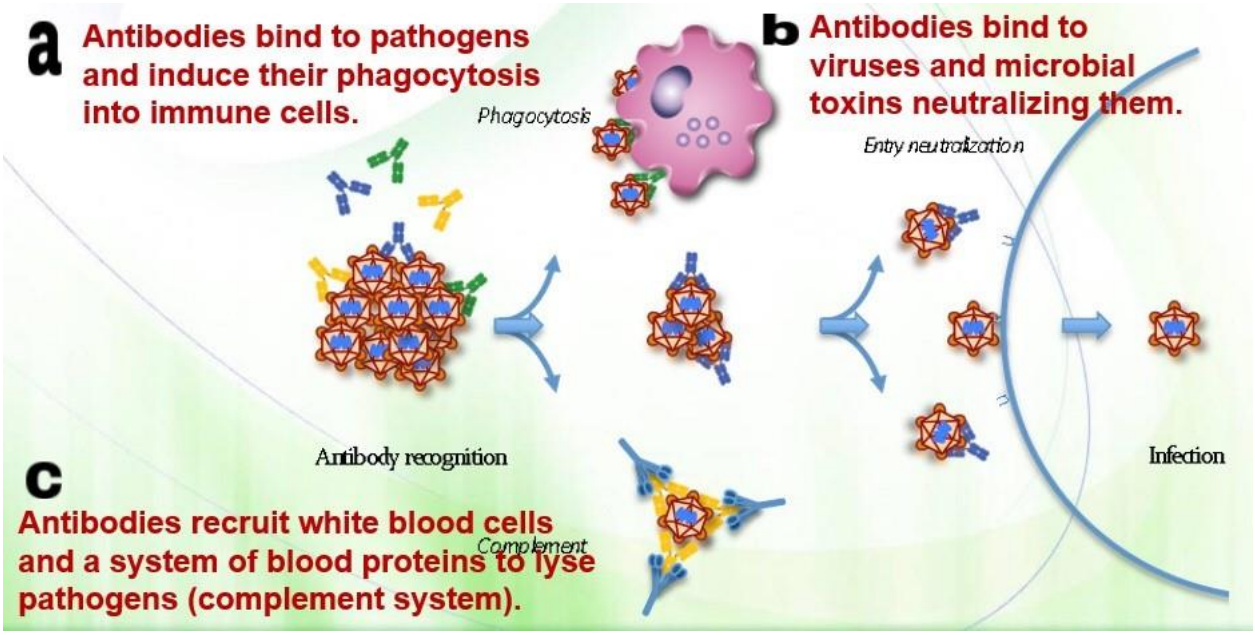
- The adaptive defense has two further sub-mechanisms:
  1) Humoral Immunity: Involves B cells that originally come from the **B**one marrow, hence the name. (we'll be focusing on this one)
  2) Cellular Immunity: Involves T cells that originally come from the **T**hymus, hence the name.

  - **Now, let us take a moment to see how these B cells work:**

  They are the cells that are responsible for the production of antibodies/immunoglobulins. Generally, when these antibodies are produced, they have different mechanisms in how they fight the antigen or protect the body.

  Now let us consider some of these mechanisms:

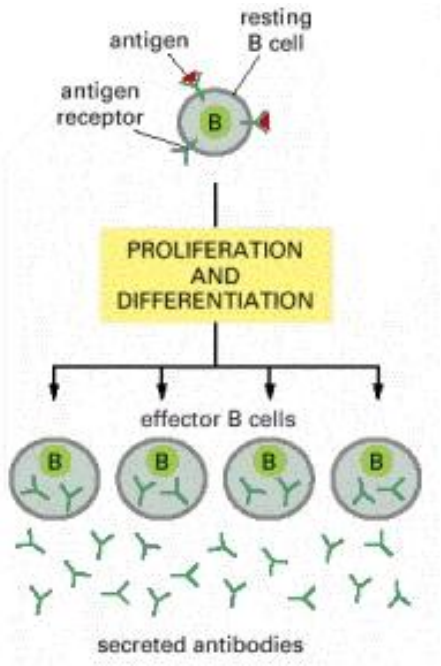
  a) The antibodies bind to and recognize the antigens specifically and then they help phagocytic cells take in the antigens through phagocytosis. (Simply the antibodies help phagocytic cells eat the antigens).
  b) The antibodies bind to the antigens, neutralizing them. What we mean by neutralizing is that the antibodies surround the antigen, either to prevent it from entering the cells in the body or to prevent whatever reaction they do.
  c) The antibodies bind to the antigens, to activate the **"complement system,**" a system in which a release of enzymes occurs that degrade these antigens.



- **How do B cells recognize antigens?**

So what happens is that when B cells differentiate in the first place, gene rearrangement takes place, which is a process by which genes of these cells are arranged differently from one cell to another, which results in producing a large number of different antibodies.

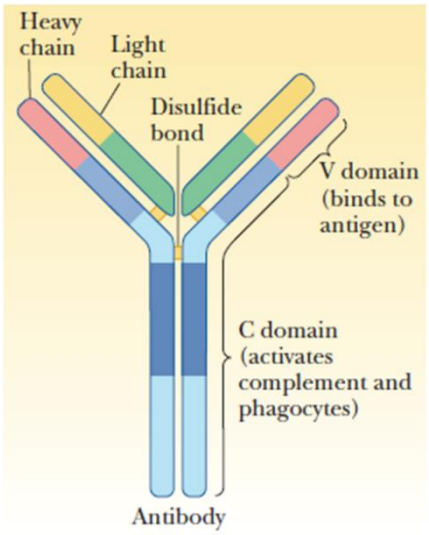
In general B cells are dormant, so basically they are asleep (silent). Once antigens get inside our bodies, they reach the antibodies on the surface of these B cells and they get recognized by the antibodies (they bind to antibodies). Such binding activates the B cells, which results in producing a more diverse number of antibodies. This occurs as B cells undergo proliferation, maturation, and diversification to increase the diversity of B cells. in conclusion, when a B cell is activated by an antigen, it proliferates and differentiates into an antibody-secreting effector cell, by making and secreting large amounts of soluble (rather than membrane-bound) antibodies at a rate of about 2000 molecules per second.




## What is the structure of immunoglobulins?

As we already know, immunoglobulins are proteins and, in general, proteins are made of one polypeptide chain or more.

In our case here immunoglobulins (Y-shaped molecules) are made of four polypeptide chains, 2 of them are identical light chains, and the other two are identical heavy chains. All of these polypeptide chains are held together by covalent disulfide (-S-S-) bonds. Additionally, within each of the polypeptide chains there are also intra-chain disulfide bonds (within-chain = between the amino acids of a single chain).



Immunoglubulins are glycoproteins (carbohydrate + protein), with oligosaccharides linked to their heavy chains.

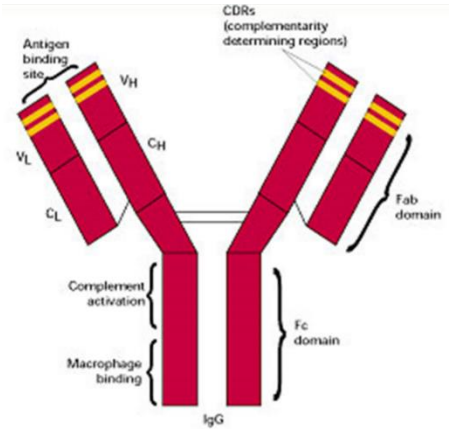
So, each of these chains, whether it's a light or a heavy chain, has 2 types of domains: a constant domain and a variable domain. The variable domain of the chain is the one responsible for recognizing and binding to antigens.

## To get more of an insight into these domains:

A light chain consists of one variable ($V_L$) and one constant ($C_L$) domain.

The heavy chain consists of one variable region ($V_H$) and three constant regions ($C_H1$, $C_H2$, and $C_H3$).

$V_L$ and $C_L$ pair with $V_H$ and $C_H$.




## Constant domains:

The constant domains are what differentiate different immunoglobulins from one another, and this increases their diversity.

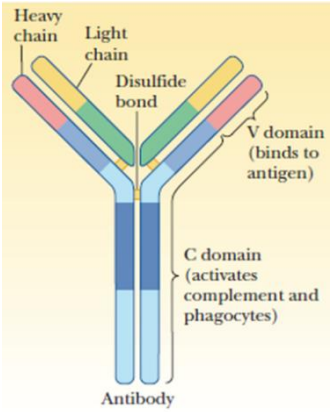
- **Light chains** have 2 genes for constant domains which are:
  a) kappa (κ) chain
  b) lambda (λ) chain
- **heavy chains** have 5 types of constant domains which are: (alpha, delta, gamma, epsilon or mu). They define five classes or isotypes of immunoglobulins: (IgA, IgD, IgG, IgE, IgM).

  Constant regions, are uniform from one antibody to another within the same isotype, meaning that all immunoglobulins that are of the IgG class, for example, have the same uniform constant region. However, they differ from one class to another, meaning that the constant regions of IgG & IgD classes, for example, are different.

The Fc domain of antibodies, which is their “tail,” is important for binding to phagocytic cells, allowing for antigen clearance. The Fc domain has two components, one for complement activation and the other for macrophage binding. Basically, these Fc domains allow for the binding of these antibodies to the surface of B cells and phagocytic cells, eventually leading to an immune response against antigens.

## Variable domains:

The variable region is found at the tips of the Y (displayed in the colors yellow, blue and red in the picture to the right), and it is the part of the antibody that binds to antigens.
The part of the antigen that is recognized and bound to by the antibody is called the **epitope**.
Looking at the shape of the antibody, you can see that it can bind to two identical antigens at a time since the two "arms" have identical variable regions.



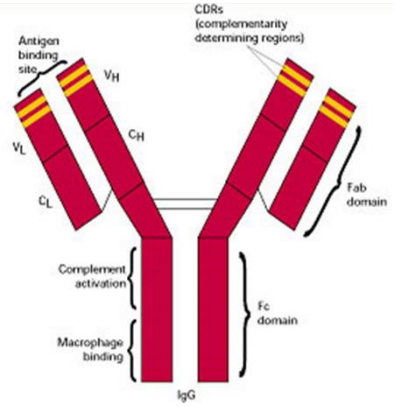

Now, we have explained earlier that B cells are initially dormant (silent) and they get activated by the binding of antigens. Then actived B cells undergo gene rearrangement, differentiation, and maturation, making B cells diverse, which results in having different affinities of binding to antigens. So, some of these B cells have a high affinity of binding to antigens so they stay activated. Other B cells have a low affinity of binding to antigens and will eventually undergo apoptosis (they die).

00:10:00

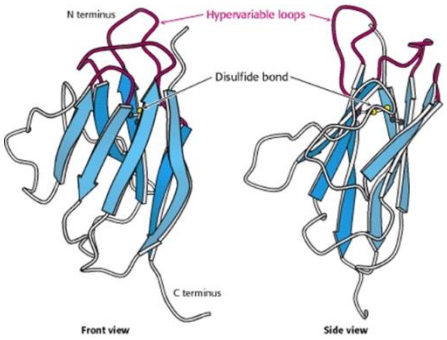
Now, returning to B cells that stay active (high affinity), after they go under maturation, they differentiate, thus they can only produce **one** type of immunoglobulin.
The primary sequences of the variable regions among different antibodies are quite distinct. (There are about 7-12 amino acids in each one that contribute to the antigen-binding site).

## What are the hypervariable regions (CDR's)?

We already know now that the variable region (in light & heavy chains) is the one responsible for the binding of antigens. But when we dig deeper, we find that it's not really the whole variable region that does the binding, rather it's a certain part of it, which is called the "Complementarity Determining Region" or "hypervariable region." These regions serve to recognize and bind specifically to an antigen with high affinity. The low dissociation constant indicates tighter binding (dissociation constant (Kd) $10^{-12}$-$10^{-7}$).

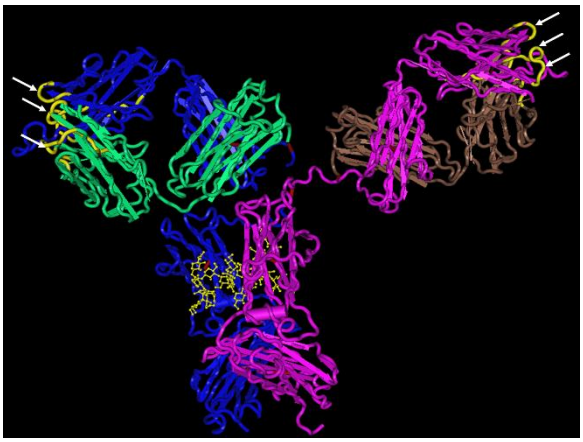



Hypervariable regions exist as a specialized domain known as an "immunoglobulin fold," which is a domain that is present in every immunoglobulin. And it is a large motif of a large number of β strands making β sheets, and these immunoglobulin folds specifically consist of three loops connecting different β sheets. Each one of these immunoglobulins folds consists of a sandwich of two anti-parallel β sheets held together by a disulfide bond making a shape of a barrel, hence it is known as "beta-barrel".




****NOTE**: a motif is a continuation of secondary structures, one following the other without the interruption of random regions. (in our case here these secondary structures are loops and β strands).

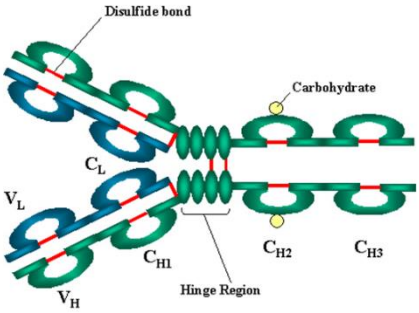
So we just said that the “immunoglobulin fold” is a motif composed of loops and β strands, so the part of this motif that is actually responsible for the recognizing and binding to the antigen is the "loop" part (pointed at by arrows in the picture to the right). These loops are small, and they contain a sequence of amino acids that can be altered and mutated to increase the number of antibodies that can bind to antigens, basically, to increase the diversity of antibodies. So in summary, the enormous diversity of antigen-

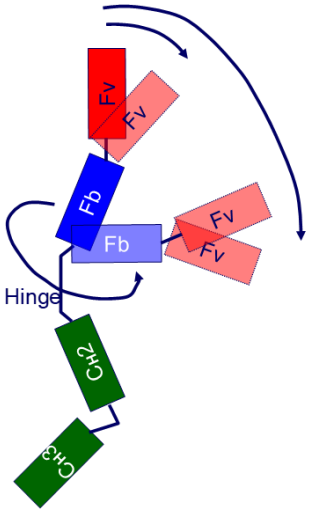


binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops. And this antigen-antibody binding that we're talking about is mediated by noncovalent interactions, never by covalent ones.

It's important to understand that the overall three-dimensional structure necessary for antibody function remains constant.

## Hinge region:




Another important region in immunoglobulins is the hinge region, which occurs between constant region no.2 and constant region no.1 of the heavy chain. This is where the arms of the antibody molecule forms a Y. So this hinge region is an amino acid sequence that gives flexibility to the antibody, allowing it to shift and move to fit when binding with the antigen, thus increasing the affinity.

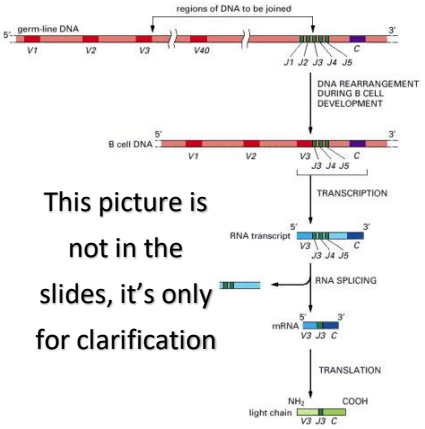
## Diversity of antibodies:

Each individual is capable of producing more than $10^{11}$ different antibody molecules.

Different ways and methods cause this diversity:

a) <u>DNA rearrangement:</u> Once B cells get activated (we already explained how), they undergo gene rearrangement, which is some kind of a mutation where a change in the native chromosome happens. It can be a deletion, an addition, a duplication, an inversion or a translocation, resulting in different mature B cells which results in producing different antibodies, thus achieving diversity.

b) <u>Imprecise joining of regions:</u> Each light-chain variable region is encoded by a DNA sequence assembled from two gene segments, the V gene segment and J gene segment, and the joining of these two segments to form the variable light chain region can differ, producing different variable regions, thus different antibodies.




This picture is not in the slides, it's only for clarification

c) <u>Addition/deletion of nucleotides during rearrangement:</u> This is one of the events that can happen during the gene rearrangement while the B cell is maturing, and each event can produce different chains, making the antibodies also different.

d) <u>Somatic hypermutation:</u> Is a mechanism performed by the immune system to adapt itself to new foreign bodies and threats, just like what happens during class switching, where the body decides what antibody should be produced and what's fit for the foreign body (antigen) that it's fighting.

**LET'S GET BACK TO CONSTANT CHAINS TO EXPLAIN AND UNDERSTAND MORE:**

- **Light chains** have 2 genes for their constant domains which are:

  c) kappa (κ) chain

  d) lambda (λ) chain

these genes exist in different regions on chromosome 2.

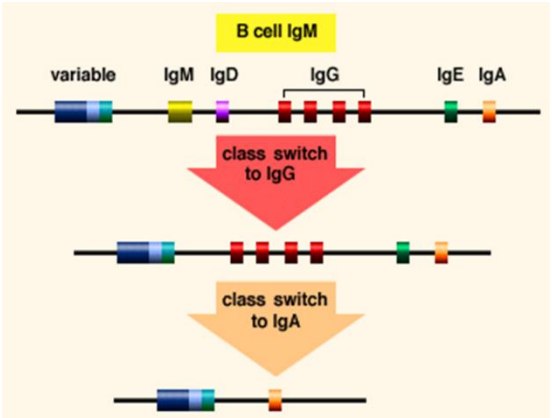
- **heavy chains** have 5 types of constant domains which are: (alpha, delta, gamma, epsilon or mu), they define five classes or isotypes of immunoglobulins: (IgA, IgD, IgG, IgE, IgM).

**All immunoglobulins that are produced from the same B cell will have the same variable region since it has already matured and it can only produce one variable region, so each B cell can give 5 antibodies differing only in constant regions. (a heavy chain is composed of variable and constant regions, if we're talking about one B cell, it can give us one variable region, and we can have 5 isotypes of constant regions, how many possibilities of heavy chains can be produced? $5 \times 1 = 5$)

For example: variable region 1 + mu = IgM

variable region 1 + gamma = IgG

## Class switching:

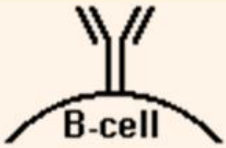
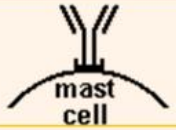



When we have a change of the constant region of the immunoglobulin, especially of the heavy chain, with one unchanged variable region, something called “class switching” occurs.

So initially when the B cell is still dormant, it
00:20:00 has only the IgM immunoglobulin on their surface. Once the antigen comes to bind to the B cell, it binds to IgM. The B cell then gets activated, then differentiated, then and only then class switching occurs (class switching refers to a DNA rearrangement changing the heavy chain constant gene) to decide exactly what isotype of the constant region this B cell is producing now. After that, the variable region can jump to other isotypes producing different antibodies. So it always has to start with IgM.

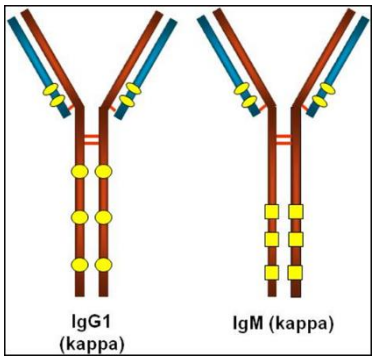
## Types of antibodies:

The doctor simply read what is written in this table:

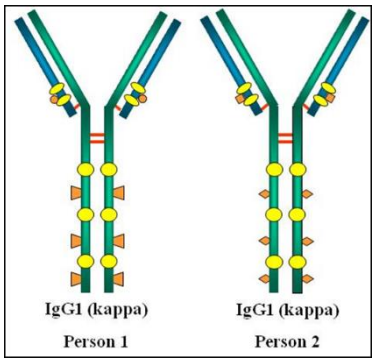
| Isotype | Structure | Notes |
|---|---|---|
| IgM |  | Contain mu heavy chains<br>Expressed on the surface of B-cells<br>Found primarily in plasma cells<br>The first antibodies produced in significant quantities against an antigen<br>Promote phagocytosis and activate the complement system that leads to cell killing<br>Appear usually as pentamers |
| IgG |  | Contains Gamma chains<br>Monomers<br>Most abundant immunoglobulins in sera (600-1800 mg/dL)<br>Promote phagocytosis and activate the complement system<br>Only kind of antibodies that can cross the placenta |
| IgD |  | Contains delta heavy chains<br>Present on surface of B-cell that have not been exposed to antigens |
| IgE |  | Heavy chains type epsilon<br>A monomer<br>Plays an important role in allergic reactions |
| IgA |  | Contain alpha chains<br>Found mainly in mucosal secretion<br>The initial defense in mucos against pathogen agents<br>Appear usually as dimers |

## Idiotypes Vs Isotypes Vs Allotypes:

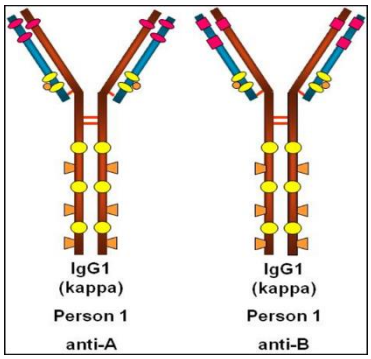
- immunoglobulin molecules that have different variable domains of both their light (VL) chains and heavy (VH) chains and the same constant regions are said to share an **idiotype**.
- The different classes of immunoglobulins that are determined by their different CH (constant heavy) regions and have the same variable regions are called **isotypes**. (just like when we talked about class switching)
- Immunoglobulins of the same class but different among individuals of the same species due to different genetics, basically from different alleles but they still can recognize the same antigen are called **allotypes**.




isotypes



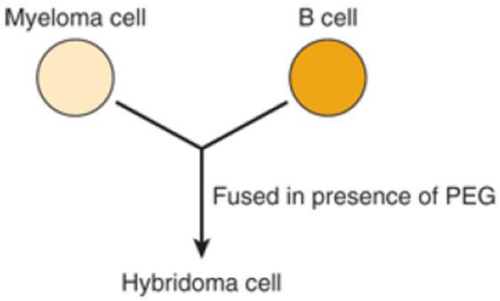

allotypes




idiotypes

## Hybridoma and monoclonal antibodies:

What scientists have taken advantage of is the production of monoclonal antibodies, which is important in treatments, like cancer for example, or even beyond that.

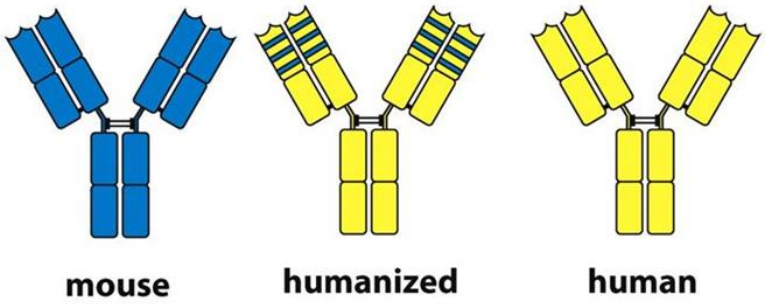



Scientists would inject an antigen in the subject's body (animal). Of course, there would be many B cells that will recognize and bind to this antigen, some with high affinity, and some with lower affinity, and the resulting antibodies are polyclonal, meaning they are directed against a number of different epitopes on the antigen (so different antibodies are produced). To "create" an immortal B cell that produces a single antibody (monoclonal), a B cell hybridizes with a B cancer cell (myeloma), so it will start dividing as a cancer cell, uncontrollably. Therefore, we will have many identical B cells, which will in turn produce one specific antibody

00:30:00

repeatedly, so we will have monoclonal antibody activity rather than polyclonal activity. Besides, these B cells will all have the same affinity for binding to this antigen (whether it is high or low). They apply this method in laboratories, so they can use the medium that the cells grow in and purify it for the antibodies so they can be ready to be given to patients.

**One problem patients would face is that their body will reject the antibody if it was from an animal, so what happens is, through genetic engineering, they can hybridize a human constant region with the variable regions they produced by B cells from mice for example, making it acceptable by the human body. So they become humanized by attaching the mice CDRs onto appropriate sites in a human immunoglobulin molecule.




## Benefits of monoclonal antibodies:

a) Measure the amounts of many individual proteins and molecules (e.g. plasma proteins, steroid hormones). Since they are the same antibodies, they recognize the same antigens with the same affinity, and they have the same variable regions, they give reproducible results.
b) Determine the nature of infectious agents (e.g. types of bacteria), for the same reason as above, we can produce antibodies against infectious agents, and detect them using different techniques. One of them is known as ELISA.
c) Used to direct therapeutic agents to tumor cells.
d) Used to accelerate the removal of drugs from the circulation when they reach toxic levels. Certain antibodies can bind to certain drugs, helping them to get out of our system.

Just keep going, you will get there
فقط واصل التقدم، سوف تصل بالنهاية