IMMUNOLOGY

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Hi fellows , this will be the immunology sheet for lecture 8.

The word ''Antibodies'' has passed our hearing many times and it'll pass even more, as antibodies hold **great importance** for the immune response of our bodies, and thus we'll begin talking about the structure and function of the different classes of antibodies ,as well as their dynamics (how they increase and decrease when exposed to an antigen)

Antibodies are found in serum or plasma , and in mucus.

Antibodies are circulating proteins, found in blood, that are produced in vertebrates in response to exposure to foreign structures known as antigens.

serology: The classical name of the study of antibodies and their reactions with antigens, since they are serum proteins (serum= blood - (cells+ coagulation factors)).

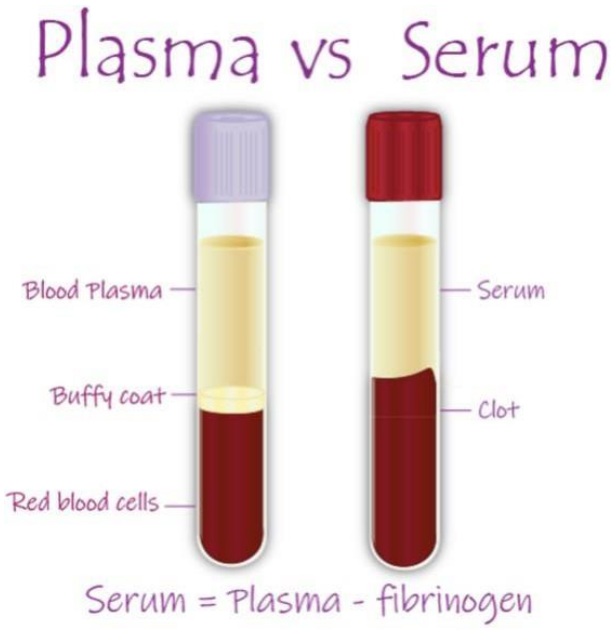
Or simply (plasma=blood-cells)

(serum=plasma - coagulation factors"fibrinogen").

It'sobtained from centrifugation of blood where RBCs are separated from the plasma, and plasma have coagulated(Fibrinogen->Fibrin).

Link of a video clarifying more about plasma, serum ,antiserum
https://youtu.be/VHpAs1sLfgs

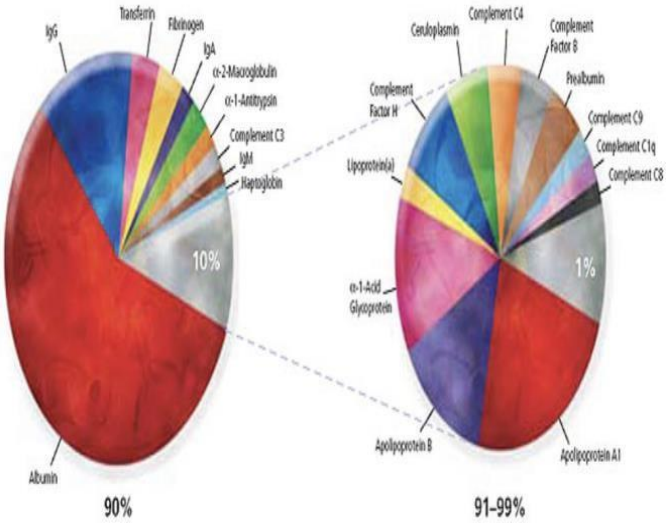
Any serum sample that contains detectible antibody molecules that bind to a particular antigen is commonly called an **antiserum**.



-plasma proteomics holds great promise for the future of biomarker discovery, as well as in vitro diagnostics.

Although plasma is readily accessible for analysis ,the study of the plasma proteome is fundamentally limited by its vast dynamic range(10 orders of magnitude)

Adults (70 kgs) produce 2-3 grams of antibodies **daily**, which is considered a **huge** amount of protein production on a daily basis, they also make a **great part** of the protein fraction of the **serum**.



Plasma proteomics=all proteins in the plasma

-Contains mostly albumin

-Contains IgG

-Contains C3(as the highest concentration of the complement proteins within plasma)

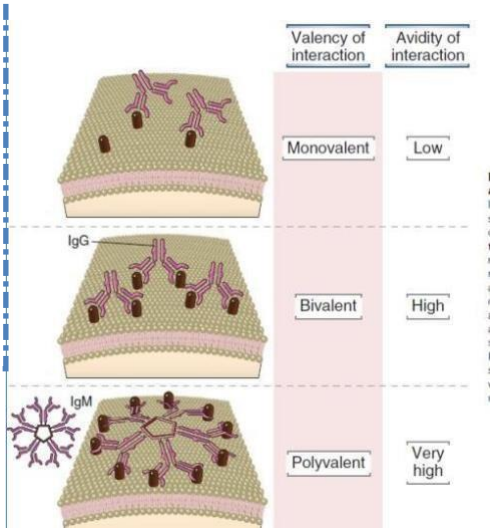
Antibodies **recognize** a part of the antigen called **the epitope,** one antibody could bind to multiple epitopes of the same type (multiple identical epitopes).

The part of the antibody that binds the epitope is called the Fab fragment, which is the antigen binding site of the antibody.

Definitions:

Affinity: the strength, and the ability of the **Fab** fragment to bind an epitope, different antibodies have different affinities to a certain epitope (antigen).

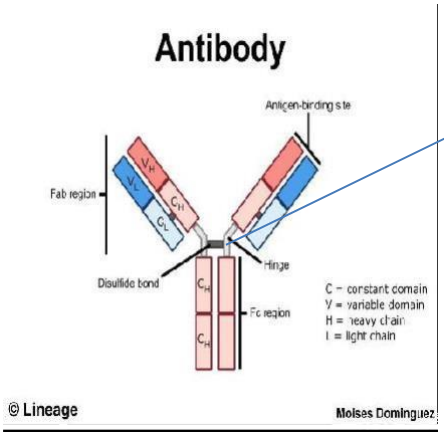
Avidity: the amount of identical epitopes (of a multivalent antigen) an antibody can bind **at once**. An antibody has at least two Fab fragments, so it can bind to more than one antigen by binding to identical epitopes.



Conclusion:

More epitopes bound> high avidity

Less epitopes bound > low avidity



This is the Hinge region.

-The avidity depends on **the hinge region** of the antibody.

This region helps the antibody to open up and bind multiple epitopes (like a big hug).

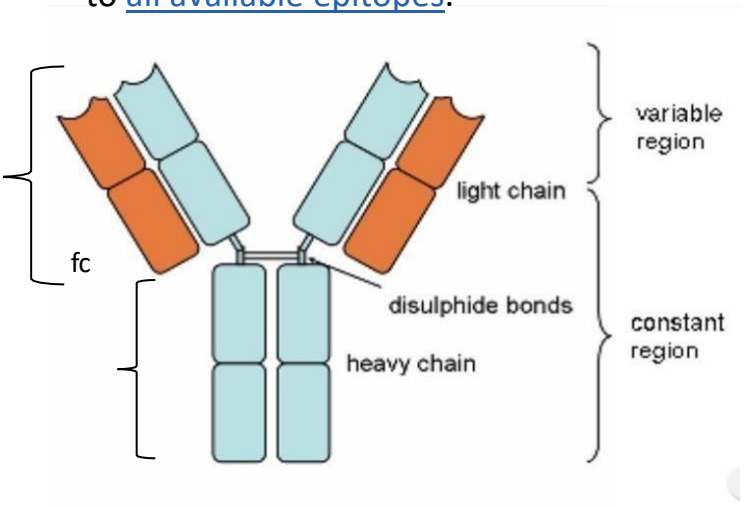
Having multiple Fab fragments allows the antibody to bind multiple epitopes. The strength of binding between the antigen and the antibody would be better, meaning that the antibody is doing its job more properly.

Over all, the avidity takes into consideration the binding of all Fab sites to all available epitopes.

Affinity	Strength of binding of one epitope to one Fab fragment.
Avidity	The number of epitopes an antibody can bind with all its Fab fragments.

The structure of the antibody:

What is an antibody made of and what are its main parts?



Antibodies are proteins, specifically, glycoproteins and immunoglobulins. What does this indicate? They are

1. : Glycosylated, they have an important glycosylation at the Fc side.
2. Globular proteins
3. Immune functioning, which we already know.

Antibodies are composed of:

Two regions formed by Four chains of protein, two **heavy** chains and two light chains. heavy chains are connected via disulphide bonds by certain cysteine residues, these bonds form the **hinge region** (refer back to avidity).

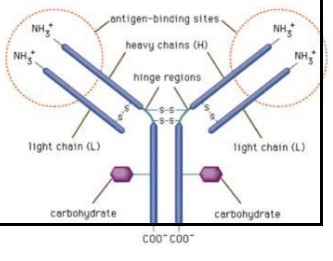
➐The two regions of the antibody are 1) the Fab region (antigen binding fragment)

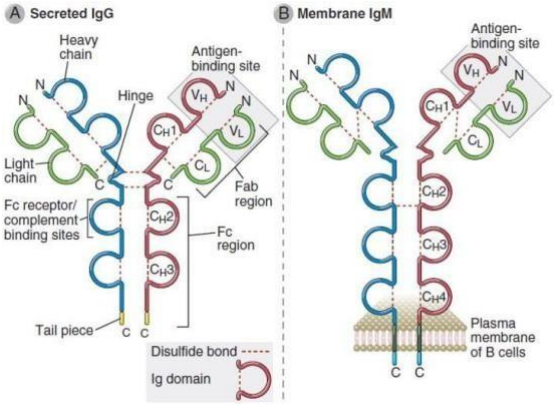
2) the Fc region (The fragment crystallizable region)

The Fc is the tail part that binds to a cell surface receptor called the Fc receptor present on phagocytes and natural killer cells, which helps in phagocytosis and opsonization

The Fc region also interacts with some proteins of the complement system(c1 complex which activates the classical pathway binds to antibody-antigen complex through Fc portion)..

➐only amine terminals are on the fab region.
The carboxyl terminals are on the Fc region.





Antibodies can be either soluble, which are secreted antibodies that reside in the circulation, tissues, and mucosal sites ,or bound to the membrane of B -lymphocytes. (aka. B-cell receptors)

that function as receptors for antigens "at first".

-When the the B cell binds it's antigen, it

becomes activated, create clones and turns into plasma cells, then plasma cell starts secreting this immunoglobulin in the form of an antibody.

What main classes (isotypes) of antibodies are there and how are they classified?

Main classes arise from classifying them according to the constant region, specificities come from differences in the variable regions.

The variable region:

❼The upper part of the antibody holds the variable regions (the tip of the fab region), composed of both **heavy** and light chains, at the amino terminal of the antibody.

It makes sense for the variable part to be variable. Remember that its role is to provide an enormous amount of different epitope complementary antibodies, and the possibilities here are endless, so it differs from one antibody to the other giving them great specificity.

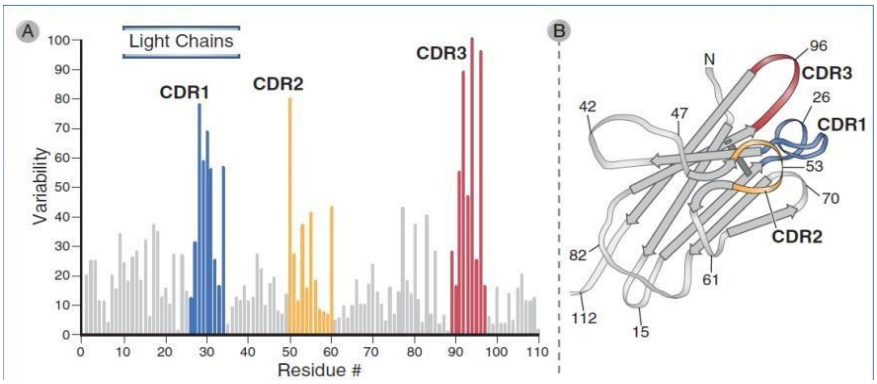
❼ Most of the sequence differences and variability among different antibodies are confined to three short stretches in the V region of the heavy chain and to three stretches in the V region of the light chain. These diverse stretches are known as hypervariable segments. Together they dictate the **affinity** of binding between the antigen and the antibody. Why do we talk about hypervariable region in variable region? Because B-cells with repeated exposure to the antigen, start secreting better antibodies (With higher affinity) and the cause of this increase is due to changes in the hypervariable region.

Because these sequences form a surface that is complementary to the three-dimensional structure of the bound antigen, the hypervariable regions are also called complementarity determining regions (CDRs). (refer forward to **affinity maturation**).

CDRs determine how complementary the antibody structure is to the antigen structure, and they affect the affinity of the interaction.

❼Variable antibodies that recognize many antigens are made by genetic recombination of genes coding for them. This happened early on during the maturation of the B cell.

Variability means that if we analyze all the residues of two antibodies and sequence them, we observe certain stretches of amino acids within the whole protein that are always more variable between the two antibodies. (More different in the sequence of amino acids within this specific region (CDR)).



The constant region:

Constant region (Fc), is totally made up of heavy chains, which extend from the Fab into the Fc.

Constant should be constant, because it recognizes receptors we already know, so no need for them to variate.

Classes differ according to their heavy chains, in other words, constant regions effect classes (isotypes)

The heavy chain, C regions, of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence. This sequence differs in antibodies of other isotypes or subtypes

Variable	Able to hold different foreign antigens.
Constant	able to hold known receptors, receptors we already have (e.g. Fc rec.)

Classes of antibodies (isotypes):

We have five classes, IgM, IgD, IgE, IgA, IgG "MAGED"

❼Different antibodies are anatomically specific in differentlocations (eg. IgA in mucus), their appearance time during immune responses differs, and these isotypes have different constant regions (threedifferences) i.e we can chang the class by changing Constant region

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Heavy chains differ in their type (amino acid sequence) only from one class to another, **IgA**'s heavy chain is called alpha (α) , the **IgD**'s is called delta (δ), **IgM**'s is mu(μ), **IgE**'s is epsilon(ε), **IgG**'s is gamma(γ) .

❼classes also have subclasses - e.g. **IgG** 1,2,3,4 - because of small differences in their heavy chains.

More details: try to recognize their structures, most important things are highlighted

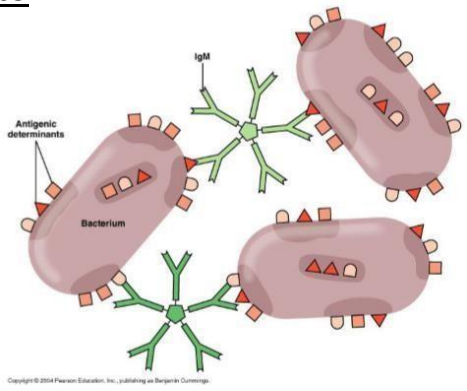
TABLE 5–2 Human Antibody Isotypes

Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	IgA (dimer) Monomer, trimer	Mucosal immunity
IgD	None (δ)	Trace	3	None	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	IgE Monomer	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	IgG1 Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5		Naive B cell antigen receptor, complement activation

IgD ❼ pre-receptor. It's present on thenaïve b cell's surface, before its interactionwithitscognate antigen.

IgE ❼**mast cells** have Fc receptors, that would bind to the Fc region of IgE. It plays an important role in hypersensitivity.

IgM ❼ five Y shaped parts connected together at the Fc region. B-cellreceptors are IgMs, and Natural antibodies are IgMs(similar to those of the primary immune response). They are produced without exposure to antigens.



They have high avidity, since they have 10 Fab regions that can bind several epitopes together.

❼IgM is good for what's called agglutination, because it has many specific binding sites. What basically happens is that it glues particles together, it limits the movement of microbes, thus, limiting the infection to a certain spot. We can use this property (agglutination) in the diagnosis of certain infectious diseases (like bacterial ones for example).

IgM is the first antibody to be secreted (primary response)and disappears (after the appearance of IgG).

IgG ❼ monomer. It is the most abundant immunoglobulin within the serum (also tissue fluids) and provides the most extensive and long-lived antibodyresponse to thevarious microbes and antigens.

• IgG antibody is characteristically formed in large amounts during the secondary response to an antigenic stimulus, and usually follows production of IgM in the course of a viral or bacterial infection.

• IgG is the only immunoglobulin class able to cross the placental barrier and, thus, provides passive immune protection to the newborn in the form of maternal antibody through transcytosis.

But these antibodies are short-lived (the new born is protected only for a short period of time) after that their body will start to degrade these antibodies.
NOTE: Also on our bodies antibodies are degraded but since we have plasma cells that continuously produce them, then we still have them for along time BUT on newborn the adaptive immunity wasn't formed yet

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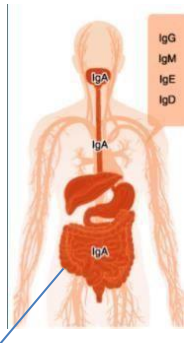
IgA ❼ dimer in its secreted form. Immunoglobulin A has a special role as a major determinant of so-called local immunity in protecting **mucosal epithelial surfaces** from colonization and infection.

Its major role is to prevent the attachment of the antigen-bearing microbes to receptors on **mucosal epithelial surfaces** (once it finds a microbial antigen it binds it, hindering the movement of the microbe, preventing it from entering the epithelial surfaces)



• At the epithelia, two IgA molecules combine with another protein, termed as secretory piece, which is present on the surface of local epithelial cells.

This complex is termed secretory IgA (sIgA).

Most of the 2-3 grams of antibodies produced daily are **IgAs** because they supplement the large area of mucosal epithelial surfaces of the **GIT** and the **RT**

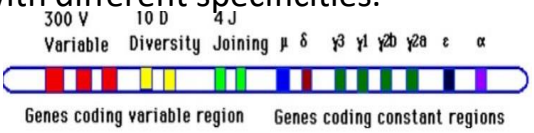
How antibodies are produced? Well, B- cells produces them.

During its development, each B-lymphocyte becomes genetically programmed through a series of gene-splicing reactions to produce a antibody molecule with a unique specificity

But again, how come we have all this diversity?

The antibody diversity comes from the random recombination of inherited germline DNA (and some base addition mutations along with it) exclusively in lymphocytes (making the **antibody repertoire**), the recombined genes are coding for **variable regions** of both the heavy and light chains. It can recognize 10^7 or more different epitopes

Antibody repertoire: the total collection of antibodies with different specificities.



Class switching:

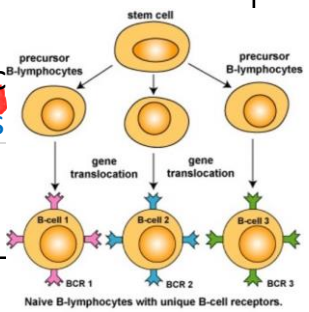
how do we have different classes of those antibodies?

❼After initial secretion of IgM, cytokines secreted by T- cells stimulate the plasma cells to switch from IgM production to the production of IgG, IgA, or IgE.

❼Class switching is accomplished by **genetic rearrangement** of gene segments **encoding the constant region**, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original antigen specificity.

❼This process of **class switching** or **isotype switching**, allows plasma cells c the same activated B cell to produce a variety of antibody classes with the s

Same antigen



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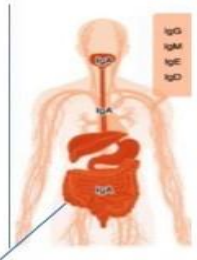

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Antibodies/ *IgD*

- **IgD (IgD)** is a monomeric antibody isotype that is expressed in the plasma membranes of immature B-lymphocytes. **IgD** is also produced in a secreted form that is

– It's function doesn't well understood
– Immature B-cells well probe it as a surface receptor and with time as it matures it start to change this IgD to IgM.
– So, why our bodies produce IgDs then IgMs? At the beginning of the disease, we need to control it as much as we can so we produce IgMs but after that we want to phagocytose them (pathogens) so we

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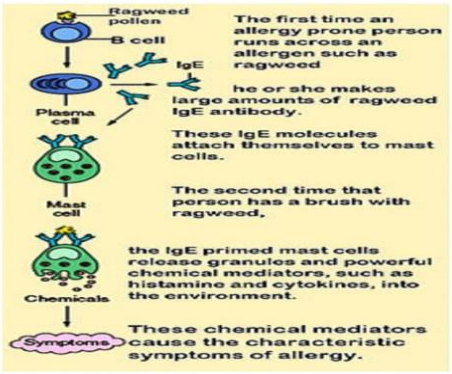
Antibodies/ *IgE*

- IgE not only provides protective immunity against **helminth parasites** but can also mediate the **type I hypersensitivity reactions** that contribute to the pathogenesis of allergic diseases such as **asthma, allergic rhinitis and atopic dermatitis**.



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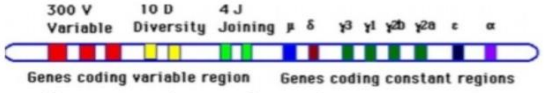

❼After initial secretion of IgM, cytokines secreted by T- cells stimulate the plasma cells to switch from IgM production to the production of IgG, IgA, or IgE.

❼Class switching is accomplished by **genetic rearrangement** of gene segments **encoding the constant region**, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original antigen specificity.

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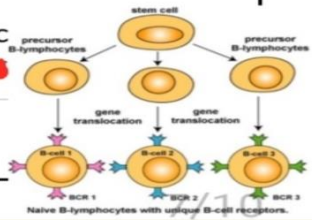
Same antigen



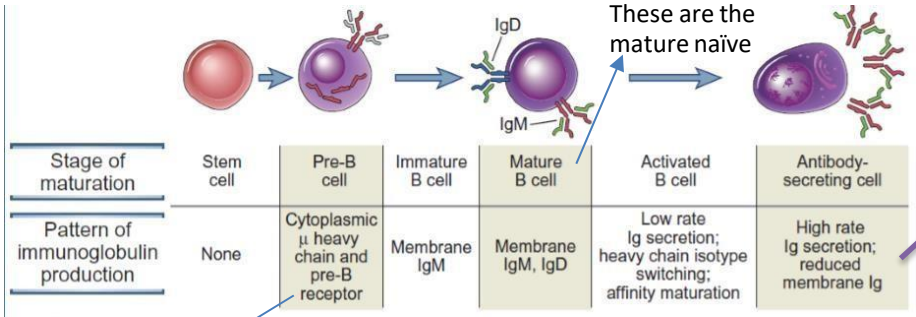
specificity. During class switching, specific sections of the gene coding the constant regions are combined together, forming whatever class is desired. (check the picture)

B cell maturation and antibody secretion:

B cells don't fully mature until they face an antigen, represented by Follicular Dendritic Cells. They remain mature naïve cells until represented. (just like us, we don't learn that that's a hole, until we fall in it -_-#)

levels of maturation:

Stem cell ❼pre-Bcell ❼immature Bcell ❼mature B-cell (here wecallits receptors B-cell receptor) ❼ activation (accompanied by class switching) ❼



Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell	Antibody-secreting cell
Pattern of immunoglobulin production	None	Cytoplasmic μ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate Ig secretion; reduced membrane Ig

FIGURE 5–10 Ig expression during B lymphocyte maturation. Stages in B lymphocyte maturation are shown with associated changes in the production of Ig heavy and light chains. IgM heavy chains are shown in red, IgD heavy chains in blue, and light chains in green. The molecular events accompanying these changes are discussed in Chapters 8 and 11.

Pay attention to this diagram and notice the types of immunoglobulins present at every stage.

So IgM precursors (mu heavy chains in the cytoplasm) and IgMs on the cell surface.

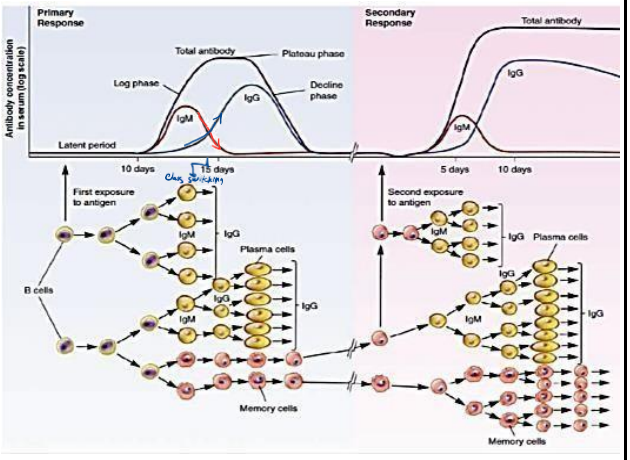
full differentiation

full maturation of B-cells:

look at this diagram:

During the Primary response, which is the first time the B cell encounters the antigen (1 week after encountering the challenge), Mature naïve b-cells are exposed to their cognate antigens (the specific antigen B cells recognize and activate them).

This antigen binds to the IgM that is bound to B cell's membrane (BCR binding). Helper T Cells then bind to the antigen displayed on the antibody of the B cell. When helper T cells recognize this antigen, Helper T cells release cytokines which activate B cells. Primarily, B cells when activated, undergo clonal expansion and the cells will differentiate into either:

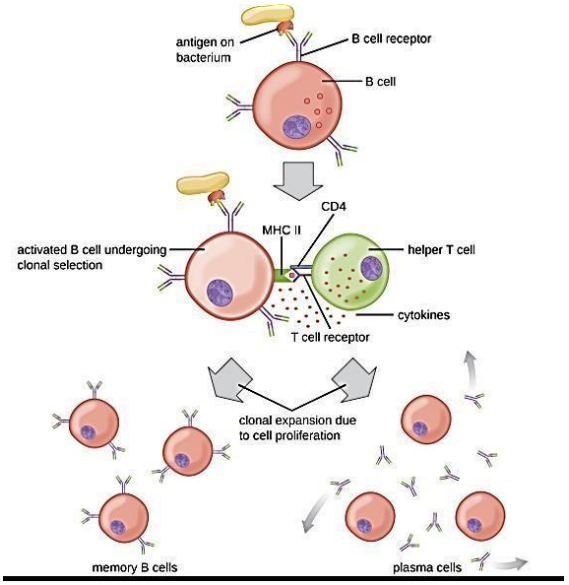


1. plasma cells that secrete IgM (approx. 10 days after encountering the antigen), which later switches to IgG (takes approx.. 2 weeks to get released) by class switching.

or

2. memory cells that don't release antibodies and primarily circulate the blood and lymph nodes, waiting to be encountered to activate the secondary response.

During the Secondary response, Memory cells get exposed to the antigen. They proliferate, reforming plasma cells, which this time have undergone **affinity maturation**, allowing the antibody to bind to the antigen in an enhanced affinity, better than primary response antibodies' affinity, in one to two days. They secrete IgM (minorly) or IgG (majorly).



Summary table : (it includes important comparisons)

	Primary response	Secondary response
Type of cell doing the recognition.	Naïve mature B-cells.	Memory cells.
Lag time between the recognition and the appearance of the antibody	Long (7 or up to 10 days)	Shorter (1-2 days)
the rate of exponential increase to the maximum steady-state level.	Slow	Rapid
Steady state level	Lower	Higher, representing larger amount of antibodies.

levels **rise exponentially** to attain a maximal steady state in approximately 3 weeks, then **decline gradually** with time.

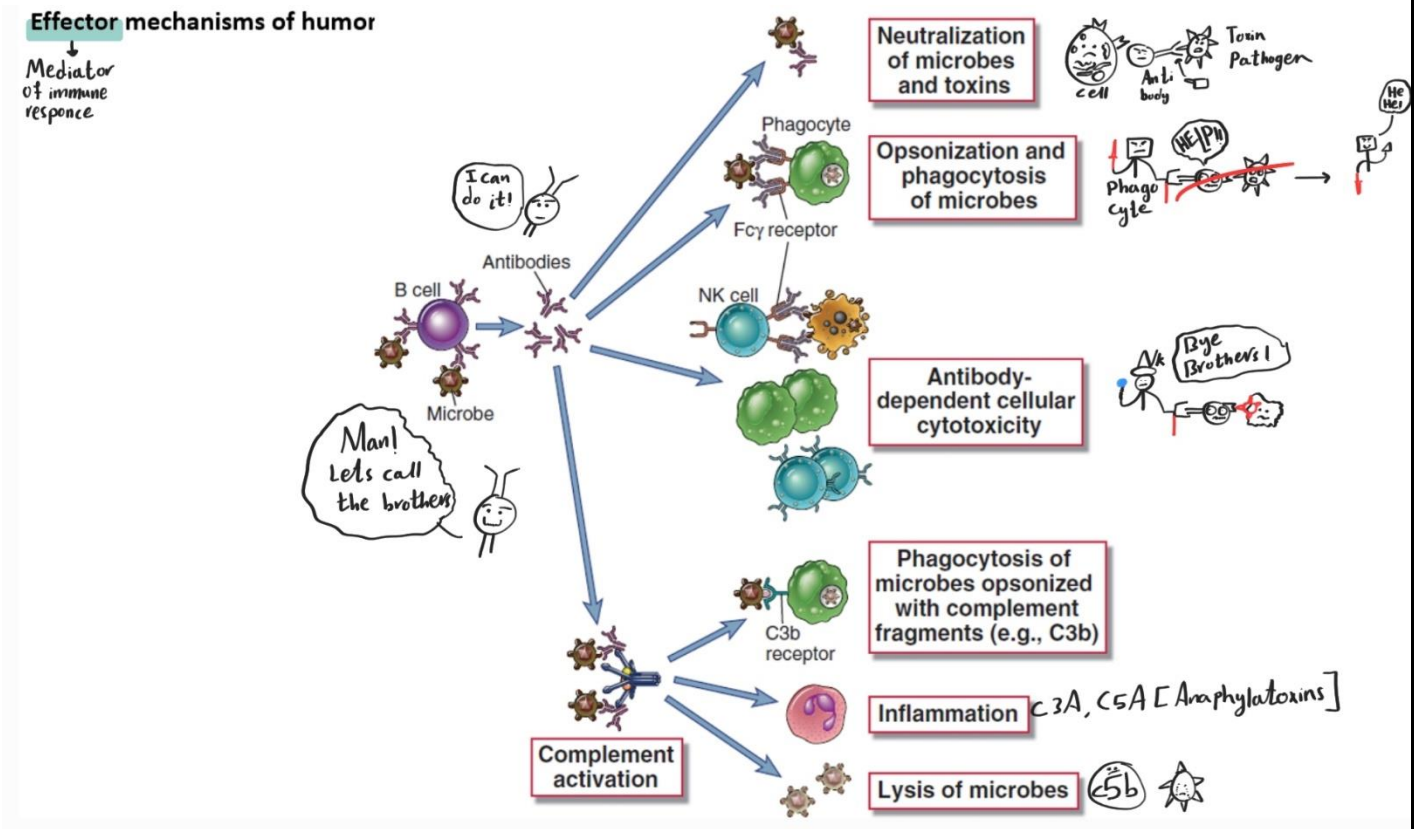
Another key factor of the secondary response is that **antibodies** formed are **predominantly of the IgG class**

AFFINITY MATURATION: is **Somatic mutations** in antigen-stimulated B lymphocytes that generate changes in their antibodies' V domain structures (**increasing the affinity** of binding antigen compared with the original V domains sometimes.). This usually occurs after the primary response, when Memory cells undergo clonal expansion and differentiate to produce Plasma cells in the secondary response.

Those B cells producing higher affinity antibodies , **preferentially bind to the antigen** and -as a result of selection- become the dominant B cells with each new exposure to the antigen. And this process is called **affinity maturation.** Though the mechanism isn't clear yet.

"At every exposure to the antigen, a type of affinity maturation takes place, but only antibodies with higher affinity will remain, propagate and clonally expand"

e.g. IgG that is produced initially has less affinity towards the same antigen, than IgG that is produced after affinity maturation is gained.



Have fun studying this beautiful ~~eight~~ nine 12 paged sheet

(it was eight but we added information upon correction so nine it is) :D