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Hi fellows, this will be the immunology sheet for lecture 9, as given on Sunday.

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 <u>different classes</u> of antibodies.

Antibodies are <u>circulating proteins</u>, 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 <u>serum proteins</u> (serum= blood - (cells+ coagulation factors)). It's obtained from centrifugation of blood where RBCs are separated from the plasma, and plasma have coagulated(Fibrinogen->Fibrin).

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

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 <u>binds the epitope</u> is called the <u>Fab fragment</u>, which is the antigen binding site of the antibody.



fraction of the serum.

Definitions:

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

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

Conclusion:

more epitopes bound \rightarrow high avidity.

less epitopes \rightarrow low avidity.



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

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

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

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

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



The structure of the antibody: Fab

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

Antibodies are proteins, specifically, glycoproteins and immunoglobulins. What fc does this indicate? They are **1**. <u>Glycosylated</u>, they have an important glycosylation at the Fc side. **2**. <u>Globular</u> 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 <u>disulphide bonds</u> by certain cysteine residues, these bonds form the **hinge region** (refer back to avidity).

 \rightarrow The two regions of the antibody are 1) the <u>Fab</u> region (antigen binding fragment)

2) the <u>Fc</u> region (The <u>fragment</u> <u>crystallizable</u> 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.

→only <u>amine terminals</u> are on the <u>fab</u> region.
The <u>carboxyl terminals</u> could be found in the <u>Fc and Fab</u> 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. <u>Bcell receptors</u>)

that function as receptors for antigens.

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

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

The variable region:

 \rightarrow The <u>upper part of the antibody</u> holds the variable regions (the tip of the fab region), composed of both **heavy** and <u>light</u> chains, at the <u>amino terminal of the antibody</u>.

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.

 \rightarrow Most of the sequence differences and variability among different antibodies are confined to <u>three short stretches</u> in the V region of the heavy chain and to <u>three</u> <u>stretches</u> in the V region of the light chain. These diverse stretches are known as <u>hypervariable segments</u>. Together they dictate the affinity of binding between the antigen and the antibody.

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

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

→ Variable 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 analyse 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 <u>between the two antibodies</u>. (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 <u>should be constant</u>, because it recognizes receptors we already know, so no need for them to variate.

Classes differ according to their heavy chains, in other words, <u>constant regions</u> effect <u>classes</u> (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

→ Different antibodies are <u>anatomically specific</u> in different locations (eg. IgA in mucus), <u>their appearance time</u> during <u>immune responses</u> differs, and these isotypes have <u>different constant regions (three differences)</u>

Heavy chains differ in their type (amino acid sequence) only from one class to another, IgA's heavy chain is called <u>alpha (α)</u>, the IgD's is called <u>delta (δ)</u>, IgM's is <u>mu(μ)</u>, IgE's is <u>epsilon(ϵ)</u>, IgG's is <u>gamma(γ)</u>. \rightarrow classes also have subclasses - e.g. **IgG** <u>1,2,3,4</u> - because of small differences in their heavy chains.

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



IgM \rightarrow five Y shaped parts <u>connected together at the Fc region</u>. B-cell receptors are IgMs, and Natural antibodies are IgMs(similar to those of the primary immune response). They are <u>produced without exposure to</u> antigens.

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

→IgM is good for what's called <u>agglutination</u>, because it has <u>many specific binding sites</u>. 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 <u>(primary response</u>) and disappears (after the appearance of IgG).

 $lgG \rightarrow$ monomer. It is the <u>most abundant</u> immunoglobulin within the serum (also tissue fluids) and provides the <u>most extensive</u> and <u>long-lived antibody response</u> to the various microbes and antigens.

• IgG antibody is characteristically formed in large amounts during the secondary response to an antigenic stimulus, and usually <u>follows production of IgM</u> 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.

 $lgA \rightarrow \underline{dimer}$ 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.

But again, how come we have all this diversity?

The antibody diversity comes from the <u>random recombination</u> of <u>inherited germline</u> DNA (and some base addition mutations along with it) exclusively in lymphocytes (making the **antibody repertoire**), the recombined genes are <u>coding for **variable regions**</u> <u>of both the heavy and light chains</u>.

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

Class switching:

how do we have different classes of those antibodies?

 \rightarrow After initial secretion of IgM, cytokines secreted by T- cells <u>stimulate the plasma cells</u> to switch from IgM production to the production of IgG, IgA, or IgE.

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

→This process of **class switching** or **isotype switching**, allows plasma cells cloned from the <u>same activated B cell</u> to produce a <u>variety of antibody classes</u> with the <u>same antigen</u>

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 \rightarrow pre-B cell \rightarrow immature B cell \rightarrow mature B-cell (here we call its receptors B-cell receptor) \rightarrow activation (accompanied by class switching) \rightarrow full differentiation



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

full maturation of B-cells:

look at this diagram:

During the <u>Primary response</u>, which is the first time the B cell encounters the antigen (<u>1</u> <u>week after encountering the challenge</u>), <u>Mature naïve b-cells</u> 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 <u>clonal expansion</u> and the cells will <u>differentiate</u> 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).



	Primary response	Secondary response				
Type of cell doing the recognition.	Naïve mature B-cells.	Memory cells.	levels rise exponentially to attain a <u>maximal steady</u> <u>state</u> in approximately 3 weeks, then decline gradually with time.			
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.	L			
Another key factor of the <u>secondary response</u> is that antibodies						

Summary table : (it includes important comparisons)

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 <u>higher affinity antibodies</u>, **preferentially bind to the antigen** and -as a result of selection- <u>become the dominant B cells</u> 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 cight nine paged sheet

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