Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 5

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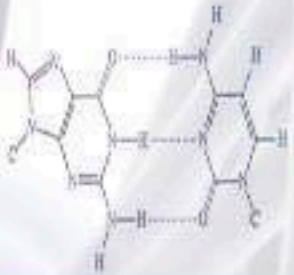
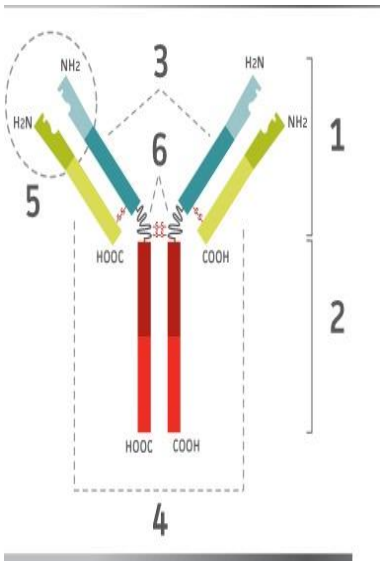
Biochemistry



Majida Al-Foqaraa'

بسم الله الرحمن الرحيم

Immunoglobulins (2)



(the easiest lecture so far ,trust me :P)

<u>As a revision</u>, the doctor asked us what we knew about Immunoglobulins:

1- They are glycoproteins; the carbohydrates are attached to the heavy chain in the Fc (the crystallizable fragment) portion (the stalk).
2- They represent the Gama band in gel electrophoresis of serum proteins .
3- Produced by plasma cells (mature B cells)
4- Y shaped molecules
5- Comprised of 2 identical heavy chains and 2 identical light chains, and that's why the two tips that bind the antigen are identical .(i.e both tips bind the same antigen, so you can't find an antibody where one tip binds one type of antigen and the other tip binds another type).
6- They have 2 Fab (antigen binding fragments)and 1 Fc (crystallizable fragment) which binds cells or complement proteins to make the immunoglobulin delivers the message .
7- The antigen binding fragment is made of 2 domains of the heavy chain and the 2 domains the light chain . (one variable and one constant)
8- The heavy chains have at least 4 domains ,while the light ones have 2.

9- Each domain adopts a Beta barrel super secondary structure. There are 7 Betas sheets in the constant regions and 8 in the variable. A disulfide bridge connects the sheets together in the core of the cylindrical barrel.

10- Heavy chains have a molecular weight of 50 KDa ,while the light ones are 25 KDa.

11- The heavy chain determines the class of the immunoglobulin ,depending on its coding gene .

12- Heavy and light chains interact with the antigen via non-covalent interactions , like hydrophobic interaction.

13- Heavy chains are composed of 1 variable and 3 constant domains (could be 4 in some Ig) , while the light ones have 1 variable domain and 1 constant domains.

14- Not all the amino acids in the variable domains are actually variable. 3 small stretches in the light chain and 4 in the heavy chain are the ones that keep changing and they are called hypervariable regions (each contains 7-12 amino acids).

15- The light chain may be either kappa (k)or Lambda (λ) , and in one Immunoglobulin they can never be a mixture (i.e both arms (Fab)have the same type of the light chain)

16- The hinge region is within the heavy chain , it is a part of the heavy chain and its located between constant domain 1 & 2 . Its secondary structure is a loop structure. The hinge is responsible for flexibility of the two arms. The two chains are bound by disulfide bridges at the hinge region .

17- Some Immunoglobulins (IgM & IgE .... ME ☺) have an extra domain in the hinge region which limits their movement (but doesn't prevent it). And we expect them to have higher Mwt. Than other Immunoglobulins.

18- Two enzymes degrade immunoglobulins , papain and pepsin.

- Papain degrades from the hinge region toward the amino end , producing 3 fragments , 2 Fab and 1 Fc .
- Pepsin degrades from the hinge region towards the carboxylic end , producing two fragments , the Fc and the 2 Fab that are connected as one unit by the hinge region .

❖ Note :

Slides from 17 to 21 are not required .

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Beginning of lecture 5 :

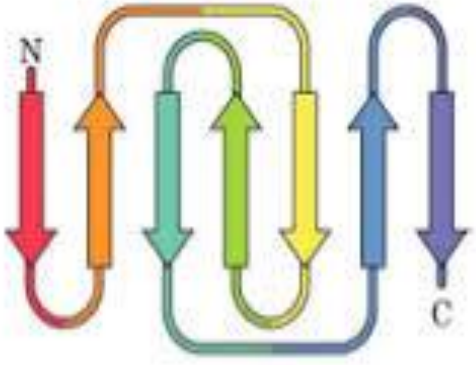
❖ Variable regions (slide#22)

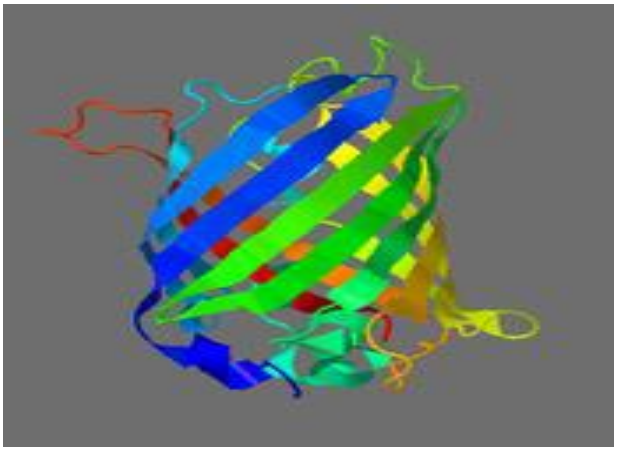
No variable regions in different humans are identical.

-Light chains have 3 hypervariable regions

- Heavy chains have 4 hypervariable regions

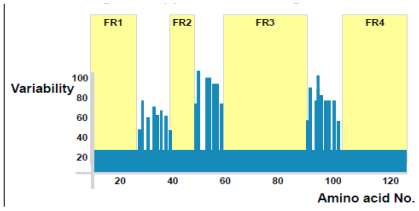
- The figure shows immunoglobulin fold in 2D and a 3D structure. It is β barrel which is composed of beta sheets that fold to form a barrel , connected by loops and in the core we have disulfide bond.





-Hybervariable regions adopt a *loop structure* to better accommodate the antigen, because as we know , the loop gives flexibility , so it will move smoothly t bind the antigen .

<u>The graph in figure to the right is really important :-</u>



<u>let's take the classroom as an example :</u>

*if we consider protein X the first chair column and protein Y is the second column , Z is the third column and so on , and every chair in each column is an amino acid.

- look at the first amino acid (chair) in each protein (column), if all the first amino acids are the same (lysine for example)→ the degree of variability between different protein (columns) = ZERO% in the 1st amino acid.

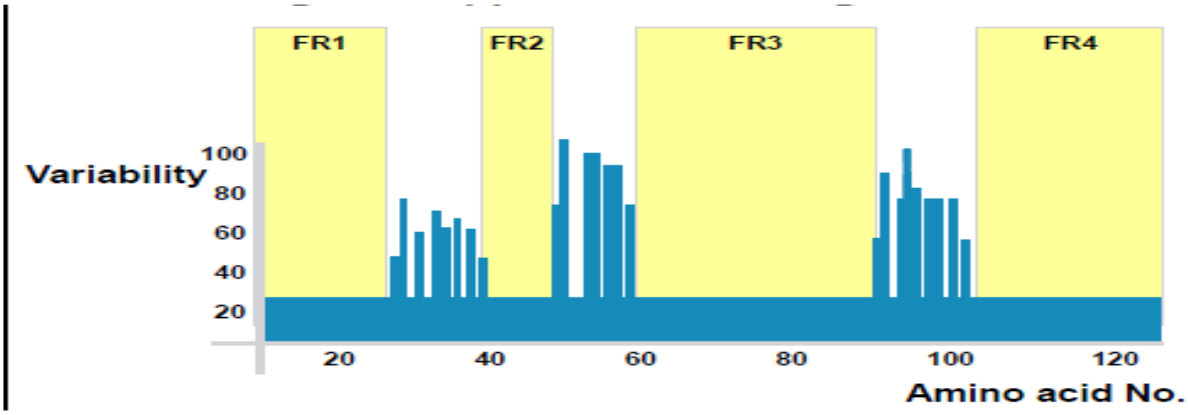
*Now, look at the second amino acid in each protein →if they are <u>all</u> different from each other ,the degree of variability in the second amino acids =100 %

-for the third amino acid, half proteins are identical and the other half are not → the degree of variability =50%

- ❖ The degree of variability is not high if it is equal to 30% or less , but it is high when it is more than 30 %.

So, if you bring different immunoglobulins and you want to know the degree of variability in the amino acid sequence of the variable domain of the light chain , you'll find it <u>30%</u> or less , <u>except</u> in three stretches of amino acids (7-12 A.A in each) ,and these A.A compose The Hypervariable Regions , where the degree of variability is very high.

Degree of variability for
→ higher than 30 %
other regions → 30% or less



Notice in the figure above :

*if the A.A are alike (when the degree of variability =30% or less), the immunoglobulins are not that much different from each other.
*they become different from each other in the hypervariable region, i.e. the three stretches (ex: the A.A from number 30 to 42 are different , so they have high degree of variability as you see)

In proteins ,When a new protein is discovered and you want to classify it in a certain family , compare its amino acid sequence with other proteins to know the degree of *similarity* between them .
- if it is more than 30% → similar
-if it is 30% or less → not similar.

Notice that in immunoglobulins , we talked about the degree of *variability*, which is very high (more than 30 %) in the hypervariable region.

Q: Is it true that immunoglobulins are hypervariable ? yes

**the variability is due to the hypervariable regions .
How to prove that this high variability is specific for immunoglobulins ?!

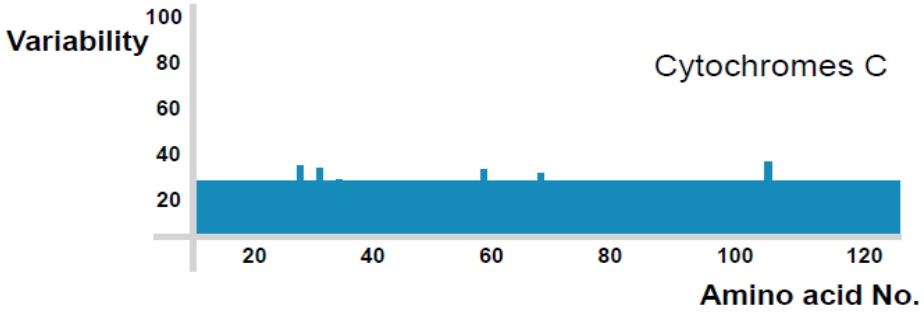
we compare it with the degree of variability between members of another group of proteins .

*In this example, we chose cytochromes C group .

Cytochromes are the proteins that have heme as a prosthetic group and they transfer electrons in the electron transport chain . Named C as they have Heme C , and cytochrome as they transfer electrons . they transfer the electrons form complex 3 (cytochrome C oxidase) to 4 (cytochrome PC1 as it contains P and C1). Other Cytochromes named P and E according to the type of heme present.

- what do you need to know that Cytochrome C family includes a huge number of different proteins , each one has a specific name (C 511, C 550 ... etc) .

-if you bring cytochrome c proteins and you follow the technique used with Immunoglobulins (the classroom example) and notice how the 1st and the 2nd amino acid for each protein change , you will find that there's no degree of variability (unlike immunoglobulin) except for sporadic amino acids. (we can say low variability)



*All of that is just to illustrate to you how much is the degree of variability between different antibodies . (remember we have 100 million Ig)

complementarity determining region CDR :

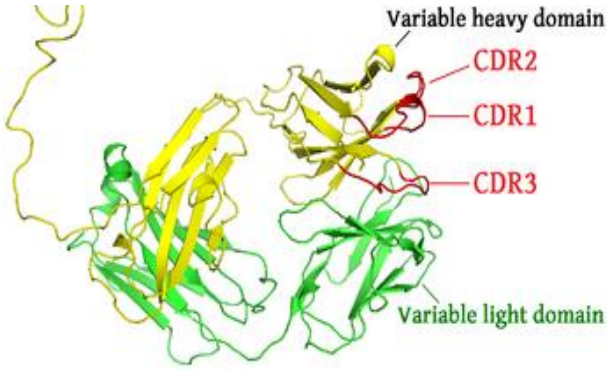
-the hypervariable region is called complementarity determining regions (CDRs)

-CDR :is the area (region) responsible for determining the complementarity between the antigen and the antibody .They are located on the small loops of the variable domain . These loops are complementary to the shape of a specific antigen . Thus, CDRs determine to which specific antigen the antibody will bind .

- the other areas between the hypervariable regions (yellow area) are called Frame work Region (they preserve the structure and the shape of the domain , As the name implies).

CDRs interaction with antigens

-Immunoglobulin binds the antigen at the tip (Fab) of the variable domain (exactly at the hypervariable region).



- If you have an antigen , does it bind to the whole CDRs(loops) or not ?

It depends on the size of the antigen, so we have 2 cases :

*large antigen: it will bind to all CDRs (which are loops) of an antibody binding fragment.

(e.g. : influenza haemagglutinin which is found in influenza virus and it is a very big protein)

*small antigen (haptens): interacts with only one or two loops (maximum) of the heavy or the light chain .

(e.g. : 5-(para nitrophenyl phosponate) pentanoic acid , which is a hatpen , binds one or two loops .

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alaah b3een , ba3raf zhe8too ☹

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Immunoglobulins Classes

*Immunoglobulins are classified according to the nature of their heavy chains.

*5 different classes of immunoglobulins, which are products of 5 different genes that produce the constant regions of the heavy chains . These genes are :

-α → IgA

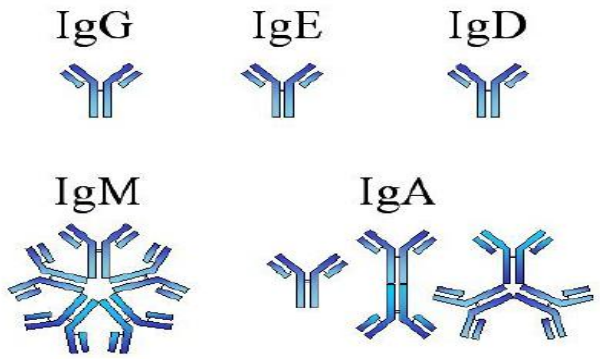
-δ →IgD

-μ → IgM

-ε→IgE

-γ→IgG

Class	Heavy chain	Chains structure	% in serum	$T_{1/2}$ (days)	Comp. fixation	Placental crossing
IgM	μ	Mono-, penta-, & hexa				
IgG	γ	Monomer				
IgA	α	Mono-, di-, or tri				
IgD	δ	Monomer				
IgE	ε	Monomer				



-<u>what should we know about these classes:</u>

<u>The names of the genes</u> responsible for the production of the constant region of the heavy chain .

<u>Their final shapes :</u>

-Some of these classes stay as monomers and continue doing their function as a –Y- shaped molecules. (no polymerization)and don't unite to form a higher structure .
These classes are (IgE, IgD and IgG)
- Other types polymerize to form higher structures , like IgM and IgA.

1. IgM can be found as monomer, pentamer and hexamer , but mainly *pentamer .*
2. IgA can be found as monomer, dimer and trimer, but mainly *dimer .*

<u>Concentrations :</u>

The highest concentration in the *serum* , plasma→IgG
The lowest concentration in the serum→IgE

The highest concentration in *secretions* (plasma, blood, tears, urine)→ IgA .

Half-life :

-Highest half life→IgG.

Ability to bind to complement proteins and fixing them :

IgM and IgG. IgM has a higher capability to fix complement proteins.

-We know from previous lectures that the antibody binds the antigen at the tips. But in order to deliver the message to the body ,the Fc portion can bind either cells or proteins (called Complement Proteins) which are part of the immune response molecules.

Q. the Dr. asked "Do all immunoglobulins have the capability of binding this complement proteins ؟ "

لحظة صمت تعُم القاعة

The answer is NO. some immunoglobulins have the ability to bind these proteins (or in other words , the ability to fix complement proteins), others don't , they just bind cells.

Crossing the placenta :

IgG is the only antibody which crosses the placenta. It transfers the immunity from the mother to the fetus.

Note : the numbers in the table are not important
عمسؤليتي☺مش مهم الارقام

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Heavy chains of the immunoglobulins :

IgD, IgA and IgG.. they have 3 constant domains and one variable.

IgM and IgE .. they have 4 constant domains and one variable.

The additional constant one is located at the Hinge region .

IgM class :

-You can find IgM :

1- Mainly intravascular : free in the plasma, blood ,and lymph (usually pentamer).
2- Bound to the B-cell surface.

*IgM only exists as a monomer on the surface of B-cells ,why ??

Because when it polymerizes , the heavy chains of the Fc portion join together , so they are no longer available to bind the cell , that's why it must be a monomer to bind its receptors via Fc portion .Also , the antigen binding portion must be exposed to the antigen to be able to bind the antigen, so polymerization doesn't occur by linking the antigen binding portions.

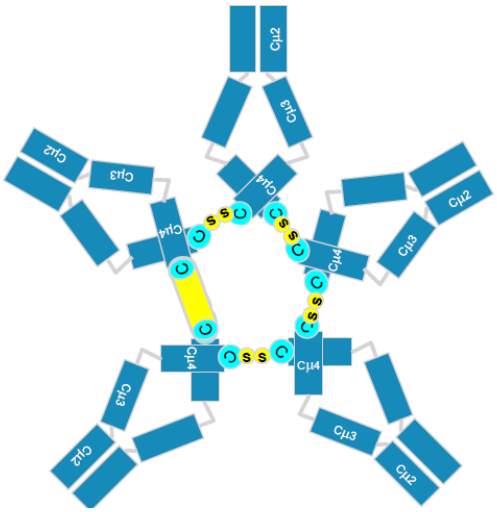
*Function of IgM :

Primary immune response.

What is the primary immune response ?

when the antigens enter the body (blood) for the first time , antibodies recognize them nonspecifically .IgM binds the antigens with

low affinity (because of the low specificity to these antigens as they have entered for the first time). After that ,IgM brakes the antigen and make it enters the antigen presenting cells and other immune cells until it reaches the immature B-cells .



Immature B-cell recognizes the composition of the antigen (lipids , carbohydrates or proteins)and changes the gene inside it to suit the antigen . Now it is able to produce antibodies that are very specific to that antigen and with high affinity to it .
When the antigen enters the body for the next time , the specific antigens are now produced and this is called secondary immune response. That happens because of the formation of the memory cell that will accelerate the secondary immune response and the antibody mediated response , which is higher in magnitude than the primary one .

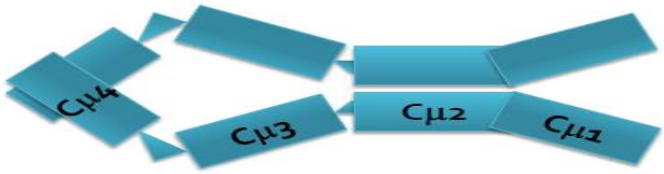
-The Process Of IgM Multemerization:

**How to connect the 5 immunoglobulins together?
By a chain called J-chain(joining chain), this chain brings two immunoglobulins closer to each other (specifically ,the fourth constant domains of two I come in close proximity). And as we know immunoglobulins have a high content of Cysteine, so when they become closer to each other ,they form a disulfide bridge between the Fc portions of the two Ig. Then the J-chain will dissociate ,and another immunoglobulin comes closer and join the complex by J-chain , then a disulfide bridge will be formed between the two close domains

and the j-chain will detach again and so on until we join the five molecules.

At the end, the J-chain Stays ,so every pentamer of IgM has only ONE J-chain.

- Mediation of the connection between the two immunoglobulins is through C μ 4 (the fourth constant domain) (sometimes C μ 3). And the j-chain fix the immunoglobulins in their places after bringing them in close proximity, it connects two , and help others to form the disulfide bridges .



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- **IgG Class:**

-found in blood ,lymph ,and intestine

-Produced in response to a wide variety of antigens.(ex. bacteria, viruses)

-responsible for the secondary immune response

-responsible for opsonization (when antibodies surround the pathogens), this process enhance phagosytosis .

-IgG is the only antibody which crosses the placenta

So it Provides the major line of defense for the fetus & during the first few weeks of newborns(because it stays in the circulation of the newborn until it is broken down)

Note :

*IgM→ Primary immune response

*IgG→ secondary immune response

* *

IgA class :

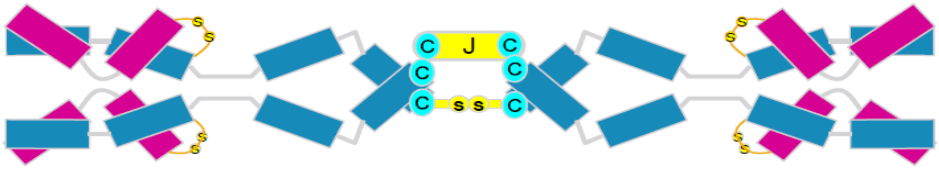
-Structure of the IgA → it can be found as monomer, dimer, or trimer in the plasma , but you can *find it in secretions only as a dimer.*
-found in secretions of the glands (tears, saliva, intestines, milk, bronchial secretion, urine , stomach acids) .

*These secretions are products of the epithelial glands, and they are secreted to the spaces found in the body , like the stomach for example . Under the epithelia glands, in the subepithelial tissue there are B lymphocytes that produce IgA as a dimer ,which will be transfered to the spaces to work there .

Functions of IgA :

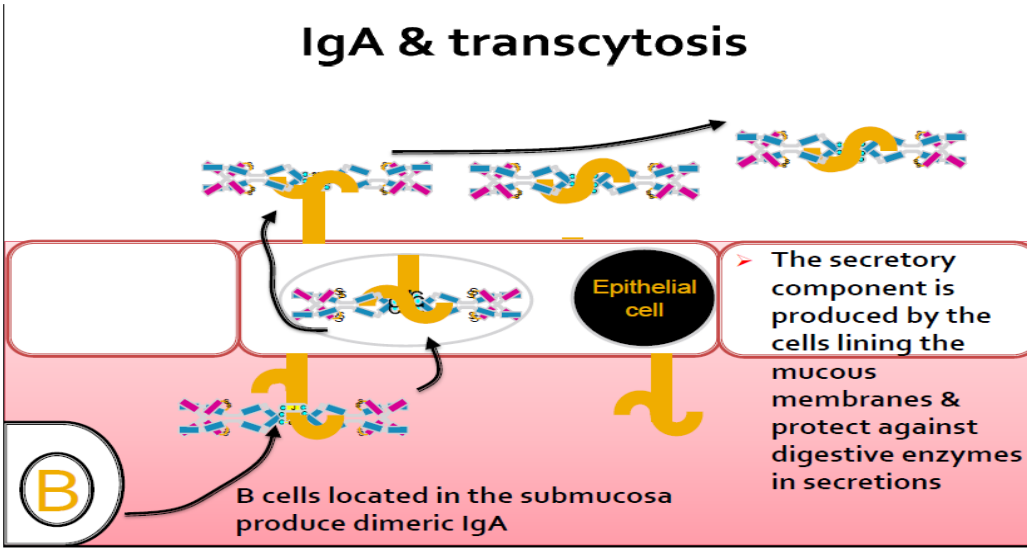
1- Localized protection . you need it in the stomach to protect you from pathogens found in some foods , in tears to protect the eye and in the skin. You needed IgA in any tissue that is susceptible to pathogens to fight them .
2- Also ,as it is found in the milk ,the fetus and newborns need it to protect the GI tract ,and it can be translocated from the GI to the circulation , then to other spaces of the body .
- Moreover , the newborn can't produce antibodies, as the plasma cells are not mature yet .But his mother will provide him with two important antibodies :IgA (From the milk) and IgG (as it can cross the placenta).

The Process Of Dimerization :



-It is the same as IgM, needs the J chain and formation of disulfide bonds.

-the disulfide bridges keep them as a dimer.



IgA transloction :

Epithelial glands secrete IgA with their secretions to the spaces , and as we said , in the subepithelial tissue there are B lymphocytes , that make IgA dimers.

-Now, the Epithelial cells produce a secretory component/ sequence (S shape) that will bind the dimer of the IgA to protect it from breaking down by proteolytic enzymes that are found in all secretions (like pepsin in the stomach for example) .The S shape component is connected to a stalk. Then the IgA will be translocated into the epithelial cells and then to the space. When it is close to the space, proteolytic enzymes will degrade the stalk, leaving the IgA dimer with the secretory component ,and now the tips of the Y shaped molecule are free and can bind the antigens found in secretions.

IgD Class :

location: B-cell surface, blood ,and lymph

-in serum :(the function is unknown)

-on B-cell surface ,IgD initiates the immune response

IgE Class :

Location: in the Blood & bound to the mast cells & basophiles which have high content of heparin &histamine, which are responsible for allergic and inflammatory response (increasing vascular permeability, skin rashes, respiratory tract constriction (wheezing), & increased secretions from epithelium (watery eyes, runny nose)throughout body

-Also IgE has a role in degrading worms *_*

-As we said that Ig Fc portion binds to receptors on cells ,The Ig that has the highest affinity to its receptor is the IgE .

نعتذر عن اي خطا سقط سهوا

اي خطأ بالشيت اعملوا حالكم مش شايفه

☺ #دفعة_2013 و اقطع

THUMBS UP FOR Abd tayyar,lujain sarrar☺

好运气全部