



University of Jordan
Faculty of Medicine



Medical Committee
The University of Jordan

Biochemistry

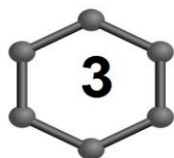


Sheet



Slides

Lecture #:



Date: **19/9/2013**.....

Title:

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Price:



Quick review of main concepts:

- The major plasma proteins are : albumin , globulin and fibrenogen
- globulin consists of 3 types: α , β and γ
- α globulin is divided into 2 types : α_1 (includes α_1 –antitrypsin and α_1 -fetoprotein)

And α_2 (includes ceruloplasmin , haptoglobin and α_2 -macroglobulin)

- β globulin includes transferrin , LDL and complement system proteins
- γ GLOBULIN INCLUDES ANTIBODIES (IMMUNOGLOBULINS)

Last lecture we've finished albumin, prealbumin and α_1 -antitrypsin.

Note to remember about α_1 -antitrypsin: if we have 1 copy of the "M" form, which is the most common, it will be enough to do the function of the protein; that's why the "MS", "MZ" forms are usually not affected by disease.

Today we will complete all globulins.

Please check the slides

Slide 26: α 1-Fetoprotein

- Continuation of α 1- group
- Synthesized in the yolk sac in fetus, then in small quantities in liver by parenchymal cells.
- We can find it in very low levels in adults (due to the reason above).
- If we have **high** amount of this protein in adults... it could refer to :

A **pathological** reason which indicates that the patient might have **either** a cancer in his liver (hepatoma) producing these high amounts of the protein; because it should be synthesized there in low quantities in normal conditions **OR** that his liver cells have been ruptured and their contents (one of them is the α 1-Fetoprotein) were released increasing its concentration... **Why** these cells could rupture? The **most common case** is because of **acute hepatitis**.

A **physiological (normal)** reason in one and only one case; which is pregnancy; Explanation: Fetoprotein will be synthesized in yolk sac – then will be reflected in the mother's blood.

- functions:
 - 1- Protects the fetus from any immunolytic attacks.
 - 2- Modulates the growth of fetus.
 - 3- Transports compounds like steroids.
- There is association between low levels of α 1- fetoprotein in feta and Down's syndrome... when α 1- fetoprotein is not synthesized in the required amounts; the possibility to be born with Down's syndrome is much higher!

Slide 25: Haptoglobin (HP)

- One of the acute – phase protein.
- α 2-Glycoprotein
- MW = 90 kDa.
- It is a Tetramer (4 polypeptide chains) or 4 subunits.
- It's a 4° structure protein because it has 2 or more polypeptides
- 4 subunits : 2 identical subunits they were named α
: 2 identical subunits they were named β
- This protein shows polymorphism (change on the amino acids)
- This change happens on the α -subunit only, B- subunit is identical in all polymorphic proteins of haptoglobin, and it doesn't change (always 2 identical B subunits) BUT α can vary.
- We will talk about 3 polymorphic types:
 - 1- Hp 1-1 : it has 2 copies of α – subunits
 - 2- Hp 2-1 : contains α -1 ad α -2 and α -1 is little bit different from α -2
 - 3- Hp 2-2 : contains 2 copies of α -2
- The function of haptoglobin : Binds free hemoglobin of the blood.

Question?!

Can we synthesize hemoglobin in the blood? Actually we produce the globin part which is (the protein part), But we can't synthesize iron (heme) – instead we get it from outside.

If we have free hemoglobin inside the blood and we don't want to lose the Fe – we try to capture the free hemoglobin inside the blood.

- In normal cases Hemoglobin is found in RBCs
If Hemoglobin was outside RBCs in high quantities (e.x: in cases of hemolysis ; rupture of RBC.) \longrightarrow high amounts of free Hemoglobin in circulatory system “ now this protein (heptoglobin) becomes important “ .
- Haptoglobin binds with free Hemoglobin forming a huge complex
Mw of haptoglobin is 90 KDa and of hemoglobin is 65 KDa... total weight is about 155 KDa.
This bigger complex can't be filtered through the kidneys as a result we will not lose the iron with urine.
- Binding between 2 proteins: can form any type of binding **except** covalent bond.

- In 4° structure :Between different subunits there is no covalent bonds
- The aim of forming this complex is not to lose iron ... So this complex can dissociate again.
- Half life of the haptoglobin is 5 days – once it binds hemoglobin- 90 min; **Reason** : when the half life is decreased then the chance for hemoglobin to dissociate is decreased and by that we decrease and even prevent the filtering of hemoglobin through the kidneys ... we9'7at ? 😊

Again :

Haptoglobin $\xrightarrow{\text{binds}}$ free hemoglobin in blood $\xrightarrow{\text{prevent}}$ iron loss in kidneys
 $\xrightarrow{\text{logic wise}}$ half life decreases $\xrightarrow{\text{faster}}$ degradation of the whole complex in liver
 and keeping Fe inside the body .

Slide 27: ceruloplasmin

- Copper containing glycoprotein/ copper binding protein.
- has six sites to bind copper (differ in their oxidation states)
- Function: binding & storage of copper, it works as a reservoir for Cu , so it has high affinity (it DOESN'T work in transport of Cu from one place to another inside the body).

Keep in mind: proteins that work in transfer should have low binding affinity, whereas proteins that work in storage should have high binding affinity.

- 90% of Cu inside blood are bound to ceruloplasmin
- 10% are bound to albumin – responsible for transport of Cu inside the plasma.
- It regulates the amount of Cu in the blood
 High Cu in plasma -: bind more cu
 Low Cu in plasma: dissociate cu to plasma
- Metallothioneins : group of proteins that work as cu regulatory proteins inside the **tissues**.

Question?! TRUE OR FALSE:

Ceruloplasmin work as cu regulatory protein inside the tissues

– False, metallothioneins work as cu regulatory protein in tissues, whereas

ceruloplasmin works in the **blood**.

- Ceruloplasmin is produced in liver: any problem in liver will affect ceruloplasmin levels.

Example: autosomal recessive genetic disease (Wilson's disease)

It affects ceruloplasmin levels : ceruloplasmin deficiency : Free cu level will increase in blood : go to tissues : start to deposit inside tissues : Tissue failure : organ failure : multiple organ failures : Death

Treated with plasmapheresis: withdrawing blood from the patient, then omitting Cu from it and then return blood back... then Cu starts to return back to blood from tissues and so on till the issue is solved.

Slide 28: the dr didn't mention anything about it in this lec.

Slide 29: C - reactive protein.

- Acute- phase protein.
- Why is it named like that?

Because they found that it can bind polysaccharide portion (fraction c) that is on the cell wall of pneumococci and it reacts against the fraction c on the cell wall

Pneumococci: pathogen affects respiratory system.

- It also has multiple roles in immune system; it helps in the defense against bacteria and foreign substances.
- In adults: the levels are very low and undetectable.
- It increases in cases of :
 - inflammation
 - Tissue damage
 - Cancer

Because it's an acute – phase protein

- It reaches its peak faster than other acute- phase proteins so if we want to detect any tissue damage or cancer we can rely on c- reactive Protein more than other proteins (it reaches the peak within 48 hours after the inflammation).
- It can work as monitoring marker for any problem that you have.

Immunoglobulins / antibodies

The last part of plasma proteins are immunoglobulins or γ -globulins

Slide 2: defense lines

This table shows general classification of the immune system, it has 2 main subsystems (specific & non-specific).

Non-specific (innate)		Specific (acquired)
➤ First line	➤ Second line	➤ Third line
✓ Barriers ✓ physical: skin, hair, mucous membranes ✓ chemical: sweat, tears, saliva, stomach acid, urine	✓ Phagocytic WBCs ✓ Antimicrobial proteins ✓ The inflammatory response	✓ Lymphocytes ✓ Antibodies

* In cases of infection, your body will start to fight that infection or pathogen through 3 lines of defense – 2 are non-specific and one is specific.

*Please refer to the table above.

Notes:

*the first line: the barriers which everybody has (physical & chemical)

*Mucous membrane lines all cavities inside the body.

*All these barriers have properties to fight pathogens and foreign bodies.

*If the pathogen crossed the first defense line, the second one will start to fight it.

* C-reactive protein is one of the antimicrobial proteins ;)

*The 1st and 2nd defense lines are general & everybody has them, whereas the 3rd line is specific: it starts producing cells and proteins (antibodies) against that specific pathogen.

*T-lymphocyte specifically and has 2 types: T-helper cells and T-cytotoxic cells

*b-lymphocyte $\xrightarrow{\text{produce}}$ plasma cells $\xrightarrow{\text{produce}}$ antibodies

Slide 3: innate vs. acquired

Innate:

* Natural immunity.

*cellular and biochemical defense mechanisms (non specific).

*non-adaptive upon infections: every time it faces the same pathogen it behaves in the same way.

* Only recognizes microbial agents.

Acquired:

*develops with time as a response to infection: not everybody has them, example: if you have an antibody against Pneumococci, then you should have been exposed to Pneumococci before... If you haven't exposed to Pneumococci then your body will not develop antibody against it.

*adapts to the infection : once you face a pathogen your body starts memorizing it and the next time you face the same pathogen ur body will produce much higher response against it .

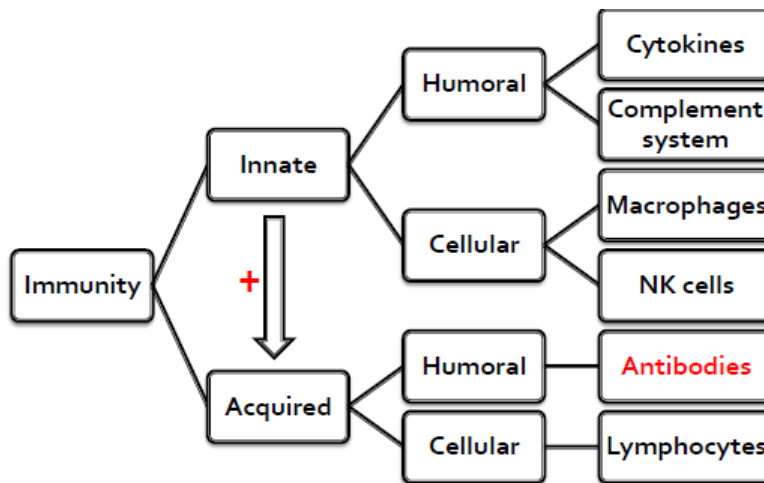
*increase the magnitude and defense capabilities with each successive exposure to particular microbe.

*recognize microbial and non-microbial substances.

Slide 4:

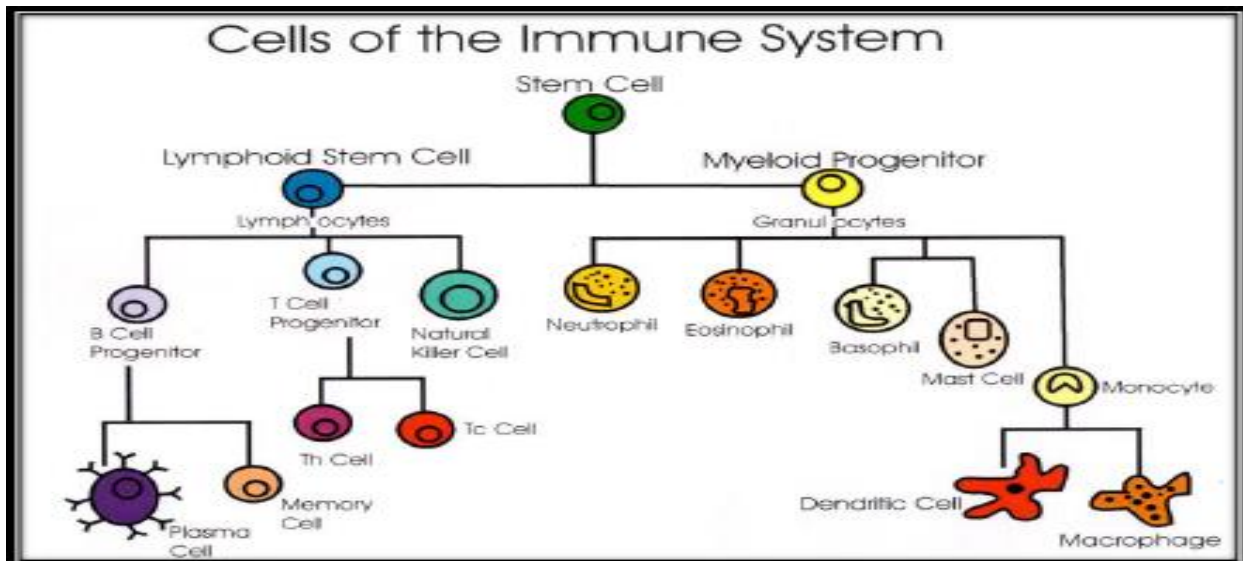
Another classification of immune system

NK cells: Natural Killer Cells



Slide 5:

Cells of immune system



Slide 6: Specific immunity

*bone marrow produce all lymphocytes , then some of them migrate to thymus gland and mature there (T-lymphocytes) and some stay in bone marrow and mature there (B-lymphocytes).

Any defense through cells is mediated through T-lymphocytes, these cells are also responsible for graft rejection, hypersensitivity reactions and defense against malignant cells and viruses.

Any defense through proteins /antibodies is mediated through plasma cells.

* The genetic deficiency is reported in each class, B & T lymphocytes.

Slide7:

- a. antibodies

Definition: glycoproteins synthesized by plasma cells and able to recognize & react against foreign molecules even if not encountered before.

Question?!

Are antibodies glycoproteins? Yes they are.

* Antibodies are γ -globulin plasma proteins and most of them are glycoproteins except for albumin.

Remember that prealbumin is glycoprotein but albumin isn't.

* Antibodies have high specificity & affinity towards their substrates.

*Synthesis of antibodies is mediated through immunogens.

*immunogen: any substance that evokes (يستحضر) immune response.

*when antibody binds the antigen, it induces the effector functions: inactivation, degradation and lyses of cells, proteins, toxins ... etc.

B. antigens:

Definition: foreign molecules which bind to the antibody.

Question?!

When do we call the material antigen? When it evokes an immune response also we call it immunogen.

*NOT ALL foreign bodies are immunogens, they should be in certain size and M.W to elicit the immune response. Some molecules by themselves can't elicit an immune response even if they were foreign because they are small and they are called: haptens.

* If Hapten binds to a bigger molecule, it can elicit an immune response.

*usually, antigens are huge molecules (proteins, carbohydrates, lipids or nucleic acids).

*any antigen will have certain places where the antibody can bind, and these are called:Epitopes

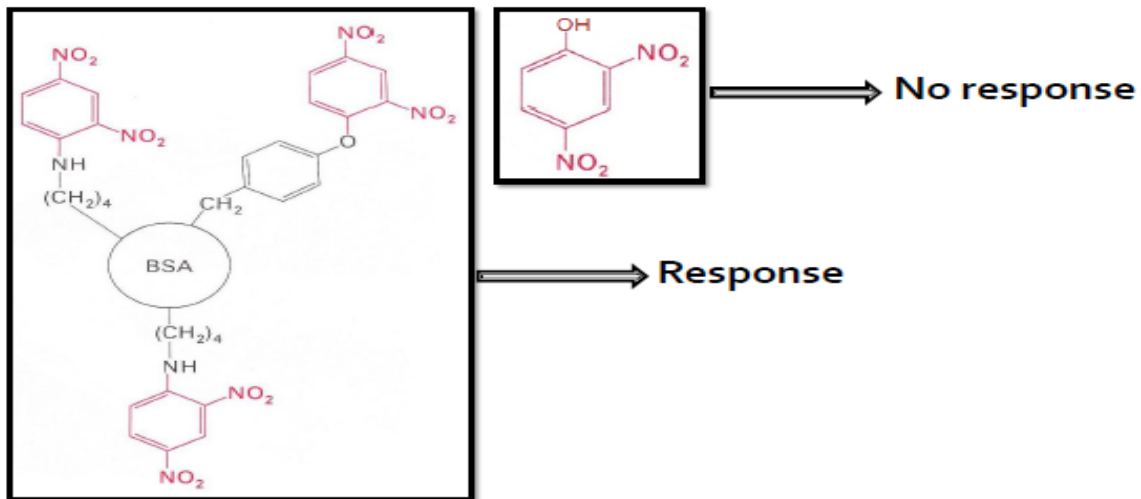
What are epitopes? epitopes are the antigenic determinant if the material is antigenic/foreign or not .

*One antigen (4 example: a bacterial cell) can have different epitopes and every different epitope binds different antibody.

Slide 8:

*This slide shows Dinitrophenol (a small foreign molecule)

*When it binds to the antibody by itself, it can't evoke an immune response, however, when those Dinitrophenols bind to bovine serum albumin (BSA), then they can be antigenic; because at that time they will be bound to proteins.



Slides 9+10: structure of antibody

- *It's consisted of 2 light and 2 heavy chains (light and dark in reference to the MW)
- *Every polypeptide chain is composed of domains (special 2ry structure).
- *Light and heavy chains are connected to each other through disulfide bonds, Also Heavy chains are connected to each other through disulfide bonds. (We have inter-chain and intra-chain disulfide bonds).
- *This huge number of disulfide bonds indicates that the structure shouldn't change.
- * Antibody recognize → antigen, so if we have high amounts of antigens then we should have high amount of antibodies to recognize them... BUT WHAT MAKES THE ANTIBODIES DIFFERENT?!

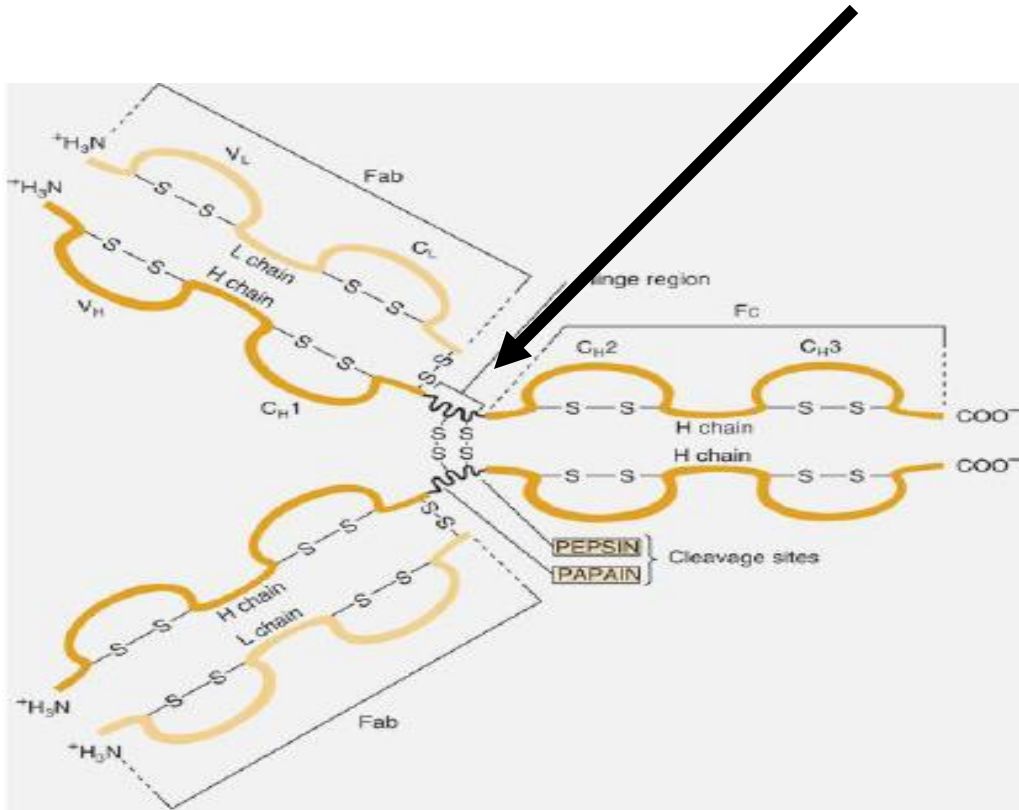
Since the antibody is a protein, we have amino acids sequence...

Any antibody has 4 polypeptide chains (4° structure)...

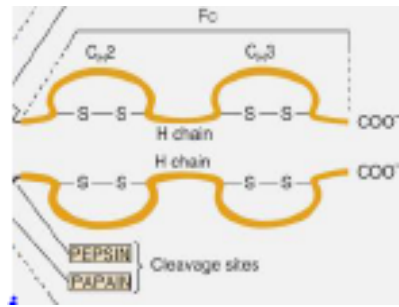
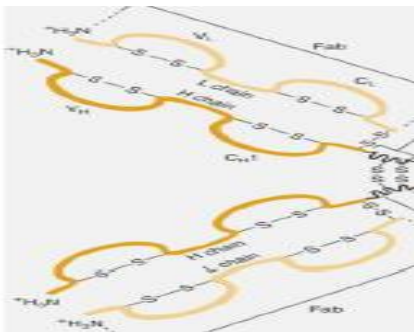
As we mentioned : we have disulfide bonds linking both of the heavy chains together at a region called hinge region , this area is a site for proteolytic enzymes , they can hit this area and result in fragmentation of the antibody molecule .

* The antibody contains 2 portions of Fab (antigen binding fragments) & 1 portion of Fc (crystallized fragment)

*If we have a proteolytic enzyme that can break the peptide bond in this place towards the free carboxylic group of the chain (before the disulfide bridges) we will get two pieces... continue on the next page..



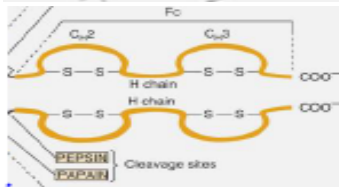
...The 2 pieces are: (Fab)₂ & Fc



And

The enzyme used is called **Pepsin**.

- **Papain** enzyme can cleave after the disulfide bond (towards the free amino group of the protein) and we will get 3 pieces : 2 Fab & Fc



- To enable the antibody to recognize different antigens the sequence of a.a should change, if we have the same sequence, the antibody can recognize only one antigen.
- The area where the a.a change (so that it can react with different antigens) is called variable domain (towards the amino groups), whereas the area in which the a.a sequence doesn't change is called constant domain (almost fixed in all antibodies; it doesn't have to change because it doesn't bind to the antigen)
- In the light chain we have one variable and one constant domains BUT in heavy chain we have one variable and at least 3 constant domains (some antibodies have 4 constant domains).
- the site of binding of the antigen is on the both tips of the Y shape molecule
- Which chains (H or L) participate in the binding to the antigen? Both heavy and light chains are responsible for binding the antigen.
- Hinge region is flexible; the sequence of amino acids in that region is arranged in loop structure, it can change its angle – the tips become closer or wider from each other - according to epitopes on the antigen.

“Life is like riding a bicycle. To keep your balance, you must keep moving.”
– [Albert Einstein](#)