INTRODUCTION TO MEDICAL

IMMUNOLOGY

SLIDE SHEET

NUMBER	2
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بسم الله الرحمن الرحيم

- The topic we are discussing has a very wide field but is also very important in immunology, you need to understand it fully 100%, and hopefully you'll enjoy it.
- There is no need to go back to the slides.

Innate Immune System

In the lab, if we want to make a plasmid (a circular extrachromosomal DNA with specific genes), one of the most organisms used is **E-coli**, we put it in agar plate then take a colony and put it in aliquid medium (LB medium) and let it mix overnight..

➔ Next day you'll find the medium became turbid; due to its fast growth!
- Their duplication time is 20 min. **But** this huge growth won't occur in our bodies even though we are always exposed to E.coli and many other pathogens and that is because of our body's**fast response of the innate immune system**!

❖ Innate immune system has three parts :

Complement system ✸ Professional phagocytes ✸Natural killer cells "NK"

1) Complement system:

- Composed of ~20 proteins that work together to destroy invaders and to signal other immune system players that the attak is On!
- Most of its components are proteins that are produced in the liver.
- Its importance is obvious as it is one of the **oldest** systems in living,for example in sea urchins which evolved around 600 million years ago

→In humans it develops very early; in the first trimester (0-12 weeks) of pregnancy, so it's very crucial.

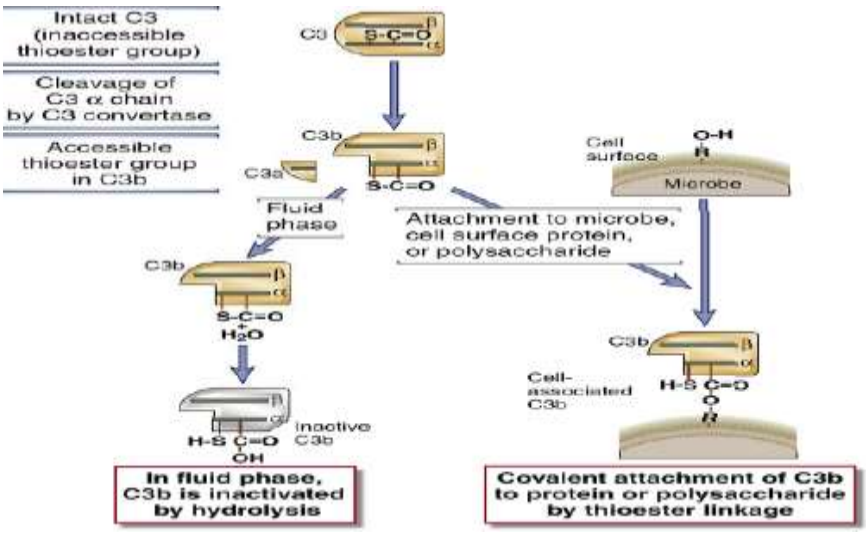
☙Methods of complement activation:-

1-Classical pathway:

→ The first one discovered, it has a relation to **antibodies**
(part of adaptive immune system) .→ "will be discussed in the next lecture"

2-Alternative pathway:C3 is the main molecule in this cascade

Specilized with its thioester group (S-C=O) inside it, which is not available to react.



C3 undergoes spontaneous cleavage ➔ giving **C3a** (a chemotactic agent) + **C3b**

C3b has an exposed thioester group, thus it's a very reactive molecule!

It has only 60 microseconds to:-

- Either find (NH2) or hydroxyl group (OH) on the surface of the microbe (normally at its surface proteins/sugars) and bind to it in order to activate the pathway

OR

- It will be hydrolyzed by water molecule to give Inactive C3b (**IC3b**) and we call it the **fluid phase.**

➔If it finds a NH2 or OH, C3b binds covalently by the thioester bond,injecting itself!

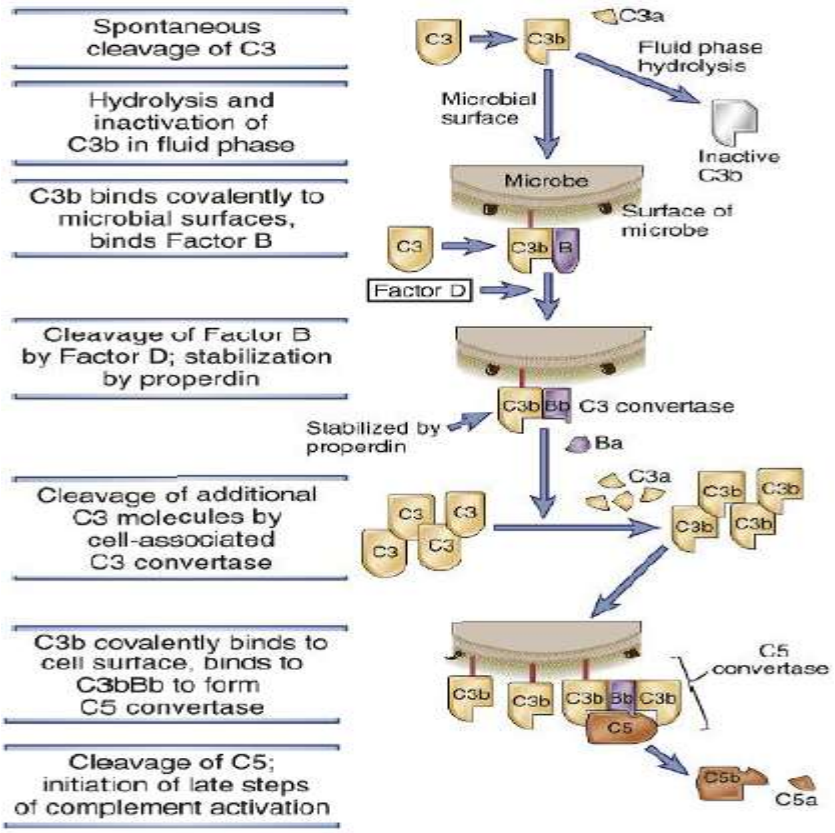
Other factors called factor B, factor D and properdin

- **Factor B** will bind to C3b, forming C3bB
- **Factor D** cleaves part of factor B (cleaves to "B" and "b"), leaving the structure **(C3bBb);** which is known as **C3 convertase**

  Note: In many different pathways, C3 convertase has many different structures, don't be surprised :p but C3 convertase (C3bBb) is the enzyme structure that is involved in the alternative pathway . C3 convertase is capable to cleave C3 molecule regardless of its structure.

- **Properdin** stabilizes C3 convertase to the surface of the microbe.

⇨ So, C3 convertase is responsible now for the cleavage of more C3 molecules (will make the process faster and more efficient).
It will cleave C3 to C3a + C3b,,, C3b will attach to the surface of the microbe, factor B then factor D will bind it to produce more C3 convertases and so on…



✸Another function of C3 convertase is to complete the formation of the **MAC system:-**

Another C3b will bind to C3bBb, making (C3bBbC3b);that can also be called **C5 convertase.**

➔C5 convertase will cleave C5 into C5a and C5b :

-C5a is similar to C3a, both play a role in inflammation

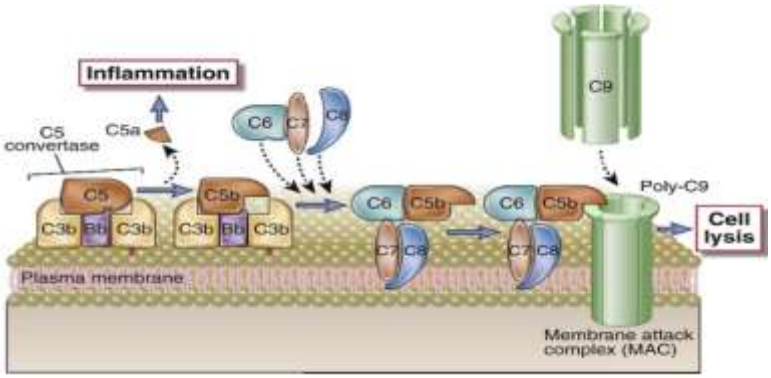
-**C5b** is a bulky molecule, will bind to other complement proteins (**C6 , C7 , C8),**

- C7 and C8 will bind in away that will dock/stick the complex to the membrane.

➔Now the full structure is ready to bind to **C9**, which is capable to do polymerizationand now this whole C5a-C6-C7-C8-C9 complex is called the **membrane attack complex (MAC).**

These MAC complexes **DRILL** holes at the membrane, creating an osmotic pressure difference, water gets inside the microbe, and the microbe will EXPLODE !

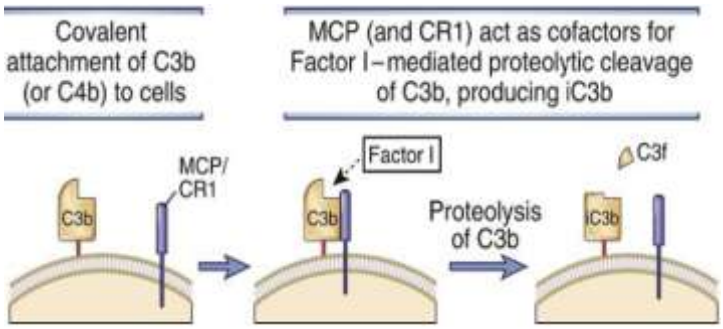
Summary: This is how alternative pathway works leading to a mechanical structure “MAC” on the surface of the microbe and just drill it ☺ !!!



Why doesn't the complement system destroy our own cells although they have many NH2and OH ??

→Because there are regulators that are able to reverse their action and protect us as:

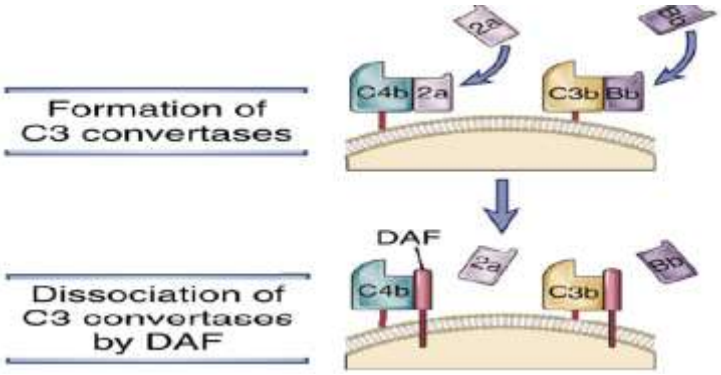
1- <u>Human cells posses surface enzymes that inactivate C3b</u>
If C3b got attached to one of our cells, there are molecules called **MCP** (membrane cofactor protein) and **CR1** (complementreceptor one), thesewill bind to the C3b and activate **factor I** to mediate immediate cleavage of C3b producing iC3b and C3f.



2- <u>We have at the surface of our cells a membrane called:</u>
<u>**Decay accelerating factor "DAF" *(CD55)***</u>

CD = Cluster of Differentiation

➔ This destroys C3 convertase (C3bBb).



Note: When C3 convertase is formed by the cleavage of 2aB, another structure called**C4b2a** is also formed and has part in the lectin pathway

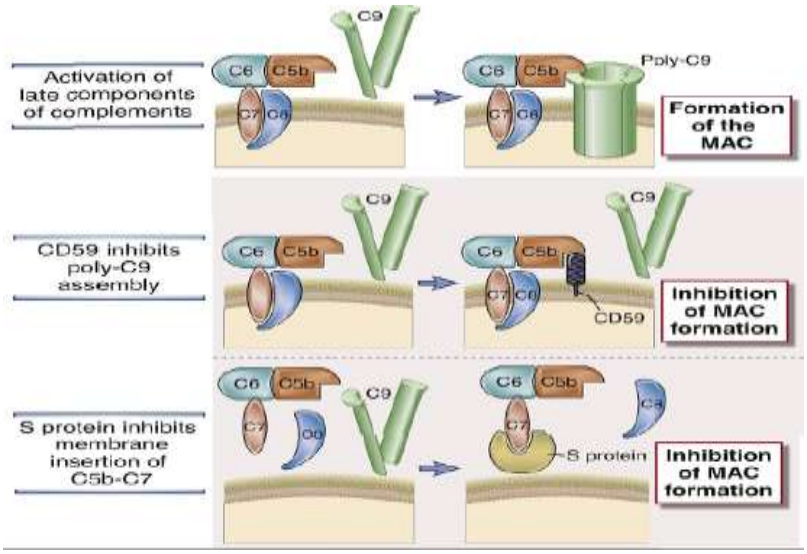
→ DAF molecule is also able to cleave C4b2a and dissociate the 2a from it .

➔ If neither of the previous mechanisms work:-

3- We have the **protectin (CD59)**
This molecule will inhibit C9 assembly to C5b-C6-C7-C8 complex by binding to C5b thus **inhibiting MAC formation in the latest step**.

** Also we have **S protein** that inhibits membrane insertion of **C5b-C7**

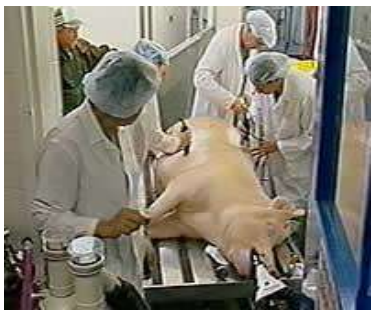
Note) S protein has a relation to blood clotting.(prevent thrombosis)



☙ Practical applications on the complement system:-

1- **PNH (paroxysmal nocturnal hemoglobinuria)**: is a disease characterized by destruction of RBCs , the patients actually have a deficiency in **CD55** and **CD59** shown in cytometry, so their cells CAN'T protect themselves from the complement system and get killed ☹

2- **Heart Xenograft experiments**: Once they tried to transplant a heart of a pig to a baboon in order to see howmuch possible heart transplant is in humans, but the result was a big failure → necrosis happened within minutes !!
Why? Because the molecules on the surface of the pig's heart that are responsible for the protection against the complement system weren't compatible with the complement of the baboon, so the complement of these primates (baboons) were able to attack the tissue of the transplanted heart.




➔We conclude that: ** **The complement system is fast**

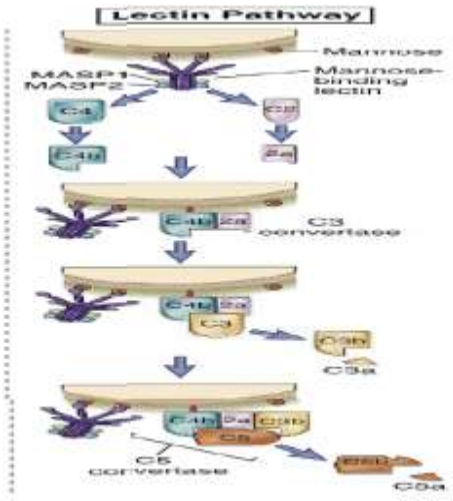
** **The complement attacks any unprotected surfaces**

2) Lectin pathway:

* What's the difference between lectin and complement pathways?

➔Hence from the name, the lectin pathway depends on the pentamer (lectin) which is able to recognize the **mannose sugar** (which is common on pathogens surfaces but not ours :D), so this pathway is**pathogen spicfic!**

Look at the figure below:-



1) The lectin will bind the mannose
2) Molecules called**MASPS** (mannose bindinglectin associated proteases) come and bind. ➔ They are proteases, so they'll start cleaving:
   - **C2** intoC2a + C2b
   -**C4** into C4a and C4b
3) Now C2a and C4b will bind together forming **C3 convertase (C4b2a)** and like other convertases will cleave C3 and so on!

➔ We conclude that:

- **Alternative pathway are just like Random Bombs**
- **While lectin pathway is a Smart Bomb,** specific by using the mannose binding lectin (MBL).

❖ Functions of complement system:-

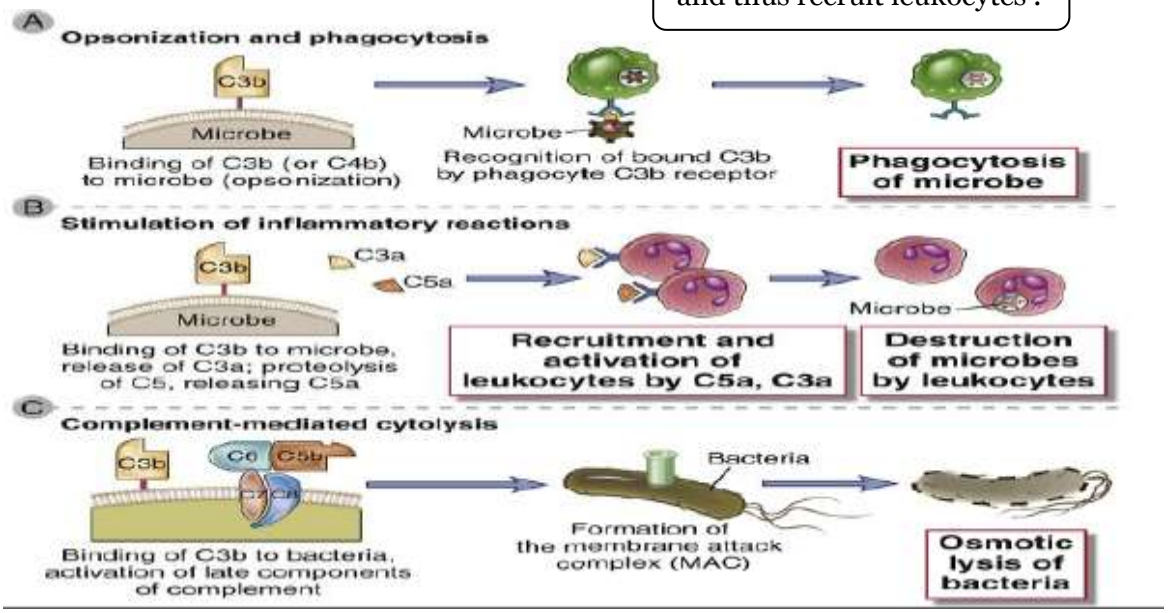
1. Osmotic lyses of bacteria through forming MAC complex.
2. Opsonization and phagocytosis:

When C3b molecule bind the pathogen,it will attract macrophagesas macrophages have complement receptors on its surface that will bind to C3b-coated pathogen and phagocytose the microbe.

"It's called the antibody of the poor "

3. Stimulation of inflammatory reactions.

C3a ,C5a are (chemokines) and thus recruit leukocytes .



❖ Professional phagocytes:

-APC: Antigen Presenting Cell.

1- **Macrophages**: APC / found in all areas of the body; skin, lungs, intestines,..

2- **Dendritic cells**: APC / found in epithelia and most tissues

- The biggest sensor of **PAMPS** (pathogen associated molecular patterns)which are molecules highly conserved in pathogens. These are excellent activators of adaptive immunity.

3- **Neutrophils**:NOT APC / short lived and mostly involving killing germs .

➔Macrophages exist in three states:-

1- Resting macrophage (garbage collector):Find dead tissues and ingest them.
They have Low MHC-II expression.

2- Primed macrophage (good APC, good killer):When there's a specific signal like (**IFN-gamma**) secreted by NK cells that indicates something abnormal is going on so these cells become primed (activated)!

→ They become a good killer and up regulate MHC-II expression

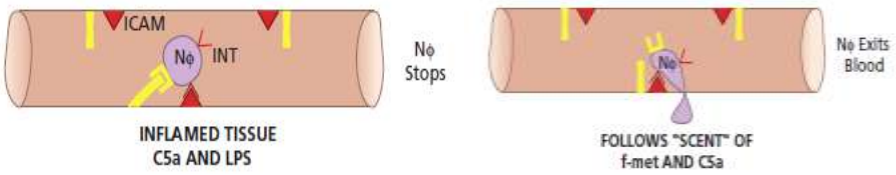
3- Hyperactivated macrophage:When there's a second signal like **LPS**
(A definitive proof that I have bacteria,it becomes angry,hyperphagocytic, develop more lysosomes, ROS, NO.

➔Neutrophils:

- If macrophages get overwhelmed, who comes to rescue? **Neutrophils.**

* They're **NOT** APC, but rather, professional killers.

* How do they know when and where do neutrophils exit the blood stream to go to inflamed tissues?**(Slow & Roll)**

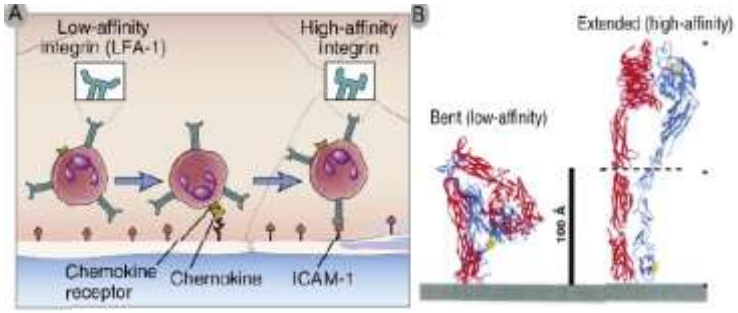


1) They have on the surface a selectin ligand (**SLIG**)
2) Normally there is no selectin on endothelial vessel walls, but when there is inflammation and the cells present pro-inflammatory cytokines like **(IL1 , TNF**) the selectin will be expressed on the endothelial cells **6 hours post tissue insult**.
3) The SLIG on neutrophils will bind to selectin on endothelial vessel wallsand thus, the neutrophils will be slowed down.
4) Then comes the turn on the **integrins**family; integrins on neutrophil walls will bind to their ligand the ICAM molecule on the endothelium, thus completely stopping the neutrophils.
5) Then the cells will go out of the circulation through (diapedesis) following the**"SCENT**" of F-met and **C5a + C3a** (chemokines) leaving from low concentration to high concentration to reach their target.

✓ Integrins first are found in low affinity integration in a closed&bent structure (LFA-1) inside the neutrophil.

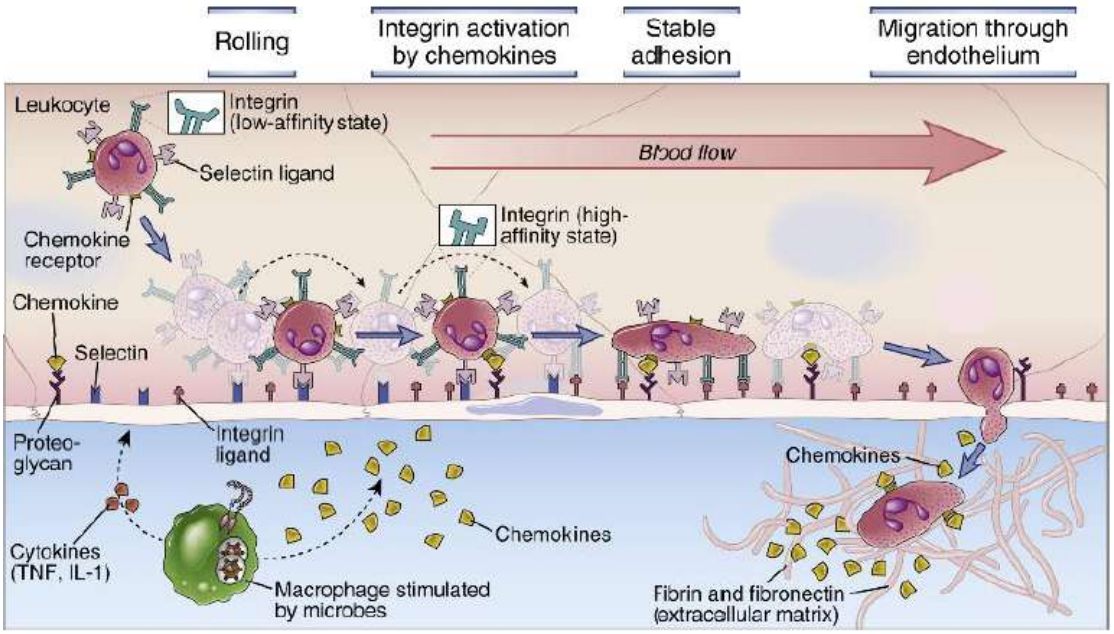
➔After damaged tissues secrete chemokines and their binding to the Neutrophils the affinity will change into high integration (will become extended structure), so that it can bind now to its ligand.

✓ **So chemokines are not only for recruitment but also they can change the morphology of integrins.**



The figure below summarizes the whole story:-

An inflamed tissue secreting chemokines – inducing expression of selectines – Neutrophils start rolling –bind to the chemokines – changing the affinity of integrins – binding to ICAM – then stops (stable adhesion) - and then get out to the site of inflammation.



➔Natural Killer (NK) cells:

- Short lived cells (1 week)
- No B or T cell receptors!
- Lymphoid origin but they are NOT B cell type or T cell .
- They are **ON CALL**, only works when really needed.
- Mostly found in blood, liver and spleen (Not in tissues)
- Once they enter the tissue they have 2 functions:-
  1- Produce cytokines (**IFN-gamma ;activates macrophages**)

2- Kill cells by forcing them to commit suicide “Apoptosis” by injecting granzymes, FasL-Fas interactions.

**NK cells has both the actions of T-helper cells through secreting cytokines and cytotxic T-cell combined.

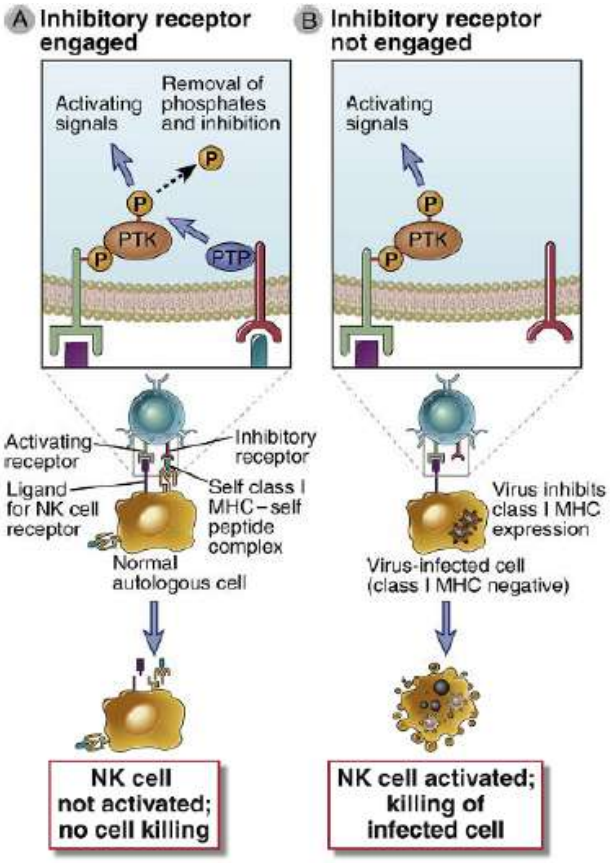
* How do NK cells recognize their target?

➔They have NK receptor **NKR**, it will bind to a ligand in a viral or cancerous cells, once they recognize it without any other signal they’ll activate protein tyrosine kinase **PTK** (activator) and kill the cell.

- But!! if they see MHC-I molecule expressed , they will activate phosphatases **PTP** (inhibitory) and it will stop. (Inhibition is due to dephosphorylation).

- Every cell in our body has an **MHC-I**molecule in order to protect it (MHC-II molecules are only in immune cells).

- Some viral and cancerous cells try to downregulate MHC-Imolecules to avoid immune cells like (T-cells)! but they weren’t smart enough to know that this is a signal for NK cells to kill :3 !!



- **Activating and inhibitory NK receptors:-**

1- Inhibitory ligands are mostly MHC-I molecules (**HLA-A, B, C**) but the most important is **KIRs** (Killer Inhibitory Receptors)
→when they bind inhibition of NK-cells occurs.

2- Activating ligands like:**CD16**

- We use it in flow cytometry in recurrent abortions females, we look after CD16 and CD56 that will make NK cells kill the fetus !!
- Their ligand is on IgG-coated cells.

Another one : **NKG2**

3- At the cytoplasmic part they have what we call **MOTIFS:** specific sites for some types of amino acids that are able to be phosphorylated like **tyrosine**, once they bind they'll be activated or inhibited.

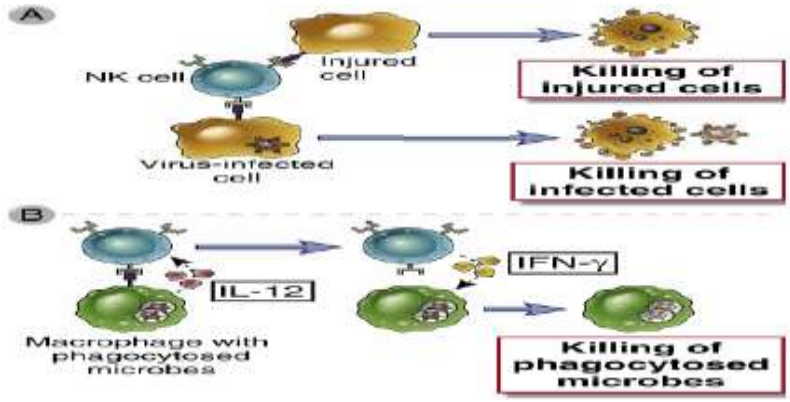
✦**The activating receptors have ITAM (**immunoreceptor-based tyrosine activating motif)

✦**The Inhibitory receptors have ITIM** (immunoreceptor-based tyrosine inhibitory motif**)**

☙Functions of NK cells:-

1- Killing of injured, infected cells and cancer cells.

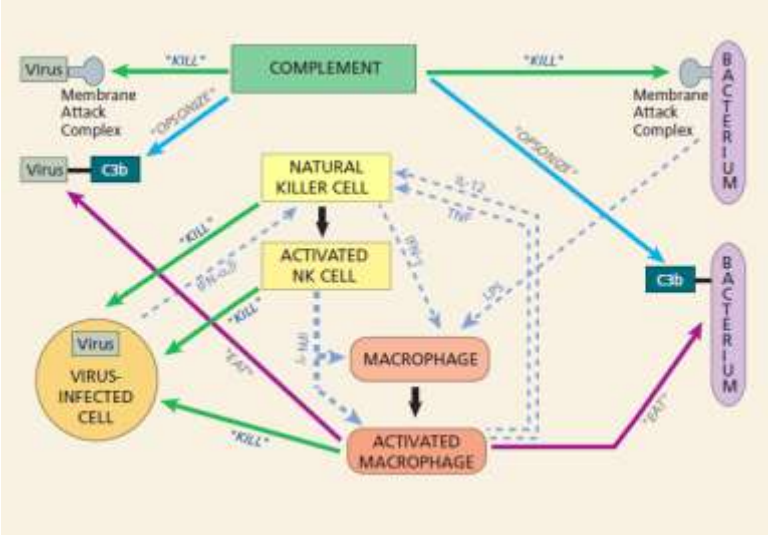
2- Secreting cytokines,**IFN-gamma** that activate macrophages, which in turn secretes **IL-12** that activates NK-cellsand kill phagocytosed microbes (A role of co-operation).



Quick Summary: The figure below shows how the complement pathway kills the viruses and bacteria throughthe MAC system and how it will bind and do Opsonization via IC3b , which will attract the macrophages to attack the viral cell and secrete IL-12 and TNF which will activate NK cells → NK are now capable to directly kill the pathogen and secrete
IFN-gamma that in turn will activate more macrophages and so on...

➢ Many viruses evolved many defense mechanisms to protect themselves from the innate immune system.

- Innate system can help contain a viral infection in early stages, but more potent weapons are frequently required! ➔ The Adaptive Immune System (Next lectures…)



Difficult roads often lead to beautiful distances :D

A special Dedication to the great Omar Mahafza.. ***Best of luck !***