بسم الله الرحمن الرحيم

Immunology course / 2017

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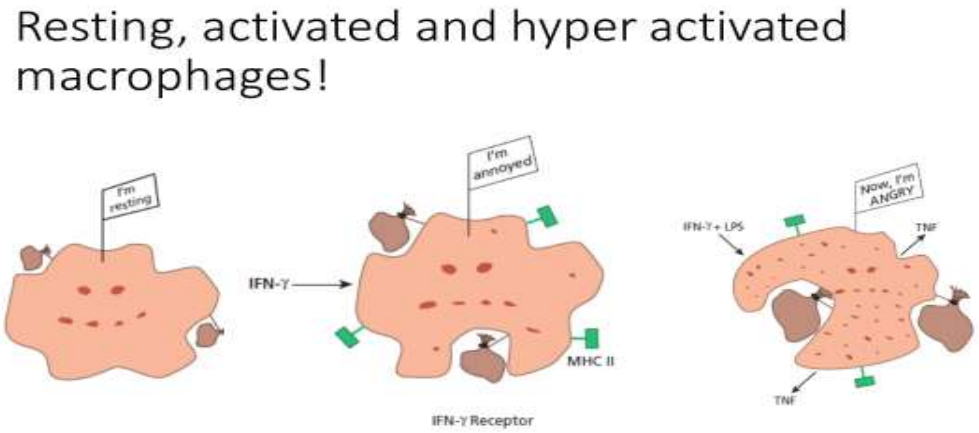
LECTURE 3/ innate immune system 2/2

Introduction

We agreed that the innate immune system is composed of: complement, phagocytes and natural killer cells (NK). We discussed the complement system last time. In this handout we will discuss the phagocytes and natural killer cells.

Phagocytes

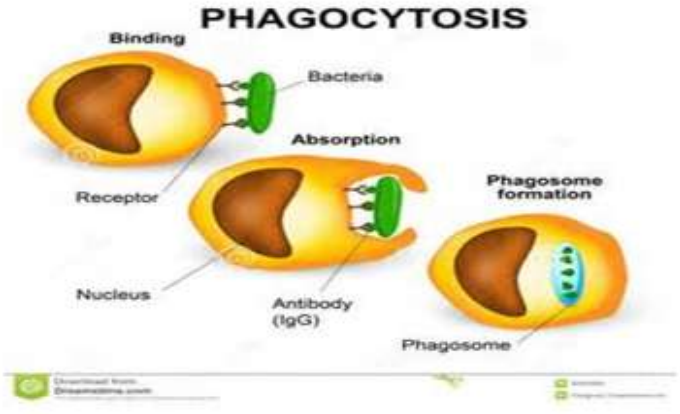
We have two professional phagocytes: **macrophages and neutrophils.**
Macrophages originate from bone marrow precursors. They circulate in the blood as monocytes
Monocytes in tissues are called macrophages or epitheloid cells.
Macrophages have three phases or levels of activation
1. Resting state
2. Activated = primed
3. hyper-activated



Resting macrophages are present beneath the skin and body surfaces , they get rid of debris and of dying cells. They just collect garbage and live a boring life!!
These express *few* MHC II

Activated macrophages = primed : are activated by several factors mainly **interferon gamma** produced from T helper and NK cells .Activation of macrophages happens in response to pathogens .
Activated macrophages **upregulate MHC II producing loads of it**
By increasing their MHC II they **can present antigens to T helper cells**.. They act as antigen presenting cells

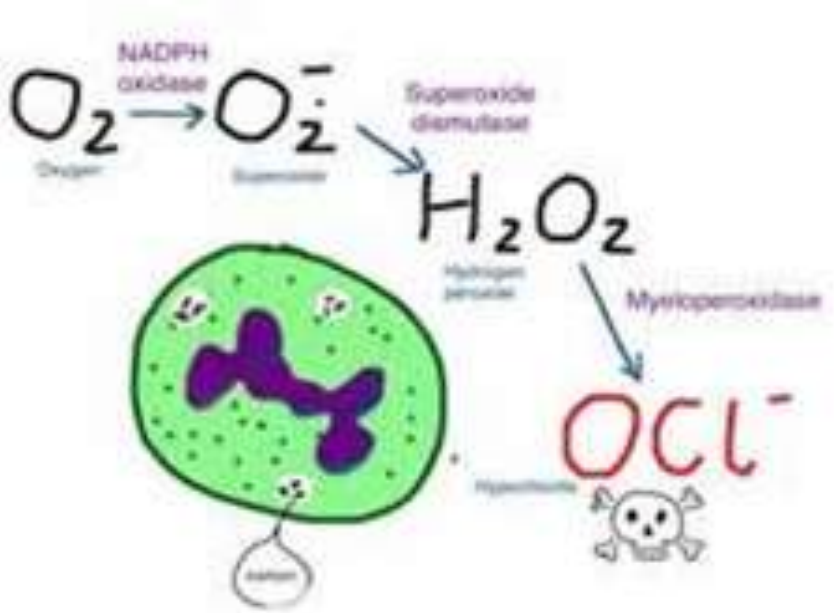
Hyper activated macrophages bosses a high phagocytic activity and they become hyper activated when they receive a **direct signal from an invader**. This signal is usually a lipopolysaccharide or a mannose
When macrophages are hyper activated they stop proliferating. Instead they become large and increase their phagocytic activity
Also they produce TNF (tumor necrosis factor).TNF Kills tumor cells and pathogens and also activates other immune cells.



Phagocytosis involves three steps:
1. recognition of the pathogen via inflammasomes or toll like receptors, both are pattern recognition receptors
2. engulfment, vis pseudopods from the plasma membrane which form the phagosome
3. fusion of the phagosome with a lysosome to form phagolysosome where killing occurs.

Killing of pathogens within the phagolysosome is achieved through
1. Lysosomal enzymes: proteases, lipases, elastase.. etc
2. Oxygen free radicals
3. Nitrogen free radicals

Oxygen free radicals are produced within the lysosome: more details in the next lecture (CGD case presentation)



NEUTROPHILS
Neutrophils originate from bone marrow precursors. They circulate in the blood where they have a life span of 5 days.
Neutrophils are phagocytic but they do not act as antigen presenting cells.
Neutrophils are present in the blood, not like macrophages which are found in the tissues. So when they are needed to fight infections, signals **from macrophages recruit them to the site of injury.**

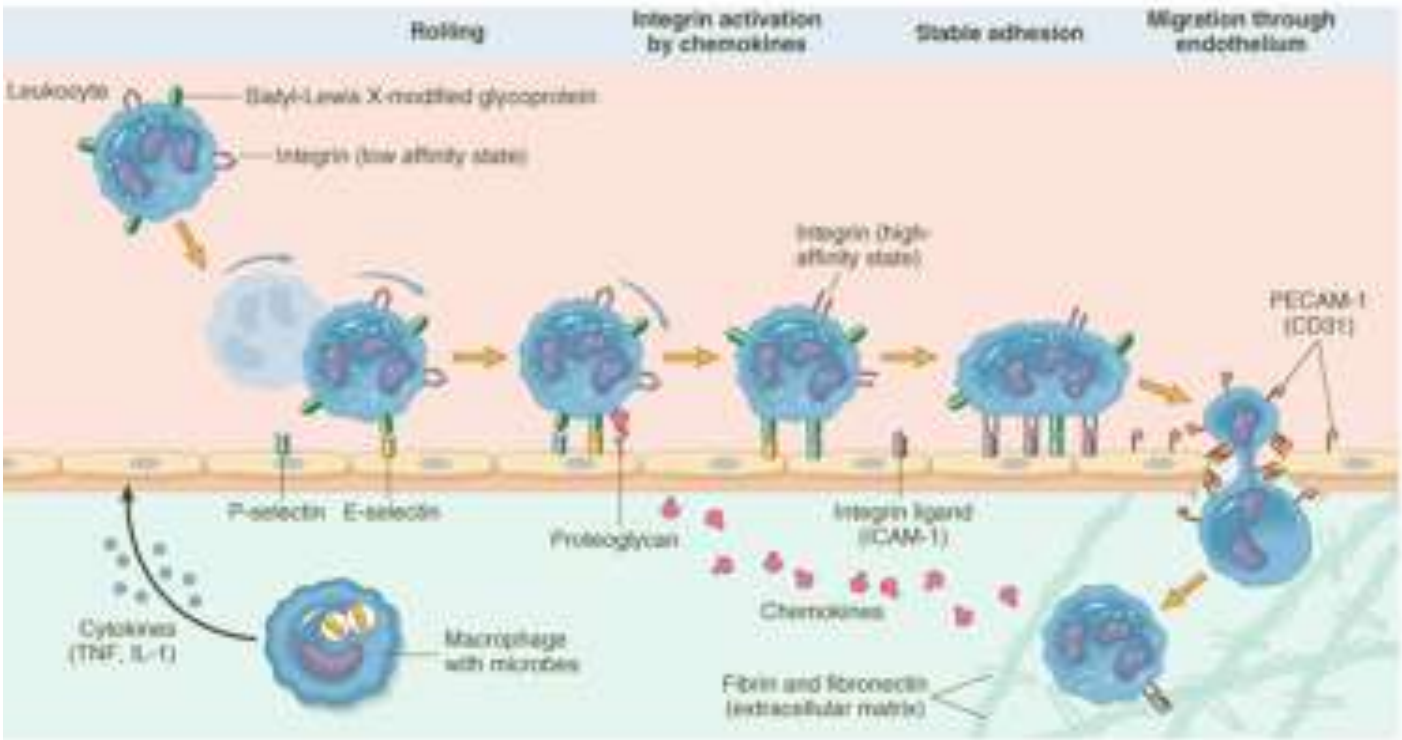
Neutrophil recruitment happens in a multistep process:
1. Margination: neutrophils move to the periphery of the blood vessels due to stasis.
2. Rolling: transient adhesion of neutrophils to epithelial lining of the endothelial cells via selectins and selectin ligands. Rolling slows neutrophils.
3. Firm adhesion: of neutrophils to endothelial cells via integrins and their ligands.
4. Transmigration through the vessel wall via PECAM and with the use of collagenase .
5. Chemotaxis towards the pathogen. This needs chemotactic agents, like IL 8, C3b,

NOTE: chemotactic agents attract neutrophils to the pathogen but are also needed for upregulation of adhesion molecules and their ligands (selectins, intergins and PECAM).cytokines also increase expression of the adhesion molecules.

Selectins: molecules present on WBCs and endothelial cells.
E selectin… endothelium
P selectin…. Platelets and endothelium
L selectin… leukocytes
Selectins bind oligosaccharides. Endothelial selectins present at low levels or not at all on resting endothelial cells.
Cytokines.. Increase expression of selectins. This overexpression occurs locally at the site of injury.

Integrin family of adhesion molecules.:resent on leukocytes and have ligands on endothelial cells.. Integrins are expressed normally on plasma membrane on a low affinity form….
Activation by **chemokines** changes the affinity. Also conformational change and clustering of integrins changes the affinity.

Transmigration: Leukocytes migrate through vessel walls by squeezing through junctions between endothelial cells. This process is called **<u>Diapedesis.</u>**



HOW DO PHAGOCYTES RECOGNIZE THE PATHOGENS??

Phagocytes recognize microbes by: **Pattern recognition receptors**, two types of these receptors exist:

1. Pathogen associated molecular patterns = toll like receptors
2. Damage associated molecular patterns = inflammasomes

Toll like receptors are Microbial sensors. There are 10 mammalian types which are located on plasma membrane and endosomes.
So: can recognize extracellular and ingested microbes
Can recognize bacterial products : endotoxins, lipopolysaccharides ,DNA, viral RNA.
They recognize a pattern: e:g liposaccharides in general, not a specific type, DNA chains not specific sequences

Inflammasomes are Multi-protein cytoplasmic complexes which recognize products of dead cells… uric acid, extracellular ATP, crystals, some microbes..
When stimulated, inflammasome activates caspase 1 which cleaves and thus activates IL- 1 which is a potent mediator.

INTERFERONS and their role in fighting viruses
Note that if **pattern recognition receptors detect virus**.. Interferon ***alpha and beta*** are produced.. **These are called Class I interferons**
Macrophages can produce interferon alpha and beta. However, **Plasmacytoid dendritic cells** are the main producers of interferon
Receptors for interferon are present on most cells. They can turn on genes to1. prevent viral replication and for 2. apoptosis if infected

NO WE DIDN'T FINISH……. SEE NEXT!!!!!!!!

NATURAL KILLER (NK) CELLS

The third component of the innate immune system is natural killer cells**.** These are lymphocytes that originate and mature in the bone marrow. Their Half-life is around one week and they are found mainly in blood ,spleen and liver

They can produce interferons and other cytokines

<u>They are activated by: interferon alpha and beta+ lipopolysaccharide</u>

NK kill cells by apoptosis.. Mainly through Fas –fas ligand or granzymes.

NK RECEPTORS

Nk cells are lymphocytes, but they do not express B nor T cell receptors. They have 2 types of receptors : **activating and inhibitory receptors**

1. **Inhibitory receptors** (killer inhibitory receptors = KIR) . These recognize MHC 1 on cells. Once these receptors are engaged they will activate *phosphatases PTP* which will *inhibit killing via dephosphorylation*.

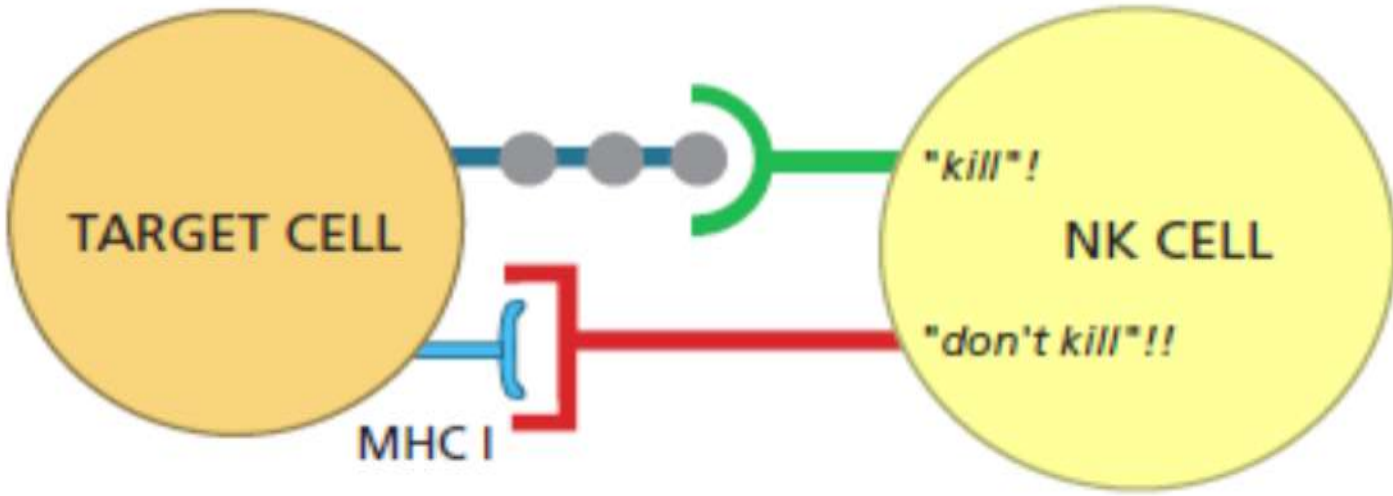
Within the cytoplasmic portion of the receptor there are amino acid sites that bind tyrosine kinase and are inhibited by dephosphorylation. These amino acid sites are called ITIMS =(immunoreceptor-based tyrosine inhibiting motif). Which simply means that once engaged these receptors have regions (motifs) that are inhibited by tyrosine kinase binding.

2. **Activating receptor**: binds to altered protein on the target cell (viral infection or cancer). Once NK cells recognize their target they send signals which will activate protein tyrosine kinase PTK and kill the cell. *<u>These contain ITAMS</u>* = immunoreceptor based tyrosine activating motifs.

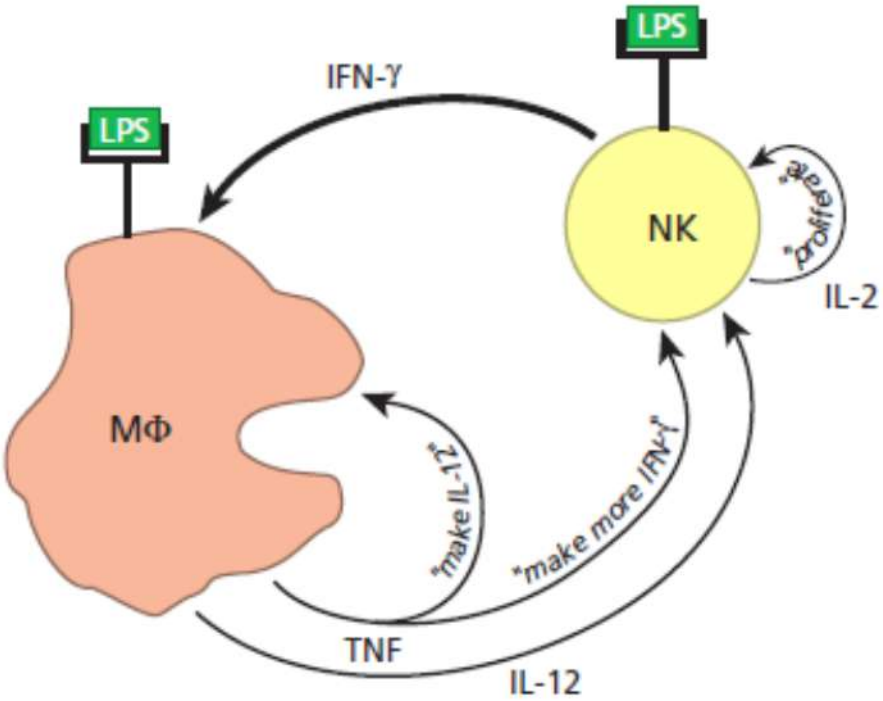
<u>NOTE THAT the balance between activating and inhibitory signals determines if the NK will kill the target cell or not.</u>

DO not be confused, the above is not complicated, it follows the usual theme of how receptors work: ligand binds to a receptor, the receptor is thus activated, second messengers activate cytoplasmic portions of the receptors (motifs), this stimulation of the receptor action needs kinases.. that's all.. but remember activating the receptor in NK depends on its role; which means if the receptor is activating receptor then activation of it will result in killing, whereas if the receptor is inhibitory then activating the receptor means inhibition of killing! I hope this isn't confusing!!!!!!!!!!!!!!

NOTE:
Activating ligands of the activating receptors include CD16, CD56
We use this in flow cytometry in recurrent abortions, we look for CD16
and CD56 that will make NK cells kill the fetus in some forms of recurrent abortion!!



Cooperation between the components of the innate immune system:
You can realize from the previous discussion that there is cross- talking between the components of the innate immune system which include:
1. Neutrophils recruited to site of injury by macrophages
2. Interferon gamma produced from NK primes macrophages
3. Macrophages produce TNF and IL 12.. That cause NK activation.. The activated NK produce. Interferon gamma .. This in turn stimulates macrophages.
4. Phagocyte and complement cooperate through opsonisation
5. Activated macrophages produce complement protein
6. Complement proteins can act as chemotactic agents to neurtrophils


LPS
IFN-γ
LPS
NK
"proliferate"
IL-2
MΦ
"make IL-12"
"make more IFN-γ"
TNF
IL-12


Thank you!