PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SIIDE

Lecture Number: 8

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Inflammation lecture 3

RECAP

In the previous lecture, we started discussing the mechanisms of inflammation, and we said whatever happens during inflammation can be categorized under the 5 "R"s

1- Recognition

2- Recruitment

3- Removal (of agent)

4- Regulation

5- Resolution/Repair

We discussed the first 2 and in this lecture we will discuss the other 3.

Recognition is done by 3 types of receptors: toll-like, sensor damage, FC receptors.

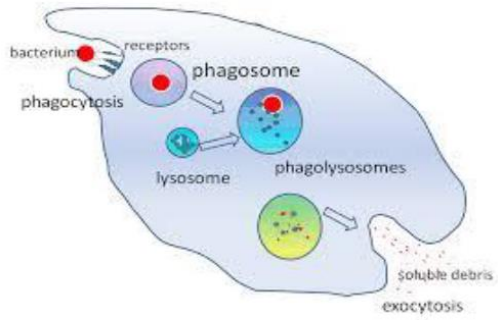
Recruitment: recruiting (bringing) white blood cells to the site of inflammation. Inflammation consists of 5 processes; *margination, rolling, adhesion, transmigration, and chemotaxis*.

Removal of the insult:

How is it done?

- By a process called phagocytosis that takes place by macrophages and neutrophils.

Written by Aseil Al Khatib



In order for phagocytosis to take place we need 3 steps must take place:

1- Recognition and attachment (by receptors)

2- Engulfment

3- Killing or degradation

Recognition and attachment:

It needs receptors. We have many types of receptors found on macrophages and neutrophils.

1- **Mannose-receptor;** mannose is a sugar found mainly in glycoproteins and glycolipids of the bacterial membrane, the "glyco" part of the glycolipid or glycoprotein is always mannose, so it is the part that can be recognized by the phagocytic cell receptors, however in our normal body cells the sugar is another type that's why the phagocyte only kills bacterial cells and not human cells.

- Mannose-receptors are **specific** for microorganisms **containing mannose or fucose** on their cell membrane.

REMEMBER THAT: Not all inflammations are caused by an infection.

2- **Scavenger receptors**: they recognize modified LDLs, LDLs are modified by either oxidation or acylation (adding oxygen or an acyl group to it).

3- **Receptor for opsonins**: opsonins are *proteins which coat bacteria/microorganisms*; the most well known opsonins are IgG, c3b and lectins. These receptors do not

recognize bacteria directly; they recognize them indirectly by first identifying these opsonins.

NOTE: the cell-receptors we discussed in the previous lecture are present in ALL CELLS, they initiate the process of inflammation and are part of the innate immune system, their main role is to alert the cell there is an invader, while the cell-receptors being discussed now are only found in microphages and neutrophils, they are highly specific forming the adaptive immune system, and they initiate the process of phagocytosis.

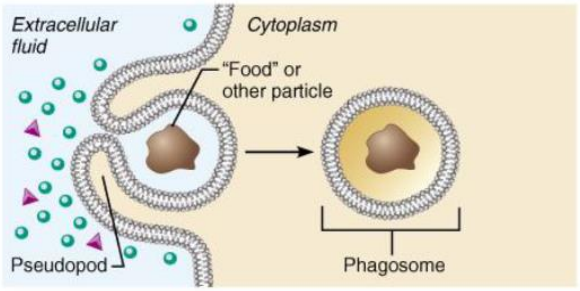
Engulfment

After the microphage/neutrophil recognizes the causative agent, it needs to engulf it, this is done by surrounding the agent and forming a phagosome, a phagosome is simply a part of the membrane surrounding the bacteria/pathogen and separates it from the rest of the cell (it's a vacuole containing the pathogen inside the microphage/neutrophil separating it from the rest of the cell).

Why do we need to separate it from the rest of the cell?

- Because we would like to kill it *in isolation* as the powerful enzymes secreted may act on the rest of the cell and kill it also.

After the pathogen is in the phagosome, the vacuole will fuse with a lysosome (an organelle containing enzymes for killing) forming a **phagolysosome**, the enzymes are now in contact with the pathogen and are able to kill it.



Second step is now done

You can see in the picture above there are some structural changes taking place in the plasma membrane in order to surround the pathogen; this action needs actin filaments activation as this process needs cell movement and separation from the rest of the membrane.

The 3rd step is killing; this is done by 3 substances:

1. *Lysosomal enzymes.*
2. *Oxygen radicals.*
3. *Nitrogen radicals.*

1. The most common way is **lysosomal enzymes**; those are found in macrophages and neutrophils.

Neutrophils contain 2 types of granules that have lysosomal enzymes; there are small granules and big ones.

- The small ones are specific and secondary, the 3 "S"s: **Small, Secondary, and Specific.** E.g. collagenase, gelatinase, histaminases.
- The big ones are **primary large azurphil granules** containing other types of lysosomal enzymes like myloperoxidase and bacteriocidal factors

* You don't need to memorize which secrets which. Just know that neutrophils have 2 different types of granule-containing lysosomal enzymes. *

Lysosomal enzymes mainly work by degrading proteins, we have 2 types of proteases: **Neutral and Acidic.**

Neutral proteases can cause collateral damage; our components are neutral hence they can be affected by the neutral proteases making those proteases more dangerous for our body than the acidic ones.

- ✓ Macrophages also have lysosomal enzymes like collagenase, elastase, hydrolases, and phospholipase.

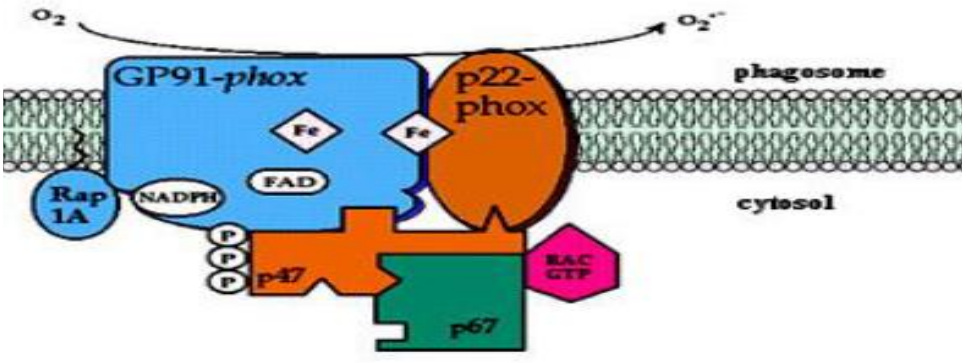
Because neutral proteases can be harmful to us, we have **anti-proteases,** the most well known one is **alpha 1 anti-Trypsin**, it protects us from damage. If someone has alpha 1 anti-Trypsin deficiency, he will develop emphysema and won't be able to fight the damage caused by the protease.

2. **Reactive Oxygen species**, how are they formed?

- ✓ We have an oxidase enzyme in microphages which changes oxygen to oxygen superoxide (a highly potent killer for both pathogens and our cells).

NADPH-Oxidase is formed of at least 7 units, they are not fully assembled in normal body conditions, some units are found in the plasma membrane of the phagosome and some are found in the cytoplasm of the cell, when they are unassembled they are not harmful, so as long as they are separated nothing happens, - oxidase is not functional - however when there is a stimulation by cytokines, cytoplasmic components assemble itself and move towards the membranous components of the phagolysosome forming a functional oxidase enzyme.

- Phagosome is a part of the membrane, this part of the membrane contains some of the components of the oxidase (the cytoplasm of the phagosome contains part of components of oxidase). As long as they are separated nothing happens (no function of the oxidase). Keep in mind the enzymatic components are found the plasma membrane binding the phagosome only and not in the whole cellular plasma membrane, since this enzyme can harm our body cells too, so we need to restrict the exposure of the enzyme to the phagosome only. Both components (cytoplasmic components and membranous components of the oxidase) come together and the enzyme becomes activated.



To put it in simpler words, imagine the cell is a room, the walls are the plasma membrane, one of the walls is red, it is in contact with a closet (the phagosome) this wall has 4 bricks, it needs 3 more bricks for the wall to be functional (in other words 3ashan yseer manzaro mnee7) those 3 bricks are present in the room

(cytoplasm) when we place those 3 bricks next to the 4 bricks on the red wall, it is now functional, and the enzyme can act on the phagosome (the closet in contact with the red wall). If all of the room had red walls (if all of the cellular plasma membrane had enzymatic components of the oxide) then the whole cell would assemble the oxide enzyme, we do not want that as the enzyme would harm the organelles.

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Note: the doctor mentioned that the enzyme is composed of 7 units and it's also written in the slides that it has 7 units, but in the figure above the enzyme has 6 units and Wikipedia states that the enzyme has 6 units. We will ask the doctor about it.

Superoxide decays quickly forming hydrogen peroxide (H_2O_2), which is not as potent as superoxide, therefore our cells have another enzyme to convert hydrogen peroxide to hypochlorite OCl^{-2}, this enzyme is called **myeloperoxidase.**

- ✓ Hypochlorite is bleach, we use it in our homes for cleaning, and it is a potent killer for microbes.

All these enzymes are present in the phagolysosome.

Extra note "uric acid crystals in the phagolysosome burst it, this results in exposure of enzymes to the whole phagocyte, thus killing it. This is one of the mechanisms of what happens in chronic inflammations".

3.**Nitrogen radicals**

The first step is the production of Nitrogen Oxide. It's produced in our bodies in 3 places; endothelial cells, neurons and wherever there is inflammation; each of these is produced by a different enzyme for a different purpose.

- In endothelial cells we have nitrogen oxide synthase, known as eNOS, it synthesizes nitrogen oxide causing relaxation and vasodilation.

- In neurons, we have nNOS, it secretes nitrogen oxide which acts as a neurotransmitter.
- In inflammation, we have **inducible nitrogen oxide synthase** (it does not act unless there's an inflammation, "i" stands for inducible not for inflammation, it's induced when there's an inflammation). It forms nitrogen oxide which reacts with superoxide, and forms ONOO- (peroxynitrite) this is the potent killer.

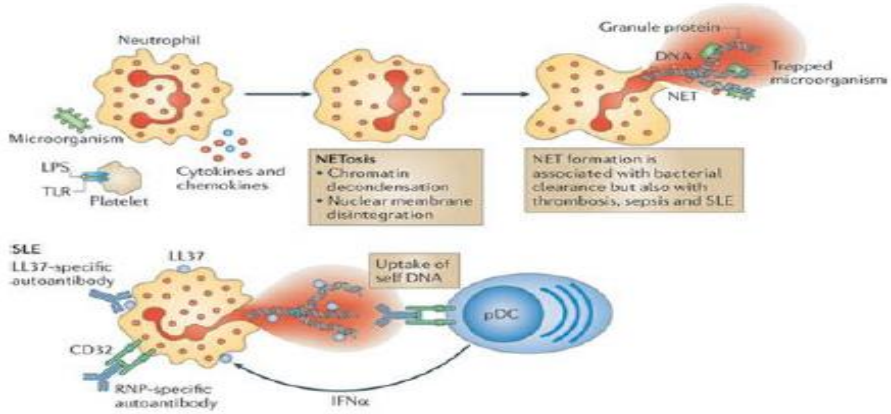
*__Superoxide__ is very important; it's a potent killer, it's converted into H_2O_2 which will be converted into another potent killer (hypochlorite), and with the presence of nitrogen oxide it's also converted into a new killer (peroxynitrite).

We have another mechanism used for killing called NET (**neutrophil extracellular traps**)

The neutrophils form a net to trap the microorganisms, how does this happen?

- ✓ The neutrophils are activated by chemical agents (cytokines mainly or by the microorganisms directly) causing condensation of chromatin in the nucleus and disintegration of the membrane, allowing the chromatin and contents to leave from loads of cells <u>forming a network of fibrillar material.</u>

<u>In conclusion: it is a net with toxins as lysosomal enzymes also leave when the neutrophil disintegrates, so the fibrillar net traps the bacteria and the leaked components kill the bacteria.</u>



Regulation:

If we're talking about chronic inflammation we stop here. There's no regulation in chronic inflammation.

Regulation is only present in acute inflammation

Why do we need it?

- ✓ Inflammation causes damage to our cells, so we need to regulate it and control the killing of cells.

We have more than one regulatory mechanism:

- ➢ *half life of leukocytes*, leukocytes when it's in the blood, it has a life span, when it goes outside the blood to the tissues its life span is shortened, it can be only hours, it cannot work more than several hours and this stops the process.
- ➢ *mediators (chemical agents)* are short lived, they are secreted only when needed and decay quickly, they can also be degraded by enzymes, this also stops the process at certain points.

The previous 2 mechanisms are considered passive mechanisms, something is working then it stops after a certain point.

Active mechanisms (telling the cell to stop the inflammatory reaction)

- ➢ **Switching of arachidonic acid metabolites**; arachidonic acid is one of the important mediators of inflammation which will be discussed in further detail next lecture, it forms 2 types of pathways, one which causes inflammatory mediators, and the other which causes anti-inflammatory mediators, in the beginning of inflammation arachidonic acid forms the inflammatory mediators, but with time it switches to forming anti-inflammatory mediators to stop the inflammation.
- ➢ **Anti-inflammatory cytokines,** when there are too many inflammatory mediators those are secreted to slow the process down, those include

transforming growth factor beta TGF-B and **interleukin 10,** interleukin 10 differs from all other interleukins as it is an anti-inflammatory chemical.

It was experimentally proven that there are some neural impulses which prevent NETs formation, again slowing the process of inflammation.

Reminder: inflammation is a protective mechanism, however it can cause damage to our cells and that's why we need regulatory mechanisms to stop the process when it needs to be stopped, this includes several mechanisms and we have mentioned them before.

** if the body can get rid of the causative agent we will move to step 4 (regulation) and this would be *an acute* inflammation and all the other steps will continue normally, however in *chronic* inflammation we never reach step 4, as we cannot get rid of the causative agents, we stop at step 3.

Resolution and repair is the last step, it is a very big lecture and will take us 4 lectures to fully cover it, so we will not discuss it now.

Vascular changes taking place locally during inflammation

2 main vascular changes happen during inflammation: *vasodilation and increased permeability.*

Vasodilation happens in response to mediators (mainly histamine); it occurs early in inflammation, its main aim is to increase blood flow.

Which blood vessels are affected the most and why?

- ✓ Arterioles and arteries since they have a muscular wall, *arteriols are the first to dilate*; the smooth muscle relaxes in the wall of the arteriols causing an increased blood flow (this *results in the hotness and the redness of the skin).*

So vasodilatation occurs firstly in inflammation, its aim is to increase blood flow to the area with influx of cells and mediators, arterioles are the first to be affected, it causes hotness and redness.

The other change is the **increased permeability** which happens in capillary and postcapillary venules, it causes the escape of protein rich fluid to the extracellular space, this is not a selective process, in other words everything can leave e.g. fluids, proteins, cell debris, etc…

What is edema?

- ✓ Presence of extra-cellular fluid

There are 2 types of edema

Exudate and transudate

What is the difference between them? ** This is a very important question **

Exudate happens when there is an increased permeability due to the destruction of the epithelium, **thus everything leaves** (including proteins and cells –cell debris-). This process has a high specific gravity.

It mainly occurs in inflammation

Whereas in transudate edema there is an increase in the pressure of venules and capillaries, whether oncotic or hydrostatic, this allows **only water to leave** with very little protein and no (or little) cell debris. This process has a low specific gravity due to not having any or very little proteins in the extracellular fluid.

What is specific gravity?

- ✓ A ratio of the density of a substance (protein for example) relative to the mass of another substance (water and proteins for example). It's a ratio for the density between a fluid and water, if the fluid has a lot of proteins and cell debris, it will be denser than water, exudates is denser than water so it has high specific gravity, transudate has low specific gravity because it does not contain proteins or cell debris.

These 2 vascular changes cause **stasis**: slowing down of blood.

Why does blood flow slow down?

- ✓ As proteins and fluids leave, we are left with viscous (heavy) red blood cells, thus the movement would be slower than normal.

Stasis was important for migration and recruitment

How does permeability increase?

- ➢ Either the gaps between endothelial cells widen causing efflux of fluids (influx to the extracellular). This process is transient and short-lived but happens quickly.
- ➢ Or by destruction of endothelial cells, e.g. in burns or injury, when they're destroyed there's nothing to stop things and fluids from going out so by these two processes permeability increases.

It's the things we love the most, that destroy us – Mockingjay

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