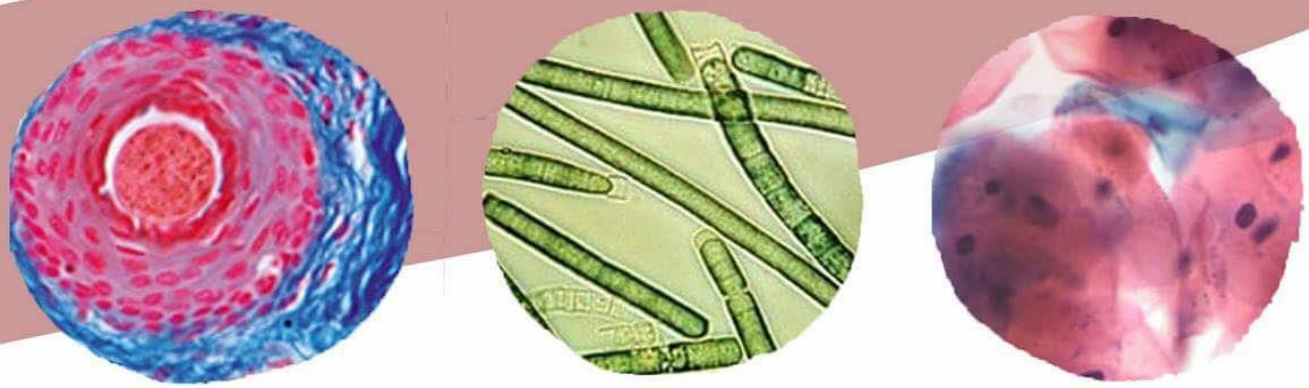
INTRODUCTION TO PATHOLOGY



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Sheet# 8

There are three things that play a role in inflammation:

1. Chemical mediators.
2. Host / inflammatory cells (host means that they are from your own body).
3. Blood vessels.

In the previous lecture we discussed the vascular changes that happen when the cells recognise an injury, in this lecture we intend to clarify the cellular changes that happen.

Inflammatory cells are considered the soldiers of the whole inflammation process but most of them are present within blood vessels whereas the majority of inflammatory reactions happen outside these vessels (in the interstitium) so in order for them to actually carry out the process they have to reach the sites of infection.

We already know that the vascular changes increased the number of WBCs but how do they get to the infected tissue?

✓ Leukocyte recruitment

The process of driving the WBCs towards the infection site which is carried out by a group of steps:

1. Margination
2. Rolling
3. Firm adhesion
4. Transmigration
5. Chemotaxis

I don't care how lame this might sound as long as it does the job and helps you memorize.
Using the doctor's example:

Let's consider that you represent the WBCs and the cafeteria represents the site of infection, if you want to leave the class room and go to the cafeteria first of all you will move towards the (Margin) of the room towards the door and when you reach it you will slow down (Rolling) then you will hold the door knob (firmly) after that you will (Transmigrate) towards the cafeteria driven by (Chemotaxis).

1- Margination.

WBCs have to move towards the margins of the blood vessels or they will never be able to reach the tissues (sites of infection) to be more specific they have to be close to the endothelial cells.

How can the WBCs move towards the margins? What causes Margination?

WHAT → Due to the vascular changes (vasodilation occurs) permeability increases so a protein- rich fluid moves into the extravascular tissues this causes the increase in the number of RBCs inside the blood vessels which increases the viscosity of the blood, thus slowing the circulation and that results in the development of Stasis.

HOW → since the vessels are packed with RBCs, WBCs are heavier so they move towards the margins.

Keep in mind that Margination is a **purely physical process** related to stasis **not** chemical mediators.

2- Rolling.

- The process through which WBCs **adhere loosely** to the endothelial cells at the margins.

  After margination happens the WBC keeps moving in order for it to leave the vessels it has to slow down its motion so it loosely adheres to the endothelial cells (reversible adherence) thus resulting in the **Rolling** of the WBCs across the endothelial lining, the aim of this motion is to decrease the speed of the WBCs so that when it reaches a specific point it can stop and actually leave the blood vessel.

  This loose adherence causes friction which helps the WBCs lose some of their kinetic movement gradually.

- Rolling is caused by **chemical mediators**.

  Adhesion molecules present on both WBCs and endothelial cells; they have ligands on the endothelial cells so they complement each other forming a loose adhesion.

  Selectins: adhesion molecules that cause the loose adhesion between the endothelial cells and the WBCs.

  Naming: lectins are sugars, this family has sugars on WBCs which are complemented by other sugars on the endothelial cells.
  - **E**-selectin: **E**ndothelium
  - **P**-selectin: **P**latelets and endothelium
  - **L**-selectin: **L**eukocytes

Why don't our WBCs stay loosely bound to the endothelial cells since selectins are always present, even without the presence of an infection?

The main reason is that there is no margination because the blood flow is normally fast, it is also caused by low affinity of selectins and some of them need to be synthesized or secreted from granules during inflammation, for example P-selectin is found primarily in intracellular Weibel-Palade bodies and they leave these vesicle-like structures after being stored in the cytoplasm and are expressed on the surface only during inflammation, and this helps regulate the amount of WBCs in our blood flow.

For your information:

Weibel-Palade bodies: are vacuoles inside the cytoplasm which contain many molecules most importantly p-selectin and other molecules which are important for platelet aggregation thus they are significant for homeostasis.

To sum up, Cytokines (a signal of inflammation) ... Increase expression of selectins making them undergo certain conformational changes to <u>increase their affinity</u> such as changing their shape or certain receptors polymerize together or they <u>activate them</u> by either synthesizing or secreting them from granules.

- Selectins help localize the inflammation at the site of the infection only.

3- Firm adhesion.

- Happens between WBCs and the endothelial cells by a family of adhesion molecules called **integrins**.

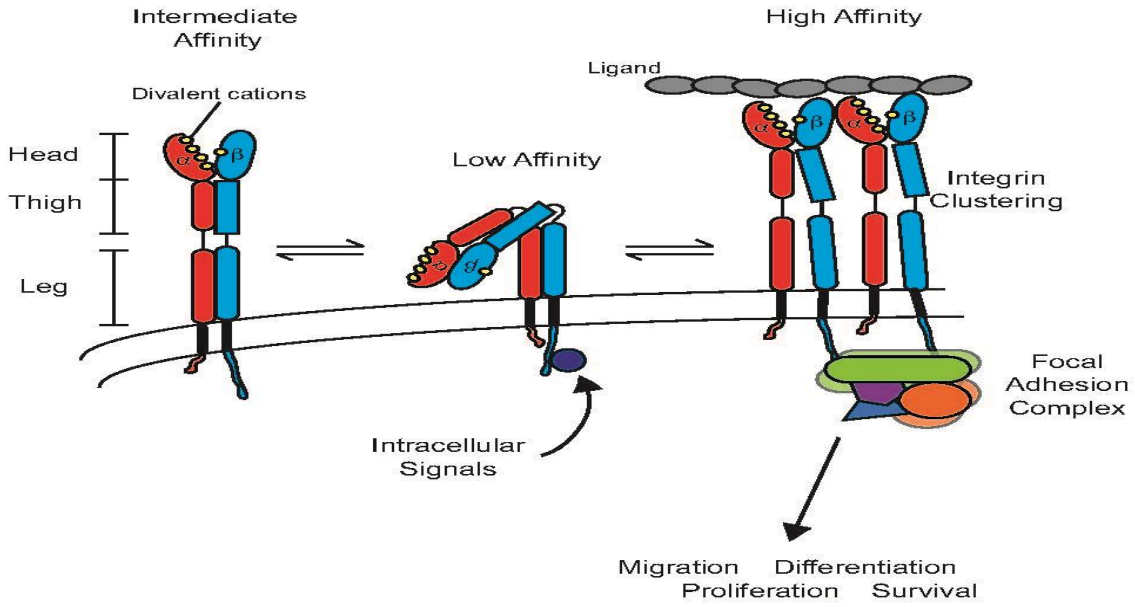
<u>Integrin on the WBCs and ligands on the endothelium and vice versa</u>

- IL- 1 and TNF activate endothelial cells to express integrin ligands.

And these molecules don't keep the WBCs and the endothelium cells firmly adhered for the same reasons as selectins (explained above)

When the integrin receives a signal (for example: cytokine) it acquires a higher affinity state by either undergoing a conformational change **and/or** polymerization.

Check this figure for better understanding of the activation of integrins.



4- Transmigration.

- The process of which the WBC leaves the blood vessel and enters the tissue.
- This occurs in the venules of systemic circulation and capillaries of pulmonary circulation.
- Transmigration is stimulated by **chemokines**.

The WBCs have come to a stop now, so they can leave the blood vessels but during that they have to penetrate two barriers which are: endothelial cells and the basement membrane.

How do they cross the endothelium cells?

By squeezing through the Gap junctions because of the Increased permeability due to the contraction of the endothelial cells caused by vascular changes, (discussed in the previous lecture), but the increased permeability isn't enough so certain adhering molecules are used,

PECAM 1 protein (aka CD31 used as an endothelial marker**)** which binds to WBCs and helps them leave the vessels through the junctions.

How do they cross the basement membrane?
WBCs secrete certain enzymes (for example **collagenase**, which is secreted from certain granules (e.g in neutrophils), degrades the collagen that exists in the basement membrane so it eats up a part of the membrane in order for the cells to leave).

How do WBCs move?

Diapedesis by forming pseudopods, WBC forms a pseudopod (leg like structure) that anchors to the interstitium and then the rest of the cell moves toward the pseudopod.

5- Chemotaxis.

- The WBCs move in a directional movement toward the injurious agent driven by certain chemicals.

MEMORIZE!!

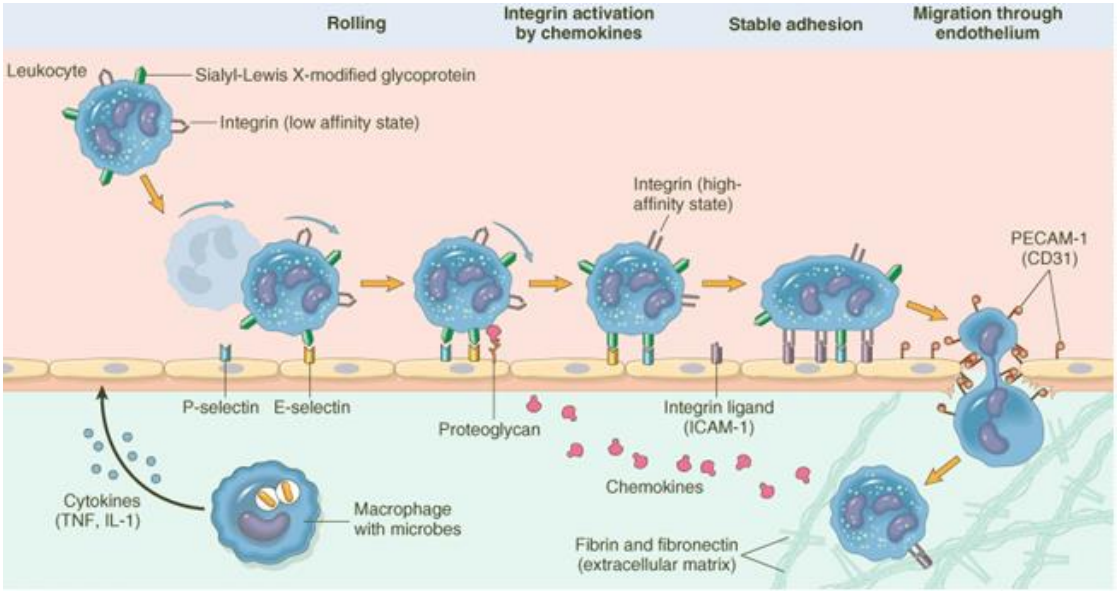
a) Bacterial products.

b) Cytokines, especially chemokines (inter leuken-8).

c) Complement components:C5a

d) Products of arachidonic acid: leukotriene B4

<u>Overview of leukocyte recruitment</u>:



- ✓ **Leukocyte activation.**
  now that the WBCs have reached the site of inflammation they are activated to get rid of the infectious agent by:

1) Phagocytosis
2) Intracellular destruction of phagocytosed microbes and dead cells.
3) Production of mediators.
4) Liberation of substances that kill extracellular microbes and dead tissues.

1- Phagocytosis:

- Macrophages are the main cells that can phagocytose also neutrophils can do so.

Phagocytosis happens in three steps:

1- **Recognition**, the macrophage or the neutrophil recognises receptors that detect microbial components, necrotic cells and host proteins = opsonins (they coat microbes which can't be recognized by WBCs to make them recognizable)

- ➢ These receptors trigger phagocytosis and they differ from the receptors that help recognise the infectious agent (TLR, inflammasomes)

2- **Engulfment** (Ingestion or absorption), the cell forms a pseudopod from its membrane leading to the formation of a vesicle around the infectious agent and then it enters the cell after that the vesicle fuses with a lysosome.

3- **Killing** and degradation, it happens due to:

- lysosomal enzymes such as Acid hydrolases, most importantly by elastase.
- Reactive nitrogen species (will be discussed later).
- Oxygen free radicals, which are unstable and highly reactive so they can kill cellular components thus killing the infectious agent. They occur inside the lysosome and in a **stepwise fashion** thus causing the least tissue damage.

- **Steps of forming these radicals:**

1. Oxygen is converted to oxygen superoxide by NADPH oxidase.
2. Oxygen superoxide is converted to hydrogen peroxide by superoxide dismutase.
3. Myeloperoxidase converts H2O2 to OCL^{--} which is extremely toxic.

The strength and toxicity of the three underlined molecules increases respectively.

This NADPH oxidase (aka phagosome oxidase) normally is inactive since it exists in the lysosome as 2 parts it's made up of 6-7 subunits of proteins some of them exist in the cytoplasm while the others are in the inner part of the lysosomal membrane, chemical mediators activate the assembly of the subunits during inflammation so the cytoplasmic subunits join the ones in the membrane.

Keep in mind that the predominant cells in acute inflammation are neutrophils although macrophages come in later and in chronic inflammation they are macrophages but all other cells can play a role even the neutrophils.

Certain inflammations caused by certain causes differ in the type of cells that first react.

- ✓ In viral infections lymphocytes are the first to react.
- ✓ Eosinophils in allergic reactions even if they were acute.

What determines which type of cells reacts to a specific infection?

- According to the cause which triggers specific mediators.

Check doctor Manar's slides for details that weren't mentioned in the record.

- If you are into medicine to be ordinary, it's not worth it … you have to do it properly.

Pain is TEMPORARY … GLORY is FOREVER.