Doctor 021

PATHOLOGY

Sheet no. 3

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TRANSMIGRATION:

An active process where the neutrophils or macrophages move outside the circulation, due to the CD31 role utilizing collagenase, destroying the basement membrane, and helping cells to reach the site of injury.

CD 31 (PECAM-1), platelet endothelial cell adhesion molecule expressed both on leukocytes and endothelial cells.

CD 31: also known as cluster of differentiation 31

WBC pierce through wall by collagenases.

CHEMOTAXIS:

The whole movement of WBCs from blood to the site of injury. And it occurs after extravasating from the blood. It is considered as an active process, and is induced by a certain group of mediators called, **Chemoattractants**.

Chemoattractants: strong mediators that induce and stimulate the movement of leukocytes, and they can be either: exogenous (from outside the body) or endogenous (produced from inside the body) , including the following:

Exogenous	**Bacterial products** -**Particularly** Peptides **of N-... termini**
Endogenous	**Cytokines:** a big family of chemical mediators produced mainly by lymphocytes and inflammatory cells like macrophages. -especially those of **Chemokine family**, which is the strongest family
	Complement system (part of plasma proteins) components -particularly **C5a**, which is the strongest → active .
	Products of Lipoxygenase-pathway of arachidonic acid (AA) -particularly leukotriene B4 (**LTB4**), which is the strongest product of AA metabolites.

- C5a: Complement component 5a

WBCs infiltrate in tissue:

The movement of WBCs from the intravascular to the tissue, Depends on the age of inflammatory response and the type of stimulus as shown in the table below:

Neutrophils (PMNs)	6-24 hours **acute inf.**
Macrophages, Lymphocytes and Plasma cells	24-48 hours and may stay longer
Eosinophils	Allergic Reactions

They are the HALLMARK of Acute inflammation
If we see neutrophils, this indicates a **recent Acute inflammation**.
Neutrophils are short-lived that they don't stay more than 6-24 hours.

They are chronic inflammatory cells
They come later on, **after** neutrophils death by apoptosis, after 24-48 hours of infection, and they could stay for couple of days or weeks. And in specific conditions -like viral infections- Macrophages, Lymphocytes and plasma cells may be the first cells to arrive instead of neutrophils.

Eosinophils are seen in specific conditions, which are considered as allergic/hypersensitivity reactions.

These cases are mentioned by the doctor in the lecture enjoy reading them:

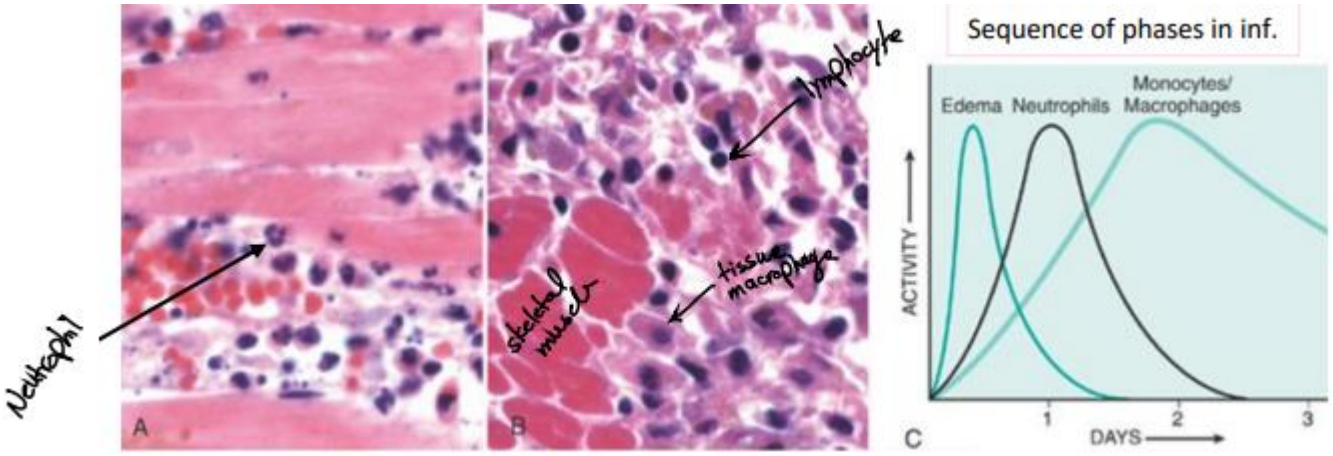
1- A 15 year old girl came to the clinic suffering from pain around the umbilicus, then it radiated to right iliac fossa. She was diagnosed with acute appendicitis, and she managed by appendectomy.

The sample (appendix) was examined by pathologists, they found neutrophils in it, this proved the diagnosis (acute appendicitis). الدكتورة شطورة

2- A 39 year old man came with spasm and the doctor gave him ibuprofen (مسكن) and let him leave, after 6 hours the patient died ,by investigations pathologists took section from the heart and they found neutrophils which indicates that he suffered from acute myocardial infarction.

الدكتور يلي روّحوا راح فيها

Let's have a small discussion about the Nature of leukocytes infiltrating in Inflammatory Reactions, through those histological photomicrographs of an inflammatory reaction in the myocardium after an ischemic necrosis (infarction):



A. a lot of neutrophils, so this is an acute inflammatory process which indicates that those morphologic changes took place within the last 24 hours, Early (neutrophilic) infiltrates and congested blood vessels.

B. Mononuclear cells infiltration is shown, which indicates that this is the chronic phase of the inflammation, Later (mononuclear) cellular infiltrates.

C. The approximate kinetics of edema and cellular infiltration. For simplicity, edema is shown as an acute transient response, although secondary waves of delayed edema and neutrophil infiltration also can occur.

-Edema: the first phase, the vascular phase, where edema ensues.

-Neutrophils: the second phase, were neutrophils infiltrate the tissue then die

-Monocytes/Macrophages: chronic inflammatory cells peak at day 2 and may remain in the tissue and take longer time to disappear.

Some notes on the diagram:

1. The upper line depends on the intensity.
2. There are some overlaps but that doesn't change the arrangement of the steps: edema, Neutrophils, Monocytes/Macrophages.

please guys don’t be confused with the next image you don’t have to know a lot about it:

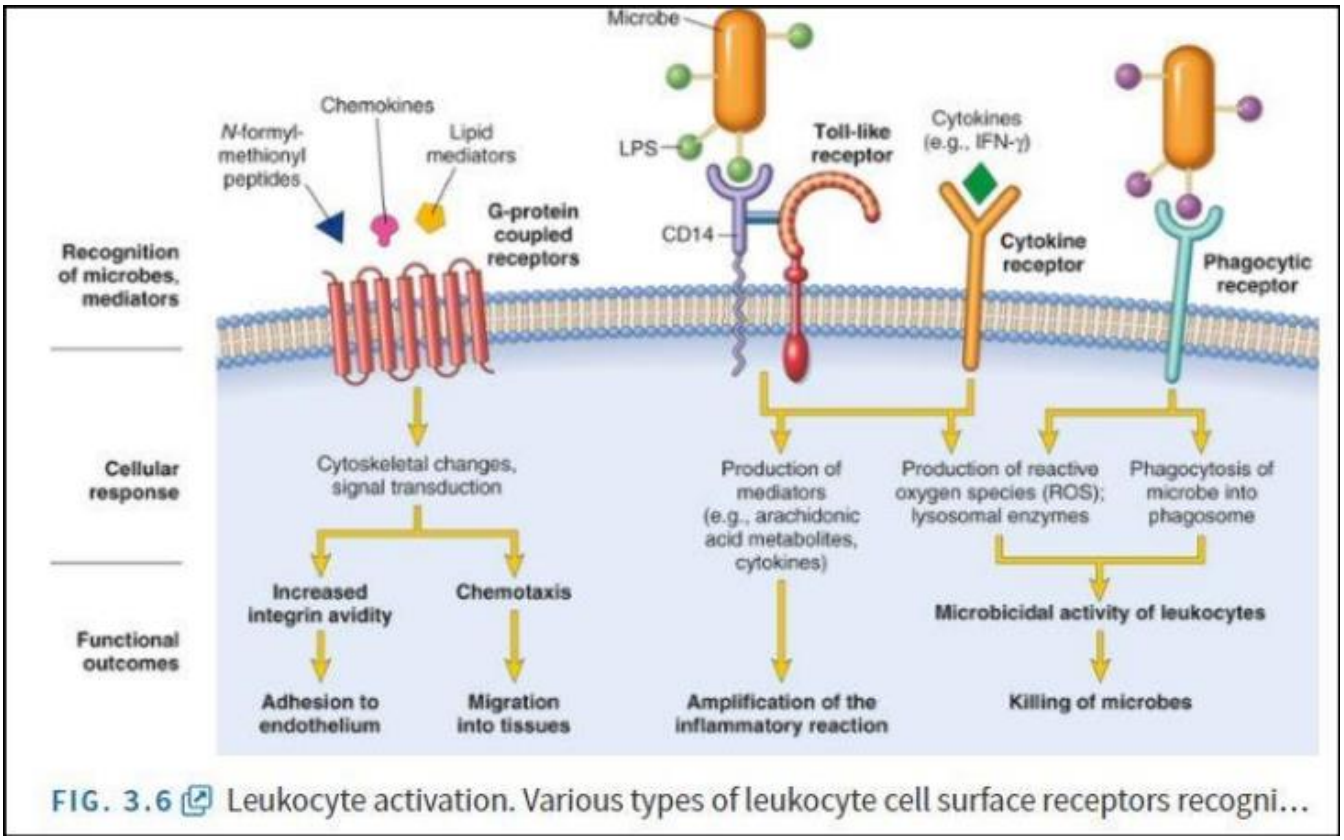


FIG. 3.6 Leukocyte activation. Various types of leukocyte cell surface receptors recogni…

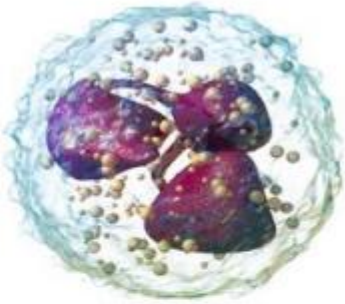
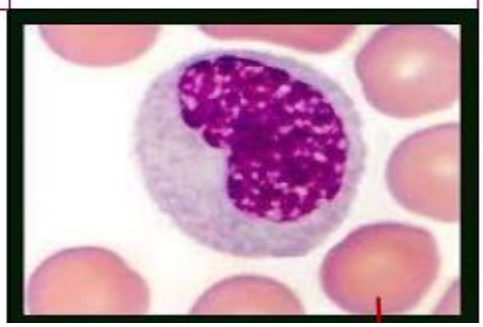
1. Firstly: recognition of offending agents (bacteria,virus,..)
2. Stimulation through certain receptors for example: Toll-like receptor, it has 3 domains (extra cellular domain, intramembranous domain, intracytoplasmic domain).
3. Then the message enters
4. Activation
5. Chemotaxis
6. Killing microbes

LEUKOCYTE ACTIVATION:

Once leukocytes have been recruited to the site of infection or tissue necrosis, they must be activated to perform their functions. The two major cells that contribute in the initial phase of inflammation are:

Neutrophils: abundance cytoplasmic (nuclear cytoplasmic ratio is low), it has multiple nuclei connected to each other by small threads, it has a lot of granules, its size is 12-15 mm(micro meter).

Monocytes (in the circulation), it differentiates when it moves to the tissue (called macrophages) and becomes bigger (the nucleus becomes smaller and the cytoplasm bigger).

Neutrophils	PMN(polymorphonuclear leukocyte) "micky mouse" with 3 nuclei. They have granules containing enzymes → when ruptured → enzymes are released and they digest the internal material (foreign bodies).	
Monocytes	The resident monocyte in the tissue is a macrophage. The monocyte circulating in the blood is not differentiated yet -have a phagocytic effect with a bean or kidney shaped nucleus. And it has less granules than neutrophils.	

So, Leukocyte activation mainly results in:

- Phagocytosis.

- Intracellular killing/destruction of phagocytosed microbes and dead cells by substances produced in phagosomes: NO, ROS.

- Liberation of substances that destroy extracellular microbes and dead tissue such as: NETs.

Remember:

R1: recognition

R2: chemotaxis

R3: removal of the enemy (third step of inflammatory response after chemotaxis)

NOTE: the perfect cell of reference in size is the lymphocytes cells because it has constant size.

RBCs aren't a good reference because if you have microcytic anemia the cell will be small, but if you have spherocytosis the cell will be big.

PHAGOCYTOSIS:

(the cell in this image either neutrophil or macrophage cell).

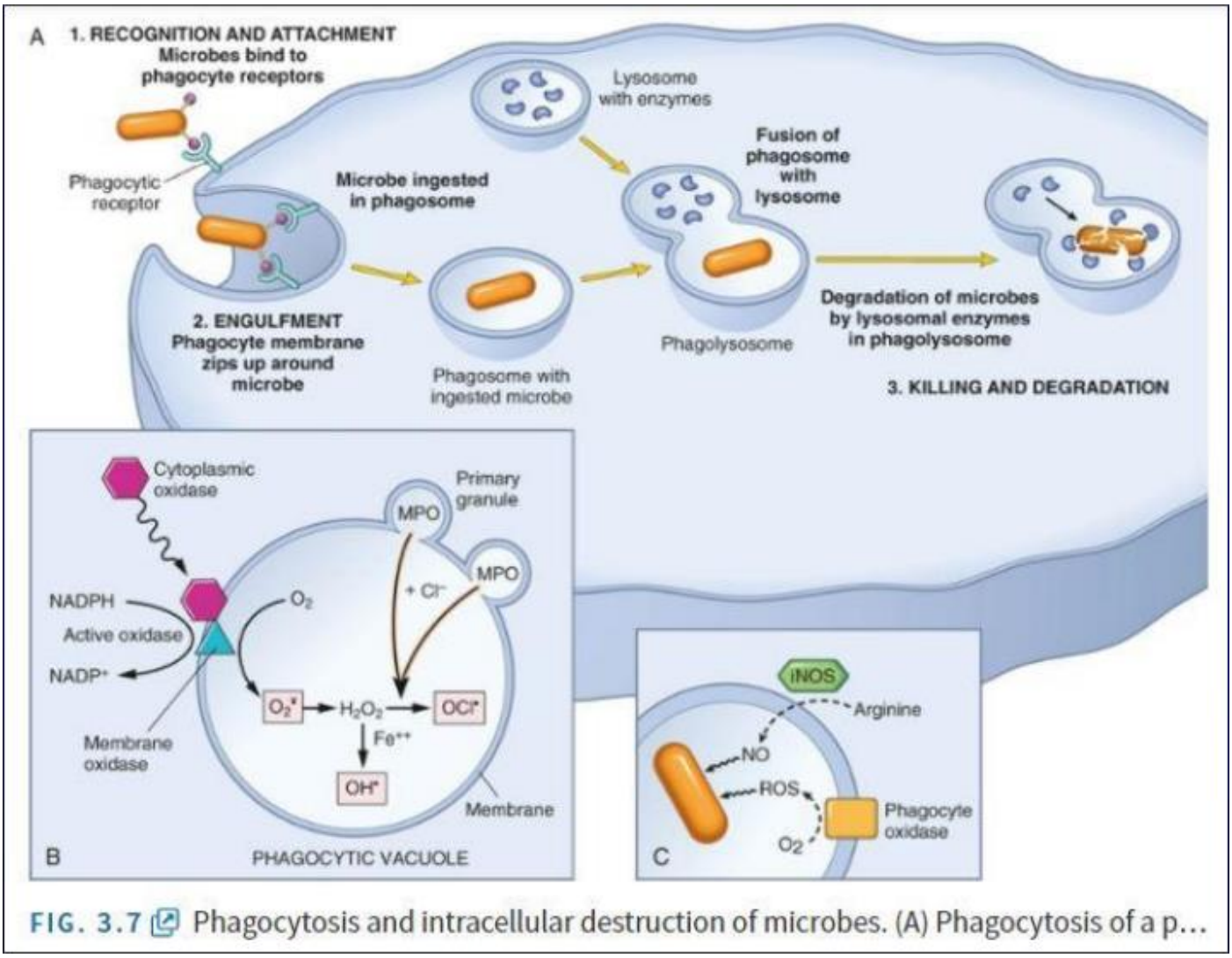


FIG. 3.7 Phagocytosis and intracellular destruction of microbes. (A) Phagocytosis of a p…

1. **Recognition and attachment of the enemy(bacteria, virus…): mannose receptors; opsonins (IgG, C3b** ”strong opsonizing agent”**)**

   (ينثني غشاء الخلية ويحيط بالجسم الغريب وبعدها تغلق الاطراف)

   Opsonization: increasing the efficiency of phagocytosis (intracellular killing, R3 in inflammatory response)

   Without the opsonizing agent (IgG,C3b) , the intracellular killing will be weak , so disease prolongs and makes more recurrent infections.

   ملاحظة : في أشخاص يعانون من أمراض نتيجة نقص في opsonizing agent

2. **Engulfment forming phagocytic vacuole: phagosome**

phagosome: is a part of the membrane of the neutrophil or macrophage that surround the bacteria or virus.

3. **Killing & degradation: reactive oxygen species (ROS); NO. H2O2- MPO-halide is the most potent bactericidal system of neutrophils**

NOTE:

Every step of these steps has a chemical mediators that stimulate it, in other words every step needs activation.

NITRIC OXIDE (NO)

- NO is the underlining mechanism of Viagra (Viagra is a kind of medicine that contains NO).

• Soluble gas (عشان هيك كان صعب يكتشفوه ويدرسوه) **produced from Arginine by NO synthase (NOS)**

- Arginine (one of the 21-20 amino acids) affected by NO synthase (NOS enzyme) to produce NO soluble gas.

• NOS 3 types: eNOS, nNOS, iNOS .

Dr.Mousa said that eNOS is an abbreviation for enveloped NOS, nNOS for neutrophil NOS & iNOS for intracellular NOS but he said he isn't sure about this information , so we found it for you.

eNOS→ endothelial NOS

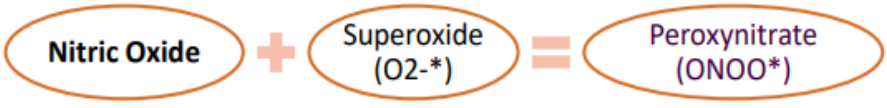
nNOS→ neuronal NOS

iNOS→ inducible NOS

• iNOS: intracellular killing stimulated by cytokines mainly IFN-ℽ (Interferon-γ IFN-γ is the main activator for NO)

• NO reacts with superoxide (O2-*) to form ONOO* radical peroxynitrite.

- When NO is produced by NOS , it will react with additional oxygen radicals (O2-*) to form ONOO* radical peroxynitrite (ONOO* is an important mediator that enhances intracellular killing).



GRANULE ENZYMES

• Present in PMNs and monocytes *(in general, granule enzymes present in neutrophils and macrophages)*

• In PMNs: 2 types; large azurophil (primary) and smaller (secondary) granules.

• Primary G: MPO, other enzymes.

*MPO: Myeloperoxidase the most important primary enzyme of large primary granule of macrophages.

- **Secondary G: lysozyme, and others**

- **These are usually neutralized by anti-proteases (such as ά-1 antitrypsin: inhibits elastase)…deficiency…diseases.**

هدول الانزيمات لما يصير degranulation ببدأوا يشتغلوا ويعملوا tissue damage عشان هيك وحدة من الأشياء اللي خلقها ربنا حتى تعمل control for inflammatory response is the presence of neutralizing agent for granule enzymes called anti-proteases.

Degranulation: losing or releasing granules of a substance, typically as part of an immune reaction.

- a1-antitrypsin reduces tissue damage.

- If anyone has a deficiency of the a1-antitrypsin it will lead to a problem in Gi system.

NEUTROPHIL EXTRACELLULAR TRAPS (NETs)

ال Neutrophil حتى بعد ما تموت بتحاول تساعد أجسامنا، بعد موتها بتطلع كل ال material سواء كانت سيتوبلازم أو DNA وبتكون هاي ال material مثل الصمغ mucoid , viscous and thick بتلصق ال injurious agent سواء كان فيروس أو بكتيريا فبتمنعهم من الحركة وبتسهل عمل new neutrophils & new macrophages هاي العملية كلها اسمها (NETosis).

- **Viscous meshwork of nuclear chromatin binds peptides and anti-microbial agents after PMN death (NETosis).**

- **Sepsis** *تسمم الدم*

NETosis involved in sepsis or septicemia.

The most dangerous septicemia is gram negative septic shock.

- **Maybe involved in SLE *(Systemic Lupus Erythematosus)*.**

SLE (مرض التذائب الاحمراري) infects young females (15-25 years).

سمي المرض بالتذائب الاحمراري لأنه خدودهم بتكون حُمر، وسمي systemic لأنه ما في system بالجسم مش داخل فيه.

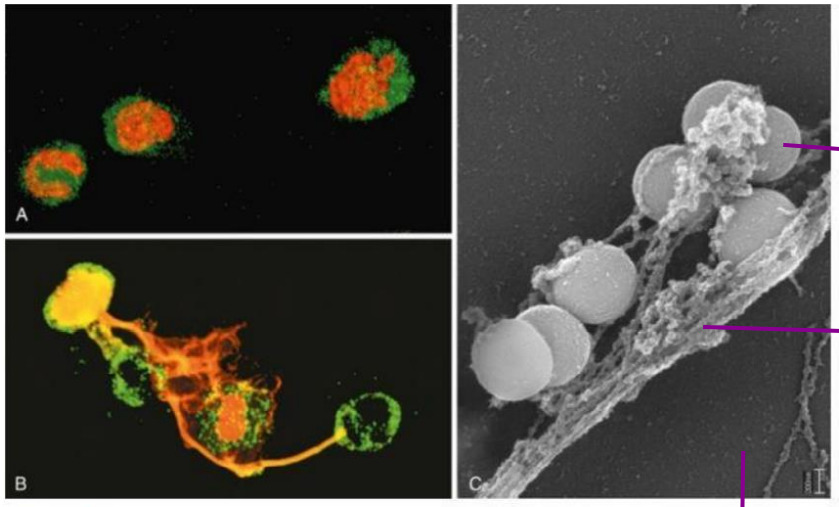


FIG. 3.8 Neutrophil extracellular traps (NETs). (A) Healthy neutrophils with nuclei stain…

Electron microscopic scanning picture

*Coccus bacteria is a spherical b*acterium.*

LEUKOCYTE-MEDIATED TISSUE INJURY *(WBC activation injury)*

- **A. Prolonged inflammation (TB** *"Tuberculosis"* **an Hepatitis)**
- **B. Inappropriate inflammatory response (auto-immune diseases)**
- **C. Exaggerated** *(too much)* **response (asthma and allergic reactions)**

OTHER FUNCTIONS OF ACTIVATED WBCs

- **Amplify *immune reaction* or limit reaction (*done by* cytokines)**
- **Growth factors secretion (repair)**
- **T-lymphocytes has also a role in acute inflammation** *by producing cytokines*.

e.g: **(T-HELPER-17); produce cytokine IL-17** *"Interleukin-17"* **(deficiency** in IL-17 **cause disease)**

THE END