7

Pathology

Doctor 2017 | Medicine | JU

◉ Sheet ○ Slides

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Chemotaxis

After exiting the circulation, leukocytes (WBCs) move along a chemical gradient in the tissues toward the site of injury by a process called **chemotaxis**.

Chemoattractants

Substances that **induce Chemotaxis** are called Chemoattractants, they can be:

1) **Exogenous**
   Bacterial Products, particularly **peptides** with **N-** formyl methionine **termini**.

2) **Endogenous**
   - **a-** Cytokines, especially those of the **chemokine** family.
   - **b-** Components of the complement system, particularly **C5a.**
   - **c-** Products of the lipoxygenase pathway of arachidonic acid (AA) metabolism, particularly **leukotriene B4** (LTB_4)

Leukocyte (WBCs) infiltration

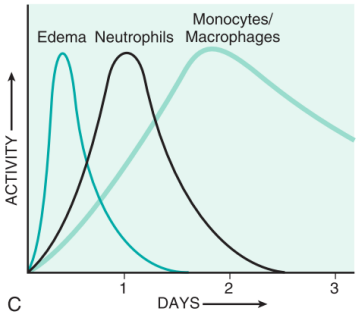
Leukocytes **migrate** toward the **inflammatory stimulus** in the direction of the **locally produced** chemoattractants and **infiltrate** that tissue. The type of the leukocyte (WBCs) infiltrate depends on the **age** of the inflammatory response and the **type** of stimulus.

In **the acute Phase**, during the first **6-24** hours of the inflammatory response	**Neutrophils** (PMNs) predominate.
Then during the next **24-48 hours** of the acute phase	Neutrophils are gradually replaced by **Monocytes** *(which differentiate into Macrophages)*, Lymphocytes in some cases.
Chronic Inflammation	**Macrophages** and **lymphocytes** predominate
In allergic reactions (Skin rash, Anaphylactic shock, etc.)	**Eosinophils** predominate

The following plot shows the steps in an inflammatory response in a tissue.

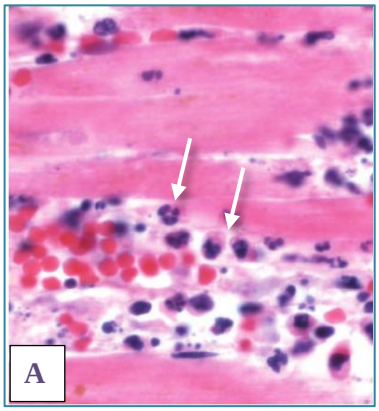
Please **learn** the steps on the plot and the **order** of them:

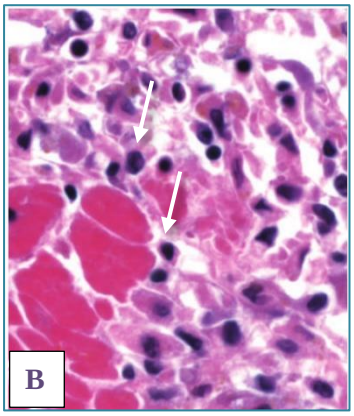
1- **Edema** occurs.
2- **Neutrophils** infiltrate.
3- Monocyte derived **macrophages** and **lymphocytes** infiltrate.



C

Inspect the following pictures:





A- In this lontitudal section, **Neutrophils** *(Multi-nucleated)* can be seen inflitarting the skeletal muscle tissue indicating an **acute inflammation** in the first **6-24hrs.**

B- In this cross section, **Lymphocyte** *(mononuclear)* can be seen inflitrating the tissue indicating a that the inflammation lasted **24-48hrs**, or a **chronic** inflammation.

Leukocyte Activation

Recognition of microbes or dead cells **induces** several responses in leukocytes that are collectively called **leukocyte activation.**

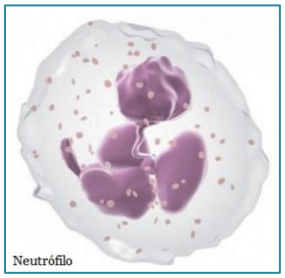
The Leukocyte activation is **most** important for **destruction of microbes** by :
(other functions of activation will be discussed later in this sheet)

1- Phagocytosis.
2- Intracellular killing.

Leukocytes **induced** for **Phagocytosis** and **Intracellular killing** are:

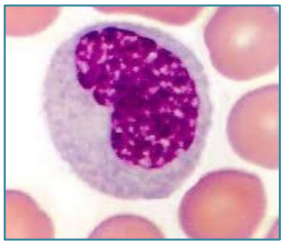
1- Neutrophils

They are **multi nucleated** (3-5) and have a **granulated** cytoplasm.
They are almost **twice the size** of RBCs (RBCs ≈ 7µm)

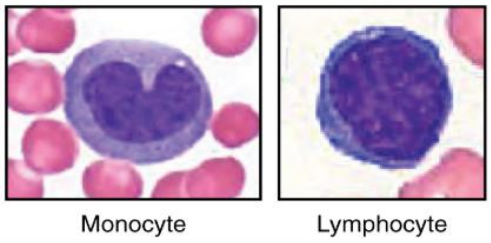


2- Monocytes

They have a **kidney-shaped** nucleus, **less granulated** cytoplasm.
They differentiate into **Macrophages**.
They are **10-12** µm in size.



Note 1: *Lymphocytes may look like Monocytes. However, a lymphocyte has a* ***big rounded*** *nucleus, while the monocytes has a* ***kidney shaped*** *nucleus.*



Note 2: *You must learn to* ***differentiate*** *between the given leukocytes since the dr. said he may bring up pictures of tissues infiltrated by them and we have to know what stage of inflammatory response it is.*

Phagocytosis

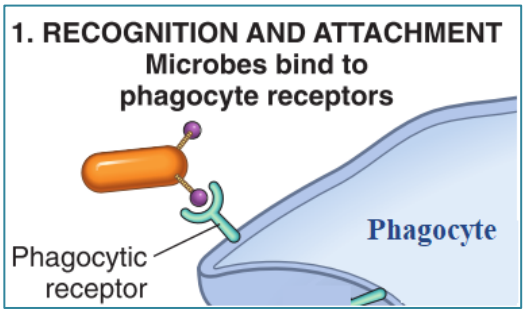
Phagocytosis is triggered by **activation** of phagocytes by **microbes** , **necrotic debris** *(which is the scattered pieces after a cell dies through necrosis; as taken before)* and **various mediators** .

It involves three sequential steps:

1) **Recognition** by Neutrophils/Monocytes and **attachment** of the microbe.

   The efficiency of phagocytosis is greatly **enhanced** when microbes are **opsonized** (coated) by specific proteins (Opsonins) for which the phagocytes then express **high-affinity phagocytic receptors** for these microbes (e.g. Mannose receptors).

   The **major** Opsonins involved are: **immunoglobulin G (IgG)** antibodies and **C3b**.



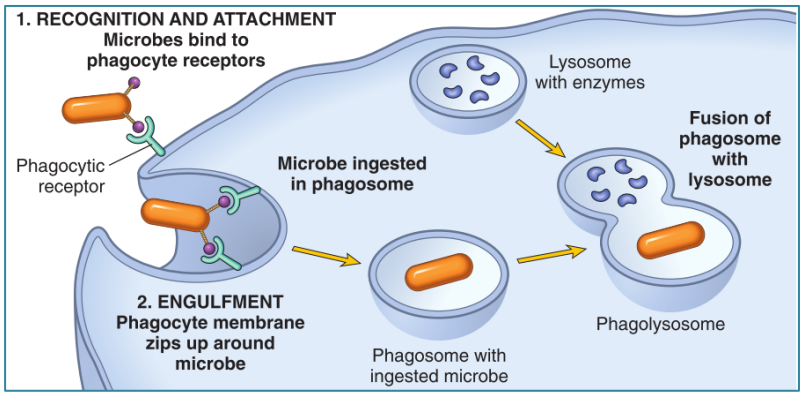
Note*: In the Complement System,* ***C3b*** *is a* ***strong opsonizing*** *agent and* ***C5a*** *is a strong* ***endogenous chemoattractant****.*

Additional Information: *Glycoproteins & glycolipids found in* ***microbial cell*** *walls contain* ***terminal mannose****. Whereas* ***mammalian*** *glycoproteins and glycolipids contain* ***terminal sialic*** *acid or N-acetylgalactosamine. That's why* ***Mannose receptors*** *are used as* ***phagocytic receptors*** *since they recognizes* ***microbes*** *and* ***not*** *host cells.*

2) **Engulfment**, with subsequent **formation** of a **phagocytic vacuole**.

After a microbe is bound to a phagocyte receptors, **extensions** of the **cytoplasm** (pseudopods) **flow around** the microbe, and the plasma membrane **pinches off** to form a **phagocytic vacuole** (phagosome) that **engulfs** the particle.

The phagosome then **fuses** with **lysosomes** (it is then called phagolysosome), resulting in the discharge of lysosomal contents into it.



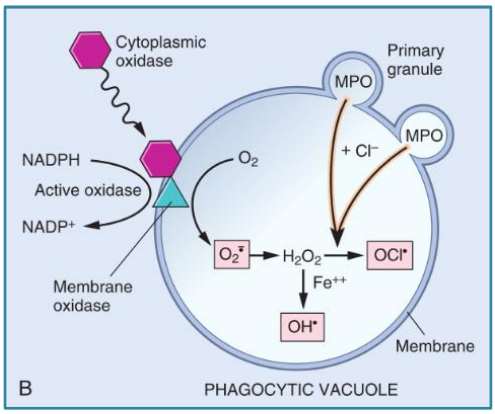
3) **Killing** & **degradation** of the ingested material.

This step is accomplished by **reactive oxygen species** (ROS), reactive nitrogen species mainly derived from **nitric oxide** (NO), and lysosomal enzymes.

A- Reactive oxygen species (ROS)

This may be additional but it's better to understand it

There is an **oxidative reaction** that is tightly **linked** to **phagocytosis**, which is **NADPH oxidation** (oxidized by a Phagocyte oxidase enzyme). As a result of this reaction, O_2 is **reduced** forming a superoxide anion O_2• (which is a ROS). O_2• is then **converted** into H_2O_2.



H_2O_2 is not able to kill microbes efficiently by itself, so it gets **converted** by the enzyme **myeloperoxidase** (MPO) with the presence of a **halide** (Cl^-) into OCl_2^- , this is called the **MPO-Halide** system which is the **most efficient bactericidal system** (agents that kill bacteria) of neutrophils.

Note 1: *the ROS are produced* ***within the phagolysosome****, where they can act on* ***engulfed*** *microbes without* ***damaging*** *the host cell.*

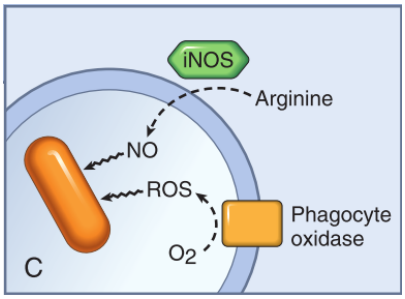
Note 2: *Myeloperoxidase (MPO) enzyme is found in the granules of the phagocyte.*

B- Nitric Oxide (NO)

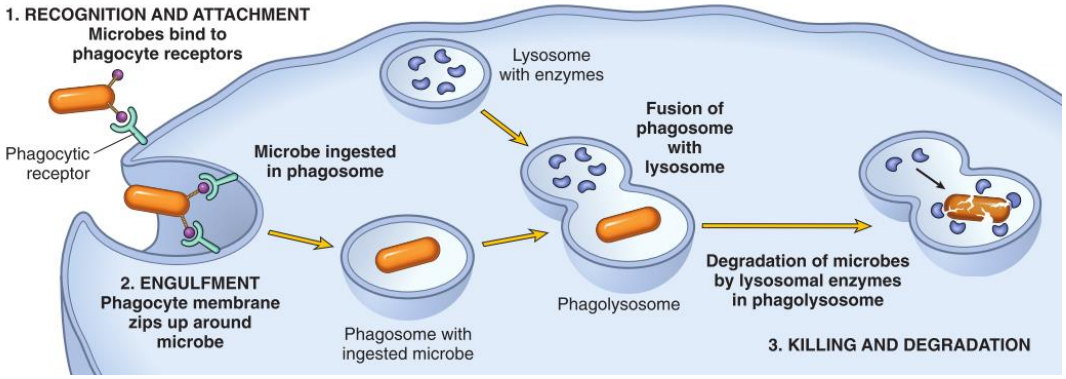
NO is a **soluble gas** produced from **arginine** by **nitric oxide synthase** enzyme (NOS), also participates in microbial killing. There are **3** different types of NOS:

- **Endothelial (eNOS)**
  eNOS generates NO for maintaining the **vascular tone.**
- **Neuronal (nNOS)**
  nNOS generates NO that acts as a **neurotransmitter.**
- **Inducible (iNOS)**
  iNOS is the type that is involved in **microbial killing**, it is expressed when macrophages are **activated by cytokines** *(mainly IFN-γ)*.

  NO reacts with superoxide (O2•) and **generates** the **highly** reactive free radical peroxynitrite (ONOO•).



A summary of the Phagocytosis process



Granule Enzymes

Neutrophils and Monocytes contain **granules** packed with **enzymes** that **degrade microbes** and dead tissues. There have **2** main types of granules:

1) **Primary Granules** *(Large azurophil):* They contain **MPO**, **elastase** and other enzymes.
2) **Secondary Granules** *(Small):* They contain **lysozyme** and other enzymes.

Because of the **destructive effects** of granule enzymes for their potentiate of further inflammation by damaging tissues, they are controlled by a system of **anti-proteases**.
(e.g. α-1 antitrypsin, which is a major inhibitor of neutrophil elastase).

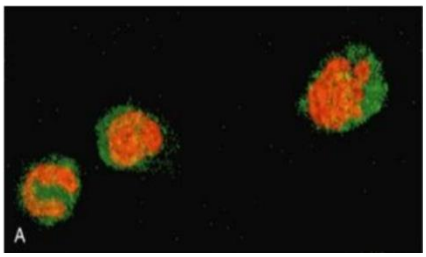
A **deficiency** of these **inhibitors** may lead to a **nonstop** action of Leukocyte enzymes, as is the case in patients with **α1-anti-trypsin deficiency** developing **emphysema** *(as taken in Biochem).*

Neutrophil Extracellular Traps (NETs)

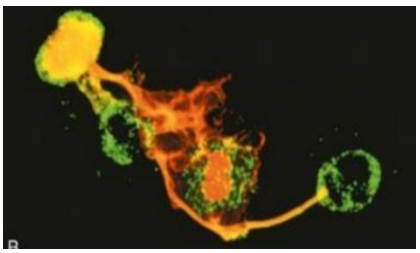
NETs provide an **additional mechanism** of **killing microbes** that does not involve phagocytosis. In the process of NET formation, the nuclei of the neutrophils are **lost**, leading to the death of the cells because this it is sometimes called **(NETosis).**

Neutrophil extracellular traps (NETs) consist of a **viscous meshwork** of nuclear chromatin that **concentrate anti-microbial** substances and **enzymes** at **sites** of infection and prevent the **spread** of the microbes by **trapping** them in the **meshwork**.

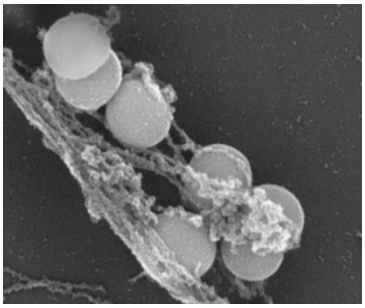
Observe the following pictures showing the formation of NETs:



This section shows **healthy** neutrophils **with nuclei.**



Release of nuclear material from neutrophils, note that two have **lost their nuclei**, **forming** extracellular traps.



An electron micrograph of **bacteria** (staphylococci) **trapped** in NETs.

It is said that NETs may play a role is **Sepsis**, since they have been **detected** in the **blood** during sepsis. *(Sepsis is a life-threatening illness caused by a body's response to an infection)*

Also, the nuclear chromatin in the **NETs**, may be a **source of nuclear antigens** in systemic autoimmune diseases, particularly lupus **(SLE),** which is a disease where individuals **react against** their **own** DNA.

Leukocyte-Mediated Tissue Injury

Leukocytes may **contribute** to the **injury** of normal cells and tissues under several **circumstances**:

1) **Prolonged Inflammatory**
   It happens in some infections that are **difficult to eliminate**, such as **Tuberculosis** (TB) and certain viral diseases such as **Hepatitis**. This prolonged host inflammatory response can contribute **more** to the pathology than does the **microbe** itself.

2) **Inappropriate Inflammatory**

   When the inflammatory response is **misdirected** against host tissues, as in certain **autoimmune diseases.**

3) **Exaggerated Response**

   When the host **overreacts** against usually harmless environmental substances, as in **allergic diseases**, including **asthma**, and some **drug reactions**.

   Recall: *The **main** leukocyte infiltrate in this type of response is, **Eosinophils**.*

Other Functional Responses of Activated Leukocytes

In addition to **eliminating microbes** and dead cells, activated leukocytes play several other roles in **host defense:**

1) They **produce cytokines** that can either **amplify** or **limit** inflammatory reactions.
2) They produce **growth factors** that stimulate the proliferation of endothelial cells (Important in the repair process).
3) It is true that in acute inflammation we only mention neutrophils. However, it has become clear that **some T lymphocytes**, also **contribute** to acute inflammation (e.g. T-Helper-17, which produces the cytokine IL-17). IL-17 **induces** the secretion of **chemokines** (chemoattractants) that recruit other leukocytes.
   Deficiency of T-Helper-17, makes individuals vulnerable to bacterial infections.

> ***Note:*** *Lymphocytes **DO** have a role in acute inflammation.*

Termination of the Acute Inflammatory Response

The inflammatory response, with its capacity to cause tissue injury, **needs tight controls** to **minimize** damage. This can be managed through:

1) Mediators of inflammation are produced in **rapid bursts** and not continuous, so that the inflammation **declines** after the microbes are removed.
2) Mediators are released only as long as the stimulus persists.
   No stimulus → No mediators
3) Mediators have **short half-lives** and are **degraded** after their **release**.

4) Neutrophils also have **short half-lives** in tissues and die by **apoptosis** within hours to a day or two **after** leaving the blood.

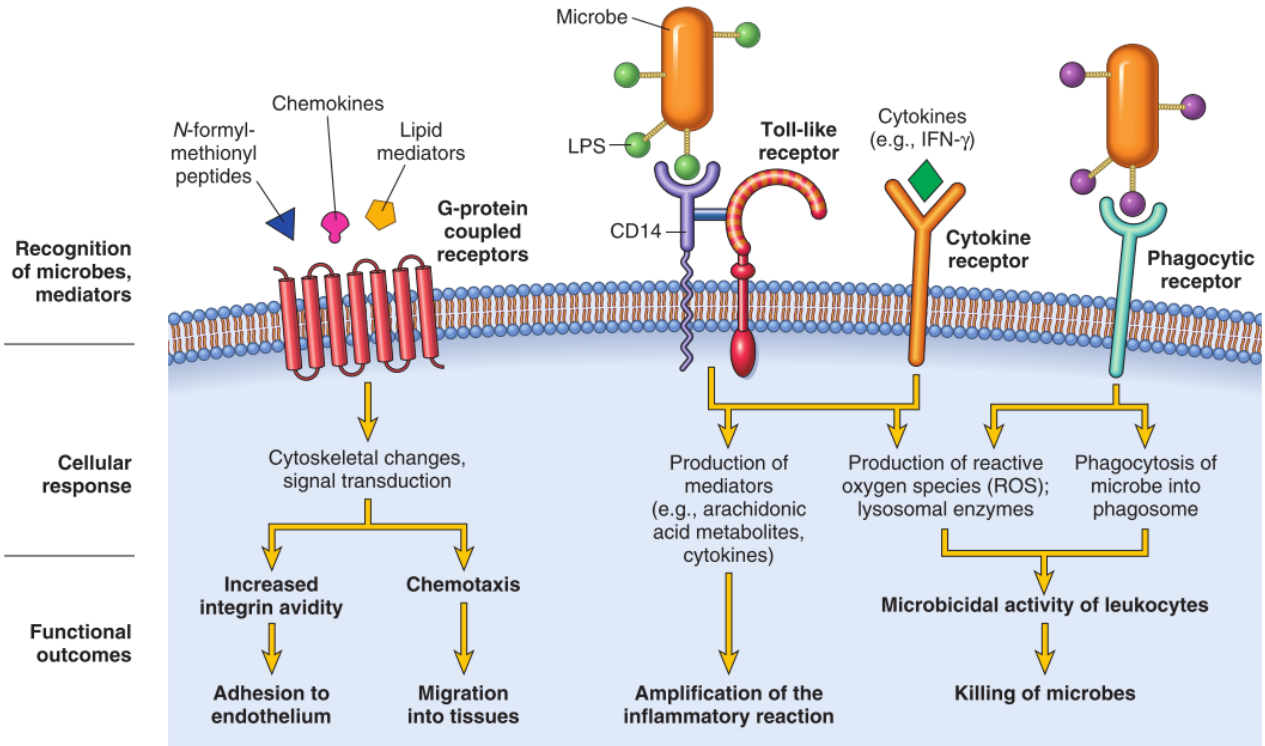
> ***Note**: In the **formation of NETs**, Neutrophils died through **NETosis,** unlike how it normally dies through **apoptosis**.*

5) As inflammation develops, the process itself **triggers** a variety of **stop signals** *(TGF-β and IL-10)* that **terminate** the reaction.

6) **Neural impulses** (cholinergic discharge), **inhibits** the production of **TNF**.

> ***Note:** Tumor necrosis factor **(TNF),** is a **cytokine** (chemoattractant) involved in **acute inflammation**.*

Various types of phagocytic cells' surface receptors, recognize different agonists. Once stimulated, the receptors initiate responses that mediate leukocyte functions. This is a summary of what was taken before, the Dr. said we should only understand this picture.



Summary of important information to know:

1) **Chemotaxis** which is the **movement of WBCs** into tissue injury site. **Induced** by **chemoattractants** that are either **endogenous** *(Cytokine; chemokines / Complement system; C5a / AA metabolites; LTB4)* or **exogenous** *(Bacterial products).*
2) Infiltration:
   Neutrophils → Acute, 6-24hrs
   Monocyte derived **Macrophages** / **Lymphocytes** → Chronic, 24-48hrs and may stay.
   Eosinophils → Allergic reactions
3) **Neutrophils**: Multinucleated, granulated cytoplasm.
   Monocytes: Kidney shaped nucleus, less granulated than neutrophils, differentiates into macrophage.
   Lymphocyte: Big rounded nucleus.
4) Phagocytosis: Recognition → Engulfment → Degradation
5) **Opsonization**, is the process of **coating** a microbe through **Opsonins** *(opsonizing agents e.g. IgG,C3b)* **enhancing** the recognition of it by **mannose** receptors for example on the phagocytic cell.
6) Granules in PMNs and monocytes are either **primary** *(contain MPO)* or **secondary** *(contain lysozyme)*, neutralized by **antiproteases**.
7) Neutrophils die through **apoptosis**. In NETs formation, the neutrophils lose their nuclei leading to its death through **NETosis.**
8) Cytokines mentioned:
   TNF – Involved in acute inflammation inhibited by neural discharges.
   IFN-γ – Activates macrophages and expresses iNOS for microbial killing.
   IL-17 – Produced by T-Helper-17, which induces the secretion of chemoattractants.
   Chemokines – They are endogenous chemoattractants.

Good Luck 😊

Ask if anything is unclear