8

# Pathology

Doctor 2018 | Medicine | JU

Sheet Slides


DONE BY

Sara Raymoony + Raghad Tayseer

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

Sara Raymoony + Raghad Tayseer

CONTRIBUTED IN THE GRAMMATICAL CORRECTION

بتول البدور

DOCTOR

Mousa Al-Abbadi

# Leukocytes

-**Leukocytes**: are white blood cells that protect our bodies from infections and foreign bodies, they include five main types: neutrophils, eosinophils, basophils, lymphocytes and monocytes.

-Monocytes are cells circulating in the blood, that differentiate into macrophages, when they go into tissue.

## -Leukocytes ROLE:

1) PMNs and Macrophages

PMN "polymorphonuclear leukocytes" (granular leukocytes): a type of immune cells that have granules (small particles) containing enzymes that would be released during infection, allergic reactions and asthma.

Neutrophils, eosinophils and basophils are examples of PMNs.

2) Recruitment and migration to tissue.

3) Elimination of the enemy (phagocytosis).

4) Migration of leukocytes from BV (blood vessels) to tissue is a multi-step process: adhesions (weak then strong), transmigration then movement towards the enemy area.

-The two predominant cells at the cellular phase are neutrophils and macrophages ⟶ they get recruited to the tissue ⟶ they are the ones that enhance phagocytosis or try to kill the invaders especially pathogens.

-The cellular phase of the acute inflammation response immediately overlaps with the immune vascular phase.

-In the cellular phase, leukocytes have to move from the intravascular compartment to the interstitial compartment (the site of injury) and this process is a multi-step process which is organized with activators.

-The following table shows the differences between neutrophils and macrophages:

*details in the table will be discussed during this sheet and the next one. Just read now

The most important period of their life.

After it, they die.

Prominent in neutrophils.

Lysosomal enzymes are prominent in neutrophils. Neutrophils have granules containing lysozymes which can be secreted to help in the digestion process.

**TABLE 3.3** Properties of Neutrophils and Macrophages

| | Neutrophils | Macrophages |
|---|---|---|
| Origin | HSCs in bone marrow | • HSCs in bone marrow (in inflammatory reactions)<br>• Many tissue-resident macrophages: stem cells in yolk sac or fetal liver (early in development) |
| Life span in tissues | 1–2 days | Inflammatory macrophages: days or weeks<br>Tissue-resident macrophages: years |
| Responses to activating stimuli | Rapid, short-lived, mostly degranulation and enzymatic activity | More prolonged, slower, often dependent on new gene transcription |
| • Reactive oxygen species | Rapidly induced by assembly of phagocyte oxidase (respiratory burst) | Less prominent |
| • Nitric oxide | Low levels or none | Induced following transcriptional activation of iNOS |
| • Degranulation | Major response; induced by cytoskeletal rearrangement | Not prominent |
| • Cytokine production | Low levels or none | Major functional activity, requires transcriptional activation of cytokine genes |
| • NET formation | Rapidly induced, by extrusion of nuclear contents | No |
| • Secretion of lysosomal enzymes | Prominent | Less |

*HSC*, Hematopoietic stem cells; *iNOS*, inducible nitric oxide synthase; *NET*, neutrophil extracellular traps.
This table lists the major differences between neutrophils and macrophages. The reactions summarized above are described in the text. Note that the two cell types share many features, such as phagocytosis, ability to migrate through blood vessels into tissues, and chemotaxis.

Can stay for days, months and sometimes more than that.

-Neutrophil extracellular traps that are networks of extracellular fibers composed of the neutrophils' DNA, which binds pathogens and microbes (they are trapped).

-Happens in neutrophils, after they die.

## -How do the leukocytes move from inside the blood vessels to the surrounding tissue? (ADHESION (WBCs to endothelium))

1) Margination.
2) Rolling.
3) Adhesion.
4) Transmigration.

-The whole process is called **diapedesis**, but some people say that diapedesis expresses the transmigration step when the cells penetrate the basement membrane and get outside.

-This process takes a little time and it has activators, proteins and mediators.

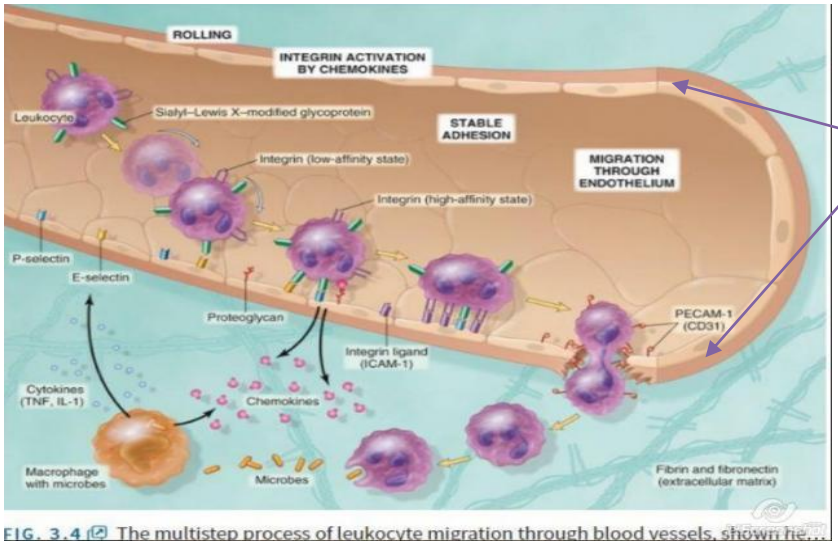



FIG. 3.4 The multistep process of leukocyte migration through blood vessels, shown he...

Both sides of the basement membrane

## The first step (Margination):

-When the flow in the blood vessels is normal (flows from the heart to the periphery and from the periphery to the heart) ⟶ most of the cells are in the center of the lumen.

-When the injury occurs ⟶ the leukocytes move from the center of the lumen towards the wall of the blood vessel.

-Margination: movement of leukocytes during the early phases of cellular response from the center of the lumen towards the wall of the blood vessel.

## The second step (Rolling):

-When the leukocytes start rolling much closer to the wall of the blood vessel.

** the rolling process is caused by the loose adhesion of the leukocyte to the endothelium, this loose adhesion is done by a protein called selectin.

## The third step (Adhering):

-Selectins and integrins attach to the leukocytes.

-The initial slowing down of the movement and rolling of the leukocytes is mediated by selectins ⟶ a big family of proteins that exist on the endothelial surface and on the leukocytes.

-Selectins (cause initial weak adhesion of leukocytes to endothelial cells) ⟶ the movement becomes slower and slower⟶ another group of proteins are activated on the leukocytes called integrins ( integrins would gain a higher affinity to endothelial cells) ⟶ firm adhesion with the endothelium.

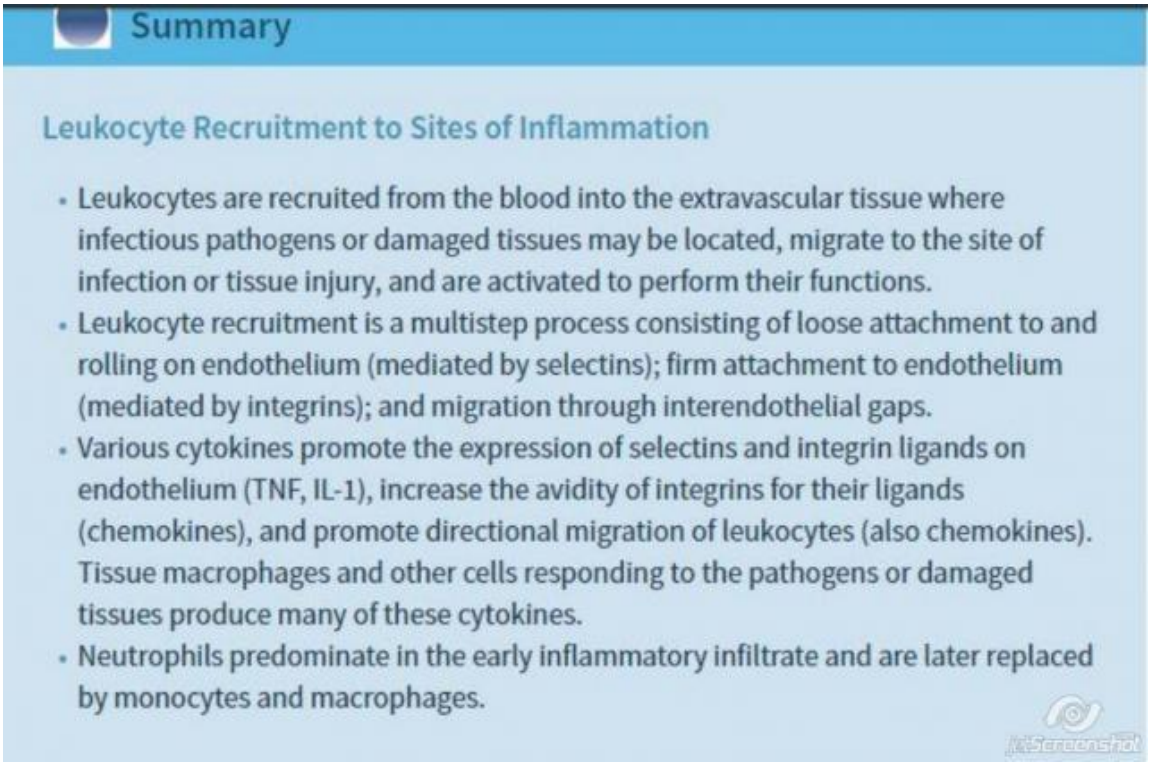
((an extra note: integrins originally exist on leukocytes with low affinity to endothelial cells, after activating them by certain mediators "chemokines" they acquire high affinity to endothelial cells))

** so there are 2 types of adhesion : **loose,** caused by selectins and **firm,** caused by integrins.

**The final step (Transmigration):**

-Stimulation by another mediator called **PECAM-1** (platelet endothelial cell adhesion molecule) or **CD31** which secretes proteins. mainly, Collagenases (which remove collagen 4 from the basement membrane), and proteases ⟶ they penetrate the basement membrane ⟶ then leukocytes would go to the site of injury. The basement membrane's main components ⟶ (Collagen 4, Laminin).

**Migration ⟶ Rolling ⟶ Selectins (weak affinity) ⟶ Movement is slower ⟶ Integrins (high affinity) ⟶ then leukocytes penetrate the basement membrane and get outside.**


Summary

Leukocyte Recruitment to Sites of Inflammation

- Leukocytes are recruited from the blood into the extravascular tissue where infectious pathogens or damaged tissues may be located, migrate to the site of infection or tissue injury, and are activated to perform their functions.
- Leukocyte recruitment is a multistep process consisting of loose attachment to and rolling on endothelium (mediated by selectins); firm attachment to endothelium (mediated by integrins); and migration through interendothelial gaps.
- Various cytokines promote the expression of selectins and integrin ligands on endothelium (TNF, IL-1), increase the avidity of integrins for their ligands (chemokines), and promote directional migration of leukocytes (also chemokines). Tissue macrophages and other cells responding to the pathogens or damaged tissues produce many of these cytokines.
- Neutrophils predominate in the early inflammatory infiltrate and are later replaced by monocytes and macrophages.

**TABLE 3.4** Endothelial and Leukocyte Adhesion Molecules

| Family | Molecule | Distribution | Ligand |
|---|---|---|---|
| Selectin | L-selectin (CD62L) | Neutrophils, monocytes T cells (naïve and central memory) B cells (naïve) | Sialyl-Lewis X/PNAd on GlyCAM-1, CD34, MAdCAM-1, others; expressed on endothelium (HEV) |
| | E-selectin (CD62E) | Endothelium activated by cytokines (TNF, IL-1) | Sialyl-Lewis X (e.g., CLA) on glycoproteins; expressed on neutrophils, monocytes, T cells (effector, memory) |
| | P-selectin (CD62P) | Endothelium activated by cytokines (TNF, IL-1), histamine, or thrombin; platelets | Sialyl-Lewis X on PSGL-1 and other glycoproteins; expressed on neutrophils, monocytes, T cells (effector, memory) |
| Integrin | LFA-1 (CD11aCD18) | Neutrophils, monocytes, T cells (naïve, effector, memory) | ICAM-1 (CD54), ICAM-2 (CD102); expressed on endothelium (upregulated on activated endothelium) |
| | MAC-1 (CD11bCD18) | Monocytes, DCs | ICAM-1 (CD54), ICAM-2 (CD102); expressed on endothelium (upregulated on activated endothelium) |
| | VLA-4 (CD49aCD29) | Monocytes T cells (naïve, effector, memory) | VCAM-1 (CD106); expressed on endothelium (upregulated on activated endothelium) |
| | α4β7 (CD49DCD29) | Monocytes T cells (gut homing naïve effector, memory) | VCAM-1 (CD106), MAdCAM-1; expressed on endothelium in gut and gut-associated lymphoid tissues |
| Ig | CD31 | Endothelial cells, leukocytes | CD31 (homotypic interaction) |

# Chemotaxis

-**Chemotaxis**: the movement of WBCs from the intravascular compartment (blood vessels) to the site of injury. It's induced by mediators called Chemoattractants.

-**Chemoattractants**: strong mediators that induce chemotaxis (stimulate the movement of leukocytes); they can be either exogenous or endogenous.

- Chemoattractants ⟶ chemotactic agents ⟶ mediators of inflammation

## Exogenous

**Bacterial products** ⟶ peptides with N- formyl methionine termini of certain bacterial products are strong exogenous chemoattractants.

## Endogenous

1) **Cytokines (chemokine family)** → a big family of chemical mediators produced mainly by lymphocytes and inflammatory cells like macrophages.
2) **Components of the complement system** (small amounts of small proteins that are circulating)
-Complement 3 → C3A (when it's active)
-Complement system molecules are very important and responsible for the pathogenesis of many inflammatory and non-inflammatory diseases.
-In the complement system → C5A is the strongest chemoattractant.
3) **Leukotrienes prostaglandins** → are major byproducts when you degrade arachidonic acid (AA).
- Leukotriene B4 → a strong chemoattracatnt from the lipoxygenase pathway of arachidonic acid (AA) metabolism.

- AN EXTRA NOTE: When strongly activated, leukocytes can cause tissue damage and prolonged inflammation → this may cause collateral damage of normal cells → to avoid this problem → scientists made some drugs that control the harmful effects of inflammation, (agents) that block TNFs (Tumor Necrosis Factors) which are major cytokines in leukocytes requirement to the tissue.

## WBCs infiltrate the tissue

-Cellular entering into tissue (infiltration) differs depending on the age of the inflammatory response and the type of stimulus.

1) **Neutrophils**: they infiltrate the tissue during the early 6-24 hours of inflammation (during the acute phase). Then they gradually disappear from the tissue due to their short life span which is about 2 days (they die by apoptosis) and are replaced by monocytes (which differentiate into macrophages) or lymphocytes.

2) **Macrophages and lymphocytes**: they infiltrate the tissue after 24-48 hours of inflammation, when all neutrophils are almost dead. They survive longer than neutrophils and may remain in the tissue, as in prolonged chronic inflammations (they can proliferate).

3) **Eosinophils**: they infiltrate the tissue in 2 situations, in allergic reactions, like asthma, or in parasitic infections.

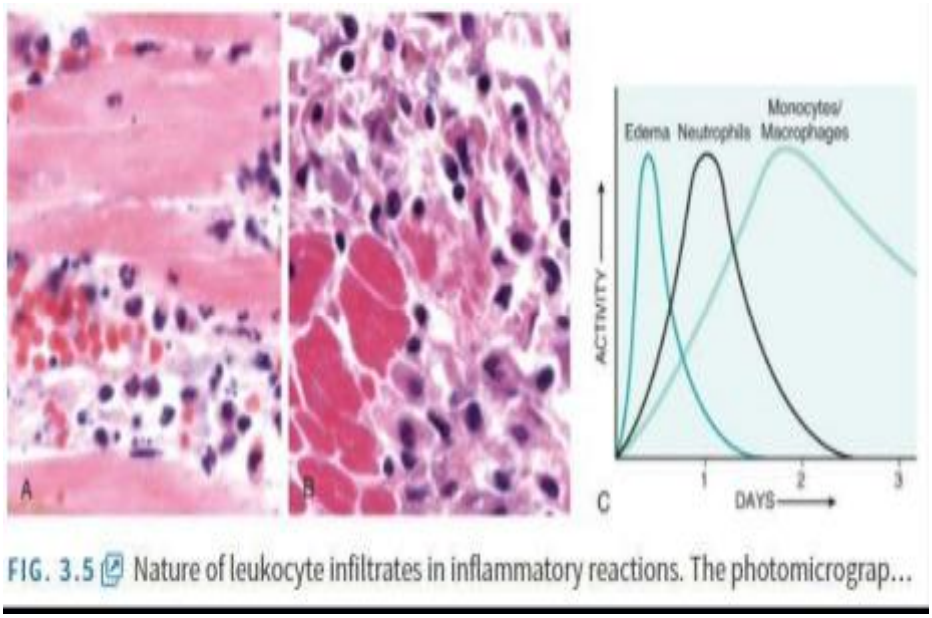


FIG. 3.5 Nature of leukocyte infiltrates in inflammatory reactions. The photomicrograp...

-**According to the above pictures**:

- Picture **A** and **B** are photomicrographs that show an inflammatory reaction in myocardium after ischemic necrosis.

-**A**: a longitudinal section shows early neutrophilic infiltrates.

-**B**: a cross section shows macrophagial infiltrates. The inflammatory tissue started to digest normal cells.

-**C**: it's a diagram that helps to know the age of the inflammatory response.

1) Edema (early vascular phase): is initiated by histamine and causes vasodilatation.

2) Neutrophils: they become recruited by chemotactic agents (chemoattractants), and then they gradually die.

3) Macrophages: they peak at day 2 and may remain in the tissue.

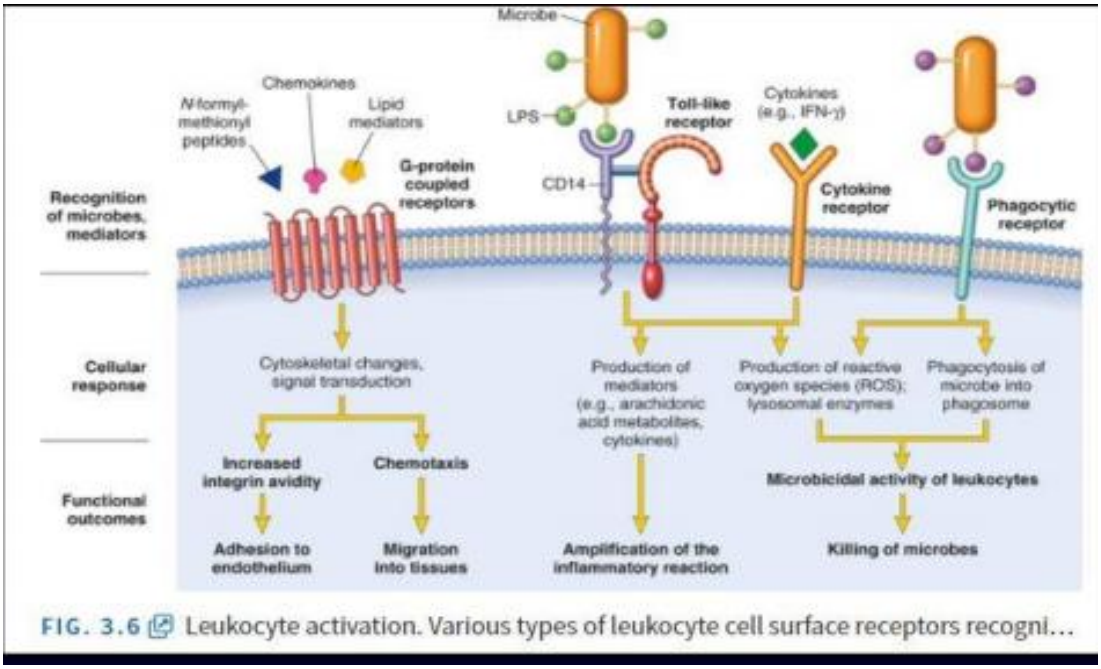


FIG. 3.6 Leukocyte activation. Various types of leukocyte cell surface receptors recogni…

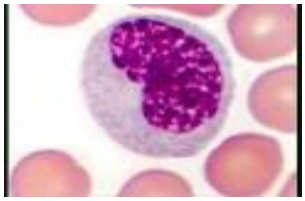
-This picture **summarizes** the leukocyte activation (several responses in leukocytes to perform their function).

**Recognition → Vascular phase → Cellular phase → Outcomes**

-The **doctor advised** to refer back to the **above picture** after his lecture.

-We must recognize the structure of **neutrophils**: they are PMN "micky mouse" (polymorphonuclear leukocyte) with 3 nuclei attached by peptides. They have granules containing enzymes → when ruptured → enzymes are released and they digest the internal material (foreign bodies).

-**Monocytes**: these cells have a phagocytic effect with a bean or kidney shaped nucleus.



-To differentiate between the two cells:

1) a Neutrophil has several nuclei, while the monocyte has one nucleus.

2) Granules are more abundant in the neutrophil than in the monocyte.

"**BOTH have phagocytic effect** "

# The Third "R": Removal Of The Offending Agent

-This is done by a process called phagocytosis and includes 3 sequential steps:

1)**recognition and attachment: Neutrophils** and **macrophages** have to be able to **recognize** and **attach** to the enemy through certain receptors:

a) Mannose receptor

b) Receptors for various opsonins which are bound to the microbe.

-The **efficiency of phagocytosis** is greatly enhanced when microbes are opsonized (coated) by specific proteins called opsonins.

-Why **high efficiency**?

Because phagocytes express high affinity receptors for opsonins.

-**Examples of opsonins**: IgG and C3B (**C3B** has the strongest opsonizing effect).

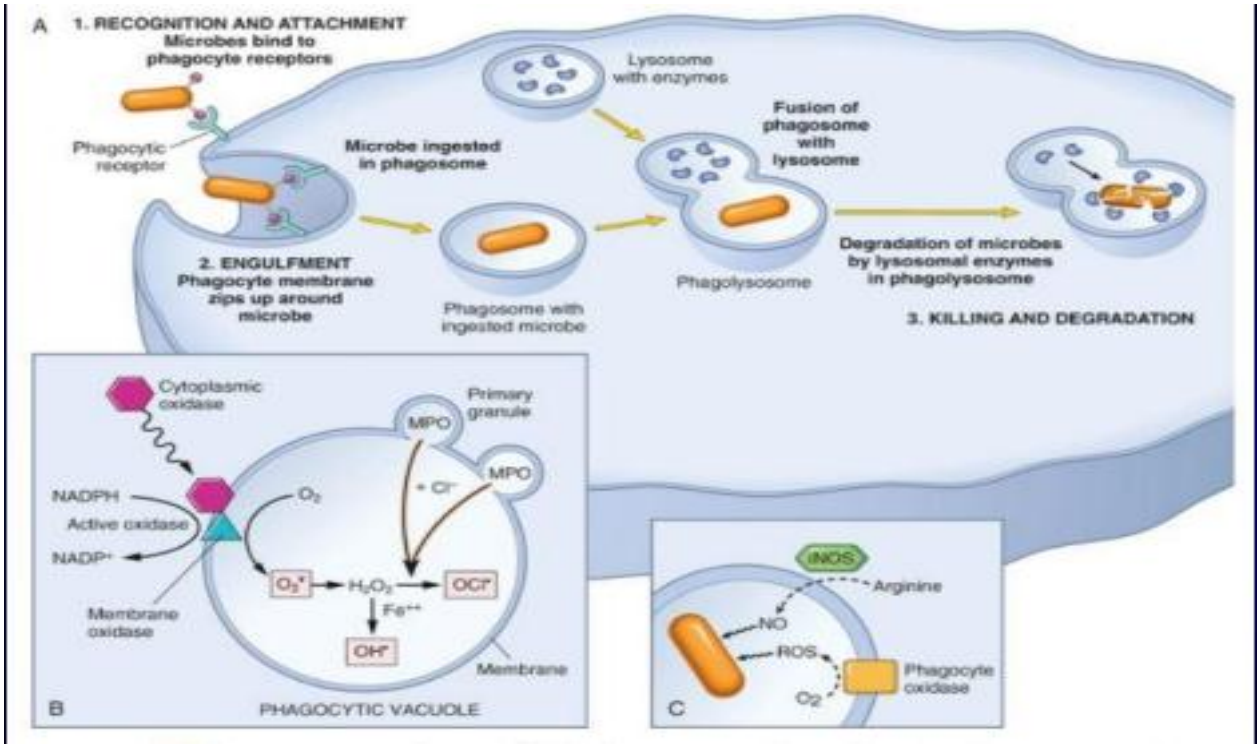
-**Opsonization**: is the process of coating microbes with opsonins (IgG and C3b).

- **NOTE**: there is a huge difference between opsonization and chemotaxis ⟶ C3A functions in chemotaxis but C3b functions as an opsonin.

-**NOTE**: when a person is vaccinated against a certain pathogen, it generates antibodies so when the body is infected with the pathogen again, it has the proper IgG antibodies produced by B cells that allow it to **respond faster** (called secondary response) -the secondary response depends on coating the microbe with the IgG antibody.

2) **Engulfment**: after the particle is bound to the phagocyte's receptor, extensions of the cytoplasm flow around it forming a phagosome. Then lysosomes are induced by mediators to attach to the phagosome and fuse with it generating a phagolysosome.

3) **Killing and degradation**: destruction of ingested materials is accomplished by ROS, reactive nitrogen species which are derived from NO, lysosomal enzymes, and enzymes found in granules of the neutrophil like lysozymes. All these killing mechanisms are normally found in phagocytes.




-**The 3 pictures above**, include the process of phagocytosis and internal reactions as follows:

**-Pic.A**: summarizes the 3 steps mentioned above.

**-Pic.B**: presents the reactions inside the phagosome, an enzyme called **phagocyte oxidase** is activated and catalyzes the conversion of oxygen into superoxide radical ( **O2 –** ), then it's converted to **H2O2** which is still not able to kill microbes efficiently. so, Myeloperoxidase(**MPO**), present in the granules of neutrophils, converts **H2O2** to hypochlorite (**OCL2-**) which is a strong antimicrobial agent that destroys microbes by defecting their lipids and proteins.

- **MPO** + **CL-** (or any other halide) ⟶ **OCL2-**

***MPO-halide is the most potent bactericidal system of neutrophils.**

**-Pic.C:** Microbicidal reactive oxygen species (ROS) and nitric oxide (NO) kill ingested microbes.

- **NO** (**Nitric Oxide**):

- A soluble gas

-Originates from arginine amino acid by the action of nitric oxide synthase (NOS).

-There are 3 types of NOS: inducible (iNOS), endothelial (eNOS), and neuronal (nNOS).

-**All these types** produce NO but only iNOS can create an NO that is capable of microbial killing (other types of NOS produce NO with different functions such as neurotransmitters).

-iNOS is stimulated by cytokines mainly IFN-ℵ (Interferon gama) to produce NO that can react with a superoxide radical to generate the highly reactive radical peroxynitrite (ONOO), a microbial killer.

EXTRA ADVICE, “The greater danger for most of us lies not in setting our aim too high and falling short,, but in setting our aim too low, and achieving our mark.”

ALWAYS MAKE YOUR DREAMS HIGH AS CLOUDS :)

**sorry for errors, if there was.

GOOD LUCK :)