PATHOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 8

Inflammation (Pt.3)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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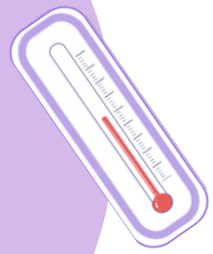


Short Quiz on last lecture (Inflammation 2):

- https://docs.google.com/forms/d/e/1FAIpQLScQRkz1q5NEX85iKsnyzaP7oR8b-5HC4HK92vBsf0JklnRhCw/viewform?usp=sf_link

Transmigration

(The previous lecture)

The last lecture we finished the initial vascular phase:

1. Vascular dilatation due to histamine
2. Increase in the vascular permeability
3. The movement of the inflammatory cells (mainly neutrophils & macrophages) from the intravascular to the extravascular:

- Margination -> Rolling -> Selectin (weak) -> Integral (strong)
- Active transmigration via CD31 (PECAM-1), **P**latelet **E**ndothelial **C**ell **A**dhesion **M**olecule expressed both on leukocytes & endothelial cells and WBCs pierce through the wall by enzymes such as collagenases.

Chemotaxis

- Chemotaxis is the movement of WBCs to injury tissue site

  For example, if there was an inflammation in the tonsils (tonsilitis), there is CHEMOTAXIS to the tonsils and so on

- It is an active process and induced by CHEMOATTRACTANTS (exogenous & endogenous)

  Produced outside the body

  Produced inside the body

More details on CHEMOATTRACHTANTS in the next slide

Major Types of Chemoattractants:

1. **Bacterial products**: Notably, the N-terminal peptides of bacterial products are potent chemoattractants.
2. **Cytokines**: This large group of mediators, primarily released by inflammatory cells such as lymphocytes and macrophages, includes the chemokine family, a subgroup known for its strong chemoattractant properties.
3. **Complement system**: Among plasma proteins, C5a (complement 5a) stands out as the most powerful chemoattractant.
4. **Lipoxygenase pathway AA metabolites**: These metabolites, components of cell membranes, include LTB4 (leukotriene B4), which is the strongest chemoattractant among AA metabolites.

CHEMOATTRACTANTS group →	An example of it
Bacterial Products	Peptides (N-…)
Cytokines	Chemokine family
Complement system	C5a
Lipoxygenase pathway AA	LTB4

WBCs infiltrates in tissue

- Depends on the age of inflammatory response and the type of stimulus

Pathologists may receive removed organs (such as appendix or tonsils), to section and stain them, to decide the type of inflammation. If we see:

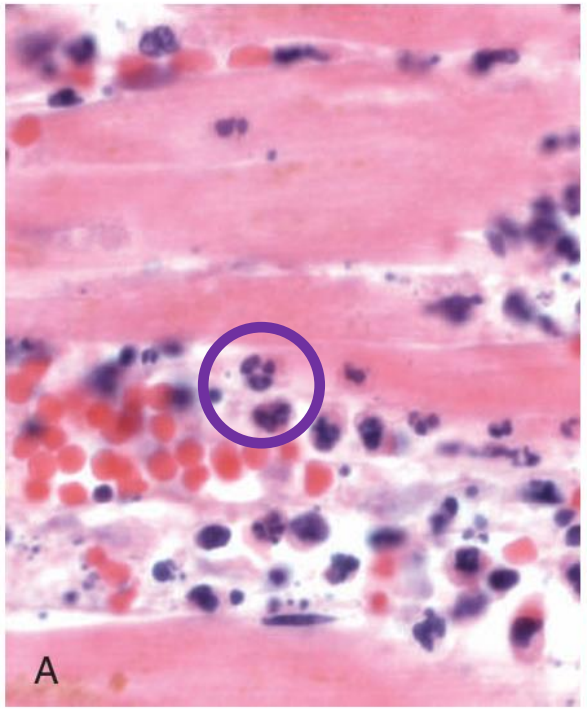
1. **Neutrophils** (Polymorphonuclear leukocytes – PMNs): finding Neutrophils means that there is an acute inflammation. Neutrophils are short living cells, which also means **recent** acute inflammation.
2. **Macrophages**, **lymphocytes** & **plasma cells**: they come after the first day and stay for days or weeks. So, they are the hallmark of chronic inflammation as they infiltrate in later stage.

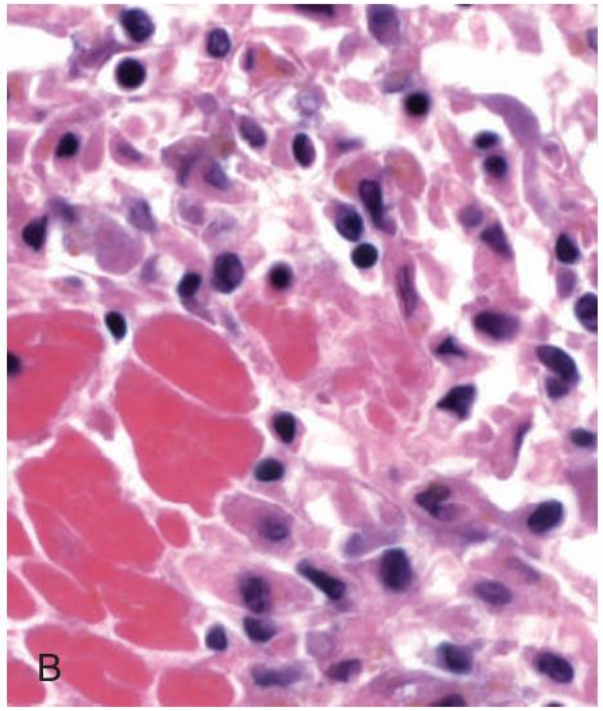
Neutrophils (PMNs)	6-24 hours, acute phase
Macrophages, lymphocytes & plasma cells	24-48 hours and then may stay
Allergic reactions	Eosinophils

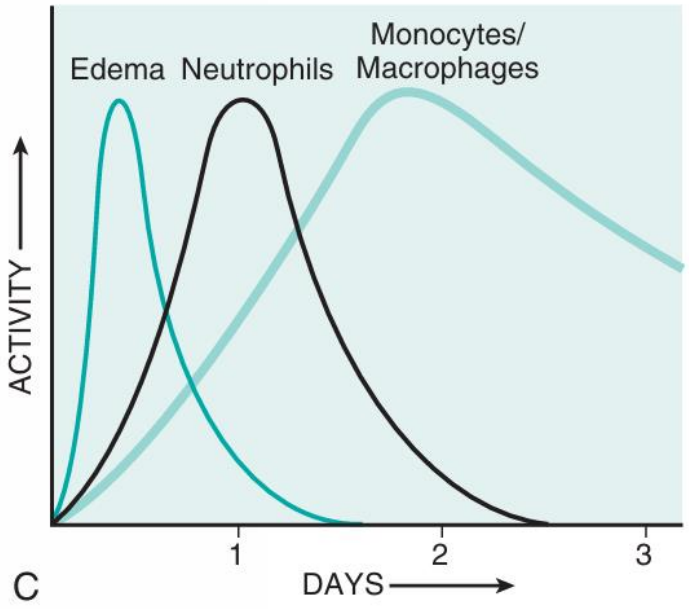
3. **Eosinophils**, now recognized as key players in allergic reactions, have been studied extensively in recent years. Eosinophilic inflammation is increasingly being considered a distinct subtype of inflammation, particularly associated with allergic conditions.

Skeletal muscle with a lot of neutrophils, which indicates acute inflammation phase

The neutrophils are followed by macrophages & lymphocytes, which indicates the chronic phase





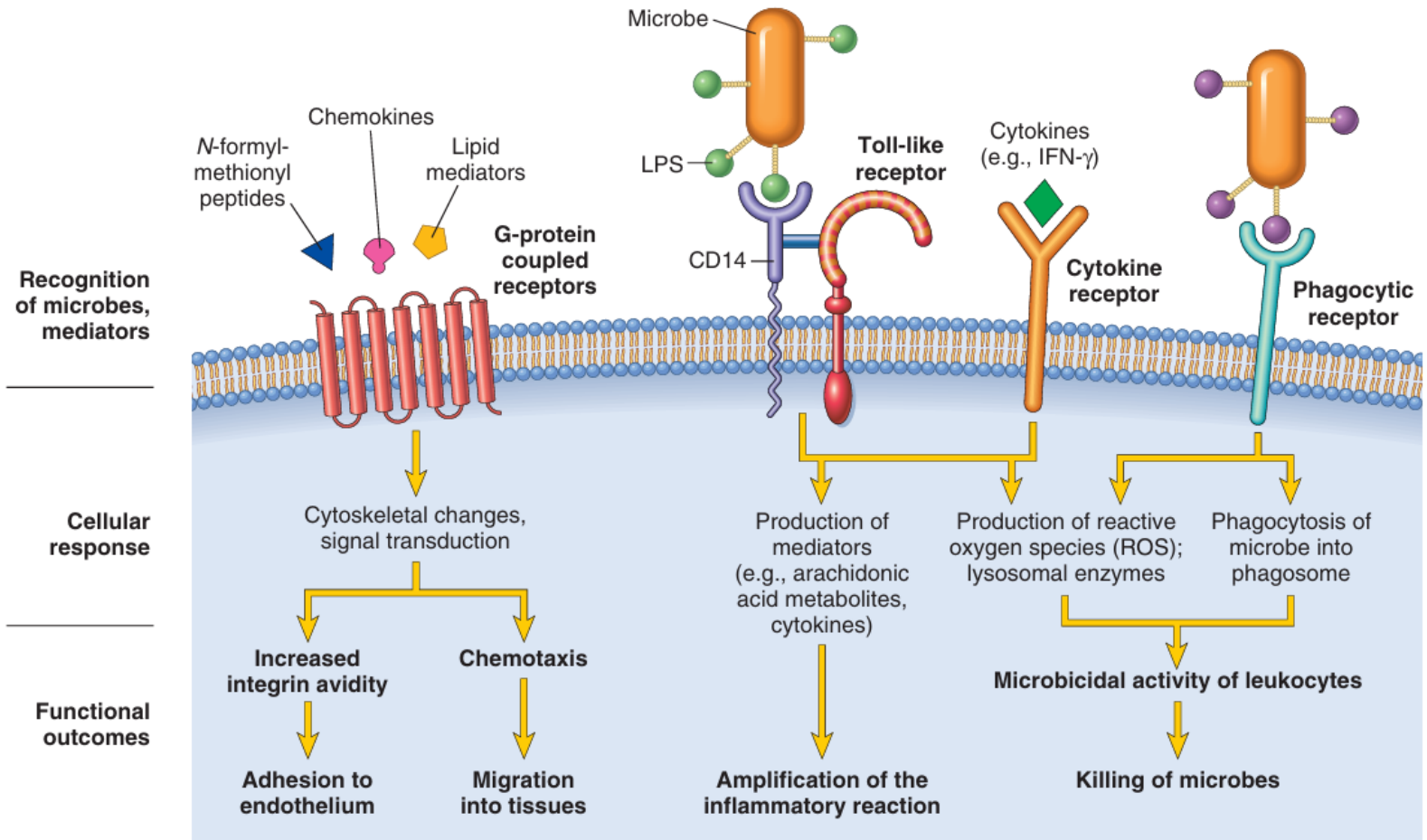


This diagram outlines the sequence of events in inflammation. Firstly, the vascular phase occurs with edema, which subsides within 1-1.5 days. This is followed by early neutrophilic infiltrations, which peak quickly and decline the second day. Finally, the chronic inflammatory cell appear taking a longer time to clear up.

This is very complex diagram. Postpone looking to it.

Dr. Mousa really said both statements 😳

The initial vascular phase is triggered by the recognition of microbes or injury by toll-like receptors, leading to a cascade of active changes and mediator release, including chemokines that stimulate chemotaxis. This process facilitates the movement of immune cells to the injury site, where they work to eliminate the microorganism. In this early phase, numerous chemical mediators are released from various sources to amplify the inflammatory response, enhancing the efficiency of microorganism elimination through active cellular processes like chemotaxis and cell adhesion.



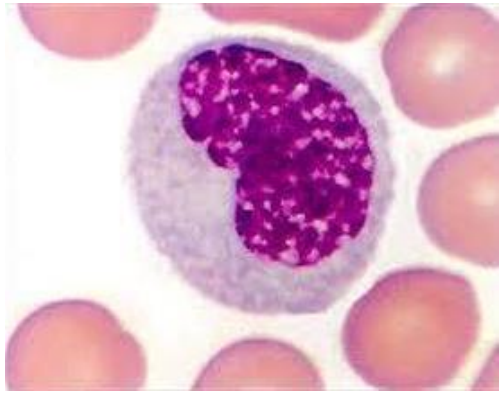
Leukocyte activation

It's an important function in the initial phase of inflammation.

The 2 major cells of the initial phase are neutrophils and macrophages.
(Macrophages ⇔ Monocytes)

Monocytes ➔ circulating in blood
Macrophages ➔ tissue resident

- **The function of both neutrophils & monocytes is** Phagocytosis and intracellular killing

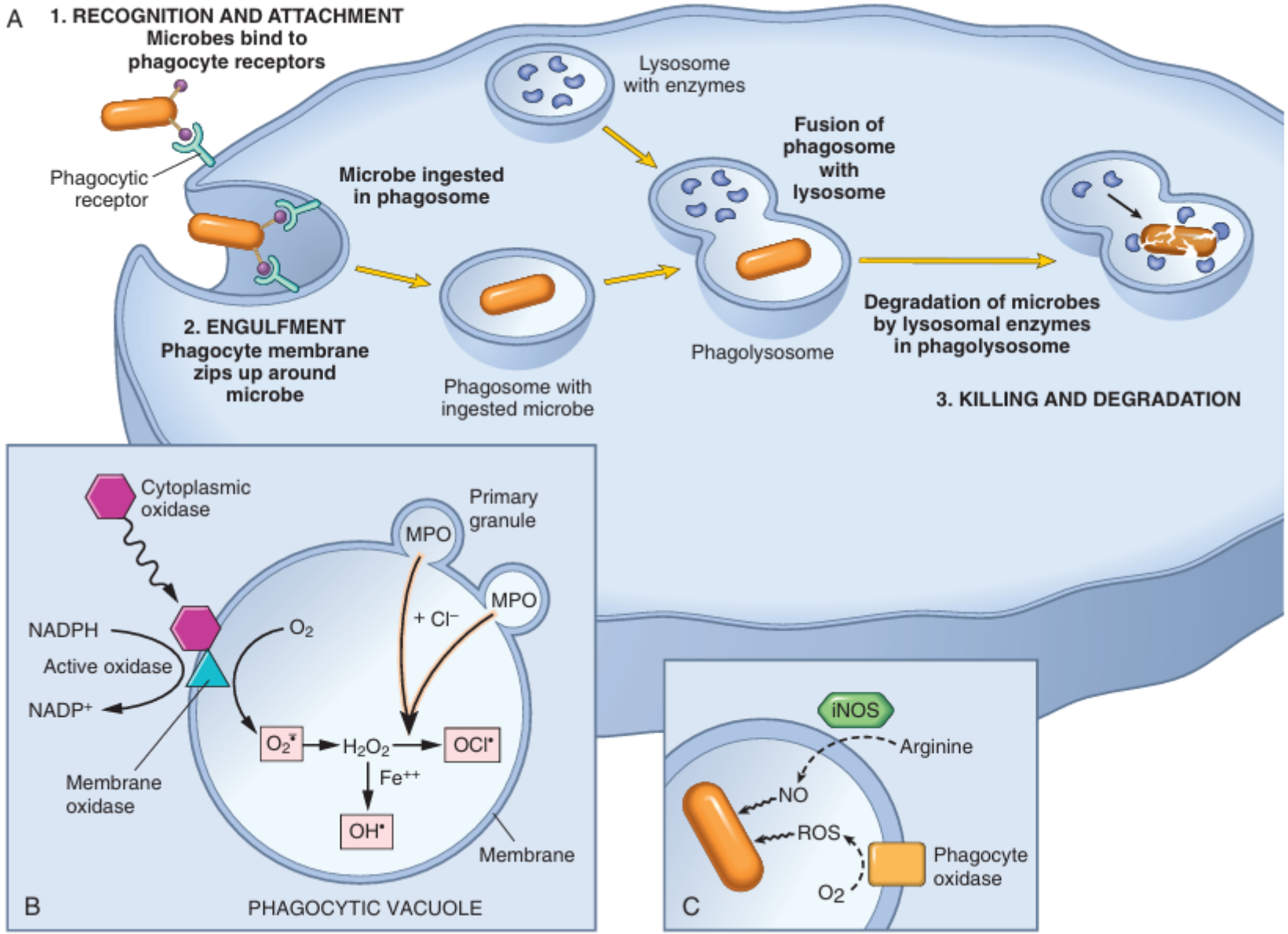


This is a monocyte in the blood with RBCs around it, kidney-shaped nucleus & less granules around the nucleus

This is the shape of a neutrophil, multiple nuclei & a lot of granules around the nucleus.

Phagocytosis A multistep process

1. Recognition and attachment of the enemy (the foreign agent): by specific receptors on the neutrophils and macrophages like (1) mannose receptors or (2) opsonins via opsonization (IgG (Immunoglobulins), C3b (Complement 3b))
2. Engulfment of the foreign agent into a vacuole forming phagocytic vacuole in the cytoplasm of the inflammatory cell (either neutrophil or macrophage) called “phagosome”.
3. Killing & degradation: its an active process through recruitment of reactive oxygen species (ROS), such as NO, H_2O_2, MPO-halide (Myeloperoxide halide) which is the most potent bactericidal system of neutrophils.


A
1. RECOGNITION AND ATTACHMENT
Microbes bind to phagocyte receptors
Phagocytic receptor
2. ENGULFMENT
Phagocyte membrane zips up around microbe
Microbe ingested in phagosome
Phagosome with ingested microbe
Lysosome with enzymes
Fusion of phagosome with lysosome
Phagolysosome
Degradation of microbes by lysosomal enzymes in phagolysosome
3. KILLING AND DEGRADATION
Cytoplasmic oxidase
NADPH
Active oxidase
NADP+
Membrane oxidase
Primary granule
MPO
MPO
+ Cl−
O2
O2•−
H2O2
OCl•
Fe++
OH•
Membrane
B
PHAGOCYTIC VACUOLE
iNOS
Arginine
NO
ROS
O2
Phagocyte oxidase
C

The figure's explanation

You may rewatch the lecture where the prof explained the figure, 16:00-18:25

The text above is hyperlinked, just press on it ☺

This cartoon illustrates phagocytosis and intracellular killing. It begins with a bacterium recognized and attached by specific receptors, like 'mannose receptors' sensing a foreign invader. This triggers an opening in the neutrophil or macrophage's membrane, enveloping the bacterium, virus, or foreign body within a phagosome.

1. **Recognition and Attachment**: The bacterium attaches to receptors on the neutrophil or macrophage.
2. **Phagosome Formation**: The cytoplasmic membrane surrounds the bacterium, forming a phagosome.
3. **Phagolysosome Formation**: Lysosomes fuse with the phagosome, forming the phagolysosome, where intracellular killing of the enemy occurs.
4. **Intracellular Killing and Degradation**: Inside this phagolysosome, chemicals, reactive oxygen species (ROS), nitric oxide, and myeloperoxidases digest and kill the organism inside.

- This process involves various granules and oxidases that generate oxygen radicals essential for intracellular killing, **following the sequence**: Recognition, Attachment, Phagosome Formation, Fusion, and Intracellular Killing.

Nitric Oxide (NO)

In the last 10-15 years, nitric oxide (NO) has attracted significant attention and research, resulting in a wealth of valuable knowledge.

Nitric Oxide is a:

- Soluble gas produced from Arginine (Amino Acid) by NO synthase (NOS)
- NOS 3 types: eNOS, nNOS, iNOS (Don't worry about their details)
- iNOS: Responsible for intracellular killing, stimulated by cytokines mainly IFN-γ
- NO reacts with superoxide (O2-*) to form ONOO* radical peroxynitrite

one of the strong reactive Oxygen radicals

iNOS: intracellular nitric oxide synthase
IFN-γ: interferon gamma

Interferons are cytokines, and there is a lot of research on them. Now interferons are being utilized as a target therapy for certain inflammatory autoimmune disease and certain cancer treatment.

The prof. advises us to read couple of review articles about the nitric oxide and its benefits, utilization for a better understanding of its involvement in carcinogenesis, atherosclerosis and inflammation.

Granule Enzymes

Both neutrophils and macrophages have granules, however neutrophil is the one who's more heavily granulated. There are polymorph neutrophils (nuclei) [PMNs] and there are two types of granules:

➔ The term "azurophil" was chosen due to the distinctive color produced by the dye reaction.

- Present in PMNs and monocytes
- In PMNs: 2 types; large azurophil (primary) and smaller (secondary) granules.
- Primary G: contain/produce → MPO, other enzymes → needed in the intracellular killing

Myeloperoxidase

- Secondary G: lysozyme, and others → They're utilized after the production of the phagolysosome in the later stages of phagocytosis
- These enzymes can be harmful to us if not properly controlled, so there are always checking and balances. As a result, they are usually neutralized by antiproteases.
- These are usually neutralized by anti proteases (such as ά-1 antitrypsin: inhibits elastase)… if deficiency in anti proteases … then diseases occur

In certain diseases, particularly alpha-1 antitrypsin deficiency which affects the GI tract, the lack of inhibition (no inhibition) of lysozymes and other enzymes released by neutrophils and macrophages can lead to cell injury and chronic disease development.

Neutrophile extracellular traps (NETs)

- "Very thick" viscous meshwork of nuclear chromatin binds to peptides and anti-microbial agents after PMN death (NETosis)

  After a neutrophil dies and ruptures, its chromatin material, along with intracytoplasmic and nuclear materials, forms a thick, viscous meshwork. This meshwork helps trap bacteria or other invaders at the injury site, allowing still-active (viable) neutrophils to target and eliminate them.

- Sepsis

  Recently, it was discovered that neutrophil extracellular traps (NETs) play a major role in the pathogenesis of sepsis, though more details are still emerging.

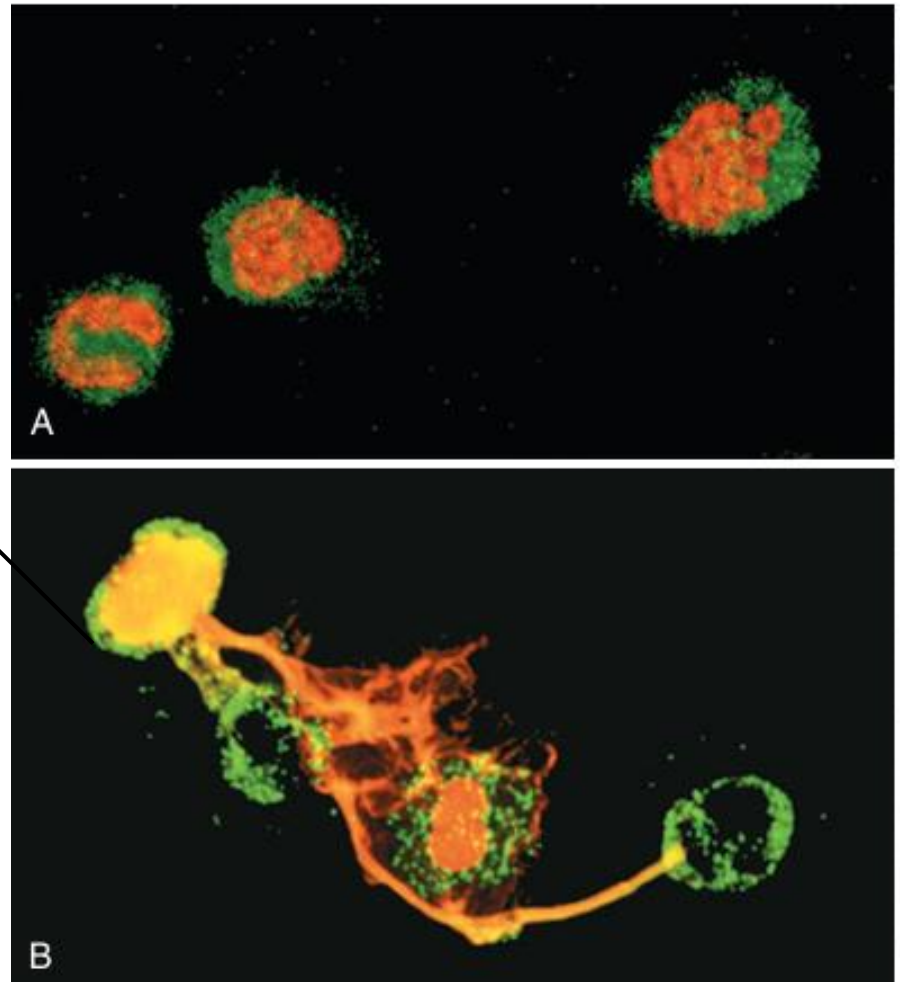
- Maybe involved in SLE

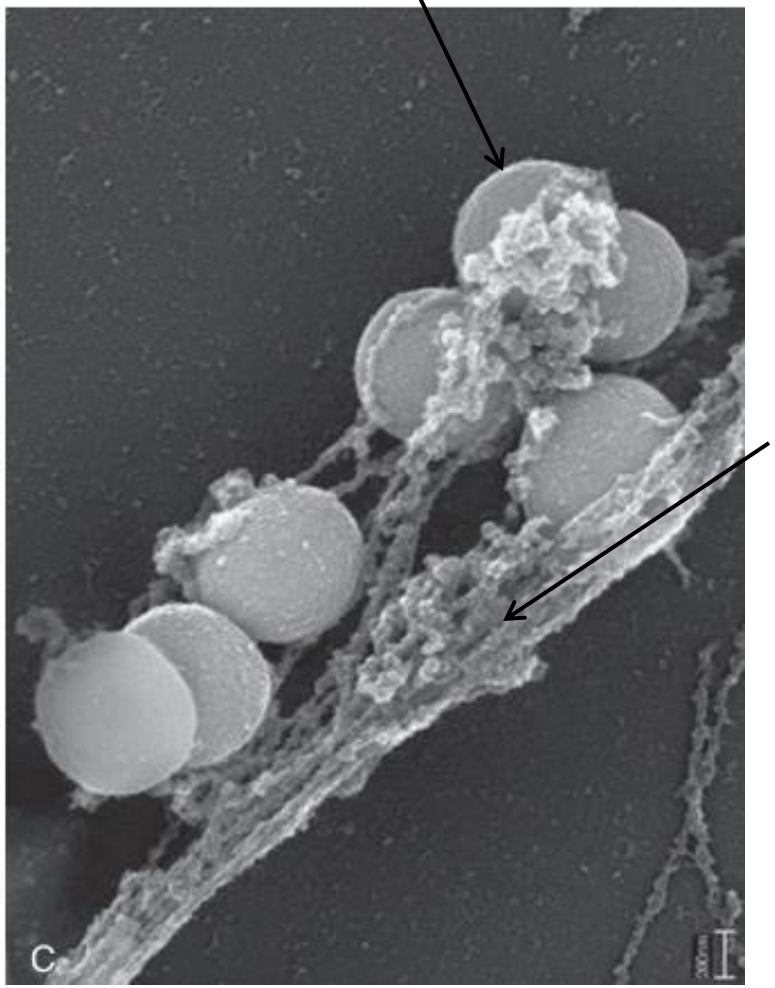
  NETs have also been implicated in an autoimmune disease called Systemic Lupus Erythematosus (SLE) (مرض التذائب الاحمراري), which primarily affects young females. This multisystem disease causes a rash on the cheeks and can affect the kidneys, heart, skin, joints, and other organs.

High scanning picture explaining those thick viscous material

Each one is a bacterial cocci

Colored picture of the same process





This is the nets of neutrophil and they stick to those organism so these organism remain in this area and will allow other inflammatory cells to come and kill them.

Leukocyte-mediated tissue injury

Although our body relies on white blood cells, such as macrophages and neutrophils, as part of our defense mechanism to eliminate harmful organisms, their activity can sometimes cause tissue injury related to the infiltration of leukocytes in tissue. Excessive immune response can lead to some injury. Each inflammatory process has the potential to leave some side effects or cause tissue injury; there are 3 mechanisms:

1. Prolonged inflammation (TB and Hepatitis)
   - Tuberculosis or mycobacterium tuberculosis is very virulent & strong organism can induce prolonged inflammation, causing tissue damage & Injury.
   - The other example is in certain types of hepatitis due to virulent virus like chronic hepatitis C infection which is the most common cause of chronic Liver disease nowadays in middle east from this prolonged inflammation

2. Inappropriate inflammatory response (auto-immune diseases)
   - A second mechanism by which leukocytes can induce tissue injury occurs when there is an inappropriate inflammatory response, which is a fundamental concept of autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis, and Mixed Connective Tissue Disease. The underlying mechanisms of these diseases are still being studied, but it is understood that the body's inflammatory response becomes excessively exaggerated, leading to damage of the body's own tissues.

3. Exaggerated response (asthma and allergic reactions)
   - The third mechanism involves an inflammatory response that is disproportionate or exaggerated in reaction to the antigenic stimuli. This is the basic mechanism in acute allergic reactions and bronchial asthma, where minor triggers like a simple flu, cold, or stress can lead to exaggerated allergic response, causing symptoms and disease.

These three mechanisms highlight how tissue injury and disease can occur due to inflammatory cell damage, indicating that although inflammation usually protects us, it can also be harmful and lead to disease in certain situations.

You're close

Other functions of activated WBCs

1. Amplify or limit reaction (cytokines)

- As we always need WBCs to be recruited to the site of injury, there are additional roles carried out by these WBCs. One of these is to either amplify or, alternatively, limit the reaction which are functions of cytokines. Cytokines can amplify the inflammatory reaction if needed. For example, in the early phases of inflammation, we usually need more cytokines to enhance the inflammatory process. This is when more WBCs –more chemotaxis– are recruited, as there may be many bacteria present. In this case, the white blood cells release cytokines to enhance and amplify the reaction.
- However, once most of the bacteria (or "enemies") are eliminated, phagocytosed and killed by reactive oxygen species (ROS) inside macrophages, additional immune cells are no longer needed. Cytokines are then released to limit, contain, and eventually terminate the inflammatory reaction.

2. Growth factors secretion (repair)

- Another major function of white blood cells is the secretion of growth factors, which play a crucial role in the later phases of inflammation when repair begins. These growth factors, released by white blood cells, initiate and support the repair process.

3. T-lymphocytes has also a role in acute inflammation (T-HELPER-17); produce cytokine IL-17 (deficiency cause disease)

The third function involves T-lymphocytes. However, it was recently discovered that a specific type of T-cell, known as the T-helper or CD4 T-cell (cluster designation 4 indicates the T-lymphocytes), plays a crucial role in acute inflammation. T-helper 17 cells (Th17) are now known to be key players in this process. Contrary to earlier beliefs that T-lymphocytes were not involved in acute inflammation, we now know that Th17 cells contribute significantly in acute inflammation by producing a cytokine called interleukin-17. A deficiency in interleukin-17 can lead to certain diseases characterized by weakened immunity.

These are three major additional functions of activated white blood cells at the site of injury, in addition to their previously mentioned roles, including recognition, phagocytosis, and intracellular killing.

Termination of acute IR

Mediators are produced in rapid bursts
Release is stimulus dependent
Short half-lives
Degradation after release
PMNs short life (apoptosis)
Stop signals production (TGF-ß, IL-10)
Neural inhibitors (cholinergic): inhibits TNF

The last slide

After we have eliminated most of the organisms—bacteria, viruses, or other pathogens—within the first few days of inflammation, it is essential to terminate the acute inflammatory response. We don't want this response to continue unnecessarily, as the enzymes and mediators involved can harm the surrounding tissue. Therefore, the next phase focuses on stopping, controlling, and ending the acute inflammatory reaction. This table outlines the main mechanisms by which our body controls and resolves the inflammatory response.
- We will discuss each of these mechanisms in detail in the next lecture.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	6	**Macrophages**, **lymphocytes** & **plasma cells**: they come after the first day and stay for days or weeks. So, they are the hallmark of acute inflammation as they infiltrate in later stage.	**Macrophages**, **lymphocytes** & **plasma cells**: they come after the first day and stay for days or weeks. So, they are the hallmark of chronic inflammation as they infiltrate in later stage.
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

تذكروا أن مهمتكم ليست ورقةً تنالونها، وإنّما أمةً تحيونها

شكوت الى وكيعٍ سوء حفظي
فأرشدني الى ترك المعاصي
وأخبرني بأن العلم نورٌ
ونور الله لا يؤتى لعاصي

Reference Used:
(numbered in order as cited in the text)

1. Robbin's Basic Pathology 11th ed, Chapter 2, pg. 30 - 33

Extra References for the Reader to Use:

1. Phagocytosis by PhysioPathoPharmaco

With acknowledgment to ChatGPT, the best AI in the world <3