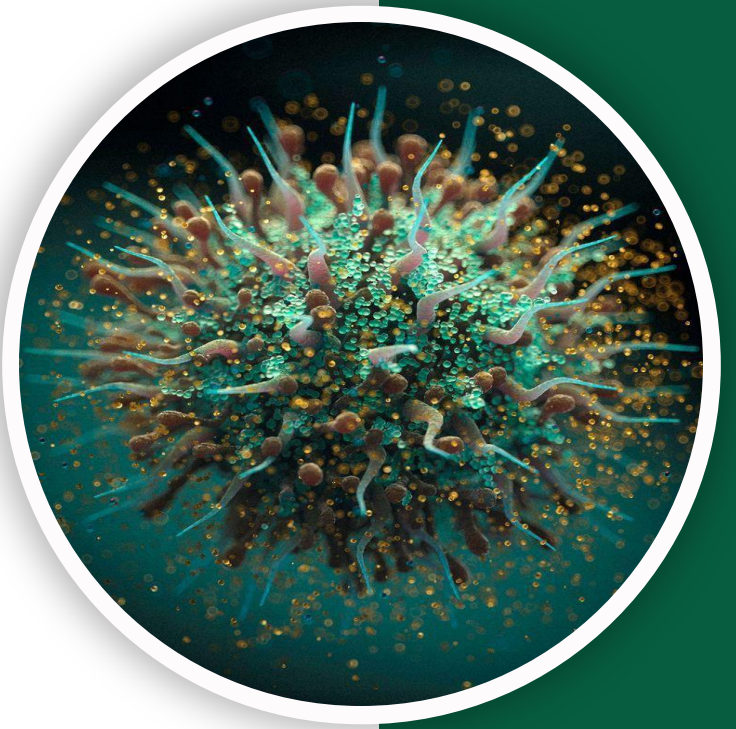
بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Pathology | Lecture 8

Inflammation (Pt.3)



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Reviewed by : NST

In the last lecture, we talked about the **initial vascular phase**:

1. **Vascular dilatation** caused by **histamine**.
2. **Increase** in the **vascular permeability.**
3. **Movement** of the inflammatory cells (mainly neutrophils &macrophages) from the inside of the blood vessels to the outside:

Margination -> Rolling -> Selectins (weak) -> Integrins (strong).

- **Active transmigration (diapedesis)** happens through CD31 (PECAM-1), a molecule found on both leukocytes & endothelial cells.
- **Note: Transmigration** occurs during the **cellular phase** of acute inflammation, **not the vascular phase.**

PECAM-1: **P**latelet **E**ndothelial **C**ell **A**dhesion **M**olecule.

- WBCs pass through the blood vessel wall by piercing the basement membrane with enzymes such as **collagenases**.

Chemotaxis

The suffix **"itis"** means **inflammation,** for example; **tonsillitis**: inflammation in the tonsils.

- **Chemotaxis** is the movement of WBCs to injury tissue site.

  For example, if there was an injury in the tonsils (**tonsillitis**), there will be a movement of WBCs (chemotaxis) toward the tonsils.

- Chemotaxis is an active process induced by **Chemoattractants** (mediators) (exogenous & endogenous)

Produced **outside** the body.

Produced **inside** the body.

Major Types of Chemoattractants:

1. **Bacterial Products:** The N-terminal peptides of bacterial products act as powerful chemoattractants that attract white blood cells to infection sites.

2. **Cytokines:** This large group of signaling molecules is mainly released by inflammatory cells such as lymphocytes and macrophages. The chemokine family, a subgroup of cytokines, is especially known for its strong chemoattractant effects.

3. **Complement System:** Among plasma proteins, C5a (a component of the complement system) is the most potent chemoattractant.

4. **Lipoxygenase Pathway (Arachidonic Acid Metabolites):** These metabolites, formed from arachidonic acid in cell membranes, include LTB4 (leukotriene B4) – the strongest chemoattractant among arachidonic acid derivatives.

Group:	Example:
Bacterial Products	Peptides (N-terminal)
Cytokines	Chemokine family
Complement system	C5a
Lipoxygenase pathway AA metabolites	LTB4

WBCs infiltrates in tissue:

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

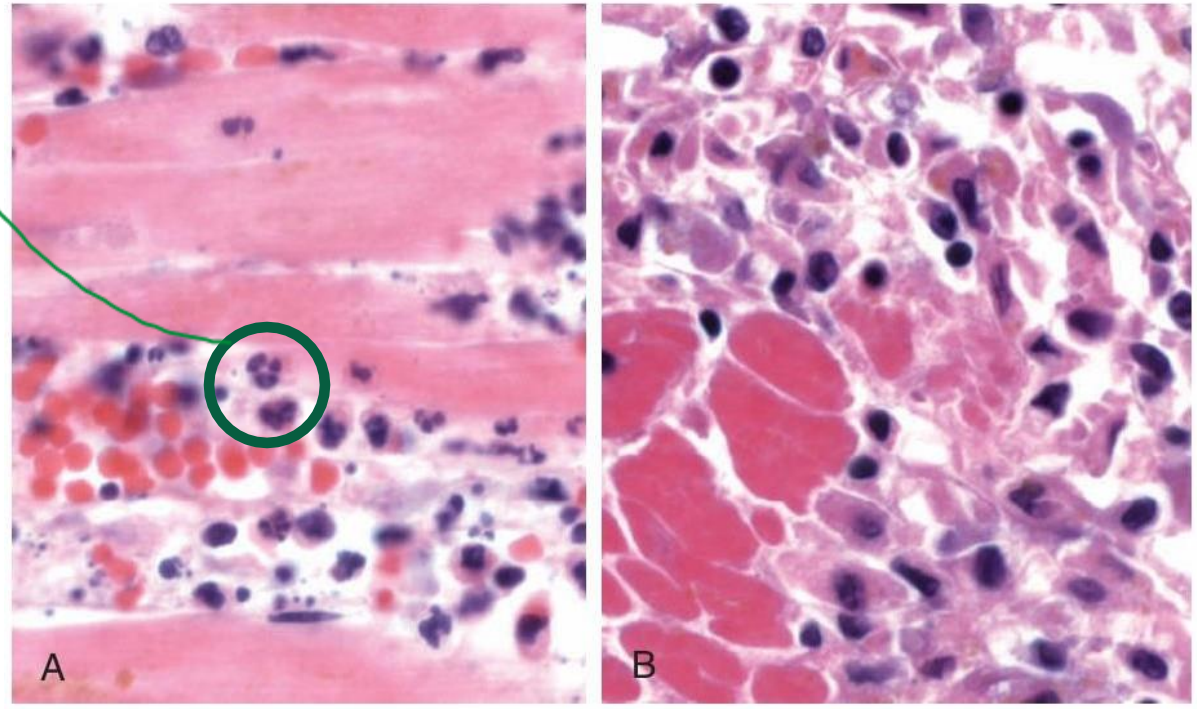
Neutrophils (PMNs)	6-24 hours, acute phase
Macrophages, lymphocytes & plasma cells	24-48 hours and then may stay
Allergic reactions	Eosinophils

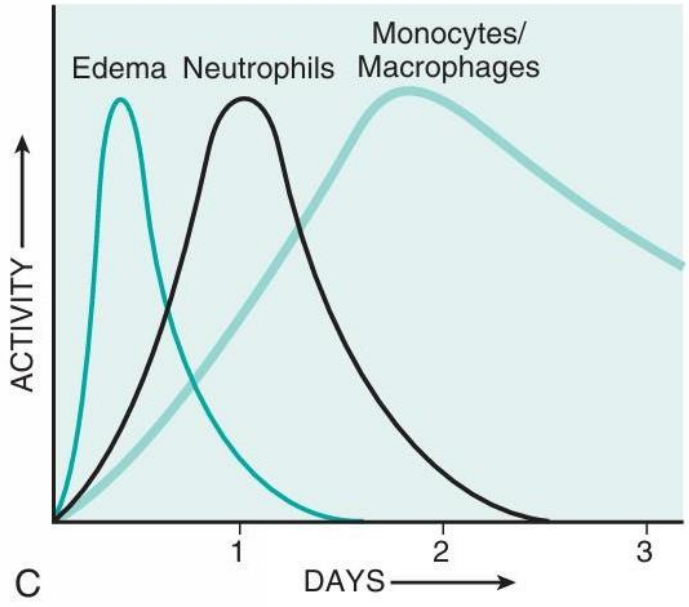
Pathologists may receive surgically removed organs (such as the appendix or tonsils) to examine and stain them in order to **determine the type of inflammation**; so if we see:

1. **Neutrophils (Polymorphonuclear leukocytes – PMNs):** The presence of neutrophils indicates **acute inflammation.** Since neutrophils are short-lived cells, their appearance means the inflammation is recent and active.
2. **Macrophages, Lymphocytes, and Plasma Cells:** These cells arrive after the first day of inflammation and may remain for days or weeks. Their presence is a sign of **chronic inflammation**, as they appear in the later stages of the inflammatory process.
3. **Eosinophils:** Eosinophilic inflammation is now recognized as a special type of inflammation mainly linked to **allergic conditions**.

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

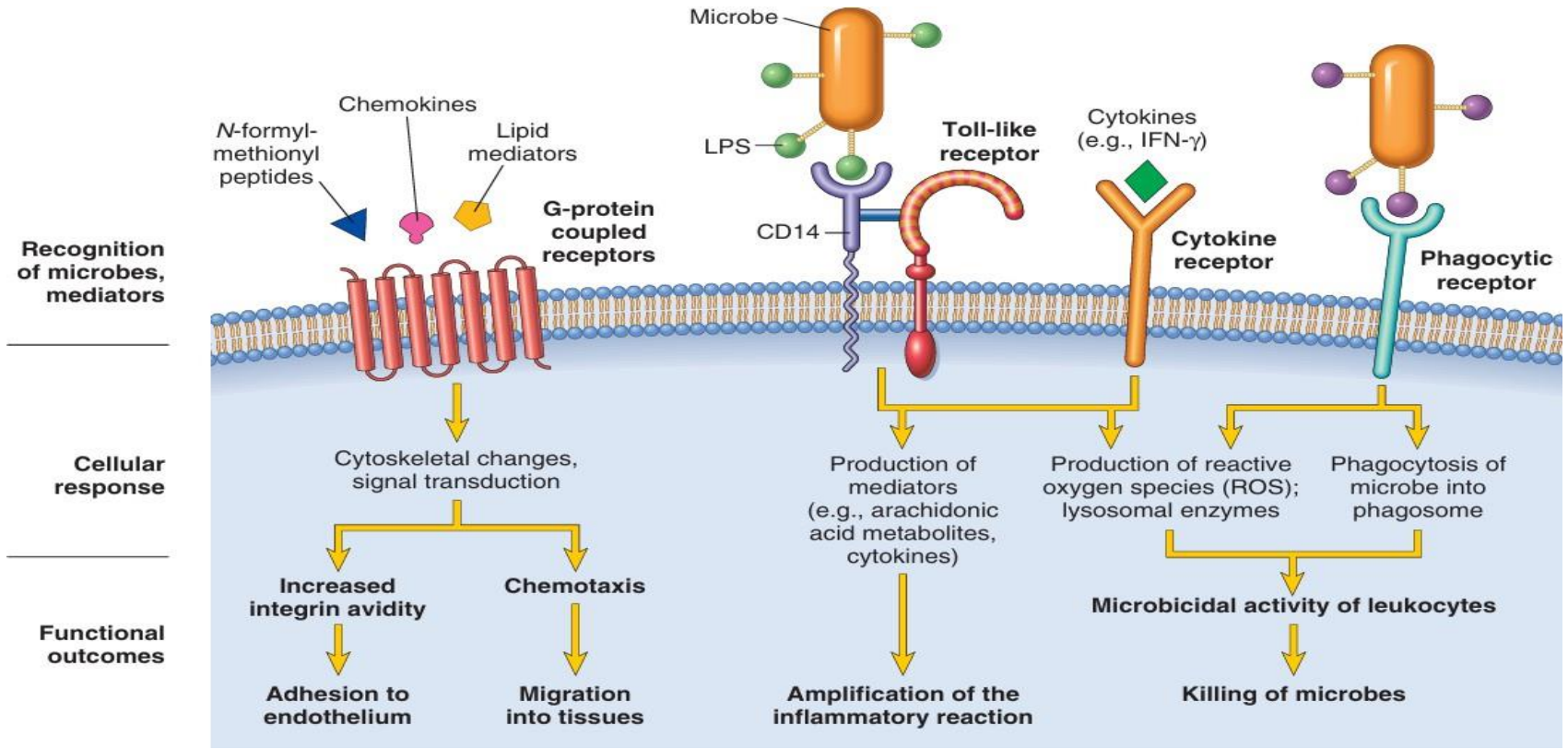
The neutrophils are followed by **macrophages & lymphocytes**, which indicates the **chronic phase**.





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.

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 **mediators 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.**



Don’t study this diagram now

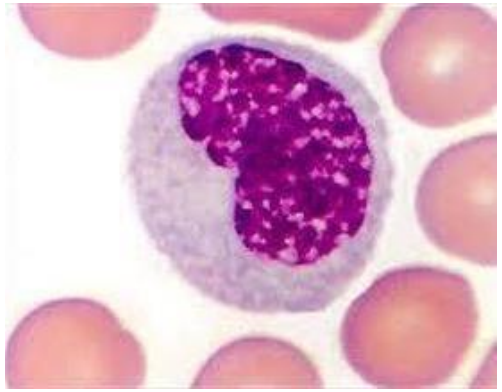
Leukocytes activation is important in the initial phase of inflammation.

Leukocyte activation

The 2 major cells of the initial phase are **neutrophils and macrophages.** (Macrophages <-> Monocytes)

Monocytes ➔ circulating in blood
Macrophages ➔ tissue resident

- **Phagocytosis and intracellular killing**
  (main functions of both neutrophils and monocytes).



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.

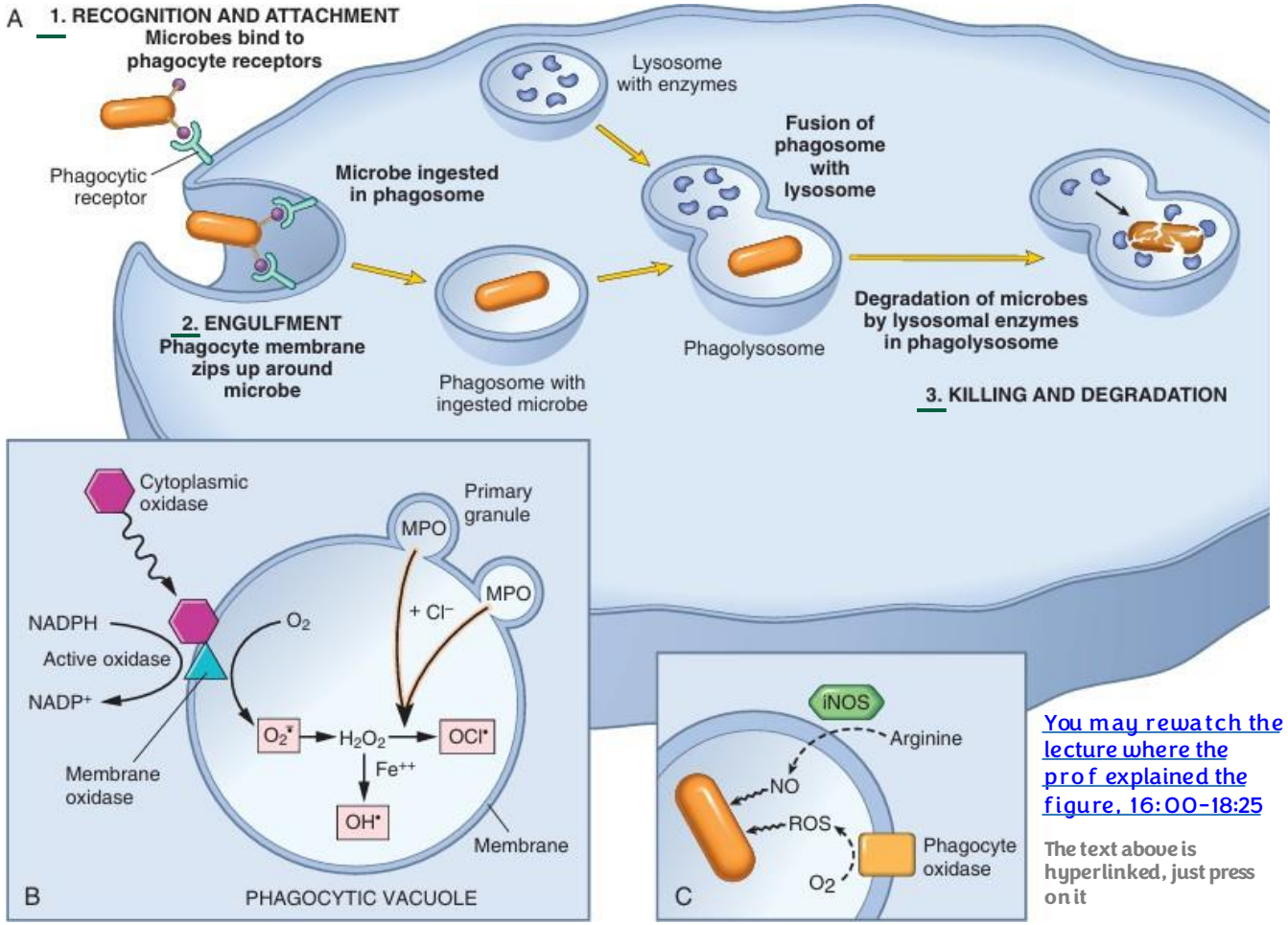
Phagocytosis:

A multistep process that consists of several stages, which are:

1. **Recognition and attachment** of the enemy (the foreign agent) by specific receptors on the neutrophils and macrophages, like:
   (a) mannose receptors
   (b) opsonins (surface receptors) via opsonization
   (examples of opsonins: IgG (Immunoglobulins) , C3b (Complement 3b))
2. **Engulfment** of the foreign agent forming phagocytic vacuole called "phagosome" in the cytoplasm of the inflammatory cell (either neutrophil or macrophage).
3. **Killing & degradation**: it's 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.

The figure's explanation

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.

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

The text above is hyperlinked, just press on it

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.

- It is a Soluble gas produced from Arginine (Amino Acid) by NO synthase (NOS)
- There are 3 types of NOS: eNOS, nNOS, iNOS (Don't worry about their details)
- iNOS is 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 diseases and certain cancer treatments

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

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

- cytoplasmic granules are present in both PMNs (polymorphonuclear leukocytes/Neutrophils) and monocytes/macrophages
  - ➢ however, neutrophils are more densely granulated, and they contain two main types of granules:
- In PMNs: 2 types of granules; **large azurophil (primary)** and **smaller (secondary)**.
- Primary G: they contain/produce MPO, and other enzymes which are needed in the intracellular killing
- Secondary G: they have lysozymes, and other enzymes, They become active after the formation of the phagolysosome, functioning during the later stages of phagocytosis.

➢ These enzymes can be harmful to the body if not properly controlled, so regulatory checks and balances are always in place to limit their activity. **SO** These are usually neutralized by anti proteases (such as ά-1 antitrypsin which inhibits elastase)… if there is deficiency in anti proteases ➔ 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)

- A "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.

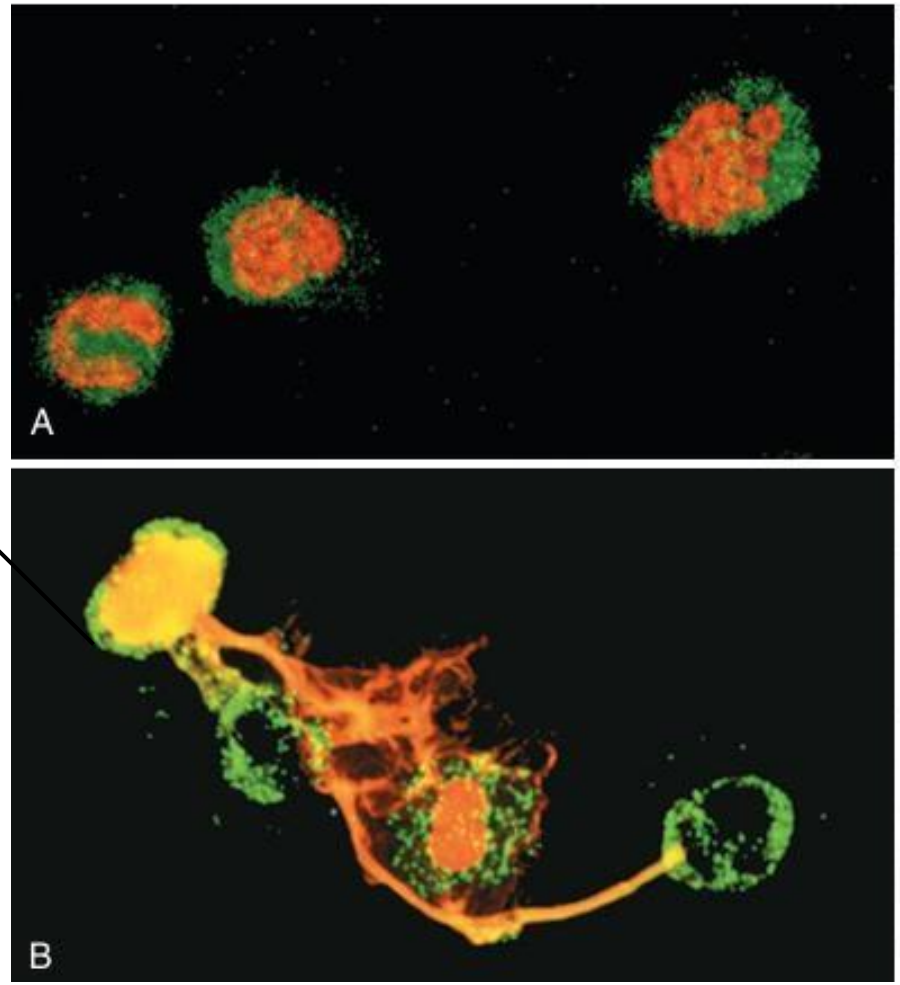
- Maybe involved in SLE (Systemic Lupus Erythematosus (SLE) / (التذاؤب الاحمراري))

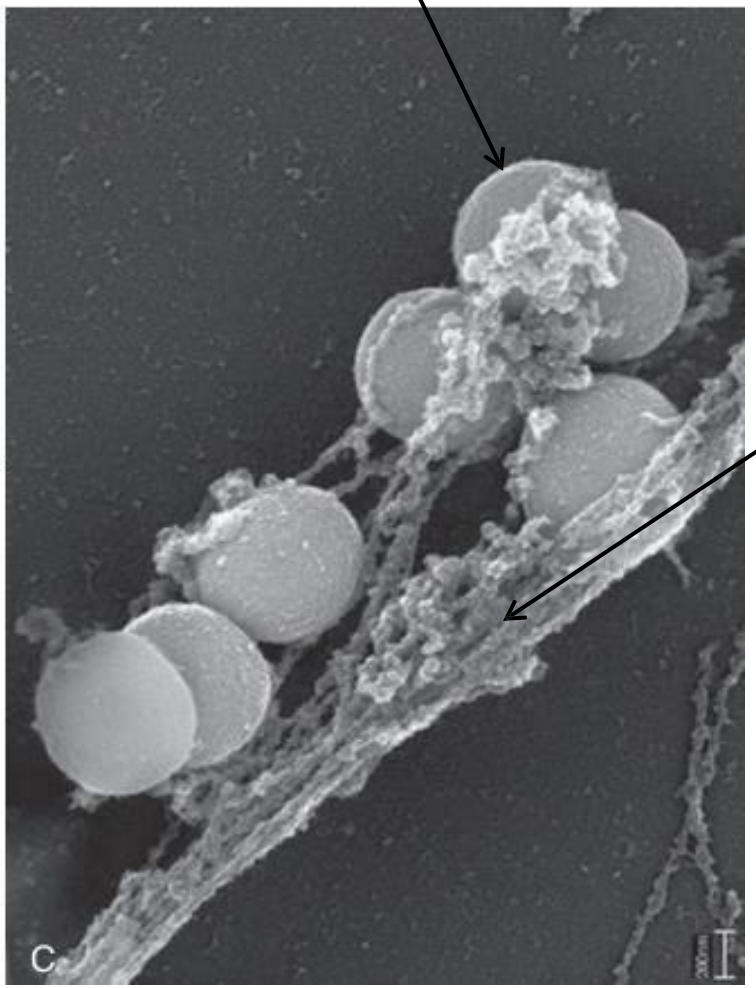
  NETs have also been implicated in an autoimmune disease called SLE which primarily affects young females. This multisystem disease causes a rash on the cheeks and can affect the kidneys, heart, skin, joints, and other organs.

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, they sometimes can cause tissue injury. Excessive immune response can also lead to tissue harm. For example, in some infections the pathogens evade some side effects or cause prolonged inflammation that harms the tissue.

1. Prolonged inflammation (TB and Hepatitis)

Tuberculosis or mycobacterium tuberculosis and virulent & strong organisms can induce prolonged inflammation, causing severe damage in tissue.

The other example is certain types of Hepatitis due to virulent virus like chronic hepatitis C infection which is the most common cause of chronic Liver disease nowadays & induce 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 reaction)

- 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 through cytokines. Cytokines act to amplify the inflammatory reaction as needed. For example, in the process of amplifying inflammation, more WBCs are recruited to enhance the inflammatory reaction.

However, once the bacteria (or "enemies") are eliminated, phagocytosed and tightly resolved, cytokines stop recruiting macrophages; additional immune cells are no longer needed. Cytokines then limit the reaction (not to continue), and eventually terminate it.

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 process of inflammation when repair happens. 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.

Additional Resources:

رسالة من الفريق العلمي:

remember to always
give your best in everything.

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			
V1 → V2			