



Cihan University/ Sulaymaniyah

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Clinical Immunology

Lecture- 3: Internal Defense System

Inflammation & Phagocytosis

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Inflammation

- Inflammation is a tissue reaction that delivers mediators of host defense; circulating cells and proteins to sites of infection and tissue damage.
- Activate the body ‘alarm’ using chemicals.
- Biological response of the immune system that can be triggered by a variety of factors:
 1. Pathogens,
 2. Damaged cells,
 3. Toxic compounds,



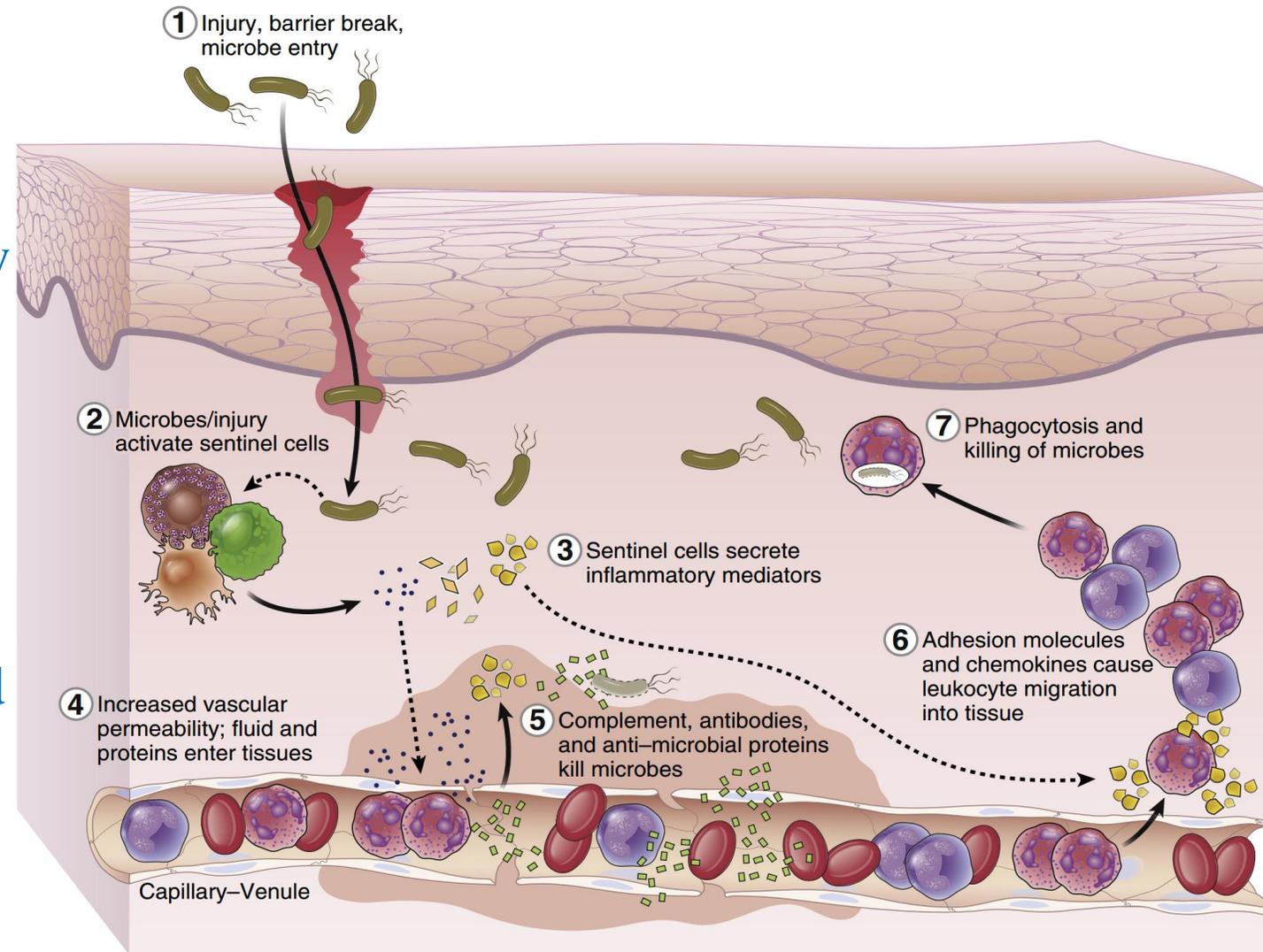


Inflammation has two Main Components

1. The **cellular component** involves the **movement of white blood cells** from blood vessels into the inflamed tissue. extravasate (filter out) phagocytes, picking up bacteria and cellular debris.
2. The **exudative component** involves the **movement of fluid**, usually containing many important proteins such as fibrin and immunoglobulins (antibodies).

Acute Inflammatory Response

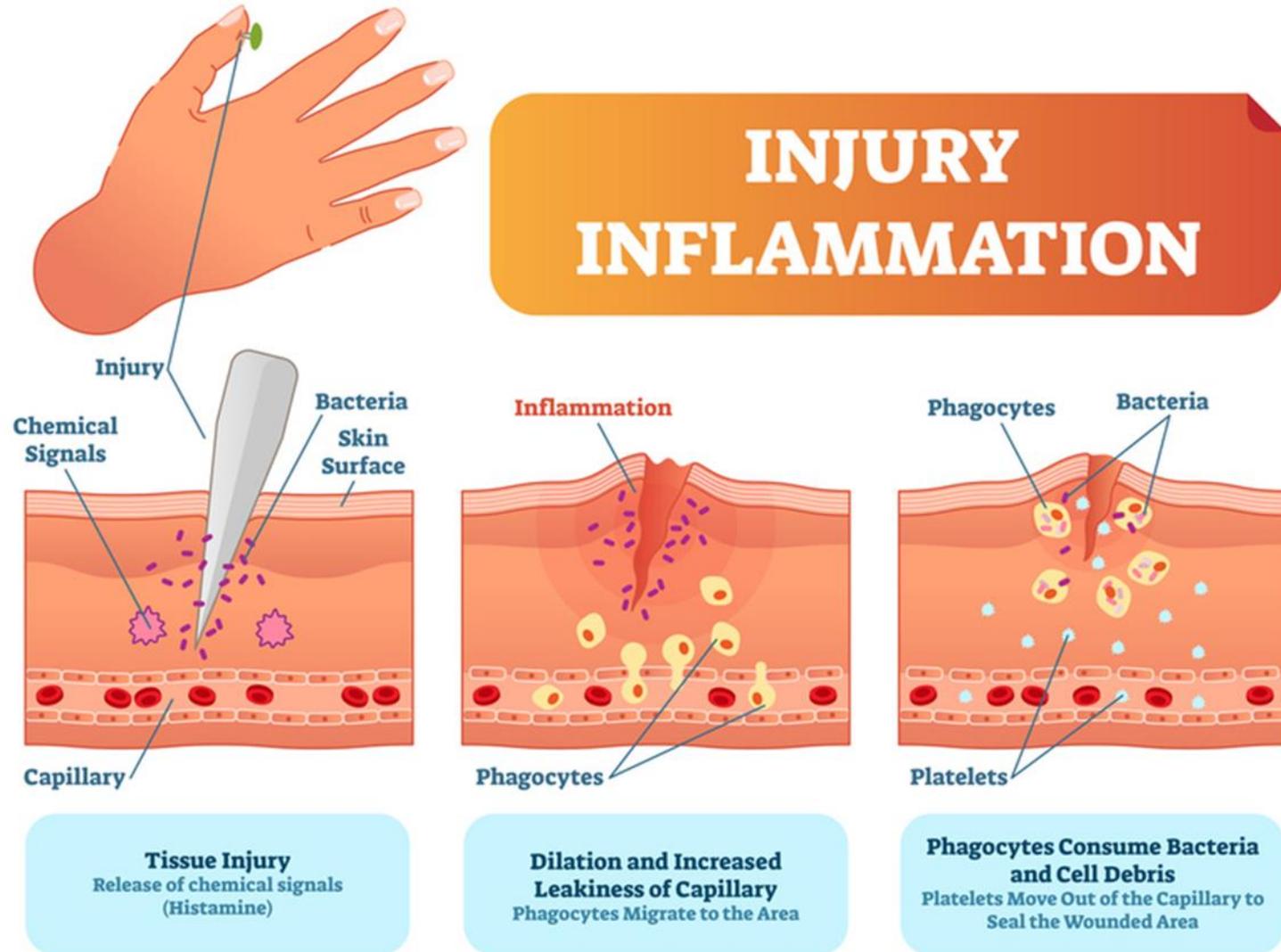
- The initial release of **histamine**, **TNF**, **prostaglandins**, and **other mediators** by **mast cells** and **macrophages** causes an increase in local blood flow and exudation of plasma proteins.
- These contribute to redness, warmth, and swelling, which are characteristic features of inflammation.



What are the Steps of the Inflammatory Response?

5 R's

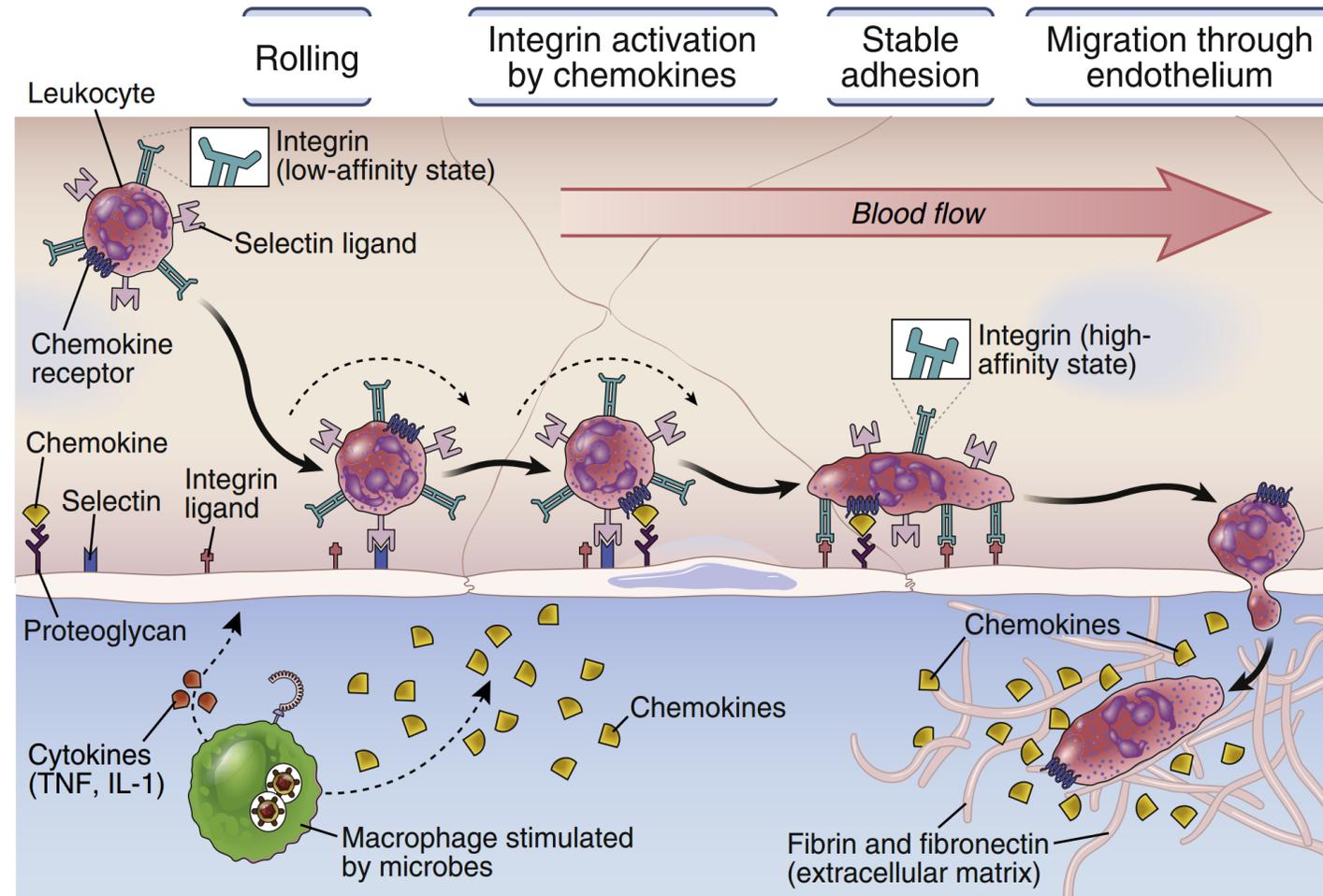
1. Recognition of the injurious agent.
2. Recruitment of leukocytes.
3. Removal of the agent.
4. Regulation (control) of the response.
5. Resolution (restoration of tissue integrity and function).



Margination: Movement of Blood Leukocytes to Sites of Infection

At sites of infection:

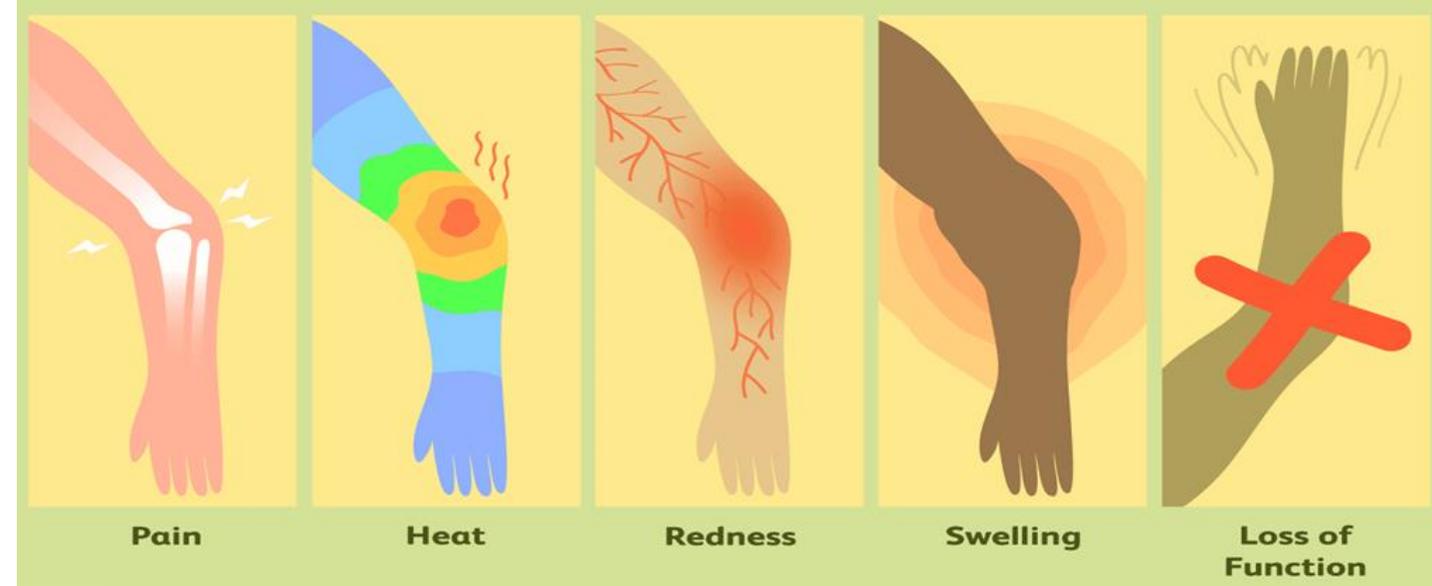
1. Macrophages, dendritic cells, produce cytokines such as **tumor necrosis factor (TNF)** and **interleukin-1 (IL-1)**.
 - This cytokines activate the endothelial cells of nearby venules to **express selectins and ligands for integrins** and to secrete chemokines.
2. **Selectins** mediate weak tethering and rolling of blood neutrophils on the endothelium,
3. **Integrins** mediate firm adhesion of neutrophils.
4. **Blood monocytes** and **activated T lymphocytes** use the same mechanisms to migrate to sites of infection.



Cardinal Signs of Inflammation

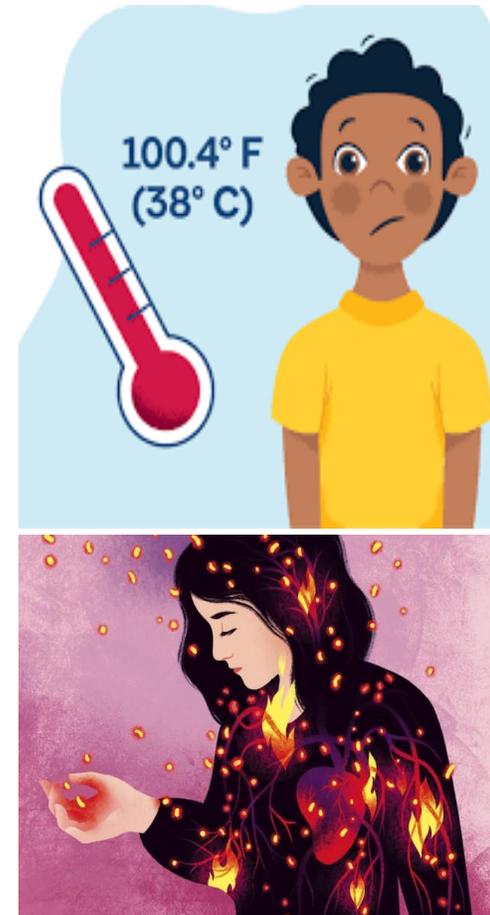
1. Redness (Rubor)- raised blood flow.
2. Swelling (Tumor)– leakage of fluids to the tissue.
3. Heat (Calor) – raised blood flow, leakage of fluids to the tissue, release of inflammatory mediators.
4. Pain (Dolor)– stimulation of pain receptors by inflammatory mediators, injury of nerve fibres, and irritation by toxic chemicals released by microbes.

- **To some extent, loss of tissue function, pain, disruption of tissue structure, fibroplasia, metaplasia.**



Fever

- If the WBCs are overrun by the invaders, such in case of major injury, highly virulence pathogens.
- Immune cells will release **pyrogens chemicals** – will stimulate hypothalamus to increase the body temperature.
- Systemic fever is like a '**fire blast**' will burn everything giving time for our more sophisticated weapon, **adaptive immune response**.
- Higher than normal body temperature in fever response **inhibits the growth of pathogens**.





Benefits of the Inflammatory Response

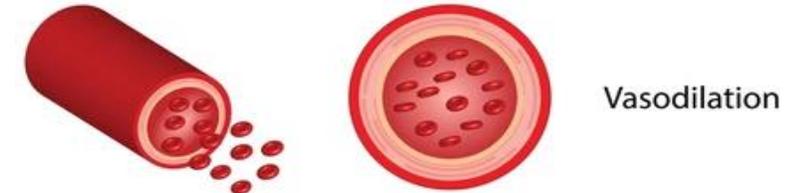
- Protective Role:
 1. Prevents the spread of damaging agents to nearby tissues,
 2. Clearance of foreign or damaged material,
 3. Encourages repair,
- The downside of the inflammatory reaction is that it is nonspecific and the inflammatory compounds released are also harmful to the host. Because of this, inflammation is under tight regulatory control in the body.

The Crucial Roles of Inflammatory Mediators

- Vasoactive substances (Inflammatory Mediators) are comprised of a chemically heterogeneous group, proteins, peptides, lipids, and nucleosides, affecting vascular tone and leading to either vasodilation or vasoconstriction.

A- Vasodilators:

- Histamine** - stimulate vasodilation and increased capillary permeability.
- Kinins** - this causes vasodilation and increased permeability.
- Prostaglandins** - increases vasodilation, increases vascular permeability, serves as a chemoattractant for neutrophils.
- Leukotrienes** - increase smooth muscle contraction, serve as a chemoattractant for neutrophils.
- Nitric oxide** - dilates blood vessels, raising blood supply and lowering blood pressure.



B- Vasoconstrictors:

- Endothelin-1 (ET-1)** is a potent vasoconstrictive peptide that plays a crucial role in vascular damage.



Introduction of Phagocytosis

- Phagocytosis; is a type of endocytosis and an important innate defense mechanism, by which extracellular particulate materials, such as **invading microbes**, **foreign particles**, **cancerous** or **diseased cells** and **cellular debris** are ingested by a cell and destroyed.
- **Neutrophils** (also called polymorphonuclear cells: PMNs), **blood monocytes** and **tissue macrophages** (also known as mononuclear phagocyte system) are the most important phagocytic cells.
- The killing of microbes is a critical function of phagocyte that is performed either **within the phagocyte called intracellular killing with include oxygen dependent and oxygen independent** or **outside of the phagocyte called extracellular killing**.

Phases of Phagocytosis

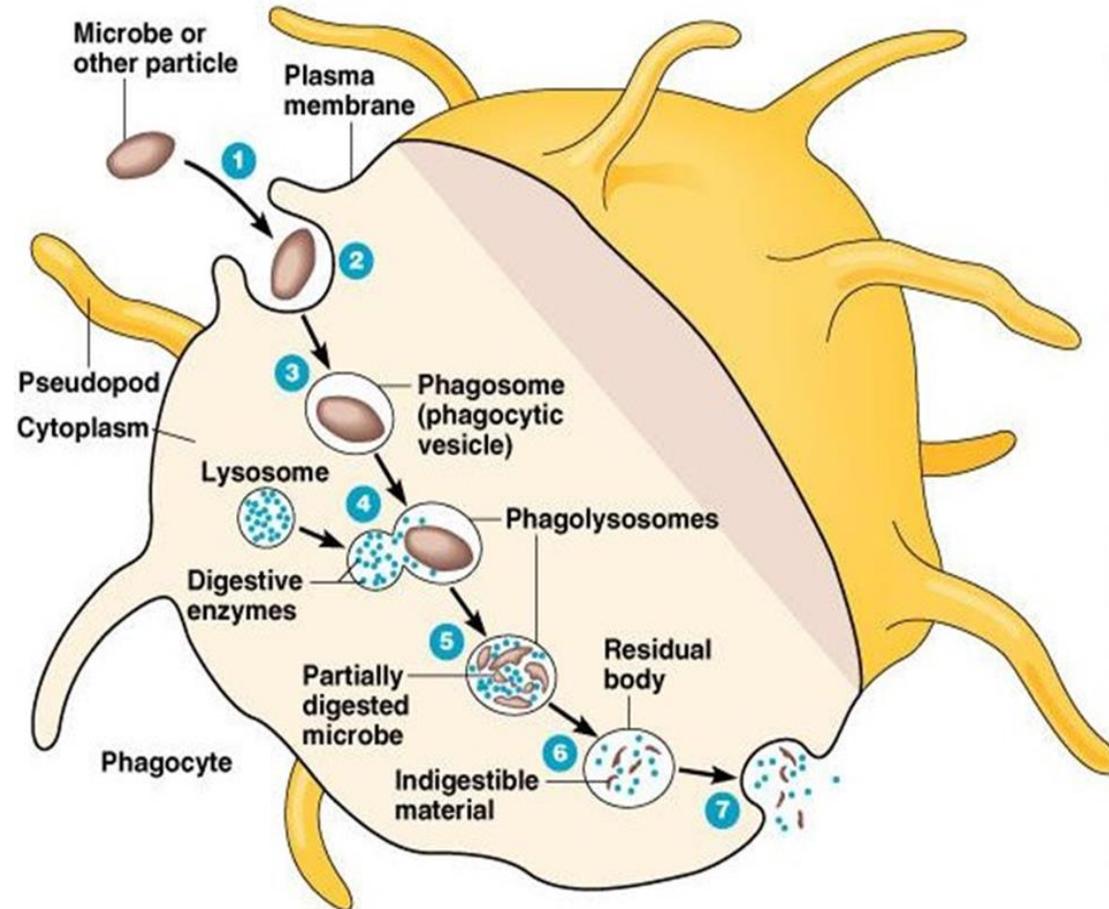
Step 1: Particle Recognition.

Step 2: Particle Internalization.

Step 3: Phagosome Formation.

Step 4: Phagolysosome Maturation.

Step 5: Digestion of Ingested Microbe.



- 1** Chemotaxis and adherence of microbe to phagocyte.
- 2** Ingestion of microbe by phagocyte.
- 3** Formation of a phagosome.
- 4** Fusion of the phagosome with a lysosome to form a phagolysosome.
- 5** Digestion of ingested microbe by enzymes.
- 6** Formation of residual body containing indigestible material.
- 7** Discharge of waste materials.



Phases of Phagocytosis

Step-1: Particle Recognition

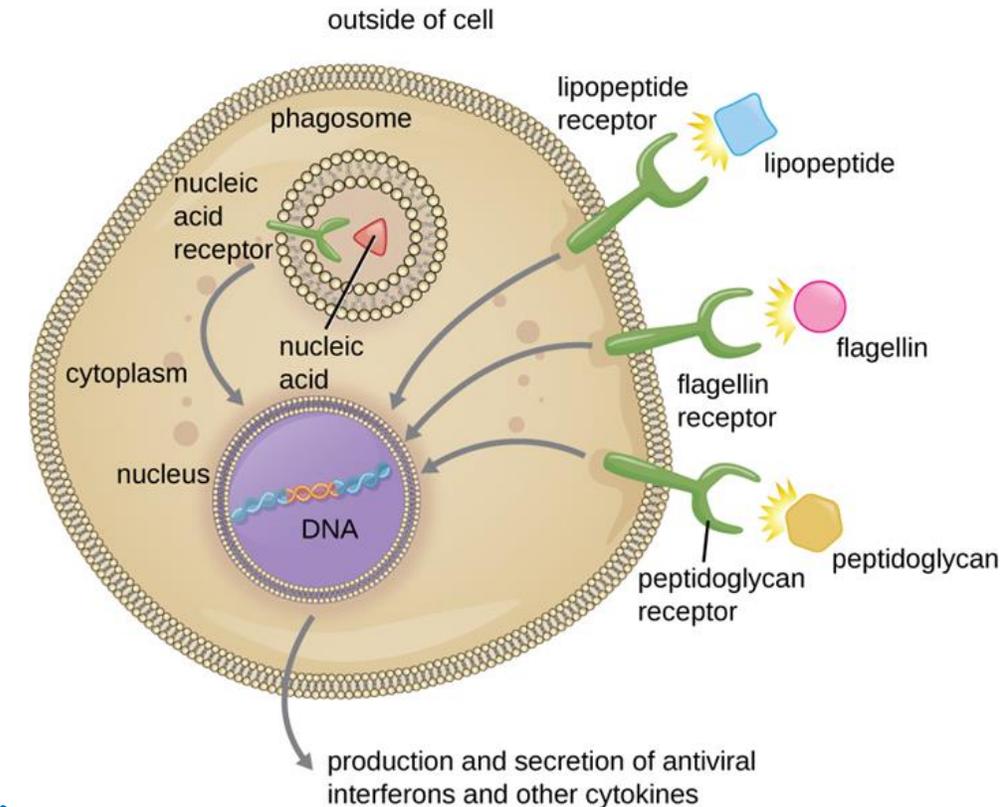
A. Receptors for Foreign Particles

1. Recognition of Pathogen by means of (Pattern Recognition Receptors):

- The internal defense system is designed to recognize molecules that are unique to infectious organisms.
- Macrophages and dendritic cells are able to distinguish pathogens from normally present molecules in the body by means of receptors known as **pattern recognition receptors (PRRs)**.
- The PRRs are also found on **neutrophils, eosinophils, monocytes, mast cells, and epithelial cells**.
- PRRs are able to distinguish self from non-self by recognizing substances, known as **pathogen-associated molecular patterns (PAMPs)**, which are only found in **microorganisms**.

Pattern Recognition Receptors- cont.

- Once PRRs bind to a pathogen, **phagocytic cells become activated** and are better able to **engulf and eliminate microorganisms**.
- Activated cells then secrete **proinflammatory cytokines and chemokines**, chemical messengers that make capillaries more permeable and recruit additional phagocytic cell types to the area of infection.
- Cytokines and chemokines also trigger the adaptive immune response.
- Some examples of PAMPs include peptidoglycan in gram-positive bacteria, lipopolysaccharide in gram-negative bacteria, zymosan in yeast, and **flagellin in bacteria with flagellae**.

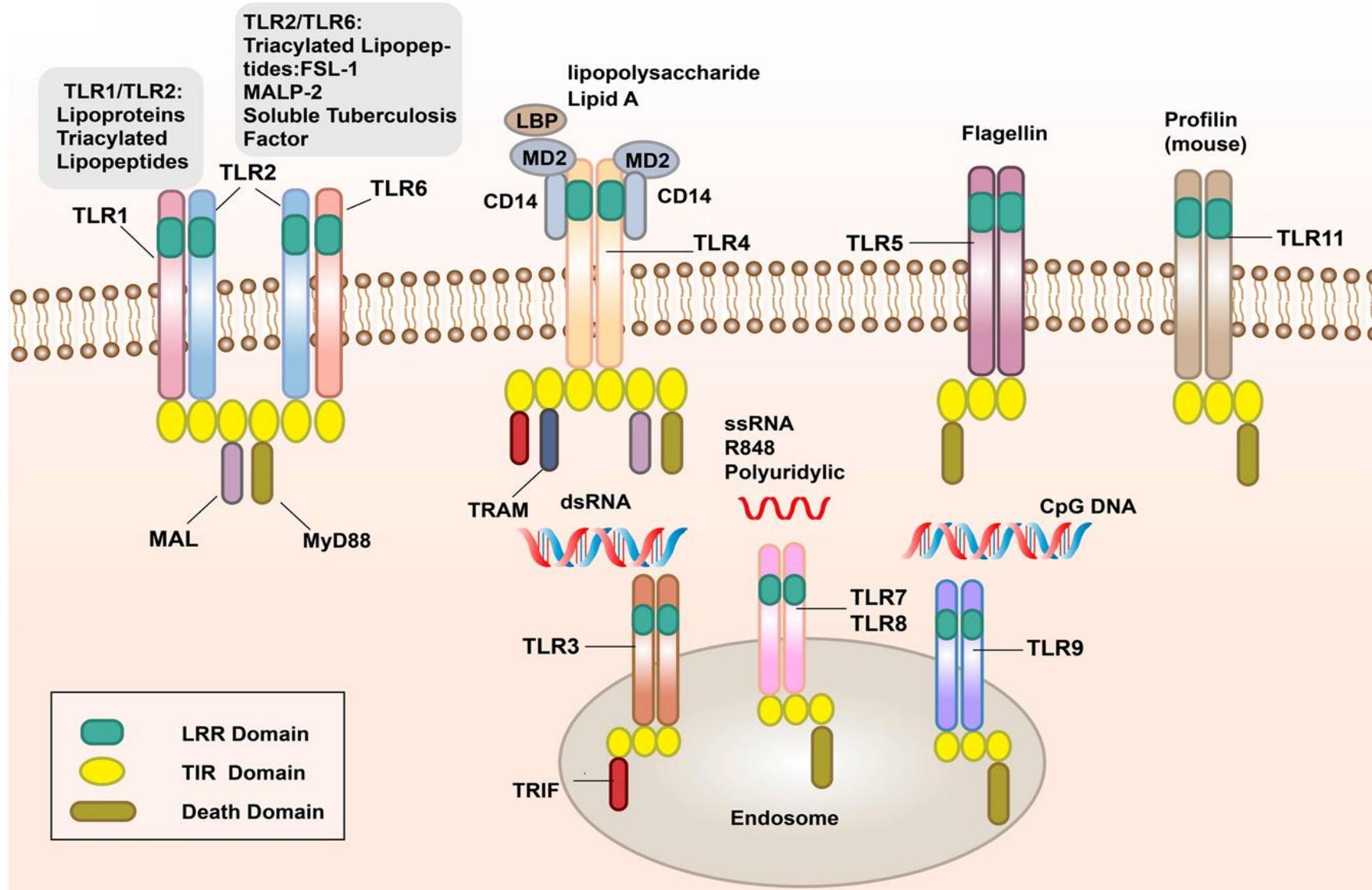


Pattern Recognition Receptors (Toll-Like Receptors)



- TLRs are membrane-spanning glycoproteins that share a common structural element called leucine-rich repeats (LRRs).
- TLRs make up a large family of receptors strategically located in various cellular compartments; some are found in the cytoplasm, whereas others are found on cell surfaces.
- TLR1, TLR2, TLR4, TLR5, and TLR6 are found on cell surfaces, whereas TLR3, TLR7, TLR8, and TLR9 are found in the endosomal compartment of a cell.
- Each of these receptors recognizes a **different microbial product**. For example, **TLR2** recognizes **teichoic acid** and **peptidoglycan** found in **gram-positive bacteria**; **TLR4** recognizes **lipopolysaccharide**, which is found in **gram-negative bacteria**; and **TLR5** recognizes **bacterial flagellin**.
- The function of TLR10 is thought to be anti-inflammatory.

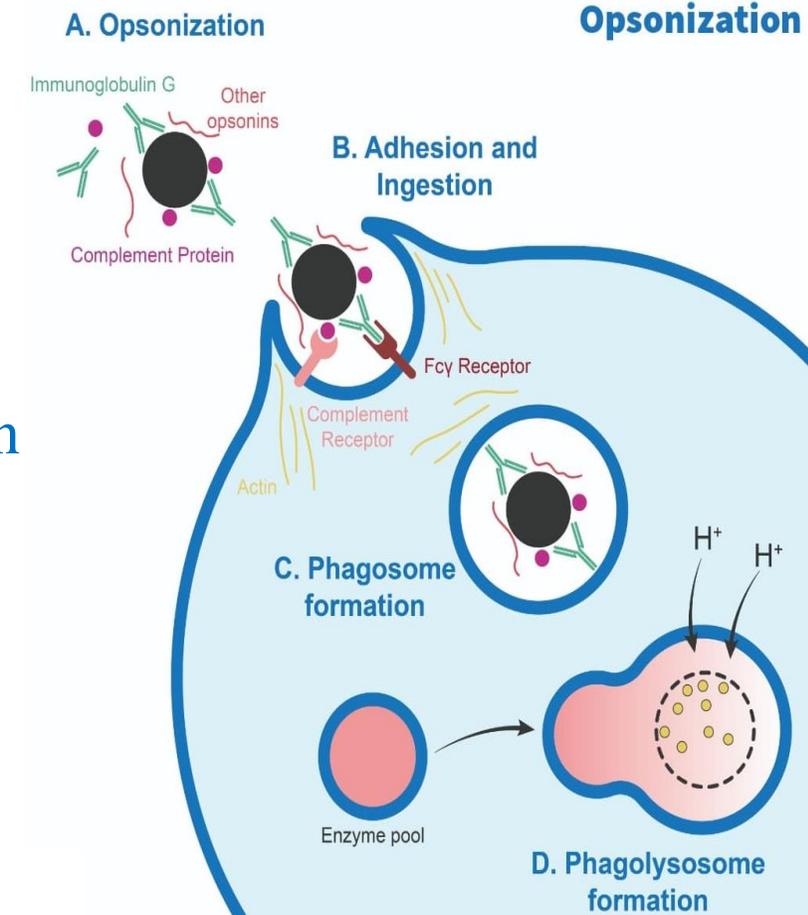
Toll-Like Receptors (TLRs)



A. Receptors for Foreign Particles

2. Recognition of Pathogen by means of (Opsonic Receptors):

- Foreign particles can also be recognized by phagocytes through soluble molecules that will bind to the particles, tagging them for ingestion.
- Once **on the surface of the target particle**, these molecules, called **opsonins**, are in turn recognized by specific receptors on the membrane of phagocytes.
- In this manner, opsonins function as a bridge between the phagocyte and the particle to be ingested.
- Antibody (IgG) molecules** and **complement components** are important opsonins that induce efficient phagocytosis.



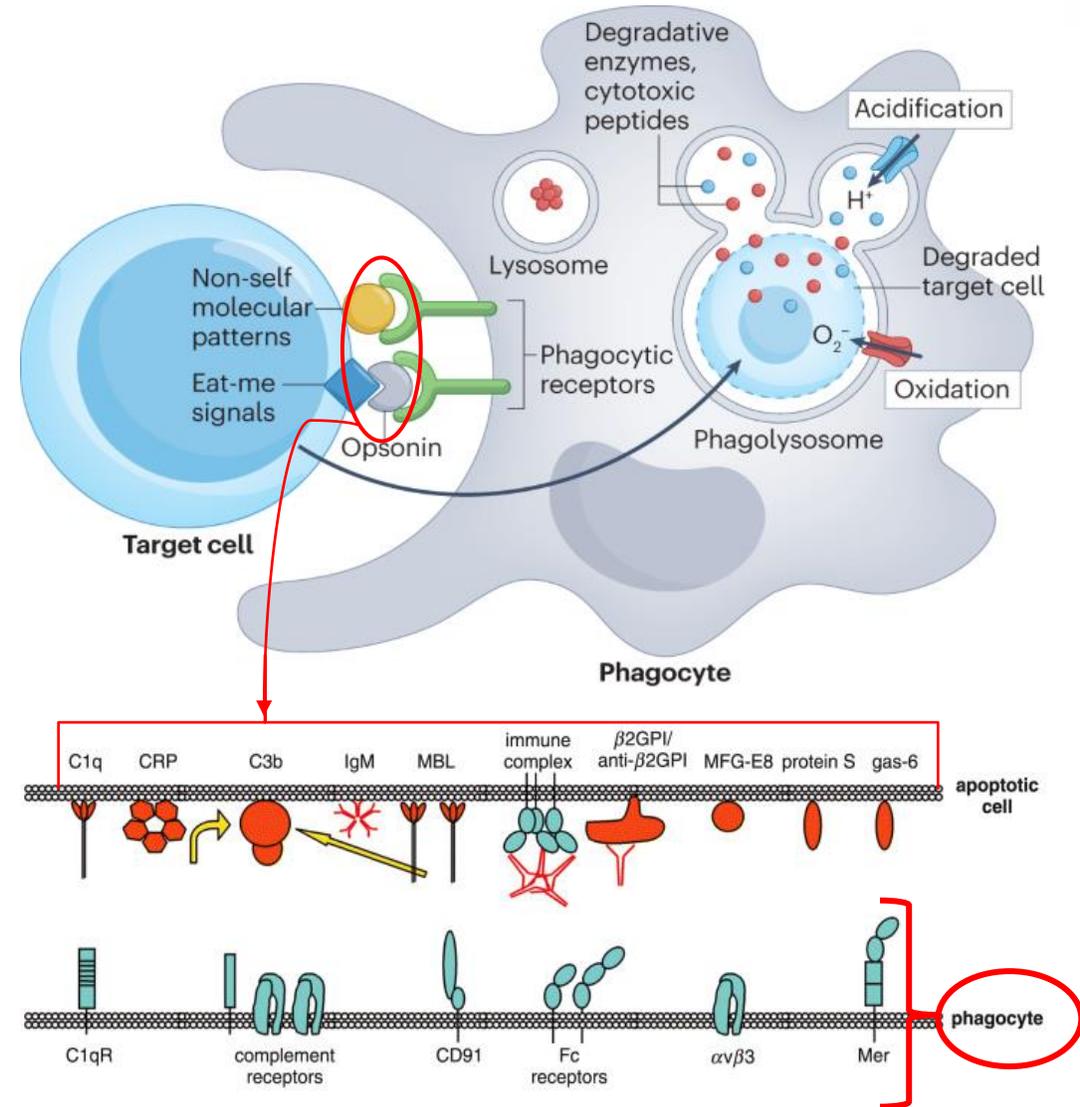


Opsonin and Opsonization

- The **opsonins** act as markers or tags that allow recognition by the immune system of the body.
- **Opsonin**; is any molecule that **enhances phagocytosis by marking an antigen** for an immune response or **marking dead cells for recycling**.
- Opsonization is the capability of antibodies, complement components and other proteins to coat dangerous antigens that can be recognized by **antibodies or complement receptors** on **phagocytic cells**.

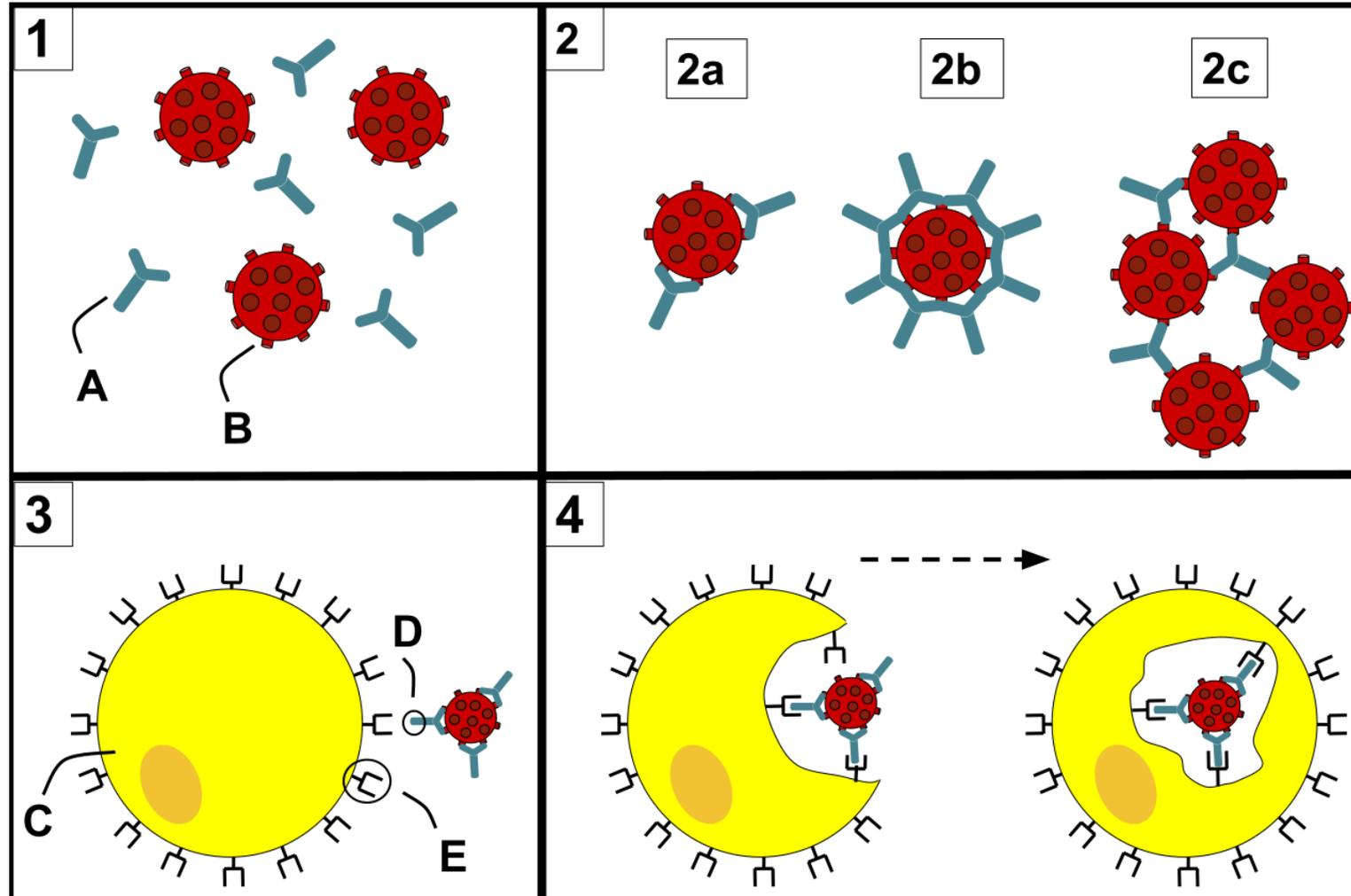
The Common Types of Opsonins

1. IgM antibodies
2. IgG antibodies
3. C3b proteins
4. C4b proteins
5. C1q proteins
6. Pentraxins
7. Collectins
8. Ficolins
9. Mannose-binding lectin (MBL)



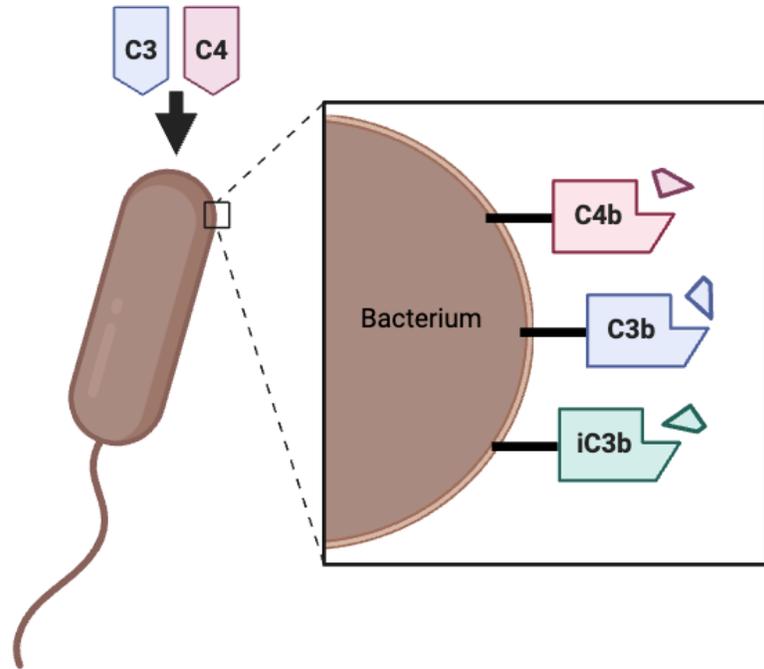
Types of Opsonization

A- Antibody-mediated Opsonization

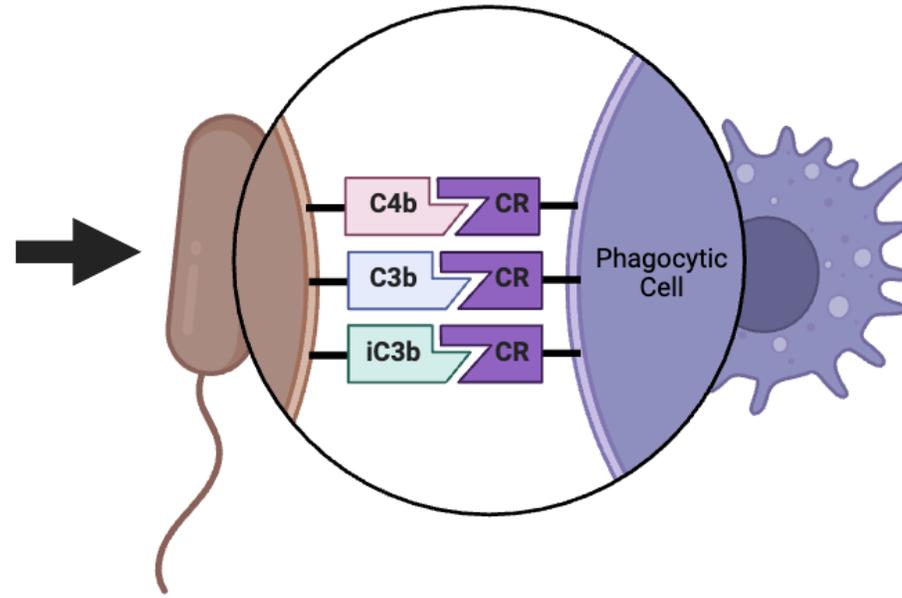


B- Complement-mediated Opsonization

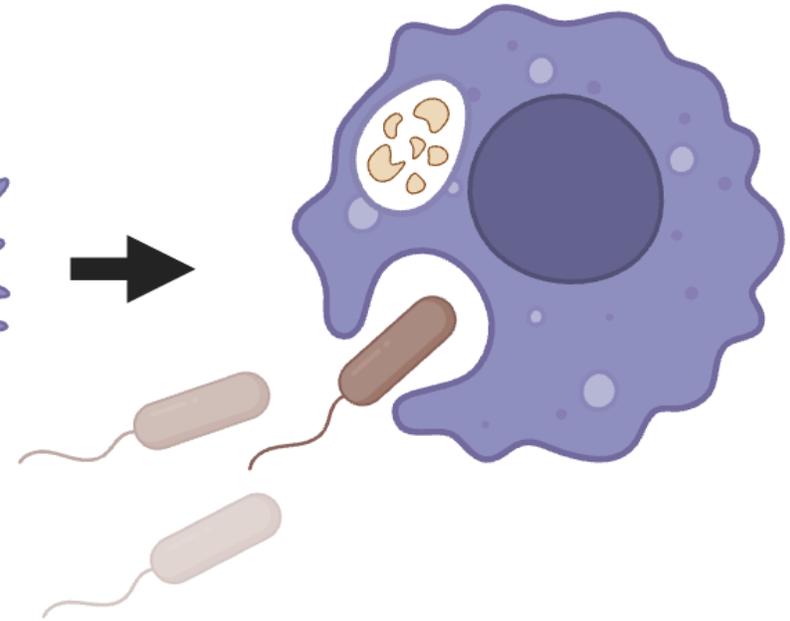
Opsonization



Binding



Phagocytosis





Step-1: Particle Recognition

B. Receptors for Apoptotic Cells (Self-particles):

- In normal organism there are millions of cells that die by apoptosis every day. These apoptotic bodies are constantly cleared by phagocytosis.
- Recognition of apoptotic bodies involves several signals.
 - ❖ First, cells in apoptosis release molecules that normally do not exist outside cells. Some of these molecules include ATP, lysophosphatidylcholine, and sphingosine 1-phosphate. These soluble molecules function as chemoattractants for phagocytes.
 - ❖ Second, apoptotic cells are displayed on their surface molecules, such as phosphatidylserine (PS) not normally present on a healthy cell. These surface molecules function as an “eat me” signal for phagocytes.

Step 5: Digestion of Ingested Microbe

1. Intracellular killing of Phagocytosed Materials

A- Oxygen-dependent Intracellular Killing:

- When a phagocyte ingests bacteria (or any material), its oxygen consumption increases.
- The rise in oxygen consumption called a respiratory burst, produces reactive oxygen-containing molecules that are anti-microbial agents.
- The oxygen compounds are toxic to both the invader and the cell itself, so they are kept in compartments inside the cell.

A- Oxygen-dependent Intracellular Killing- cont.



- 1. Oxygen-dependent production of a superoxide**, which is an oxygen-rich bacteria-killing substance.
 - The **superoxide** is converted to **hydrogen peroxide (H_2O_2)** and singlet oxygen ($(O_2 \cdot\cdot)$) by an enzyme called **superoxide dismutase**.
 - Superoxide also react with the hydrogen peroxide to produce Hydroxyl Radical ($\cdot OH$), which assist in killing the invading microbe.
- 2. Myeloperoxidase production** from neutrophil granules.
 - When granules fuse with a phagosome, myeloperoxidase is released into the phagolysosome, and this enzyme uses hydrogen peroxide and chlorine to create hypochlorite, (a substance used in domestic bleach).
 - Hypochlorite is extremely toxic to bacteria.



1. Intracellular killing of Phagocytosed Materials

B. Oxygen-independent Intracellular Killing

- Phagocytes can also kill microbes by oxygen-independent methods, but these are not as effective as the oxygen- dependent ones.
- **There are four main types of oxygen-independent Intracellular Killing:**
 1. Electrically charged proteins that damage the bacterium's membrane.
 2. Lysozymes; these enzymes break down the bacterial cell wall.
 3. Lactoferrins, which are present in neutrophil granules and remove essential iron from bacteria.
 4. Proteases and hydrolytic enzymes; these enzymes are used to digest the proteins of destroyed bacteria.

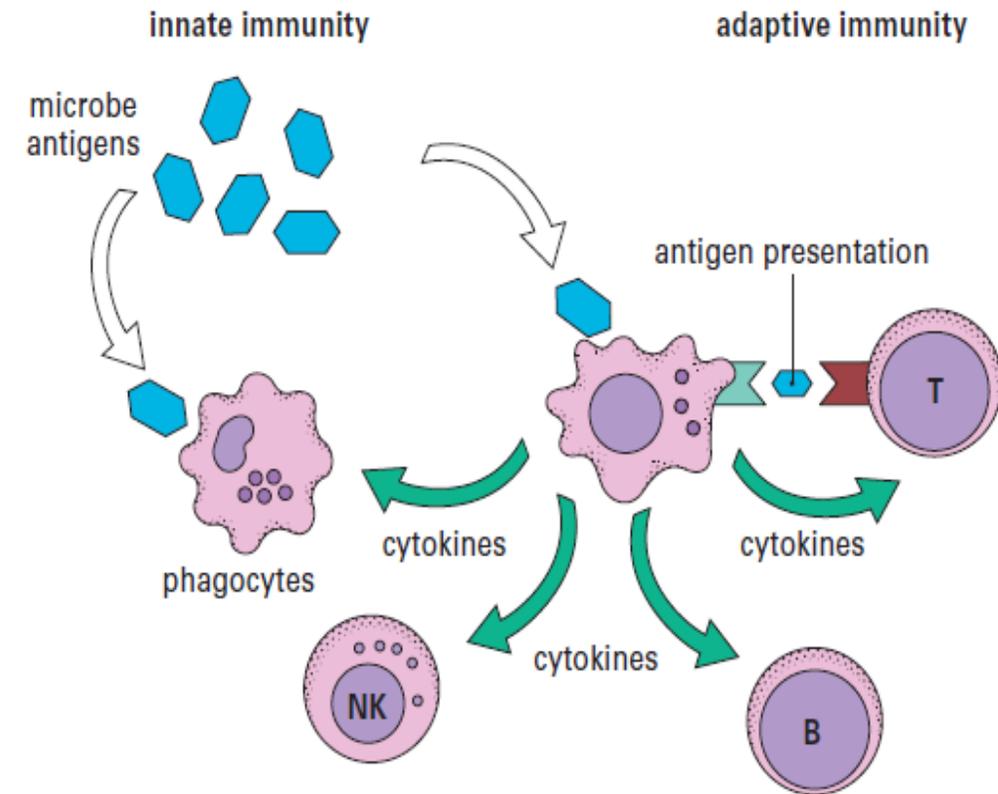


2. Extracellular killing of Phagocytosed Materials

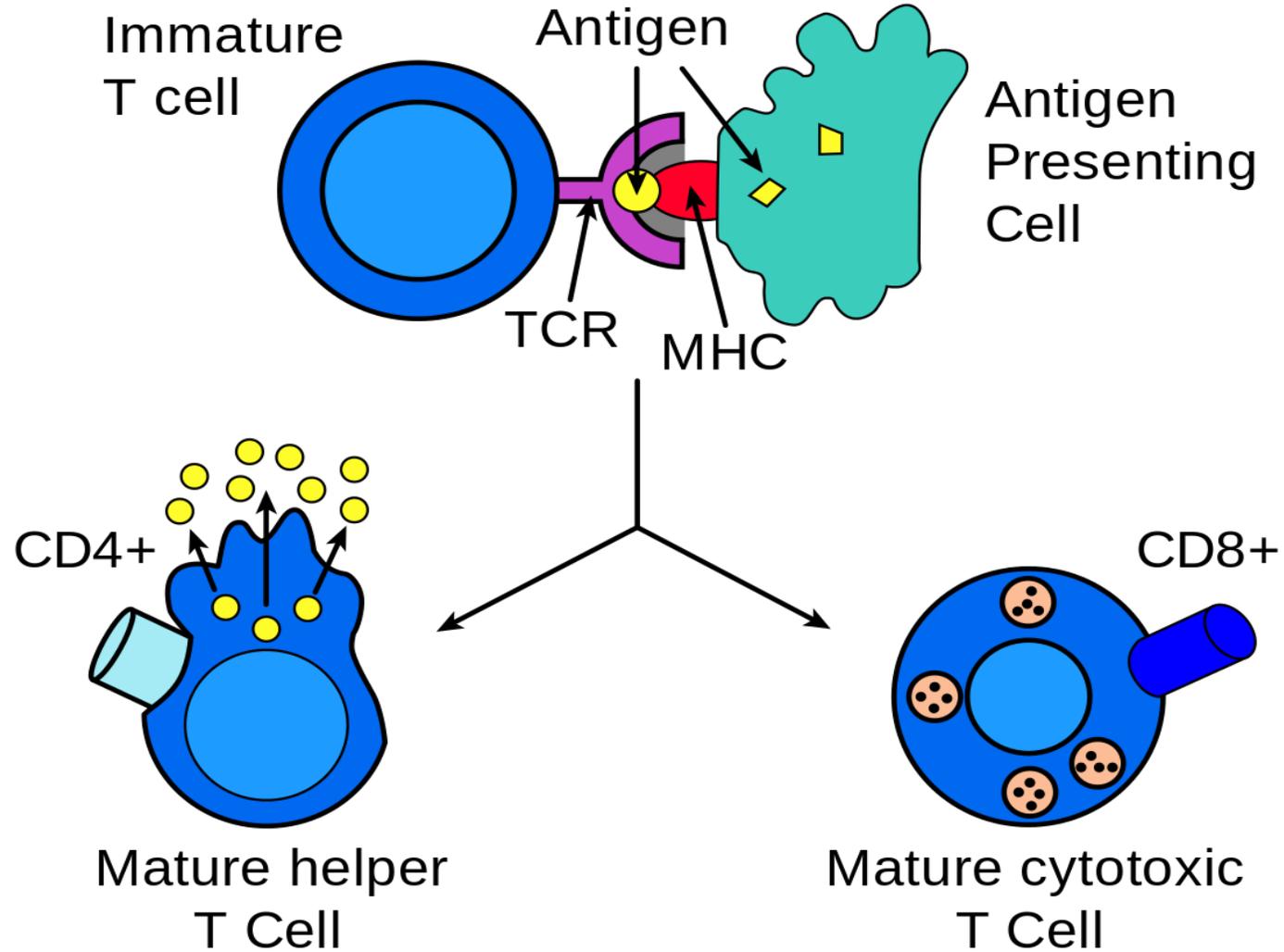
- 1. Interferon-gamma**—which was once called macrophage-activating factor—stimulates macrophages to produce nitric oxide.
 - The source of interferon-gamma can be CD4+ T cells, CD8+ T cells, natural killer cells, B cells, monocytes, macrophages, or dendritic cells.
- 2. Nitric oxide** is then released from the macrophage and, because of its toxicity, kills microbes near the macrophage.
- 3. Activated macrophages produce and secrete tumor necrosis factor.** This cytokine—a class of signaling molecule—kills cancer cells and cells infected by viruses, and helps to activate the other cells of the immune system.

Antigen-presenting cells (APC)

- Antigen-presenting cells (APCs) are a heterogeneous group of immune cells that mediate the cellular immune response by processing and presenting antigens for recognition by T cells.
- Classical APCs include **dendritic cells**, **macrophages**, and **B cells**.
- An antigen-presenting cell (APC) displays foreign antigen complex (parts of the digested invaders) with MHC (Major histocompatibility complex) II on its surface.
- T-cells recognize this complex using their T-cell receptor (TCR).

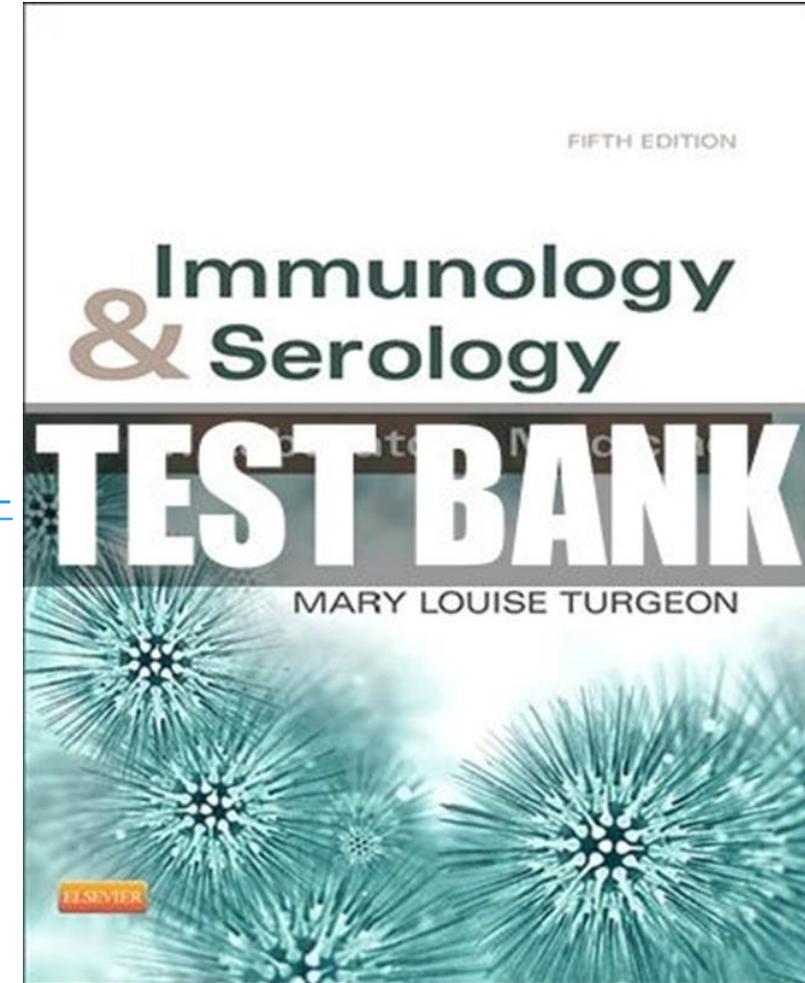


Antigen-presenting cells (APC)- cont.



References

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2. Abul K. Abbas and Andrew H. Lichtman. Cellular And Molecular Immunology 2019, 6th edition .
3. **Abbas, A. K., Lichtman, A. H., Pillai, S., & Baker, D. L. (. i. (2020).** Basic immunology: Functions and disorders of the immune system (Sixth edition.).
4. Aryal (2021) Cells Of The Immune System. <https://microbenotes.com/cells-of-the-immune-system/>
5. British society for Immunology_ What is immunology? <https://www.immunology.org/public-information/what-is-immunology#:~:text=Immunology%20is%20the%20study%20of,as%20autoimmunity%2C%20allergy%20and%20cancer>
6. <https://www.youtube.com/watch?v=GXECgTLGLtI>
7. <https://www.youtube.com/watch?v=C2Tp02VFoo0>



Here is the required reading for this lecture