Inflammation 2

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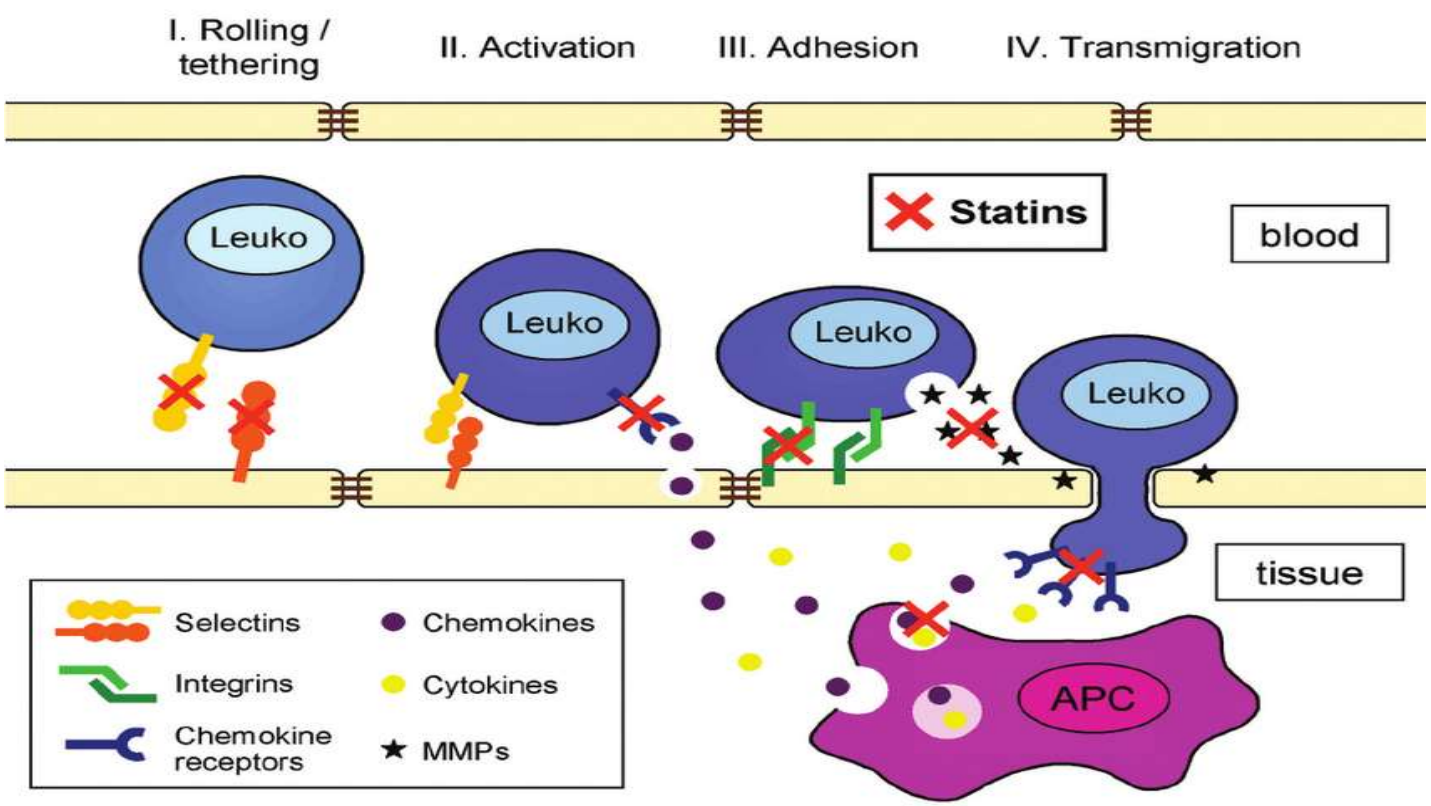


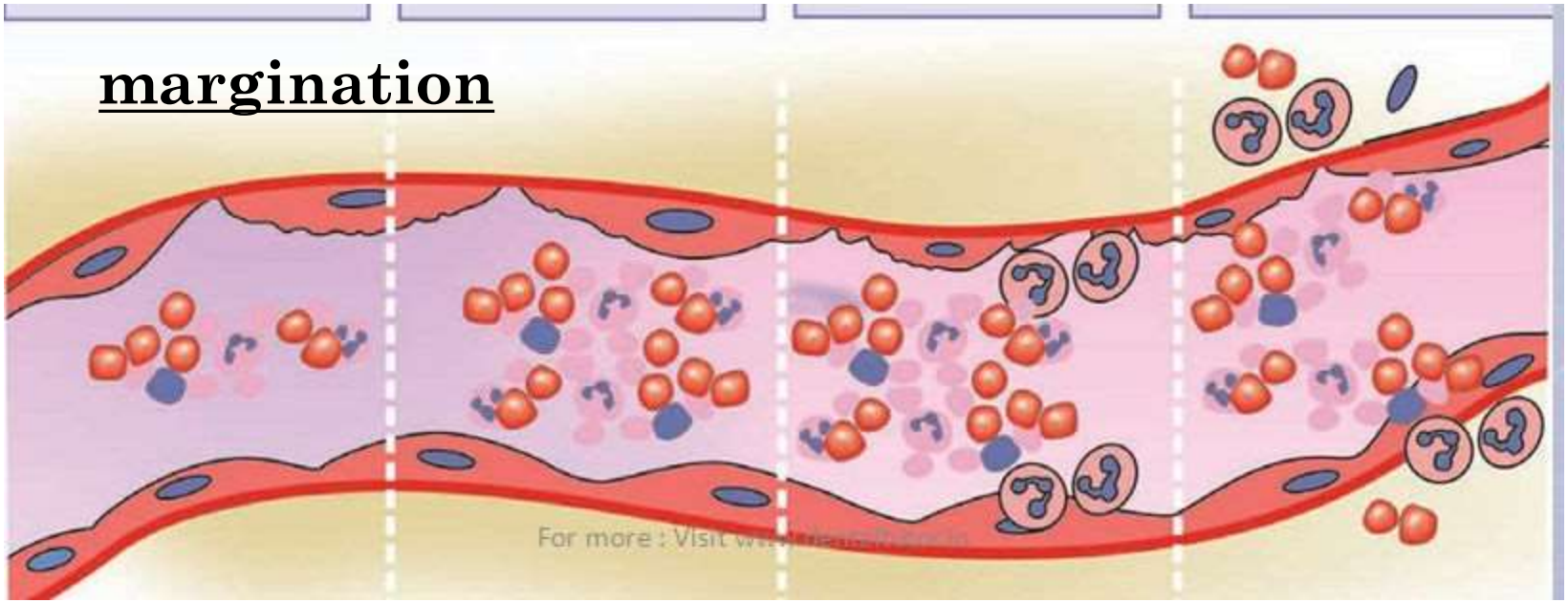
- The journey of leukocytes from the vessel lumen to the tissue is a multistep process that is mediated and controlled by adhesion molecules and cytokines, and consist of three phases:

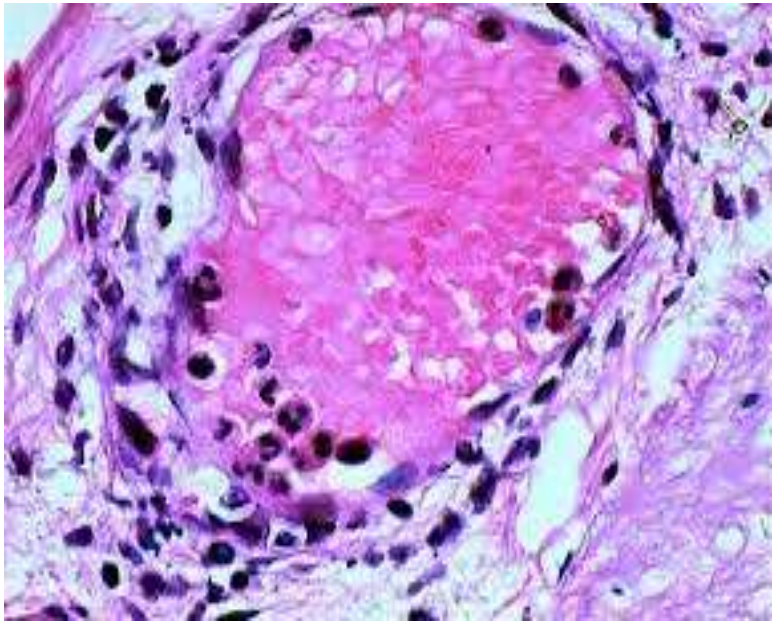
- 1. Leukocyte Adhesion to Endothelium.
- 2. Leukocyte Migration Through Endothelium.
- 3. Movement of the cells toward the offending agent

1. Leukocyte Adhesion to Endothelium

- As the blood flow slows early in inflammation (stasis), and more white cells assume a peripheral position along the endothelial surface (margination.)
- Activated endothelial cells express adhesion molecules to which the leukocytes attach loosely, then bind and detach (rolling.)
- The cells finally come to rest at some point where they adhere firmly
- The attachment of leukocytes to endothelial cells is mediated by complementary adhesion molecules on the two cell types whose expression is enhanced by cytokines, The two major families of molecules involved in leukocyte adhesion and migration are the selectins and integrins.

margination





1. Selectins

- Mediate the initial weak interactions between leukocytes and endothelium.

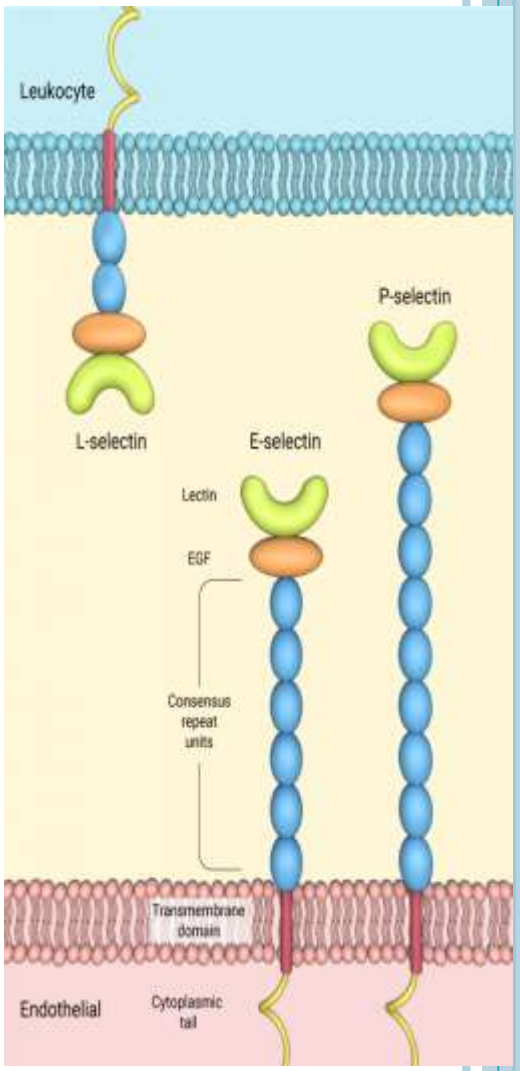
- Selectins are receptors expressed on leukocytes and endothelium that contain an extracellular domain that binds sugars (hence the lectin part of the name).

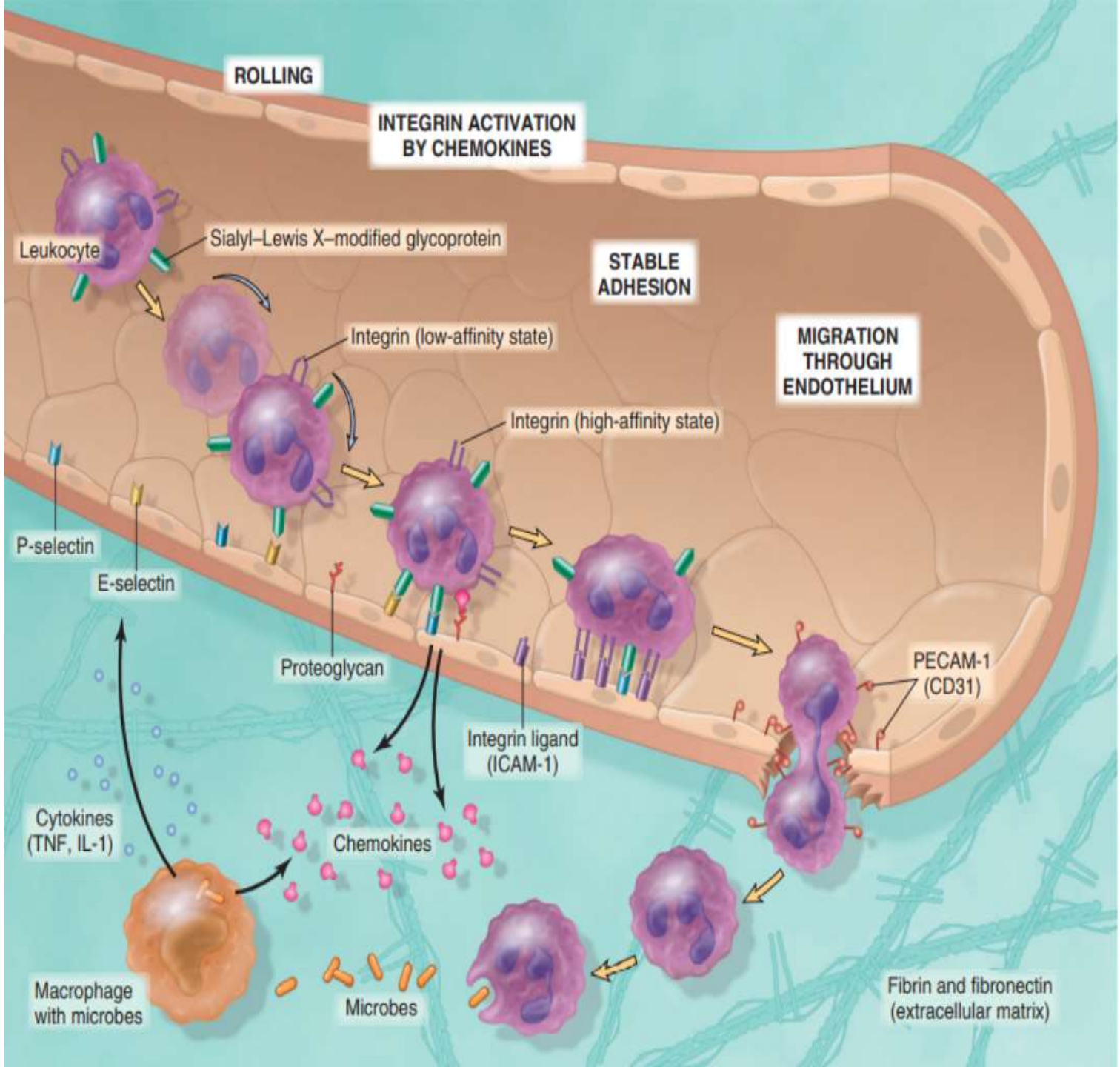
- The three members of this family are:
- E-selectin (also called CD62E), expressed on endothelial cells.
- P-selectin (CD62P), present on platelets and endothelium.
- L-selectin (CD62L), found on the surface of most leukocytes.

- The endothelial selectins are typically expressed at low levels or not at all on unactivated endothelium, and are upregulated after stimulation by cytokines and other mediators.

- Therefore, binding of leukocytes is largely restricted to the endothelium at sites of infection or tissue injury (where the mediators are produced).

- These weak selectin-mediated rolling interactions slow down the leukocytes and give them the chance to recognize additional adhesion molecules on the endothelium.




ROLLING
INTEGRIN ACTIVATION BY CHEMOKINES
STABLE ADHESION
MIGRATION THROUGH ENDOTHELIUM
Leukocyte
Sialyl–Lewis X–modified glycoprotein
Integrin (low-affinity state)
Integrin (high-affinity state)
P-selectin
E-selectin
Proteoglycan
Integrin ligand (ICAM-1)
PECAM-1 (CD31)
Cytokines (TNF, IL-1)
Chemokines
Macrophage with microbes
Microbes
Fibrin and fibronectin (extracellular matrix)

2.INTEGRINS

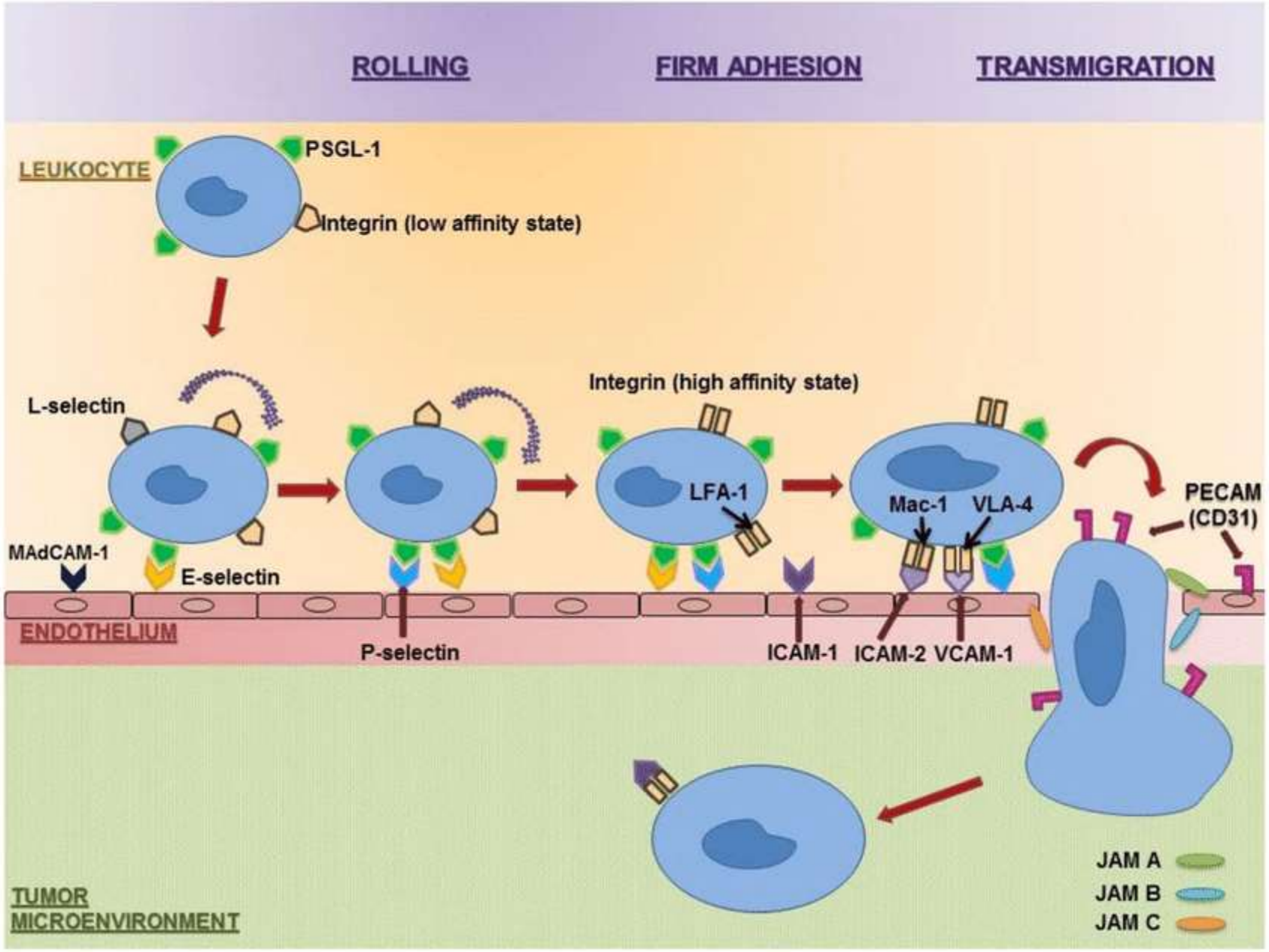
- a family of leukocyte surface proteins that mediate the adhesion of leukocytes to endothelium and of various cells to the extracellular matrix.

- They are normally expressed on leukocyte plasma membranes in a low-affinity form and do not adhere to their specific ligands until the leukocytes are activated by chemokines.

- When the rolling leukocytes encounter the displayed chemokines, the cells are activated, and their integrins undergo conformational changes and cluster together, thus converting to a high-affinity form.
- At the same time, other cytokines, notably TNF and IL-1, activate endothelial cells to increase their expression of ligands for integrins.

These ligands include

- intercellular adhesion molecule-1 (ICAM-1), which binds to the integrins leukocyte function–associated antigen-1 (LFA-1)
- macrophage-1 antigen (Mac-1) (CD11bCD18).
- vascular cell adhesion molecule-1 (VCAM-1), which binds to the integrin very late antigen-4 (VLA-4).
- The leukocytes stop rolling, and engagement of integrins by their ligands delivers signals leading to cytoskeletal changes that arrest the leukocytes and firmly attach them to the endothelium


ROLLING
FIRM ADHESION
TRANSMIGRATION
LEUKOCYTE
PSGL-1
Integrin (low affinity state)
L-selectin
Integrin (high affinity state)
LFA-1
Mac-1
VLA-4
PECAM (CD31)
MAdCAM-1
E-selectin
ENDOTHELIUM
P-selectin
ICAM-1
ICAM-2
VCAM-1
JAM A
JAM B
JAM C
TUMOR MICROENVIRONMENT

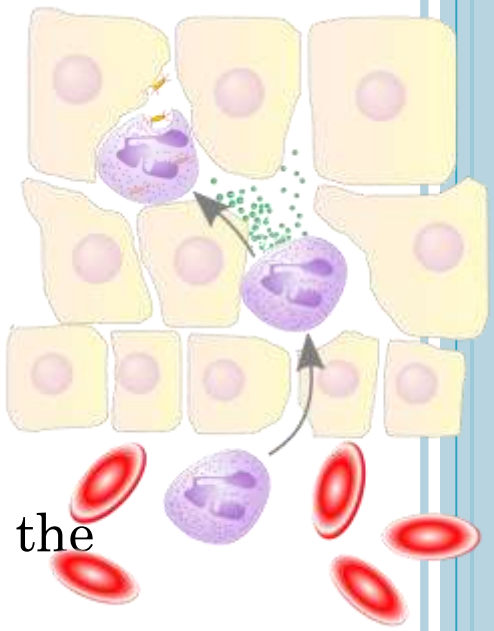
Leukocyte Migration Through Endothelium

- Occurs mainly in postcapillary venules.
- leukocytes migrate through the vessel wall primarily by:

  - squeezing between cells at intercellular junctions.
  - driven by chemokines produced in extravascular tissues
  - platelet endothelial cell adhesion molecule-1 (PECAM-1)*

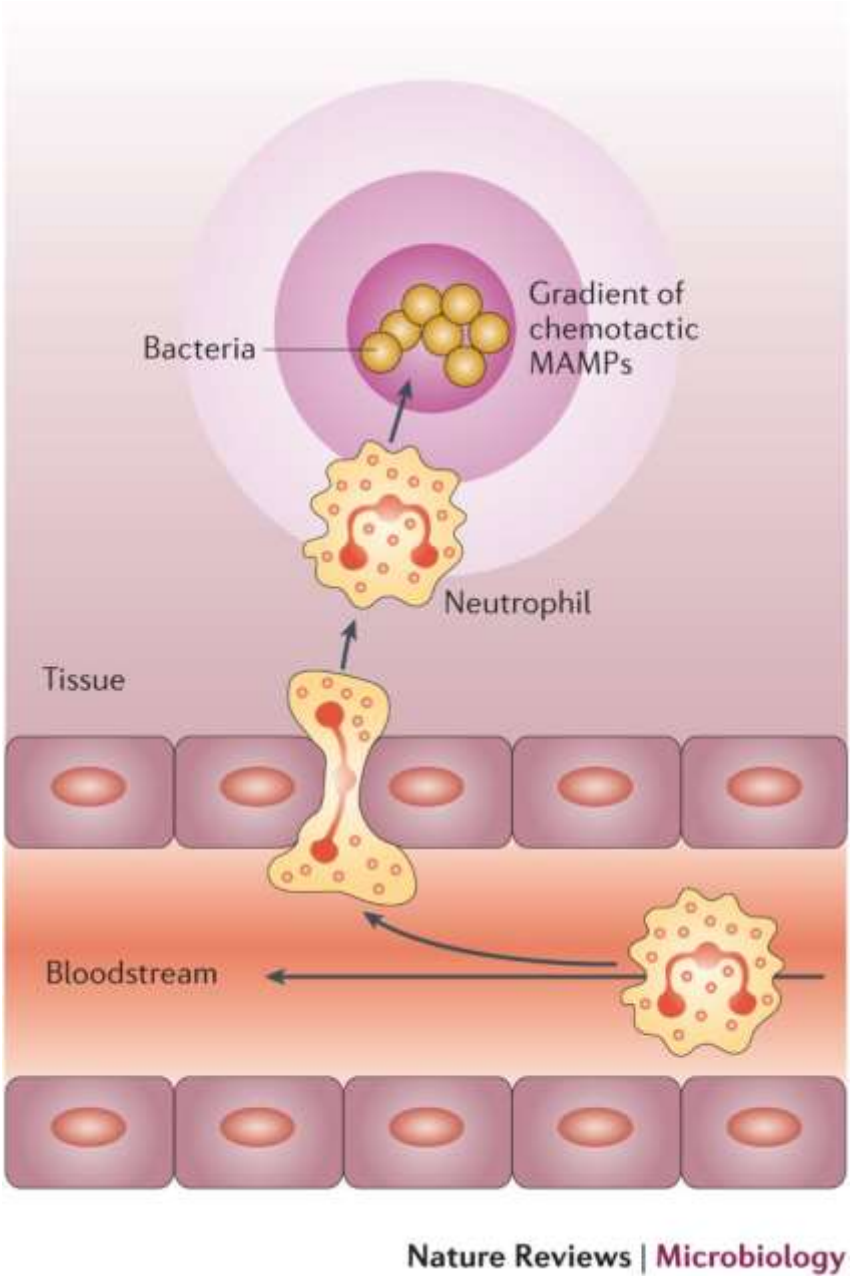
- After traversing the endothelium, leukocytes pierce the basement membrane, probably by secreting collagenases, and they enter the extravascular tissue.

Chemotaxis of Leukocytes



- Locomotion along a chemical gradient.
- Movement of leukocytes in the tissues after leaving the circulation, toward the site of injury.
- Exogenous and endogenous substances can act as chemoattractants, including the following:

  - Bacterial products, particularly peptides with Nformylmethionine termini .
  - Cytokines, especially those of the chemokine family.
  - Components of the complement system, particularly C5a .
  - Products of the lipoxygenase pathway of arachidonic acid (AA) metabolism, particularly leukotriene B4 (LTB4)

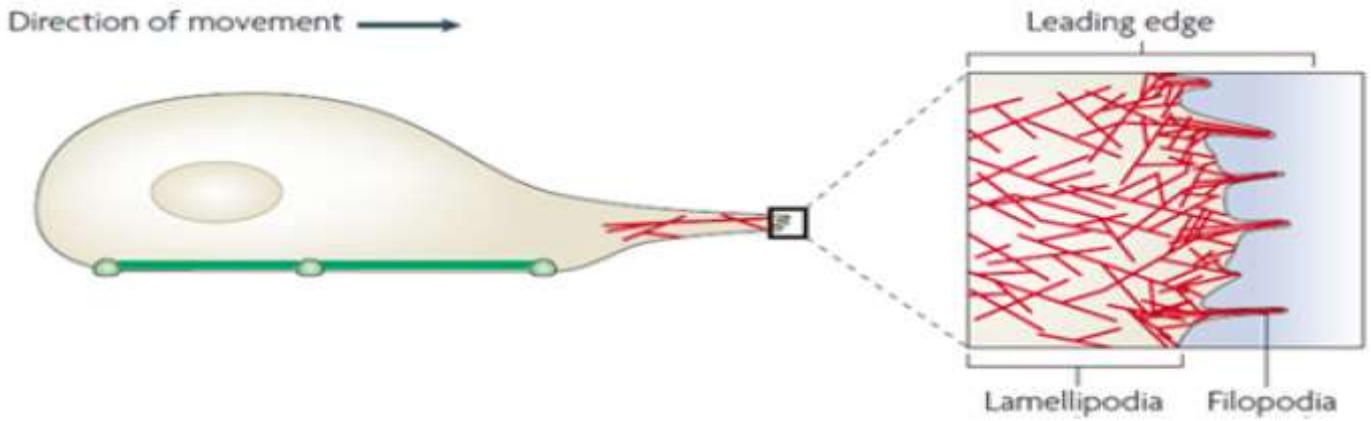


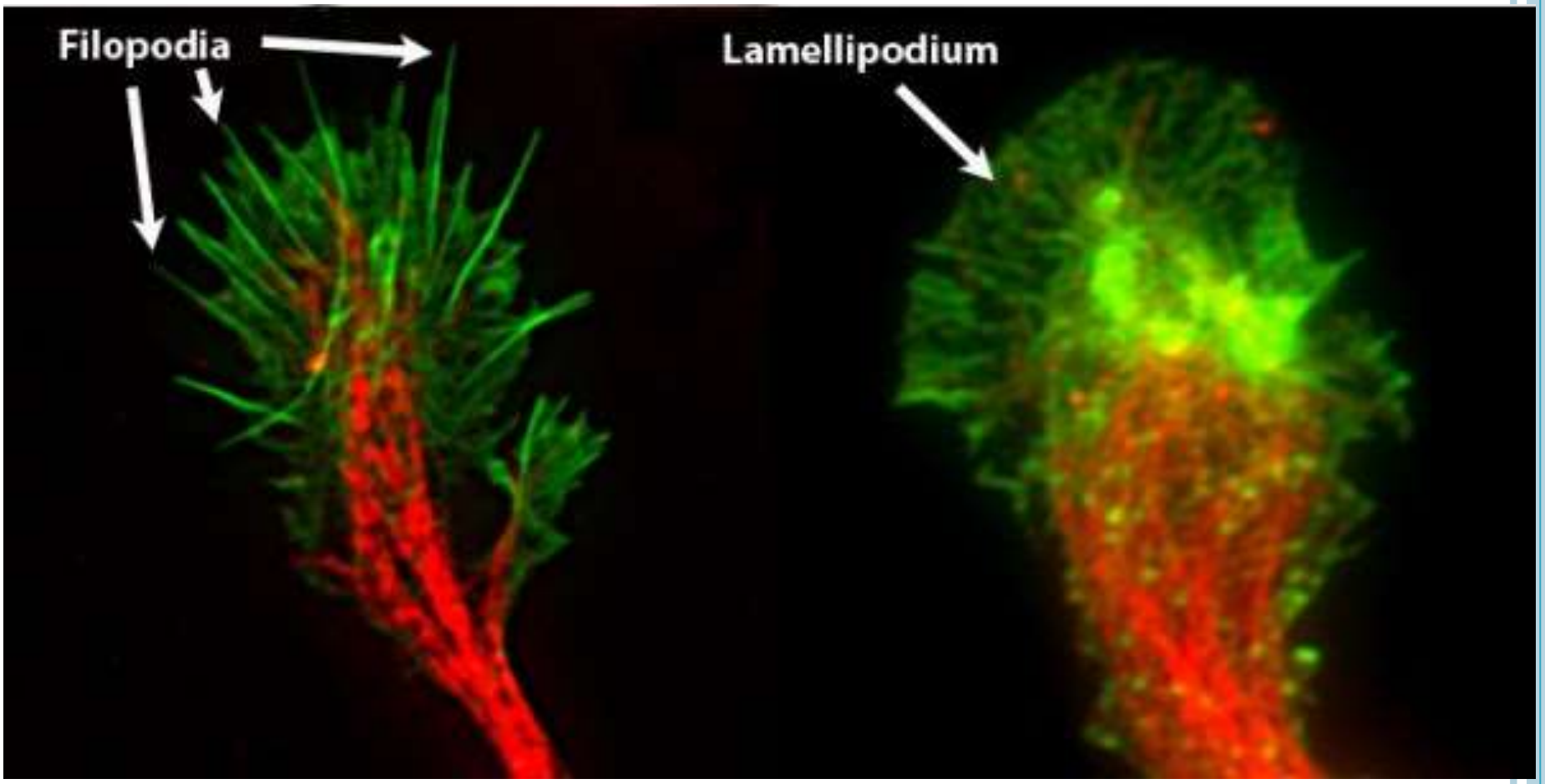
*microorganism-associated molecular patterns (MAMPs)

- These chemoattractants all act by binding to seven transmembrane G protein-coupled receptors on the surface of leukocytes.

- Signals initiated from these receptors activate second messengers that induce polymerization of actin, resulting in increased amounts at the leading edge of the cell and localization of myosin filaments at the back.

- The leukocyte moves by extending filopodia that pull the back of the cell in the direction of extension, much like the front wheels



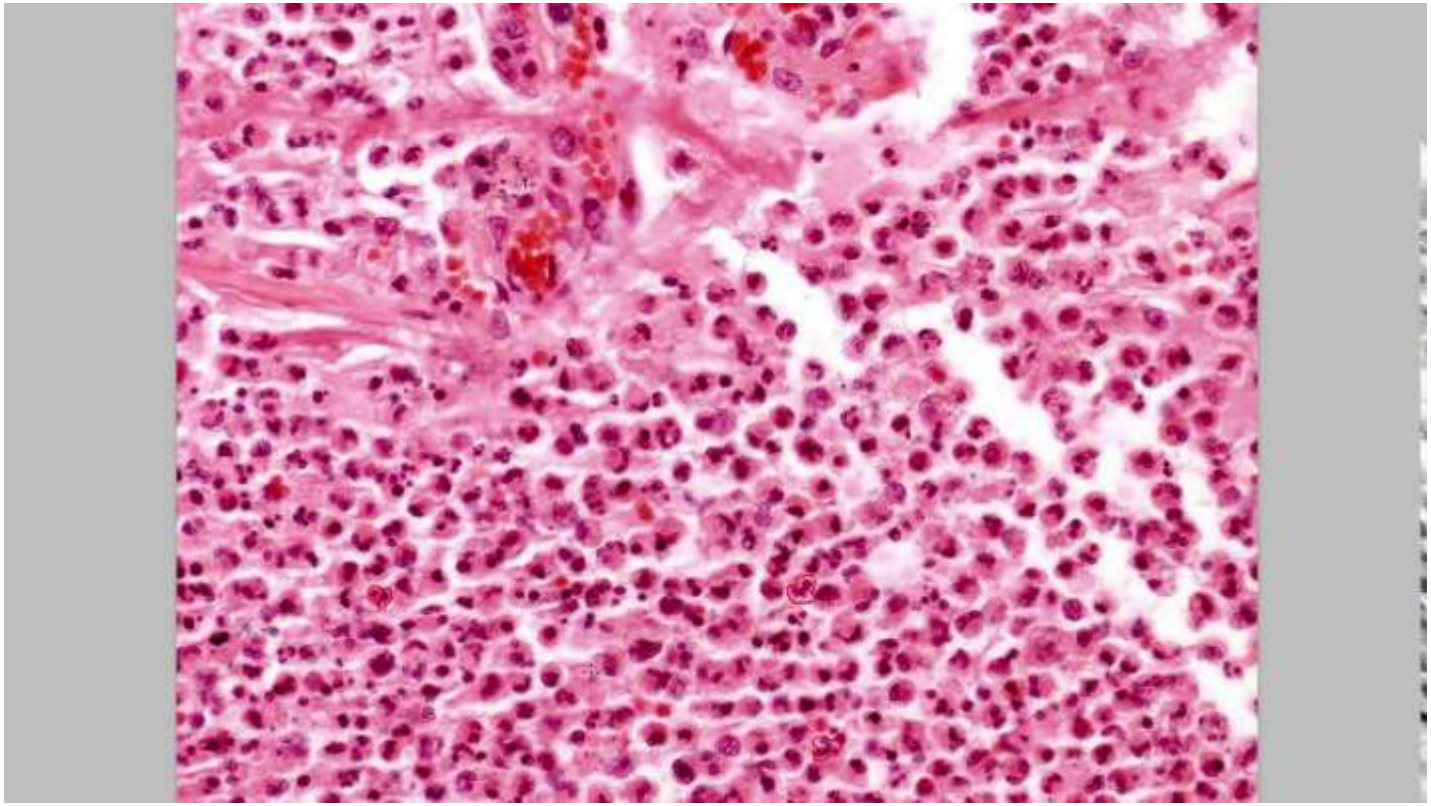

Filopodia
Lamellipodium

The nature of the leukocyte infiltrate varies with the age of the inflammatory response and the type of stimulus.

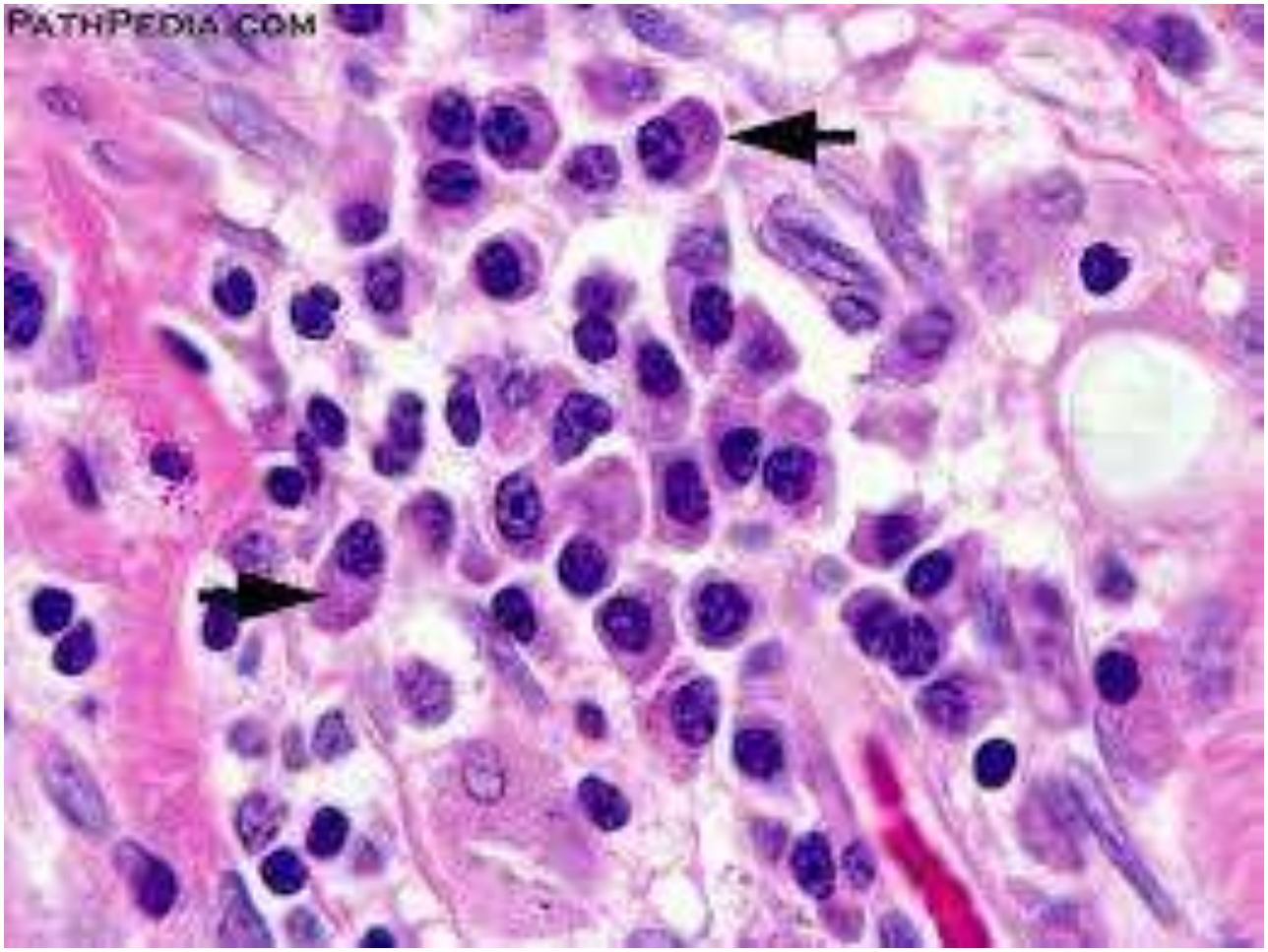
- In most forms of acute inflammation:

- Neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 hours.
- Monocyte-derived macrophages over 24 to 48 hours.

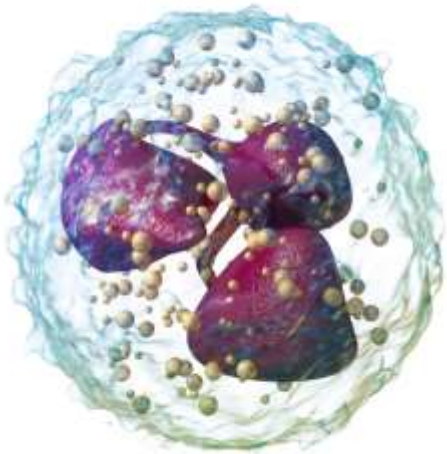
Acute inflammation



Chronic inflammation



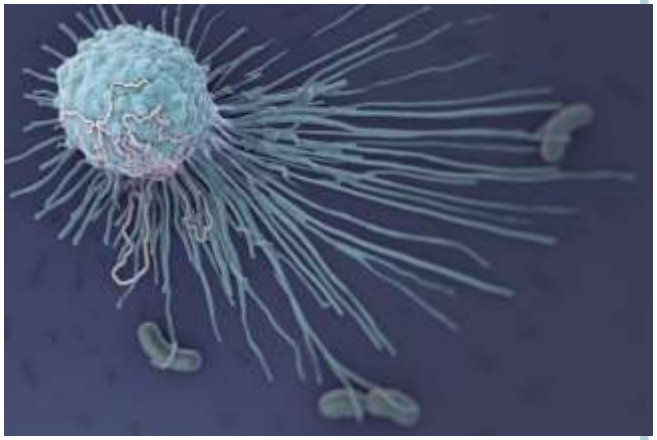
Neutrophils, why in acute?



- More numerous in the blood than other leukocytes.
- They respond more rapidly to chemokines.
- They may attach more firmly to the adhesion molecules that are rapidly induced on endothelial cells, such as P- and E-selectins.

- After entering tissues, neutrophils are short-lived; they undergo apoptosis and disappear within 24 to 48 hours.

Macrophages



- Survive longer .
- May proliferate in the tissues, and thus they become the dominant population in prolonged inflammatory reactions.

Exceptions are present?

- Inflammation produced by Pseudomonas bacteria, the cellular infiltrate is dominated by neutrophils for several days.
- In viral infections, lymphocytes may be the first cells to arrive.
- Some hypersensitivity reactions are dominated by activated lymphocytes, macrophages, and plasma cells.
- In allergic reactions, eosinophils may be a prominent cell type.

CBC	
(RBC, Hgb, Hct, platelet count)	
WBC count	13,300/mm³
WBC differential	
Segmented neutrophils	70%
Band neutrophils	8%
Lymphocytes	15%
Monocytes	4%
Eosinophils	2%
Basophils	1%

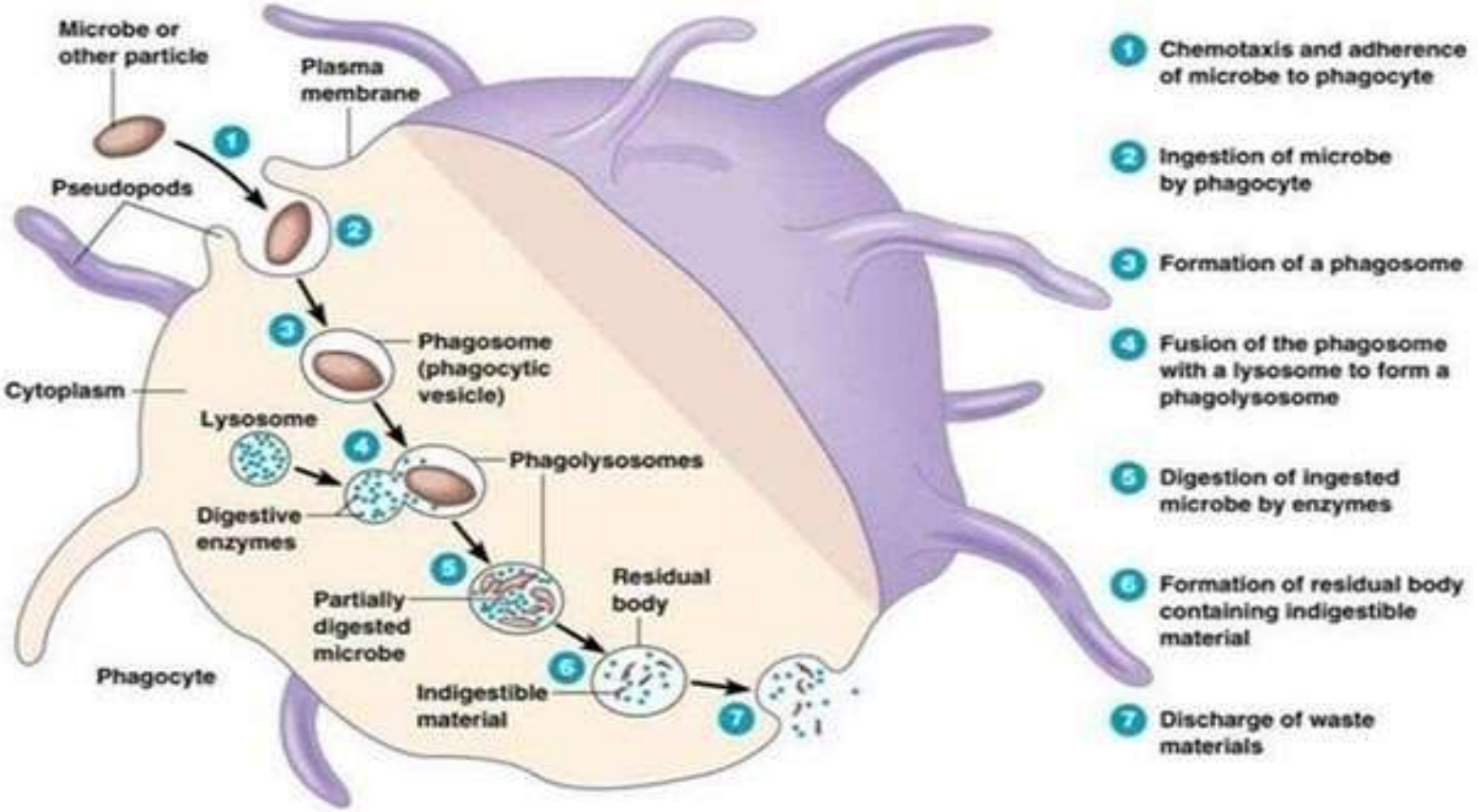
LEUKOCYTE ACTIVATION

- After leukocytes (particularly neutrophils and monocytes) have been recruited to a site of infection or tissue injury they must be activated to perform their functions.

- The functional responses that are most important for destruction of microbes and other offenders are :
- Phagocytosis.
- Intracellular killing

1. Phagocytosis

- Phagocytosis involves three sequential steps:
- (1) recognition and attachment of the particle to be ingested by the leukocyte.
- (2) engulfment, with subsequent formation of a phagocytic vacuole.
- (3) killing or degradation of the ingested material


Microbe or other particle
Plasma membrane
Pseudopods
Cytoplasm
Lysosome
Phagosome (phagocytic vesicle)
Phagolysosomes
Digestive enzymes
Partially digested microbe
Residual body
Indigestible material
Phagocyte
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

1. Recognition by Phagocytic Receptors

- Mannose receptors.
- Scavenger receptors.
- receptors for various opsonins bind and ingest microbes.

1.MANNOSE RECEPTOR

- The macrophage mannose receptor is a lectin that binds terminal mannose and fucose residues of glycoproteins and glycolipids.
- These sugars are typically part of molecules found on microbial cell walls, whereas mammalian glycoproteins and glycolipids contain terminal sialic acid or N-acetylgalactosamine. Therefore, the mannose receptor recognizes microbes and not host cells.

2.Scavenger receptors

- Scavenger receptors bind and ingest low-density lipoprotein (LDL) particles as well as a variety of microbes.
- The efficiency of phagocytosis is greatly enhanced when microbes are opsonized (coated) by specific proteins (opsonins) for which the phagocytes express high-affinity receptors.
- The major opsonins are immunoglobulin (Ig)G antibodies, the C3b breakdown product of complement activation, and certain plasma lectins, notably mannose-binding lectin, all of which are recognized by specific receptors on leukocytes.

2. Engulfment.

- After a particle is bound to phagocyte receptors, extensions of the cytoplasm (pseudopods) flow around it, and the plasma membrane pinches off to form a cytosolic vesicle (phagosome) that encloses the particle.
- The phagosome then fuses with lysosomes, resulting in the discharge of lysosomal contents into the phagolysosome .
- During this process the phagocyte also may release some granule contents into the extracellular space, thereby damaging surrounding normal cells.

3.Intracellular Destruction of Microbes and Debris

- The killing of microbes and the destruction of ingested materials are accomplished by:

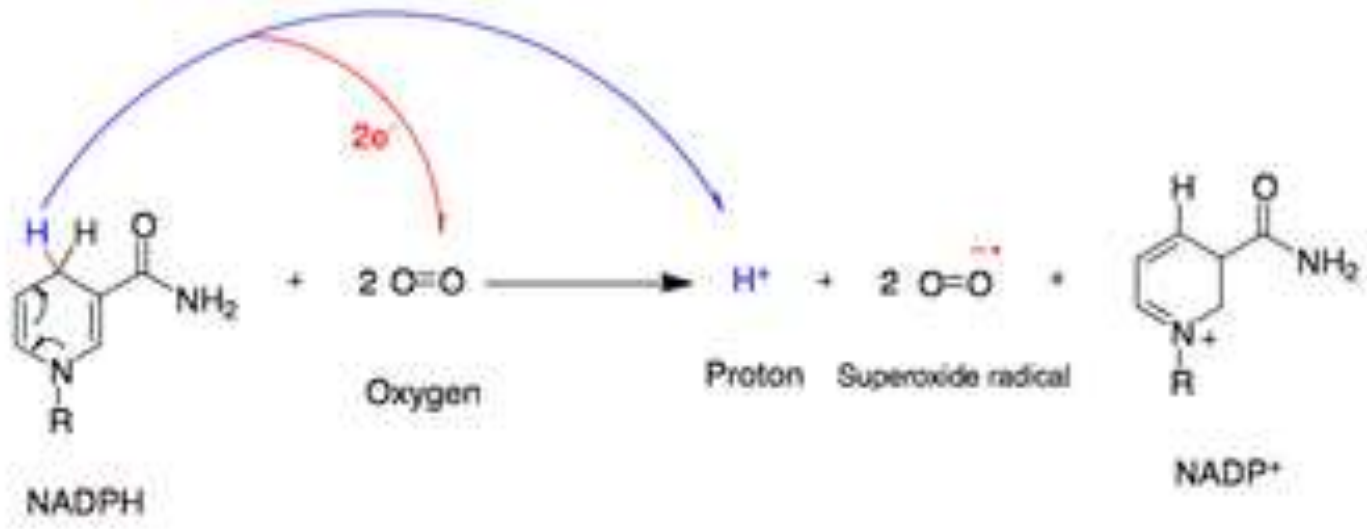
- Reactive oxygen species (ROS, also called reactive oxygen intermediates).
- Reactive nitrogen species, mainly derived from nitric oxide (NO).
- Lysosomal enzymes

Remember

- This is the final step in the elimination of infectious agents and necrotic cells.
- The killing and degradation of microbes and elimination of dead-cell debris within neutrophils and macrophages occur most efficiently after their activation.
- All these killing mechanisms are normally sequestered in lysosomes, to which phagocytosed materials are brought. Thus, potentially harmful substances are segregated from the cell's cytoplasm and nucleus to avoid damage to the phagocyte while it is performing its normal function.

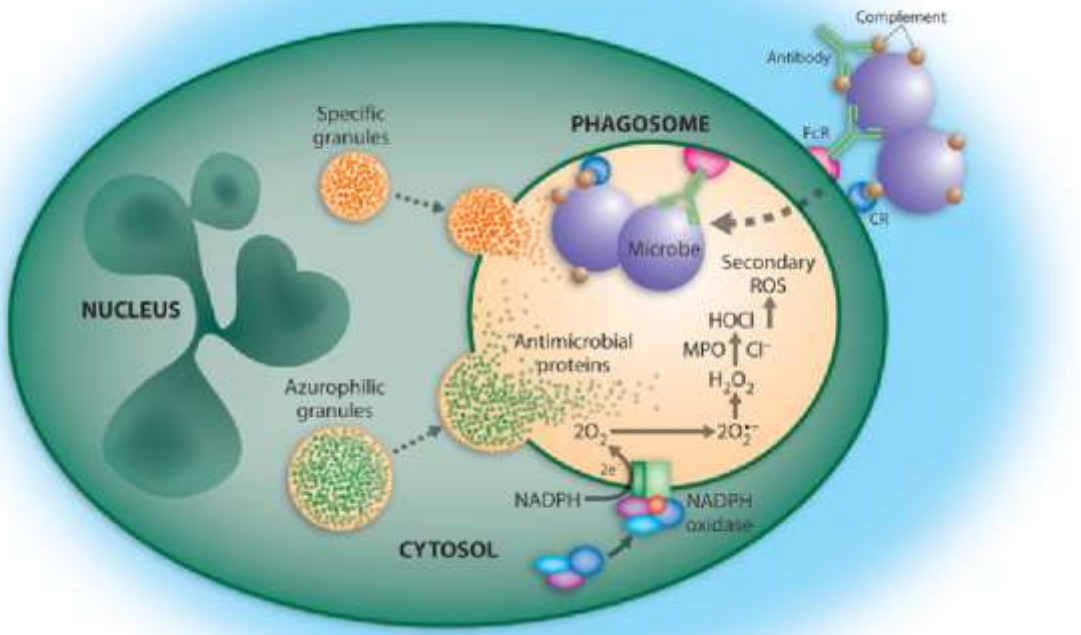
1. Reactive Oxygen Species.

- They are produced by the rapid assembly and activation of a multicomponent enzyme, phagocyte oxidase (also called NADPH oxidase), which :
- oxidizes NADPH
- reduces oxygen to the superoxide anion (O2 •)



RESPIRATORY BURST.

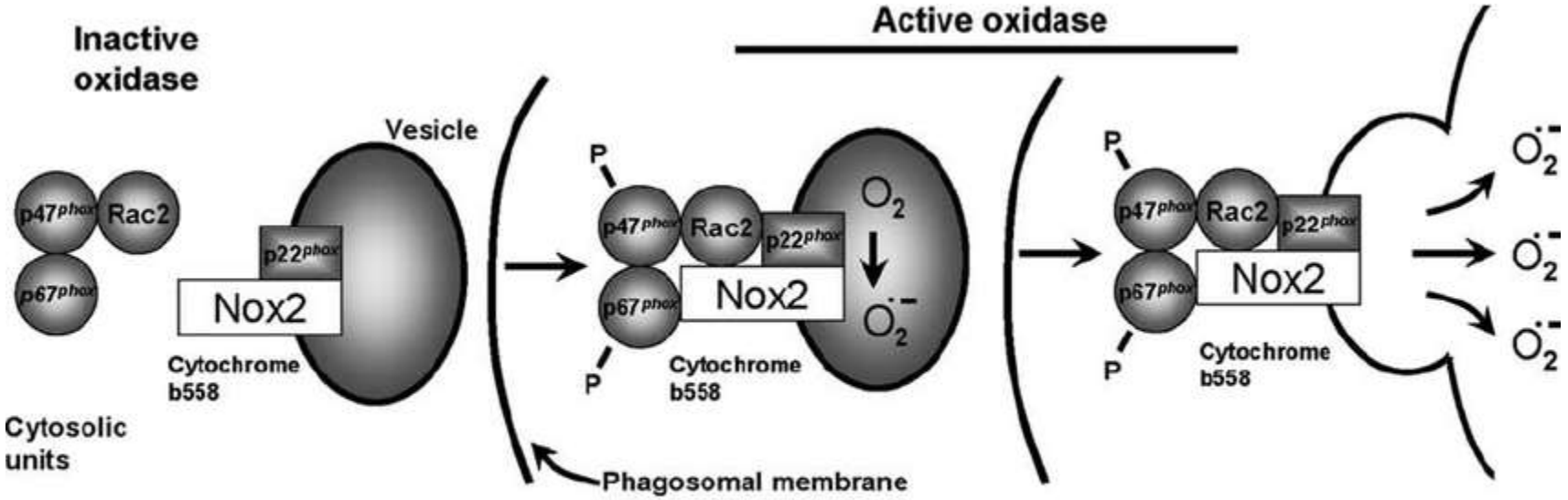
- Is the rapid release of the reactive oxygen species (ROS), superoxide anion (O2) and hydrogen peroxide (H2O2), occur in neutrophils, and tightly linked to phagocytosis.



- Phagocyte oxidase is an enzyme complex consisting of at least seven proteins.

- In resting neutrophils, different components of the enzyme are located in the plasma membrane and the cytoplasm. In response to activating stimuli, the cytosolic protein components translocate to the phagosomal membrane, where they assemble and form the functional enzyme complex.

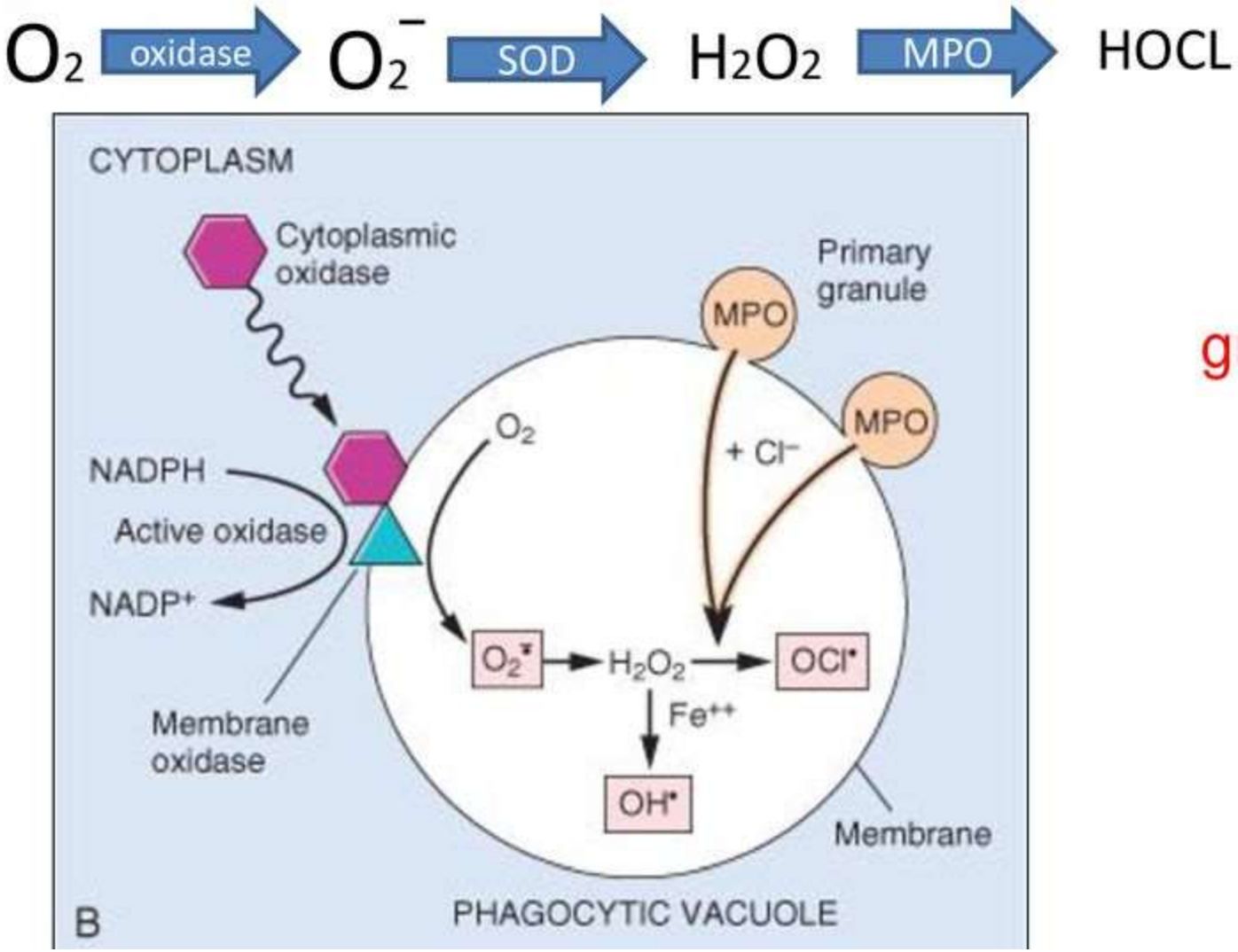
- Thus, the ROS are produced within the phagolysosome, where they can act on ingested particles without damaging the host cell



$$2O_2 + NADPH \rightarrow 2O_2^{\cdot -} + NADP^+ + H^+$$

- $O_2^{\bullet-}$ so produced is then converted into hydrogen peroxide (H_2O_2), mostly by spontaneous dismutation, a process of simultaneous oxidation and reduction.
- H_2O_2 is not able to kill microbes efficiently by itself. However, the azurophilic granules of neutrophils contain the enzyme myeloperoxidase (MPO), which, in the presence of a halide such as Cl^- , converts H_2O_2 to hypochlorite (OCl_2^-).
- The latter is a potent anti-microbial agent that destroys microbes by halogenation (in which the halide is bound covalently to cellular constituents) or by oxidation of proteins and lipids (lipid peroxidation)

The H2O2-MPO-halide system is the most efficient bactericidal system of neutrophils



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ANTI-OXIDANTS

- Serum, tissue fluids, and host cells possess anti-oxidant mechanisms that protect against these potentially harmful oxygen-derived radicals.
- <u>These anti-oxidants include:</u>

- (1) the enzyme superoxide dismutase, which is found in or can be activated in a variety of cell types.
- (2) catalase, which detoxifies H2O2.
- (3) glutathione peroxidase, another powerful H2O2 detoxifier.

- Genetic defects in the generation of ROS are the cause of an immunodeficiency disease called chronic granulomatous disease.

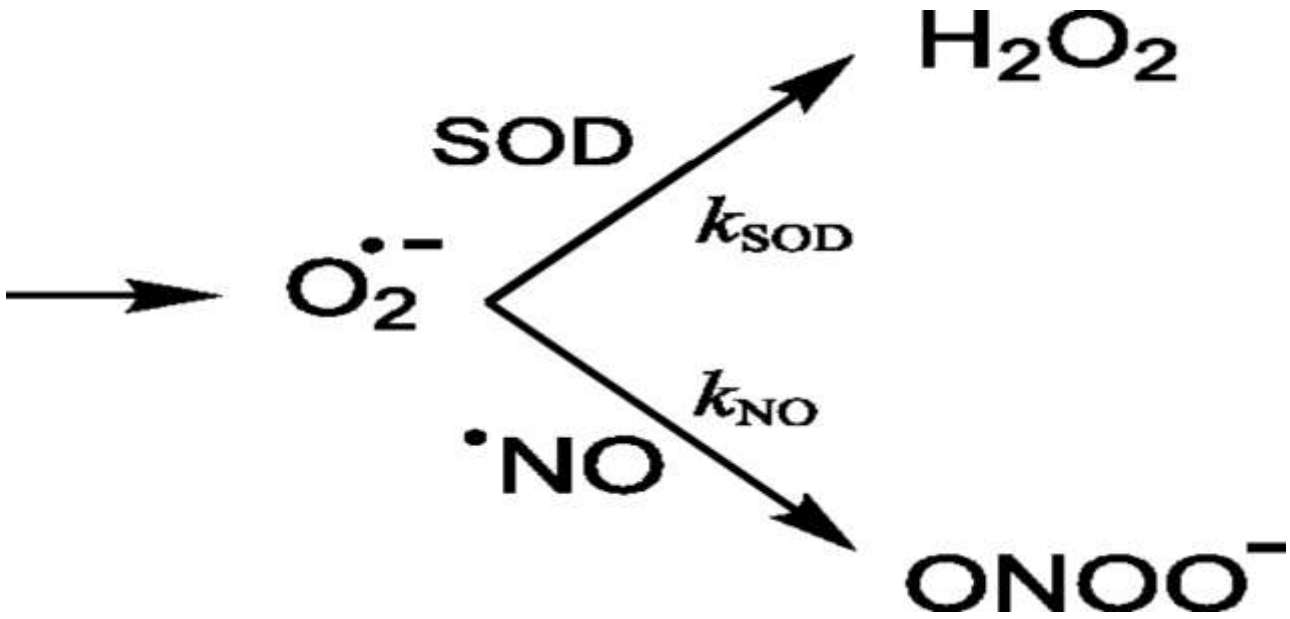
2. Nitric Oxide

- Is a soluble gas produced from arginine by the action of nitric oxide synthase (NOS), also participates in microbial killing.

- There are three different types of NOS:

- Endothelial (eNOS):
  - acts to maintain vascular tone.
  - relaxes vascular smooth muscle and promotes vasodilation
- Neuronal (nNOS): acts as neurotransmitter.
- Inducible (iNOS): involved in microbial killing, is expressed when macrophages are activated by cytokines (e.g., IFN-γ) or microbial products.

- In macrophages, NO reacts with superoxide (O2 •) to generate the highly reactive free radical peroxynitrite (ONOO•) .
- These nitrogen-derived free radicals, similar to ROS, attack and damage the lipids, proteins, and nucleic acids of microbes and host cells



Granule Enzymes and Other Proteins.

- Neutrophils and monocytes contain granules packed with enzymes and anti-microbial proteins that degrade microbes and dead tissues and may contribute to tissue damage.

- Neutrophils have two main types of granules.

- The smaller specific (or secondary) granules contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase.
- The larger azurophil (or primary) granules contain MPO, bactericidal factors (such as defensins), acid hydrolases, and a variety of neutral proteases (elastase, cathepsin G, nonspecific collagenases, proteinase 3).

Different granule enzymes serve different functions

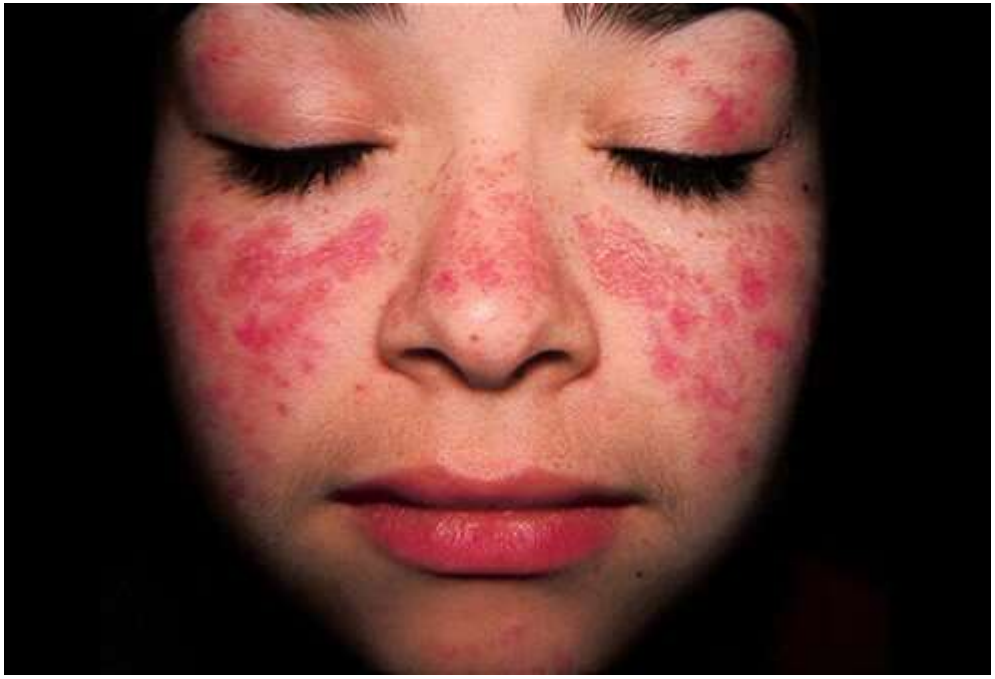
- Acid proteases degrade bacteria and debris within phagolysosomes, which are acidified by membrane-bound proton pumps.
- Neutral proteases are capable of degrading various extracellular components, such as collagen, basement membrane, fibrin, elastin, and cartilage, resulting in the tissue destruction that accompanies inflammatory processes.
- Neutrophil elastase combats infections by degrading virulence factors of bacteria.
- Macrophages also contain acid hydrolases, collagenase, elastase, phospholipase, and plasminogen activator

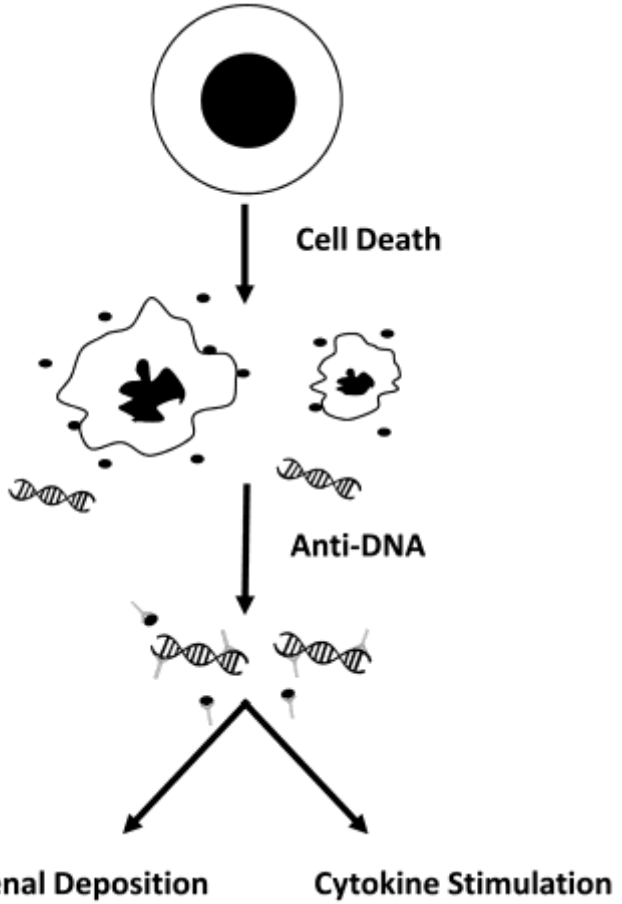
- These harmful proteases, however, are normally controlled by a system of anti-proteases in the serum and tissue fluids.
- Fore most among these is α1-anti-trypsin, which is the major inhibitor of neutrophil elastase.
- A deficiency of these inhibitors may lead to sustained action of leukocyte proteases, as is the case in patients with α1-anti-trypsin deficiency

Neutrophil Extracellular Traps

- Are extracellular fibrillary networks that concentrate anti-microbial substances at sites of infection and prevent the spread of the microbes by trapping them in the fibrils.
- Consist of a meshwork of nuclear chromatin that binds and concentrates granule proteins such as anti-microbial peptides and enzymes .
- The nuclei of the neutrophils are lost, leading to the death of the cells, sometimes called NETosis, representing a distinctive form of cell death affecting neutrophils

- The nuclear chromatin in the NETs, which includes histones and associated DNA, may be a source of nuclear antigens in systemic autoimmune diseases, particularly lupus, in which individuals react against their own DNA and nucleoprotein





Leukocyte-Mediated Tissue Injury

- Leukocytes are important mediators of injury to normal cells and tissues under several circumstances:

- As part of a normal defense reaction against infectious microbes, in some infections that are difficult to eradicate, such as tuberculosis and certain viral diseases such as hepatitis.
- In certain autoimmune diseases.
- In allergic diseases, including asthma

Leukocytes damage tissues by releasing injurious molecules.

- If phagocytes encounter materials that cannot be easily ingested, such as immune complexes deposited on immovable flat surfaces (e.g., glomerular basement membrane).

- Some phagocytosed substances, such as urate an silica crystals, may damage the membrane of the phagolysosome and also lead to the release of damaging contents

Other Functional Responses of Activated Leukocytes

- Macrophages also has many function:

- Production of cytokines that can either amplify or limit inflammatory reactions.
- Production of growth factors that stimulate the proliferation of endothelial cells and fibroblasts.
- synthesis of collagen.
- Secretion of enzymes that remodel connective tissues.

Termination of the Acute Inflammatory Response

- 1. Degradation of mediators.
- 2. Neutrophils have short half-lives in tissues and die by apoptosis within hours to a day or two after leaving the blood.
- 3. Stop signals :

- a switch in the type of arachidonic acid metabolite produced, from proinflammatory leukotrienes to anti-inflammatory lipoxins.
- liberation of anti-inflammatory cytokines, including transforming growth factor-β (TGF-β) and IL-10, from macrophages

Any question????