



# **CELLULAR EVENTS OF INFLAMMATION**

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## Learning objectives:

- By the end of this lecture student should be able to;
- Enumerate the cellular events of inflammation.
- Discuss leukocyte recruitment events.
- Discuss leukocyte activation events.



- Important function of the inflammation is to deliver leukocytes to the site of injury and to activate them.
- Leukocytes/phagocytes (neutrophils and macrophages) ingest offending agents and kill them.
- Macrophages also produce different cytokines.



- These activated leukocytes may induce tissue damage and prolong inflammation.
- So it is important that they are recruited and activated only when needed.
- Leukocyte recruitment is mediated and controlled by chemical mediators and adhesion molecules .



- Cellular events can be studied as.
- **Leukocyte Recruitment Events :**
  1. Margination, adhesion to endothelium and rolling along the vessel wall
  2. Firm adhesion to the endothelium
  3. Transmigration between endothelial cells
  4. Migration in interstitial tissues toward a chemotactic stimulus or chemotaxis.



## 1. Margination and Rolling:

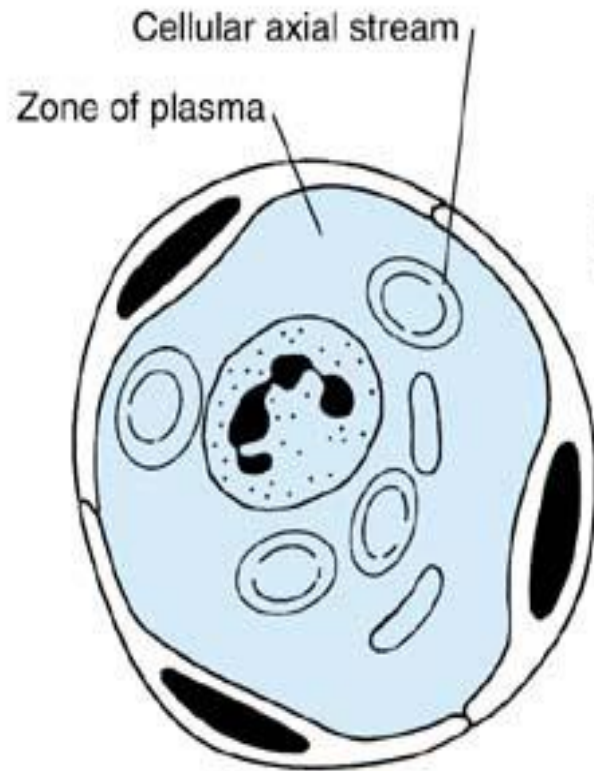
- In a normal blood vessel, the blood cells flow in the centre and are separated from the endothelial surface by a zone of plasma.
- As blood velocity in the dilated vessels decreases in acute inflammation, the flow of blood is disturbed. Erythrocytes form heavy aggregates (**rouleaux**).



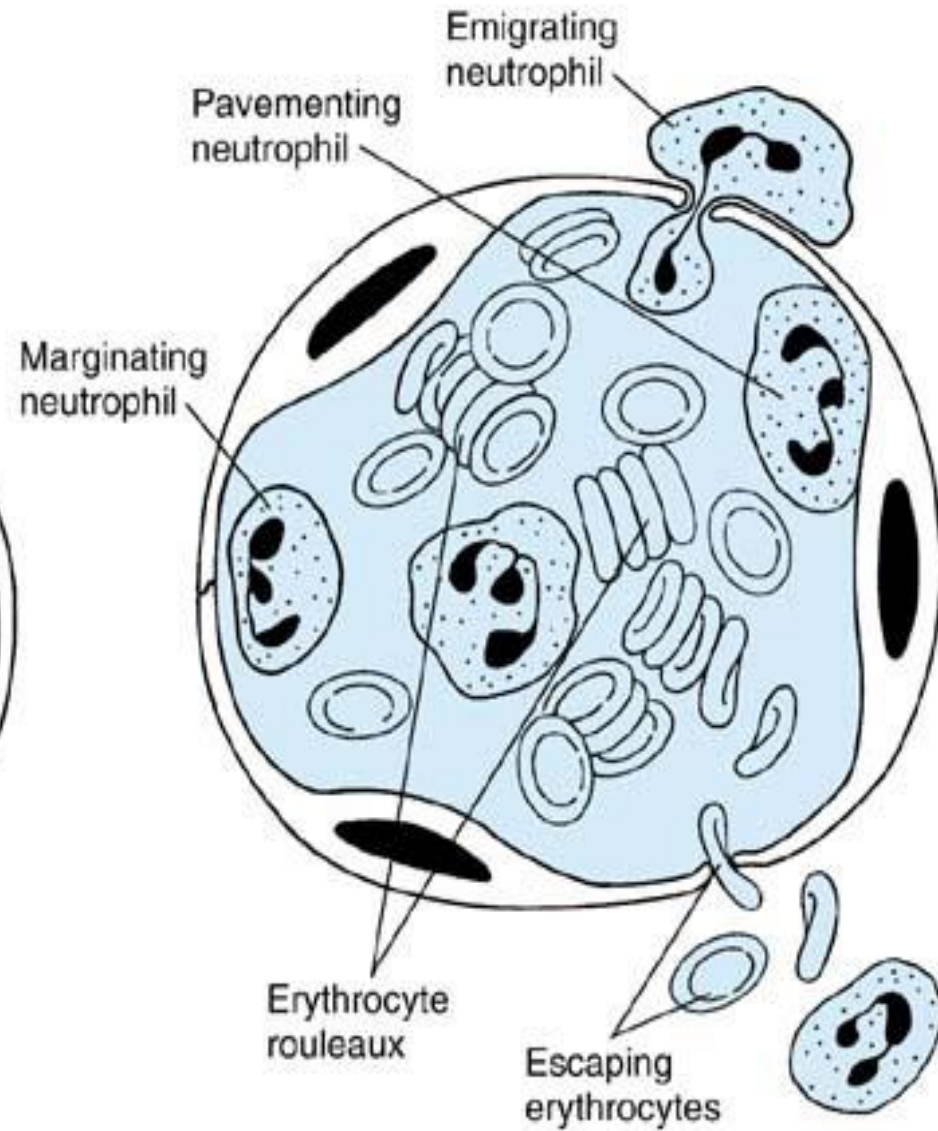
- As a result, leukocytes move to the periphery towards the endothelium. This is called margination.
- After that leukocytes loosely adhere to endothelial surface and tumble on it, a process called rolling.



**A Normal postcapillary venule**



**B Acute inflammation**

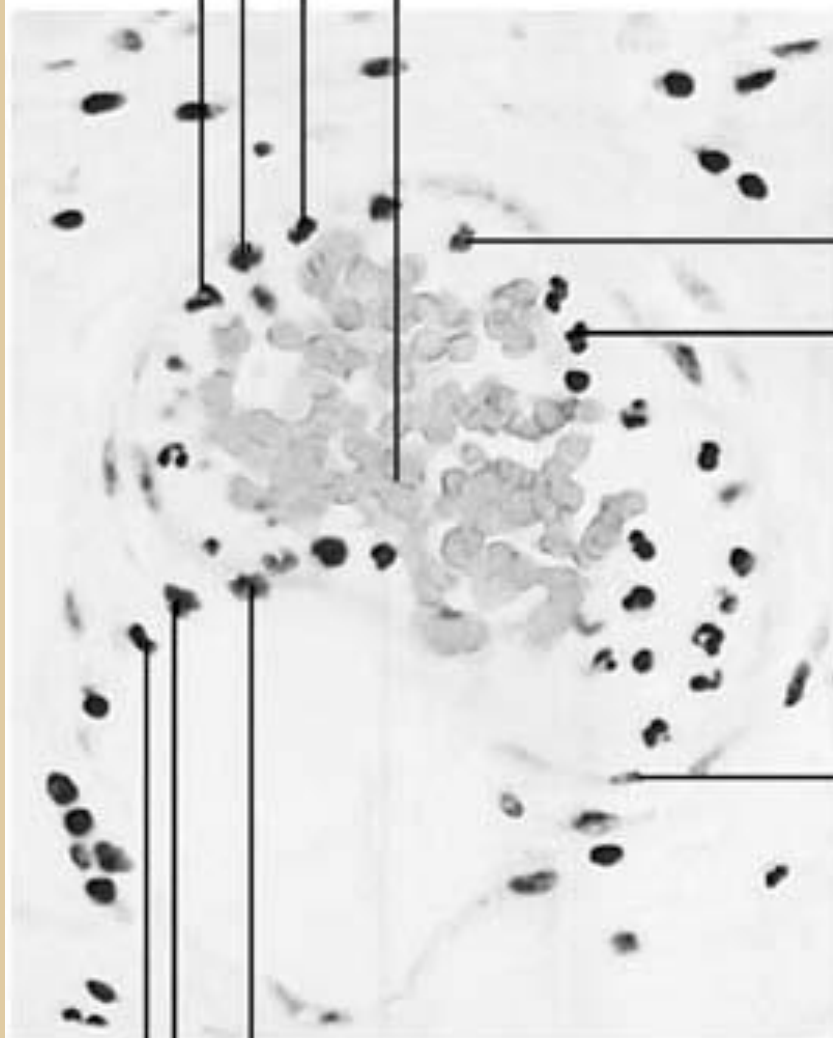






Neutrophils paved  
to endothelium

Aggregated erythrocytes  
(rouleau)



Marginating  
neutrophils

Endothelial  
cell

Emigrating  
neutrophils



## 2. Adhesion

- The next step is firm adhesion to endothelial surfaces.
- Rolling and firm adhesion of leukocytes to the endothelium is brought about by the help of certain adhesion molecules.



- These adhesion molecules are expressed in response to cytokines secreted by sentinel or guard cells after first encounter with the microbes.
- The adhesion molecules belong to two families of proteins.



1. Selectins and their ligands
2. Integrins and their ligands
  - Selectins and Integrins are expressed on leukocytes and endothelium.
  - The rolling of leukocytes takes place due to selectins (loose attachment).
  - Selectins are of three types.



- a. L-selectin (CD62L). Present on leukocytes
  - b. E-selectin (CD62E). Present on endothelium
  - c. P-selectin (CD62P). Present on platelets and endothelium.
- The ligands for selectins are sialylated oligosaccharides attached to glycoproteins.



- Ligands of L-selectins are expressed on endothelium while those of E and P-selectins on leukocytes.
- L-selectins on Leukocytes bind to their ligands present on endothelium
- E- and P-selectins bind to their ligands present on leukocytes.



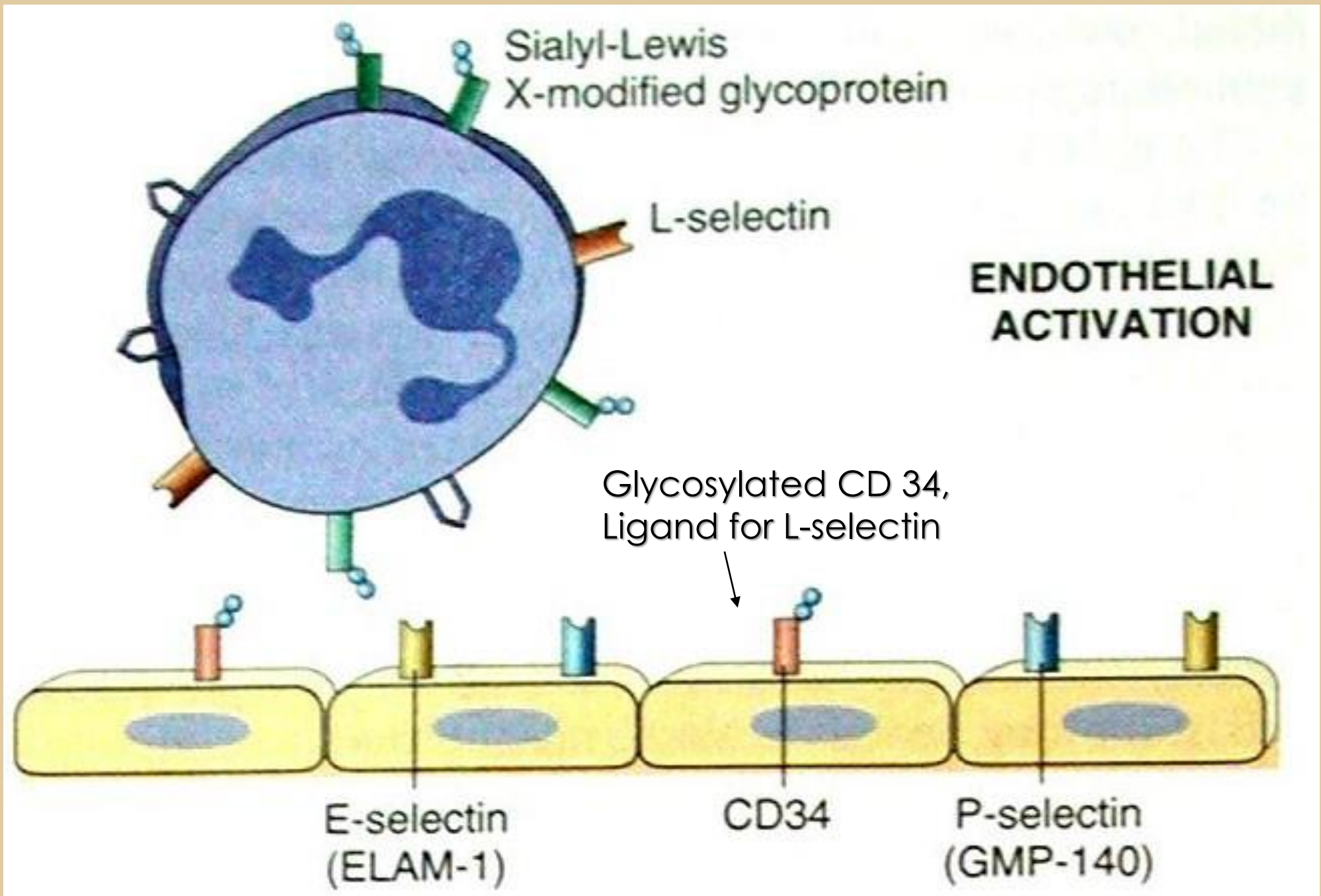
- Macrophages, mast cells and endothelial cells get stimulated by microbes and tissue breakdown products.
- These cells produce cytokines like IL1, TNF and chemokines (chemoattractant cytokines).
- These cytokines (mainly IL1 and TNF) cause

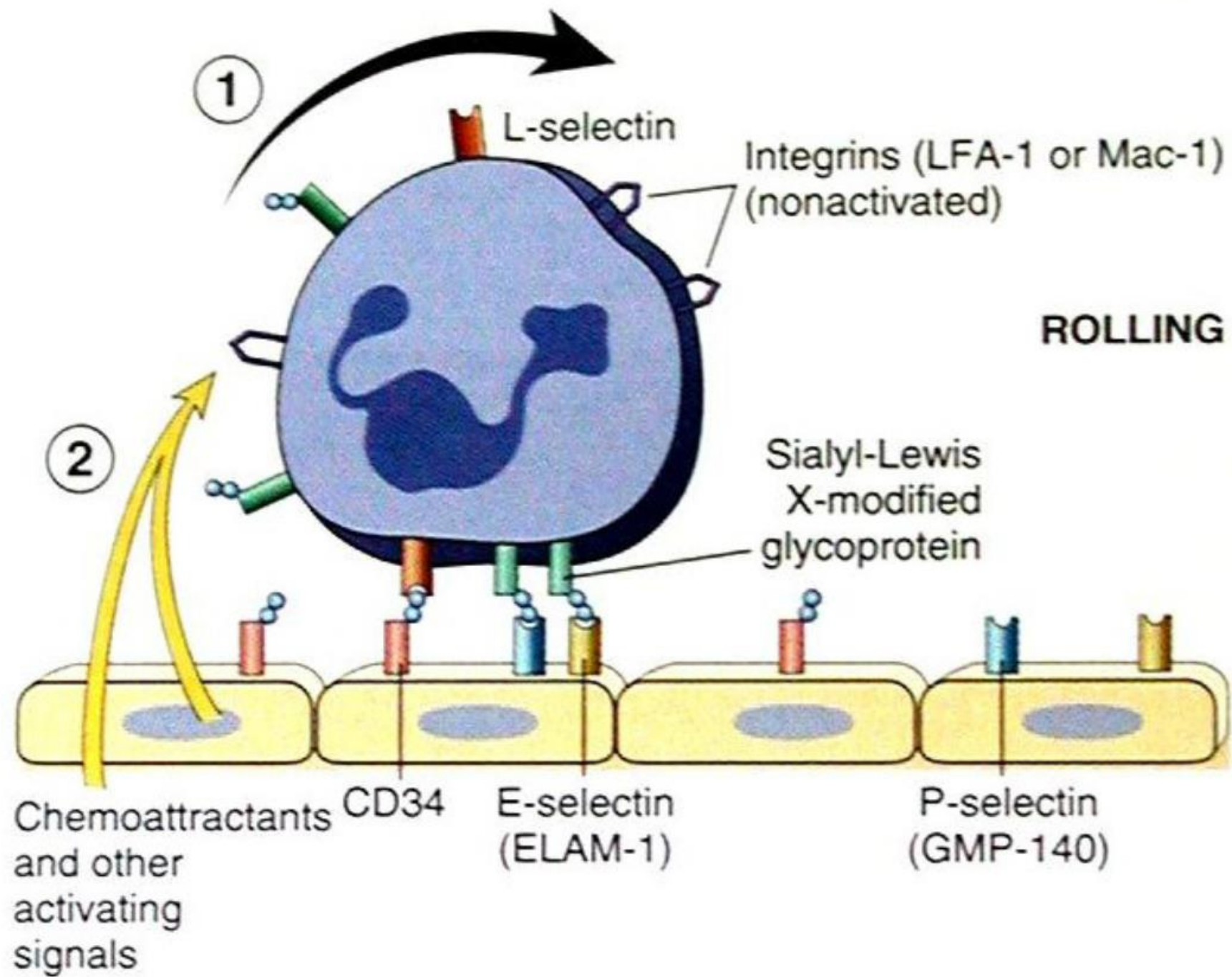


expression of selectins and ligands in post-capillary venular endothelium.

- Within 1-2 hours, endothelial cells begin expression of E selectins and ligands for L selectins.
- Ligands for L-selectins can be sialylated oligosaccharides or glycosylated CD 34.









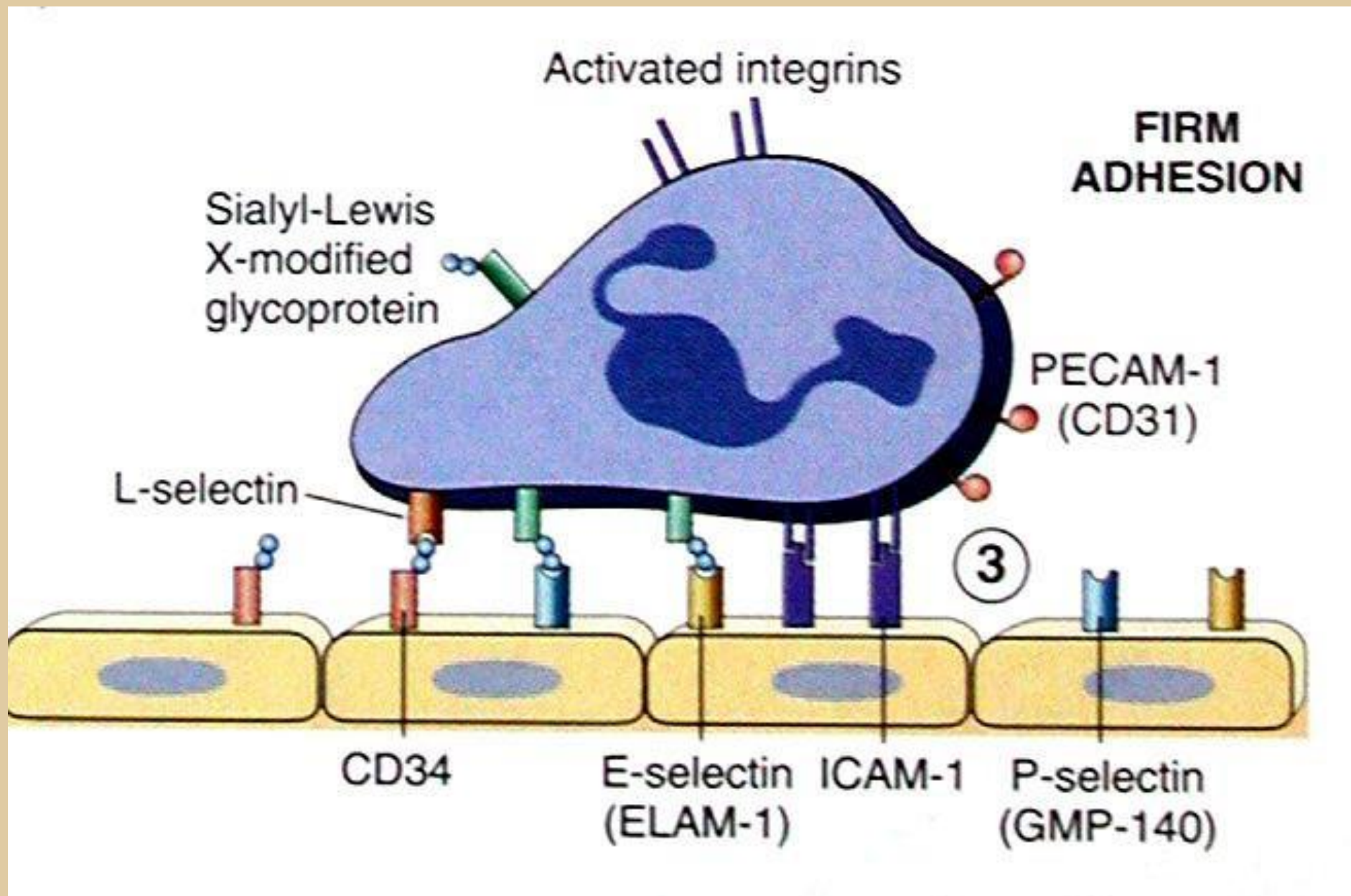
- Histamine and thrombin cause redistribution of P-selectins from inside of endothelial cells to outside.
- Selectin ligand interactions are low affinity.
- Leukocytes get attached and detached easily (rolling).

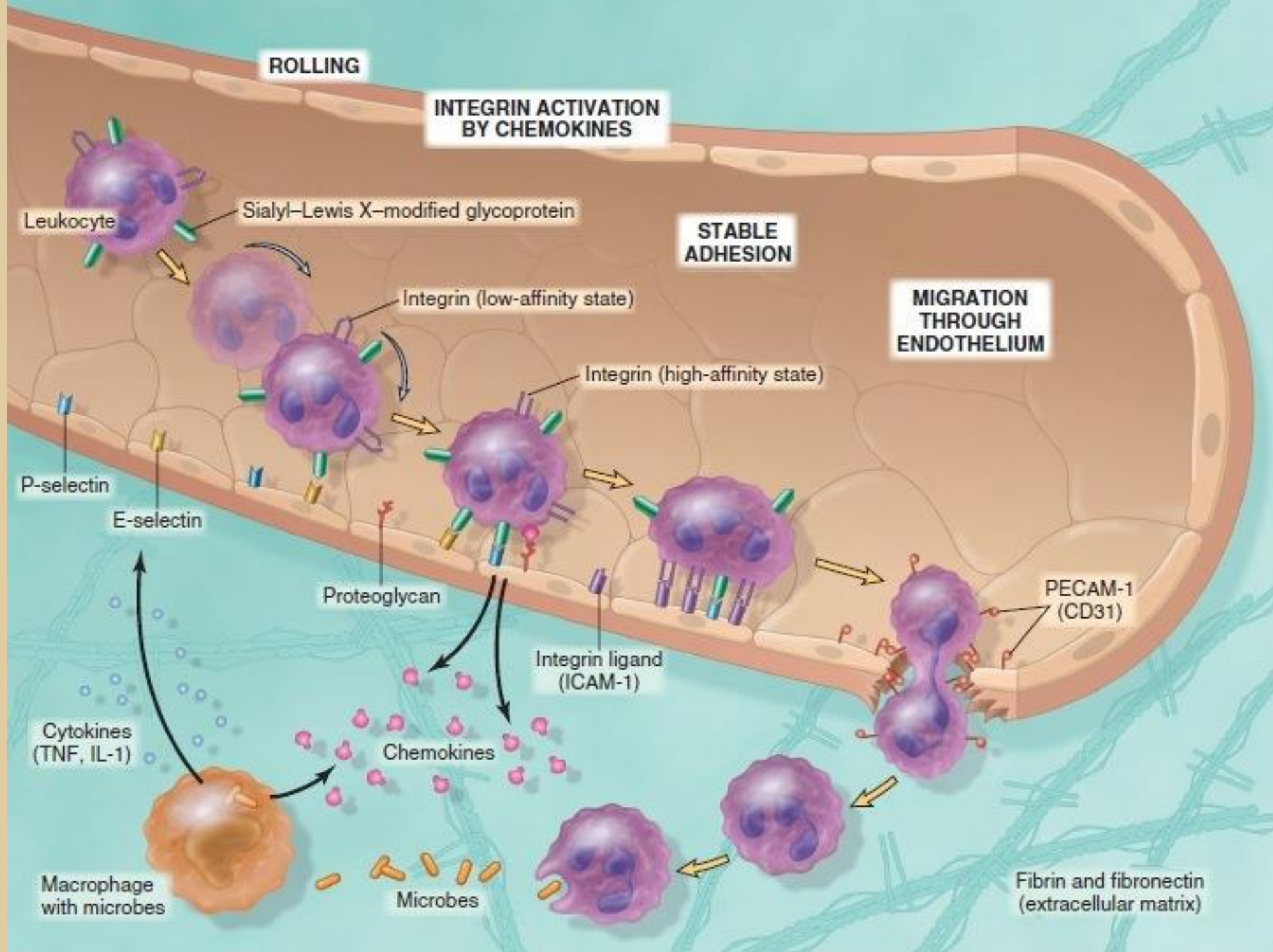


- Integrins are expressed on leukocytes.
- They help leukocytes bind firmly with their ligands.
- They are glycoproteins.
- Their ligands are present on endothelium.
- The main integrins are LFA-1 and MAC-1, and VLA-4.



- LFA-1 (leukocyte function-associated antigen-1) and MAC-1 (Macrophage-1 antigen) attach with ICAM 1 ligand (Intercellular adhesion molecule 1).
- VLA-4 (very late antigen-4) attaches with VCAM-1 (vascular endothelial adhesion molecule).







### 3. Transmigration/Emigration:

- After being adhered to the endothelial surface, leukocytes migrate through the vessel wall by squeezing between cells through intercellular junctions.
- It occurs by the movement of leukocytes, called diapedesis.

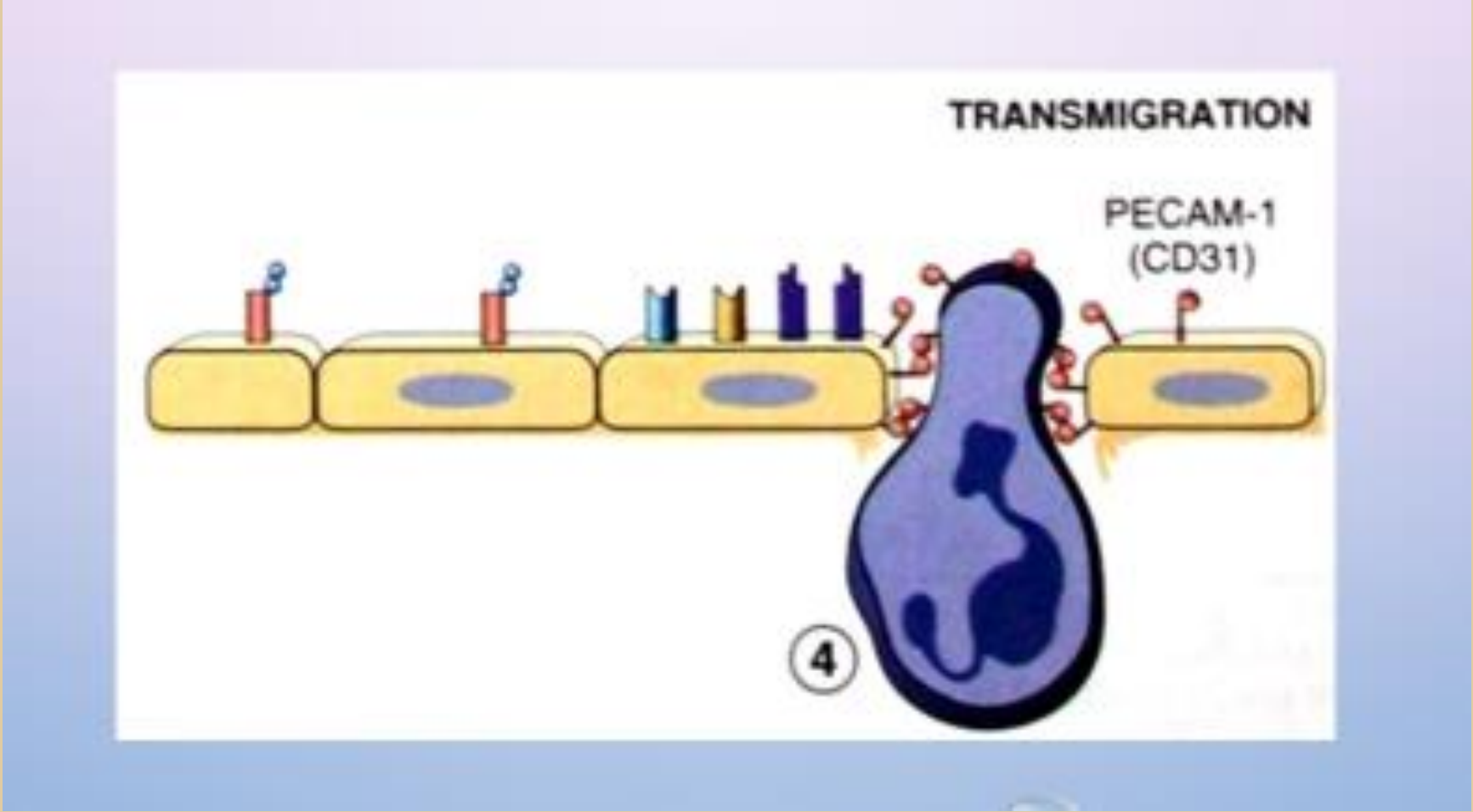




- Transmigration occurs mainly in post-capillary venules.
- Chemokines act on the adherent leukocytes and stimulate them to migrate through inter-endothelial gaps toward the chemical gradient.



- Several adhesion molecules in junctions between endothelial cells are involved in migration.
- One of them belongs to immunoglobulin superfamily called CD31 or PECAM-1 (platelet endothelial cell adhesion molecule-1).







#### 4. Chemotaxis:

- It is the process through which leukocytes are attracted towards a chemical at the site of injury after leaving vessels.
- Chemotactic substances responsible for chemotaxis are called chemotactic substances or chemotactants.
- The common chemotactants are.



## 1. Exogenous:

- Bacterial products.

## 2. Endogenous

- Complement components specially C5a.
- Arachidonic acid metabolites specially Leukotrein B4 ( LTB4).
- Cytokines like IL8 (chemokine).



## **B. Leukocyte activation events:**

### **I. Phagocytosis:**

It is a process by which microorganisms and other harmful particles are engulfed and destroyed by the phagocytes.

Phagocytosis has three distinct but interrelated steps.



1. Recognition and attachment of particle to the leukocyte.
  2. Engulfment
  3. Killing and degradation
1. Recognition and attachment :
    - The white cells identify the microorganism.
    - Recognition is brought about by the





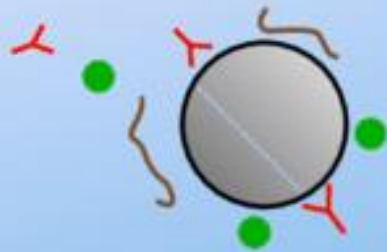
mannose receptors(lectins), scavenger receptors, and receptors for various opsonins.

- Process becomes very efficient in presence of opsonins.
- Opsonins are proteins that coat the surface of organisms and then attach to receptors present on leukocytes.

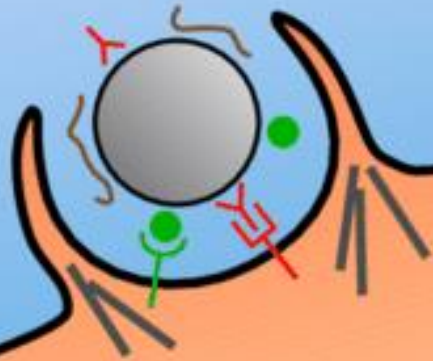


- Opsonins include complement fragments C3b and C5b, IgG, Manose binding lectin and collectins (plasma carbohydrate-binding lectins).
- MBL is also a collectin.

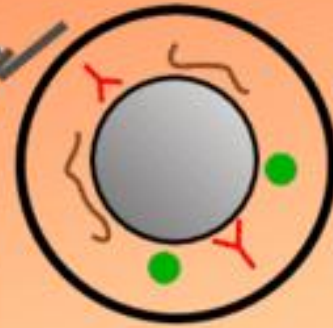
**A** Opsonization



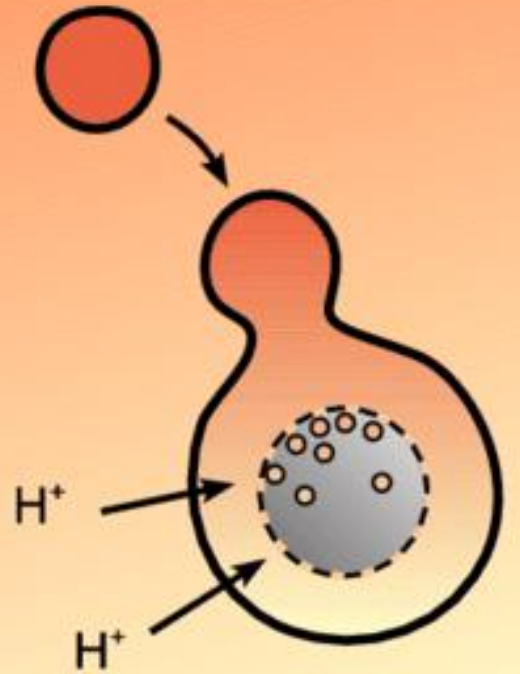
**B** Adhesion and ingestion



**C** Phagosome formation



**D** Phagosolysosome formation



Legend:

Y Immunoglobulin G

● Complement protein

~ Other opsonins

▬ Actin

Y Fcγ receptor

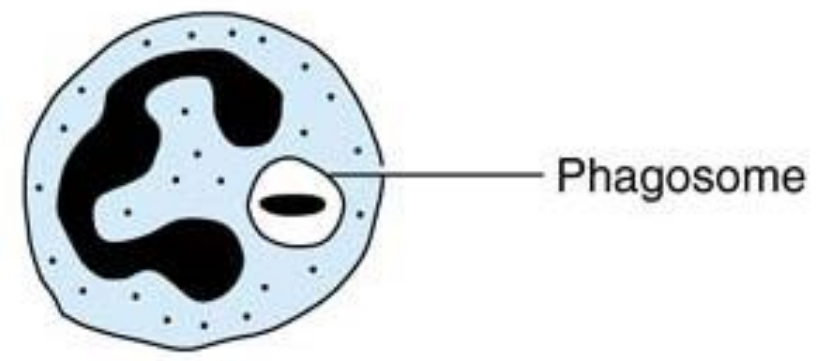
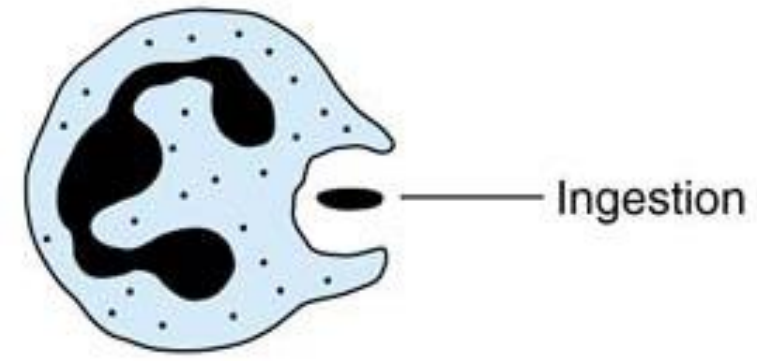
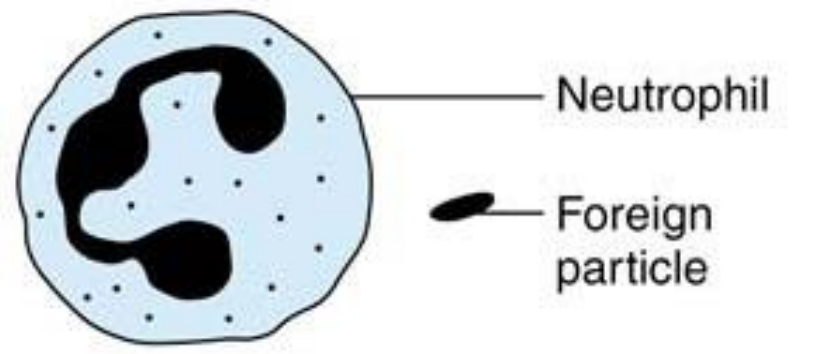
Y Complement receptor

● Enzyme pool



## 2.Engulfment:

- Once recognized by a neutrophil or macrophage, a foreign particle is engulfed by the phagocytic cell to form a membrane-bound vacuole called a **phagosome**.
- Phagosome fuses with lysosomes to form a **phagolysosome**.





### 3. Killing and degradation:

#### 1. Oxygen dependent:

- Brought about by ROS and reactive nitrogen species.
- Attachment of leukocyte and microorganism activates an enzyme NADPH oxidase (phagocyte oxidase).
- This enzyme converts NADPH to NADP + H
- During the process oxygen is reduced to superoxide (  $O_2^{\cdot-}$  ).



- In phagosome superoxide is converted into  $\text{H}_2\text{O}_2$ .
- $\text{H}_2\text{O}_2$  reacts with chloride to form hypochlorite ( $\text{HOCl}\cdot$ ) radical in the presence of MPO.
- $\text{HOCl}\cdot$  acts as anti microbial(halogenation).
- $\text{H}_2\text{O}_2$ -MPO halide system is most potent bactericidal system of neutrophils.



- $\text{H}_2\text{O}_2$  also converted to hydroxyl radical.
- Microbicidal radical.
- ROS may be released extracellularly and cause damage to adjacent cells and tissues.
- NO is a soluble gas.
- Produced from Arginine by action of NO synthase (NOS).





- There are three different types of NOS:
  - a. Endothelial (eNOS)
  - b. Neuronal (nNOS)
  - c. Inducible (iNOS)
- iNOS is involved in microbial killing.
- NO reacts with superoxide ( $O_2^{\bullet -}$ ) to generate the highly reactive free radical peroxynitrite ( $ONOO^-$ ).

### 1. RECOGNITION AND ATTACHMENT

Microbes bind to phagocyte receptors

Phagocytic receptor

Microbe ingested in phagosome

Lysosome with enzymes

Fusion of phagosome with lysosome

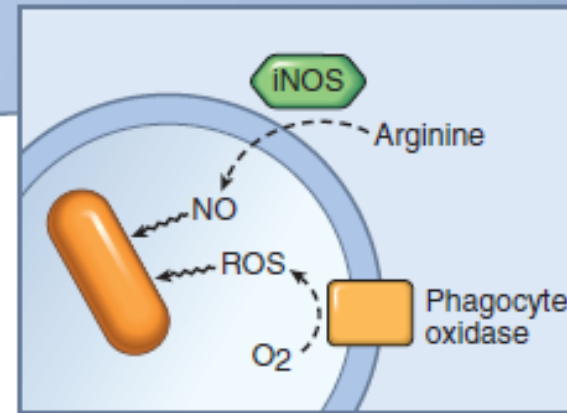
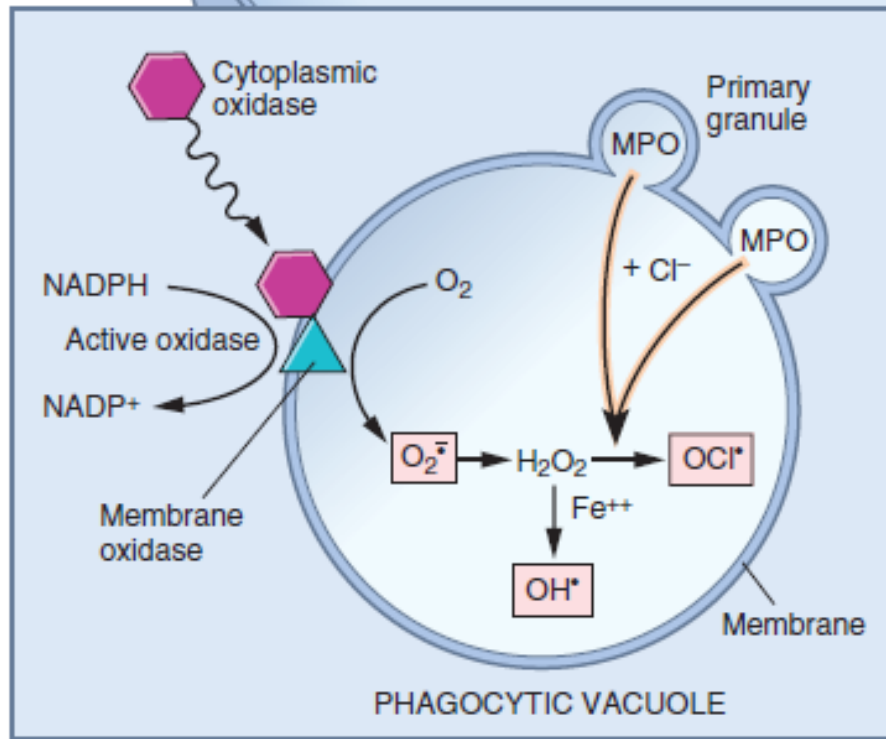
2. ENGULFMENT  
Phagocyte membrane zips up around microbe

Phagosome with ingested microbe

Phagolysosome

Degradation of microbes by lysosomal enzymes in phagolysosome

3. KILLING AND DEGRADATION





## 2. Oxygen independent:

- In the absence of oxygen, leukocytes can kill the microorganisms by the bactericidal agents present in their lysosomal granules.
- Both neutrophils and macrophages are involved.



- Neutrophils have two types of granules.
- Smaller specific/secondary granules
- Larger azurophil/primary granules
- Secondary granules contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase.



- Lactoferrin is an iron-binding glycoprotein.
- It chelates iron so competes for iron with bacteria.
- It may help generate  $\text{OH}^\bullet$
- Primary granules contain MPO, bactericidal proteins (lysozyme, defensins), acid hydrolases, and proteases like elastase, cathepsin G, nonspecific collagenases and proteinase 3.



- Lysozyme causes degradation of peptidoglycan coat of microorganisms.
- Defensins are peptides that kill microbes by creating holes in their membranes.
- All the proteases are controlled by circulating anti proteases like alpha 1 antitrypsin and alpha 2 macroglobulin.

# MCQs

1. The process of leukocyte accumulation at the periphery of vessel is;
  - A. Adhesion
  - B. Rolling
  - C. Migration
  - D. Margination
  - E. Tumbling





2. Integrins are glycoproteins and play an important role in the leukocyte recruitment events. These molecules are present on;
  - A. Endothelial cells
  - B. Mast cells
  - C. Epithelial cells
  - D. Neutrophils and macrophages
  - E. All nucleated cells





- The process of phagocytosis is essential for removing microorganisms and damaged tissues. This process is facilitated by the;
  - A. Circulating proteins
  - B. Tissue damage sensors
  - C. Opsonins attached to the surface of microbes
  - D. Other cellular proteins
  - E. Cytokine receptors



THANK YOU