INFLAMMATION AND REPAIR
Lecture 2
Cellular Events in Inflammation

(Foundation Block, Pathology)

Maha Arafah
Associate Professor
Department of Pathology
King Saud University

Email: marafah @hotmail.com

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Objectives

1. **Describe the steps involved in extravasation of leukocytes from the blood to the tissues. Know the steps at which selectins and integrins act.**

2. **Describe the meaning and utility of chemotaxis. Understand the role that chemokines play in inflammation.**

3. **Describe the steps involved in phagocytosis and the role of IgG and C3b as opsonins and receptors.**

4. **List the mechanisms of microbial killing.**

5. **Know various defects in leukocyte function.**

Reference book and the relevant page numbers..

- Robbins Basic Pathology 9th edition, pages 34-41

Acute Inflammation

CELLULAR EVENTS:

- A critical function of inflammation is to deliver leukocytes to the site of injury

LEUKOCYTE EXTRAVASATION

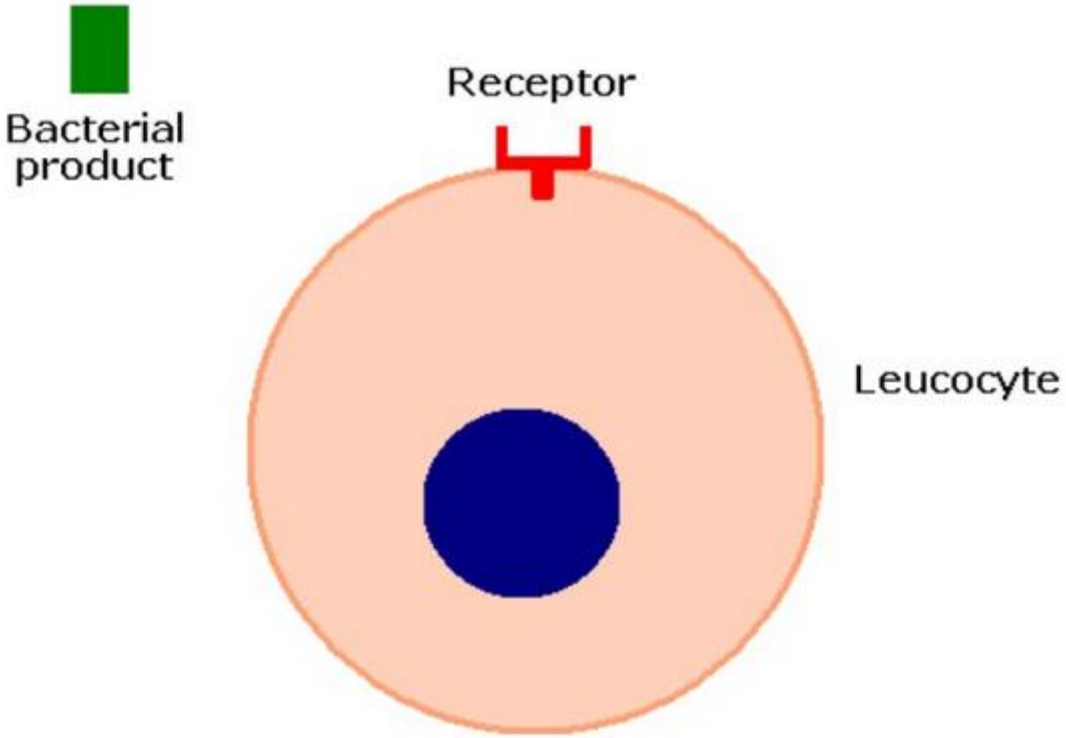
and to activate the leukocytes to perform their normal functions in host defense.

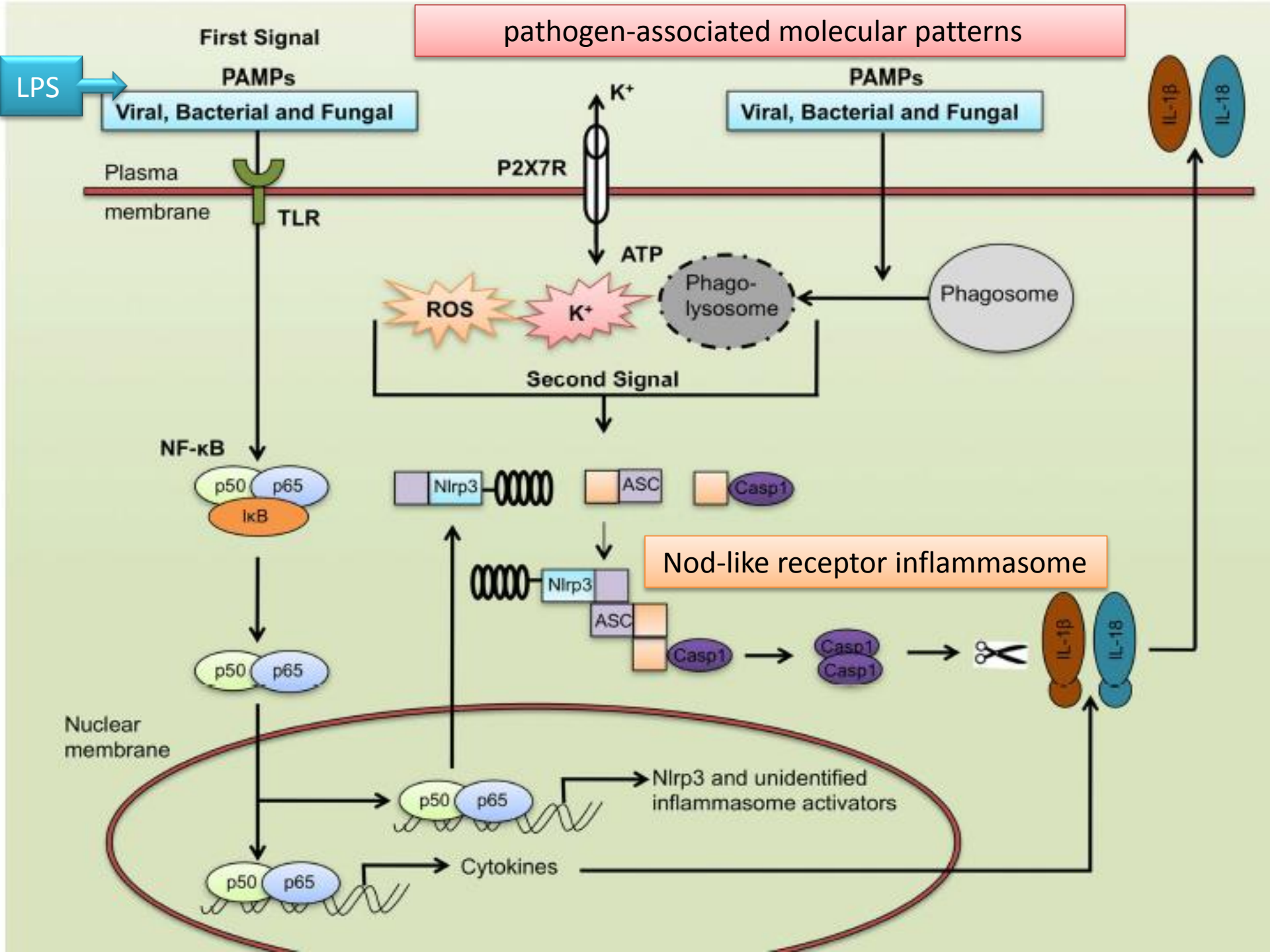
WHAT ARE THESE FUNCTION?

- Leukocytes ingest offending agents, kill bacteria and other microbes, and get rid of necrotic tissue and foreign substances.
- They may induce tissue damage and prolong inflammation, since the leukocyte products that destroy microbes and necrotic tissues can also injure normal host tissues.

Removal of the Offending Agents

1. Recognition of microbes by the receptors
2. *Leukocyte activation:*
   - Increase in cytosolic Ca2+ &
   - Activation of enzymes:
     - Protein kinase C *and*
     - Phospholipase A2
1. Destruction of microbes
   - Phagocytosis *and*
   - Intracellular killing

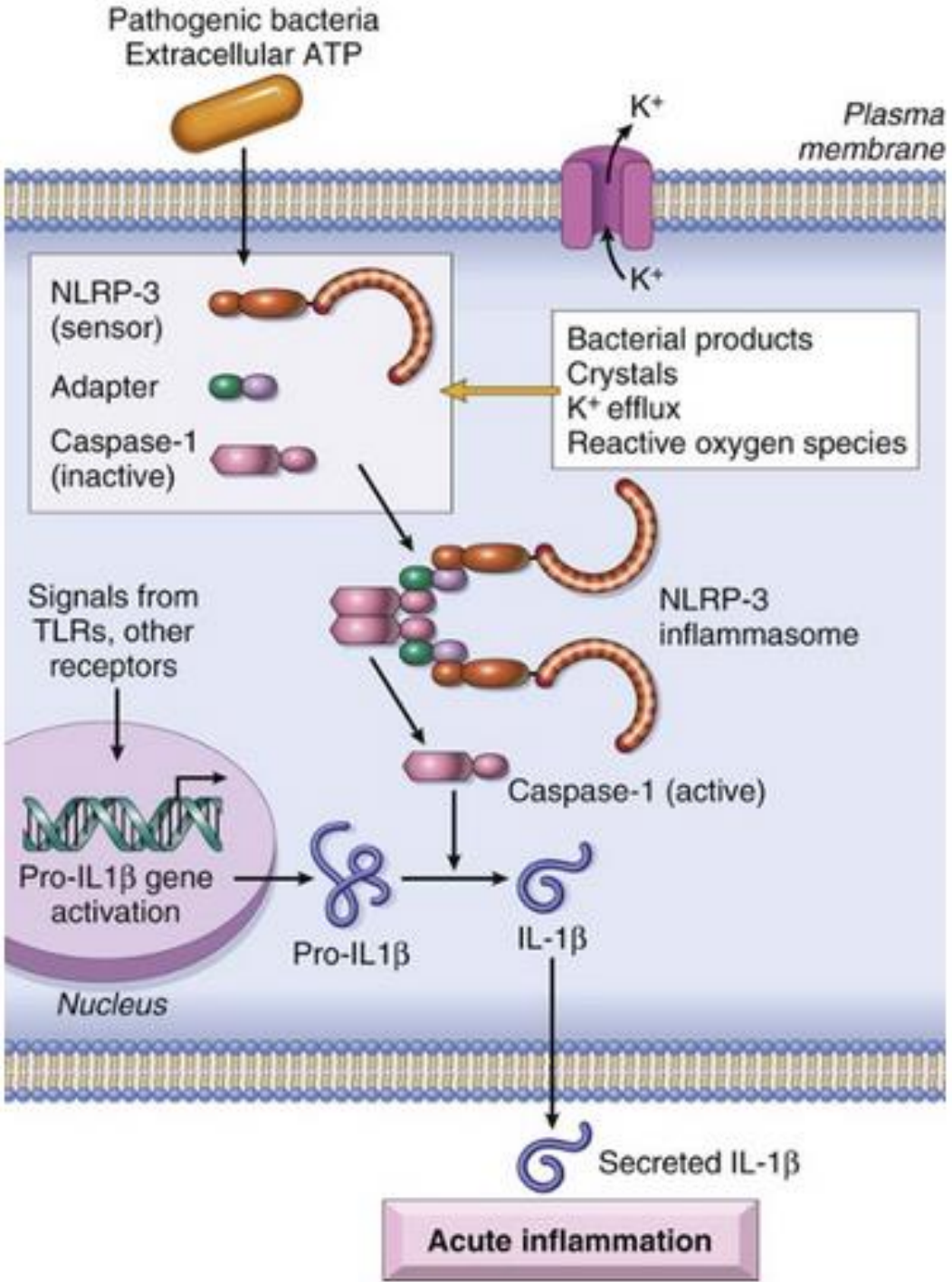

Bacterial product
Receptor
Leucocyte


pathogen-associated molecular patterns
First Signal
LPS
PAMPs
Viral, Bacterial and Fungal
PAMPs
Viral, Bacterial and Fungal
K+
P2X7R
IL-1β
IL-18
Plasma
membrane
TLR
ATP
ROS
K+
Phago-
lysosome
Phagosome
Second Signal
NF-κB
p50
p65
IκB
Nlrp3
ASC
Casp1
Nod-like receptor inflammasome
Nlrp3
ASC
Casp1
Casp1
Casp1
IL-1β
IL-18
p50
p65
Nuclear
membrane
p50
p65
Nlrp3 and unidentified
inflammasome activators
p50
p65
Cytokines

The inflammasome

The inflammasome is a protein complex that recognizes products of dead cells and some microbes and induces the secretion of biologically active interleukin 1.

The inflammasome consists of a sensor protein (a leucine-rich protein called NLRP3), an adapter, and the enzyme caspase-1, which is converted from an inactive to an active form



Recruitment of leukocytes

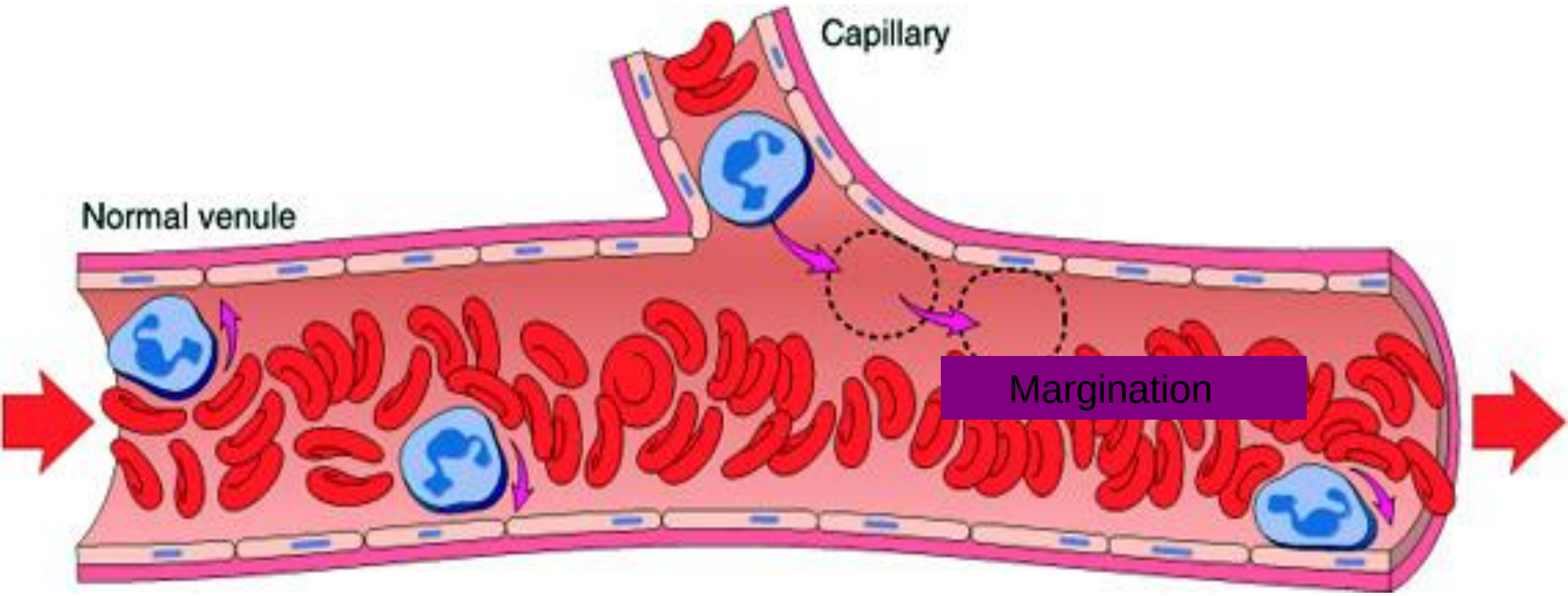
- A multistep process involving attachment of circulating leukocytes to endothelial cells and their migration through the endothelium ***(extravasation)***
- **3 steps:**

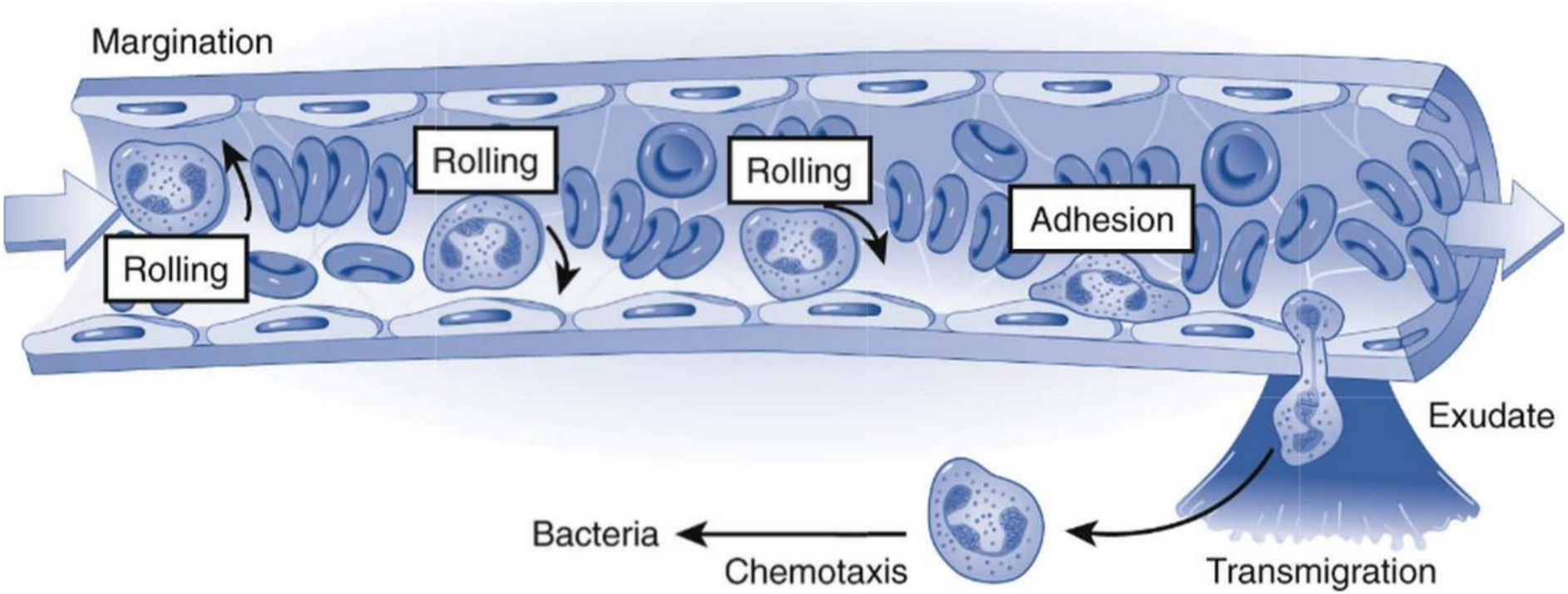
1. In the lumen:
   i. Margination
   ii. rolling
   iii. adhesion to endothelium

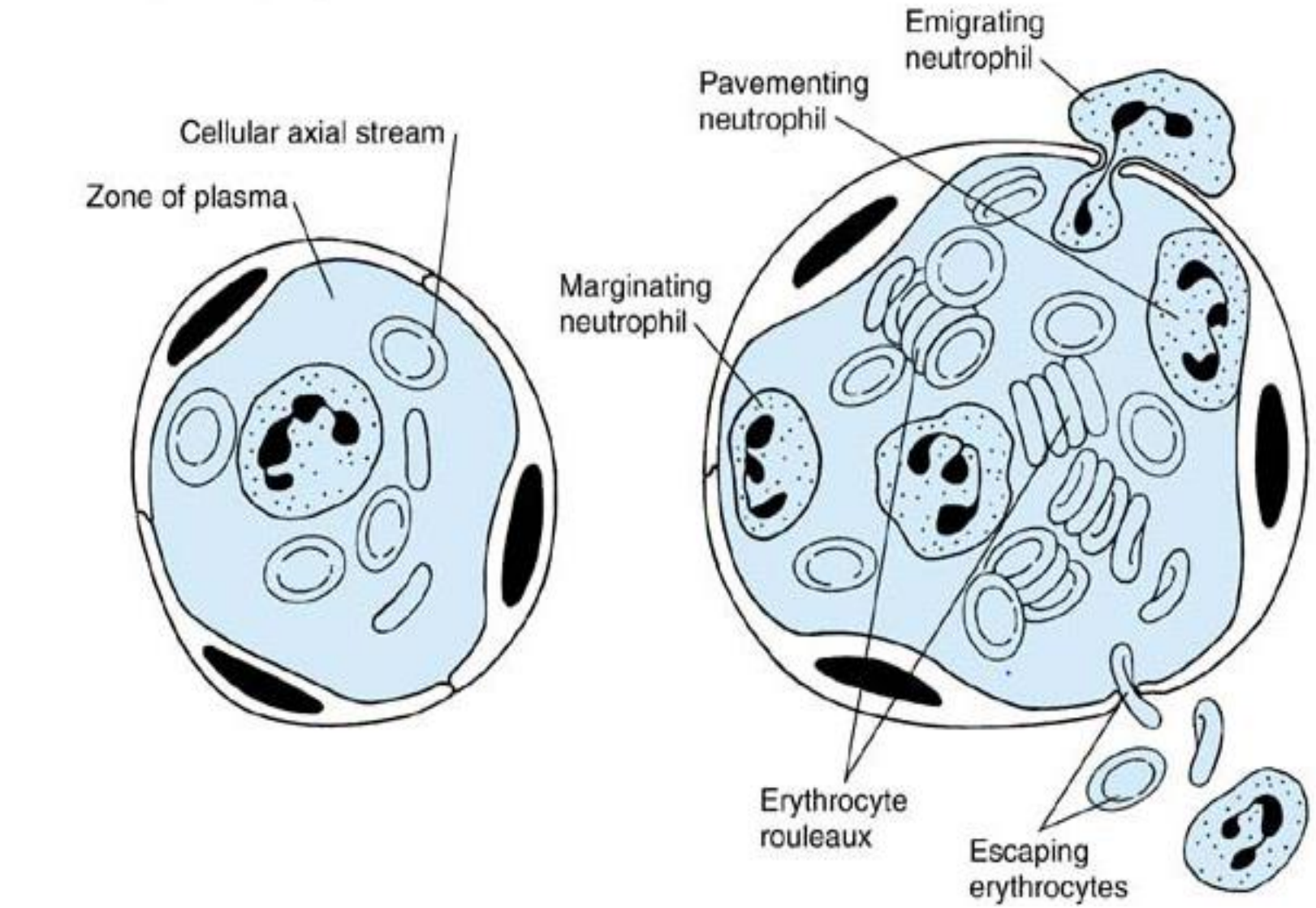
   Vascular endothelium normally does not bind circulating cells
2. Transmigration across the endothelium (also called diapedesis)
3. Migration in interstitial tissues toward a chemotactic stimulus

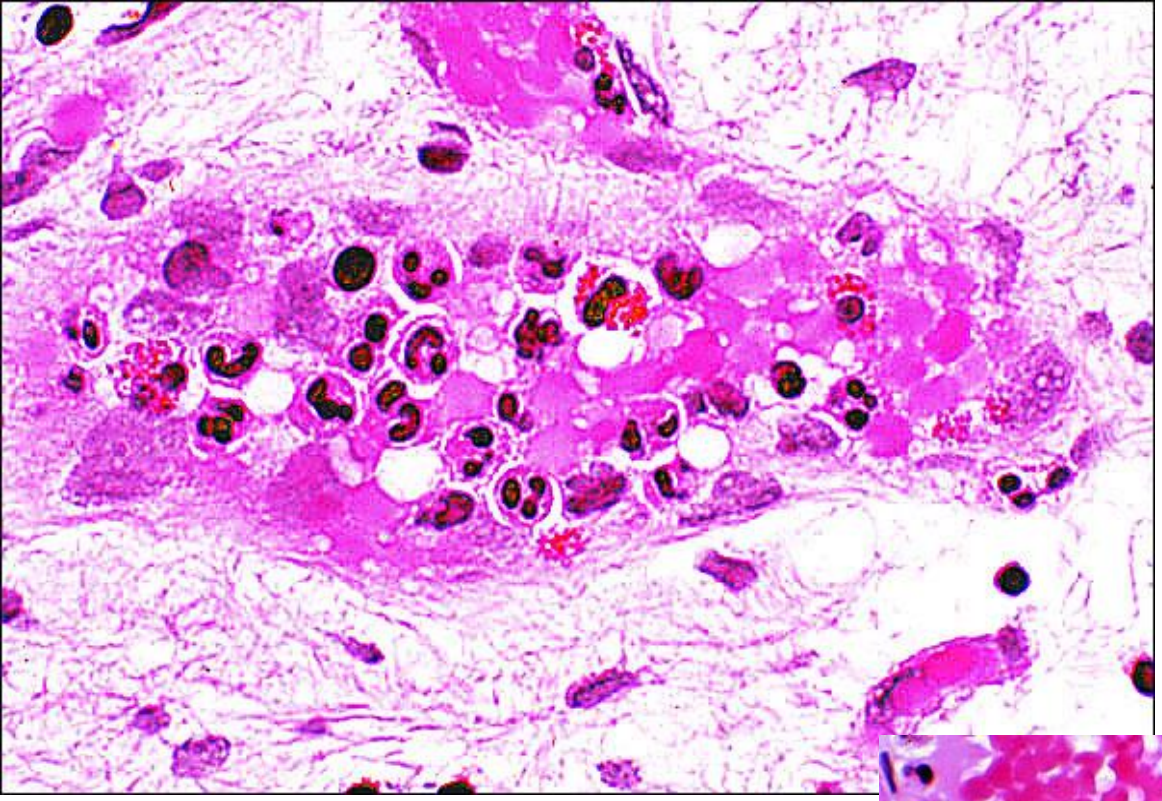
Acute Inflammation

CELLULAR EVENTS:
LEUKOCYTE EXTRAVASATION AND PHAGOCYTOSIS




Margination
Rolling
Rolling
Rolling
Adhesion
Exudate
Bacteria
Chemotaxis
Transmigration


A Normal postcapillary venule
Cellular axial stream
Zone of plasma
B Acute inflammation
Emigrating neutrophil
Pavementing neutrophil
Marginating neutrophil
Erythrocyte rouleaux
Escaping erythrocytes



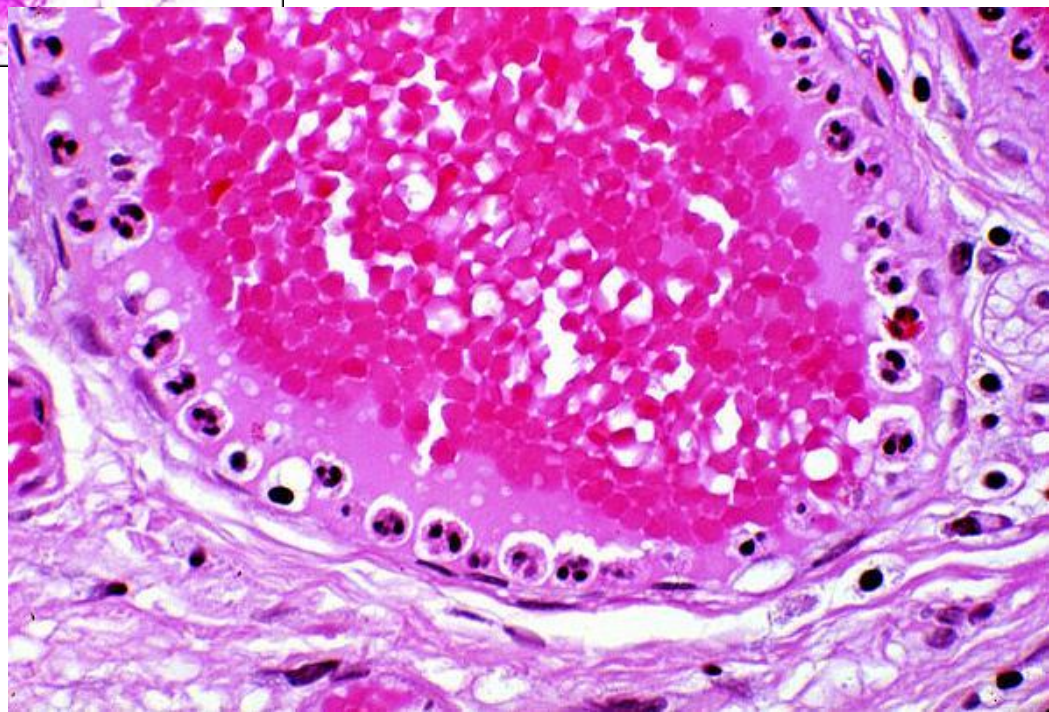
Leukocytes Rolling

Within a Venule

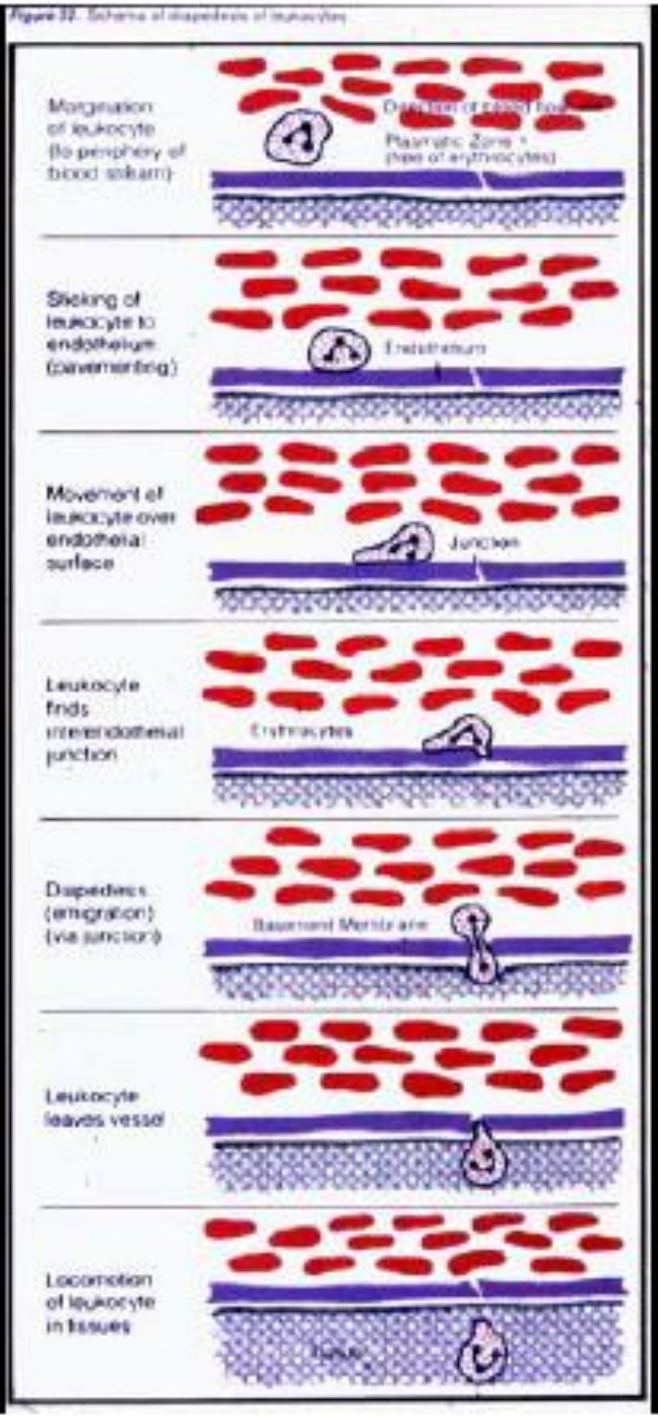
Margination

because blood flow slows early in inflammation (stasis), the endothelium can be lined by white cells

(pavementation)



leukocyte exudation



margination

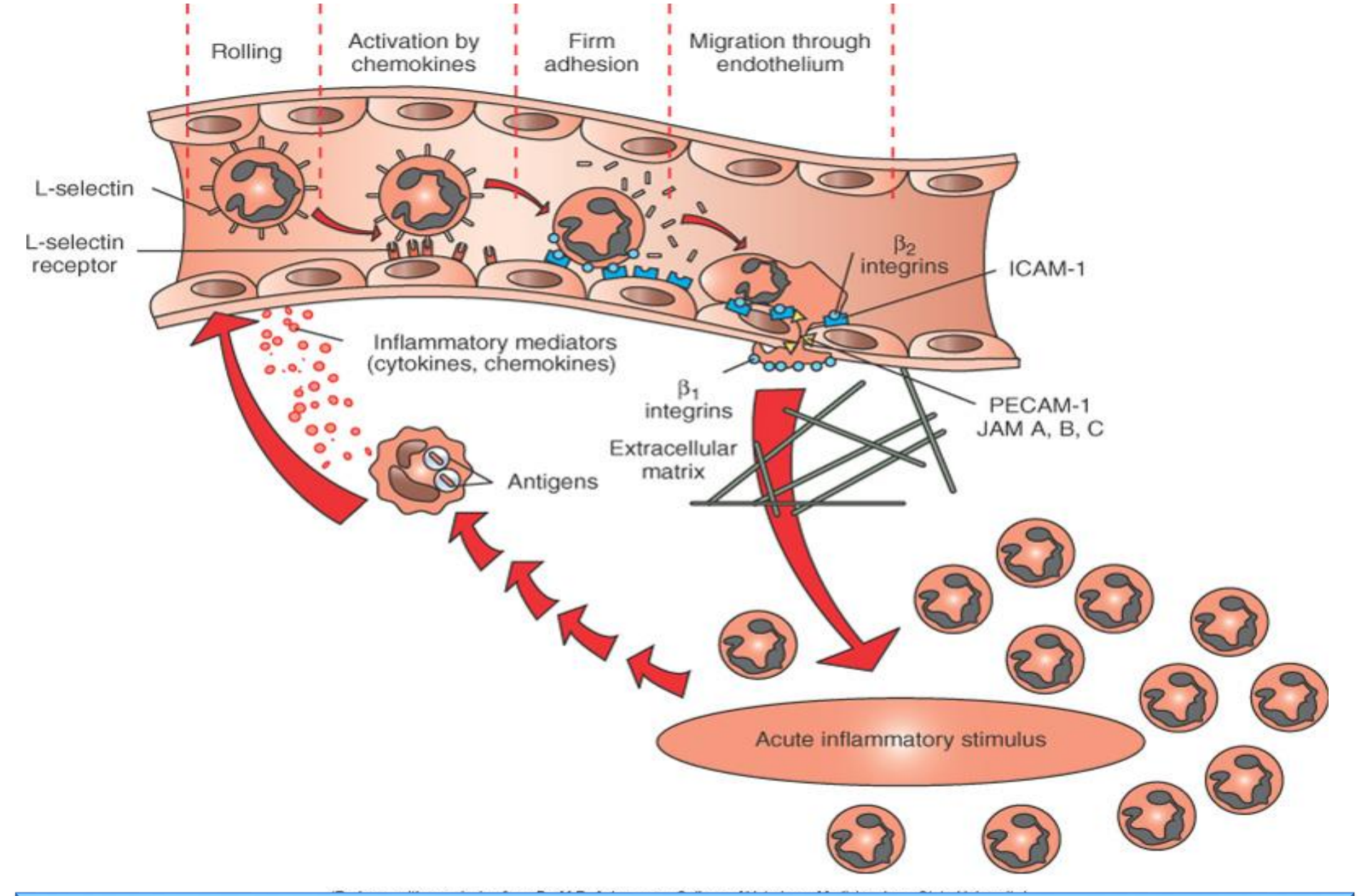
sticking

migration

insertion into jct.

diapedesis

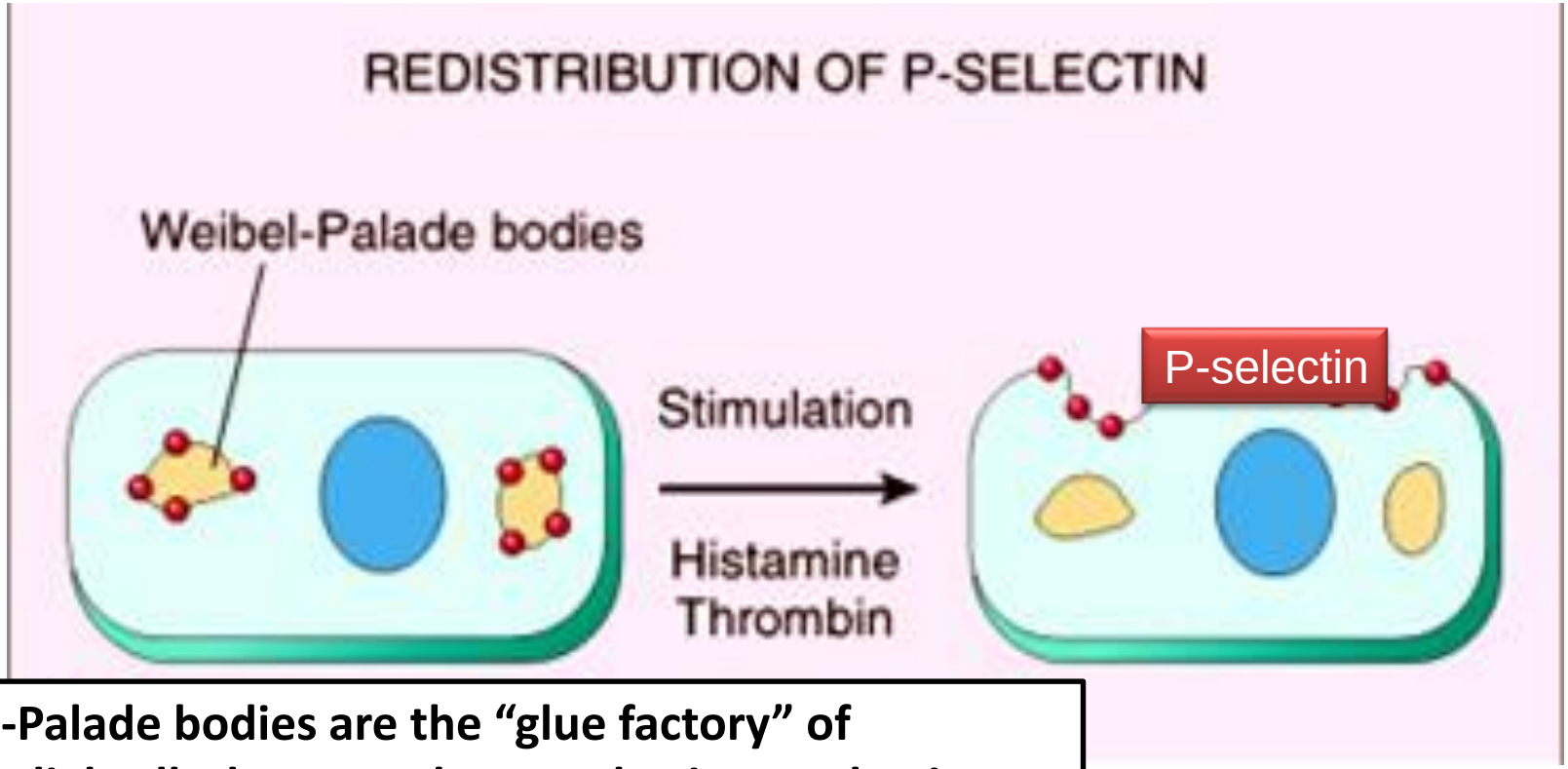
chemotaxis



Resident tissue macrophages, mast cells, and endothelial cells respond to injury by secreting the cytokines TNF, IL-1, histamine and chemokines

Leukocyte Adhesion

Mediators such as histamine, thrombin, and platelet activating factor (PAF) stimulate the redistribution of P-selectin from its normal intracellular stores in granules (Weibel-Palade bodies) to the cell surface.

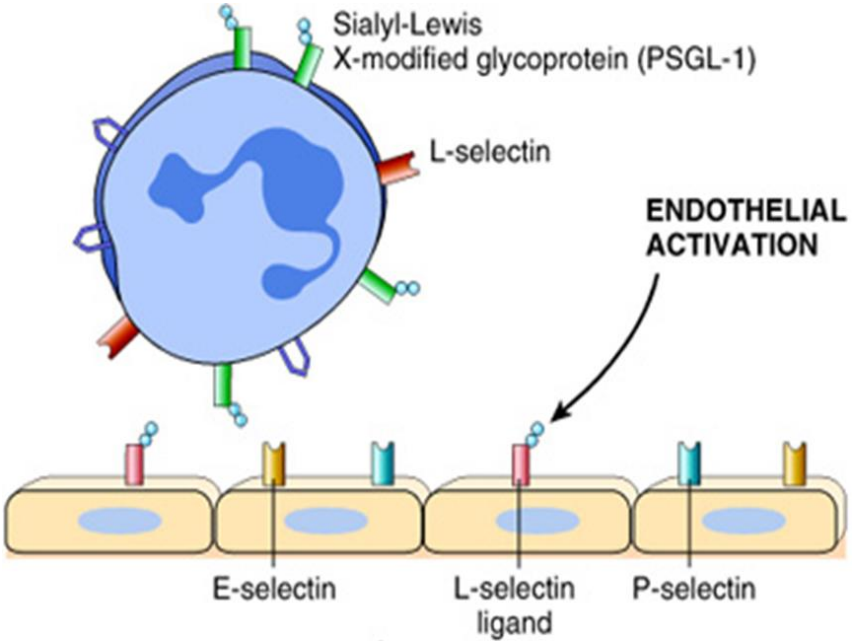


Weibel-Palade bodies are the "glue factory" of endothelial cells, because they synthesize P-selectin, an adhesion molecule for leukocytes, and von Willebrand factor, the adhesion molecule of the platelet

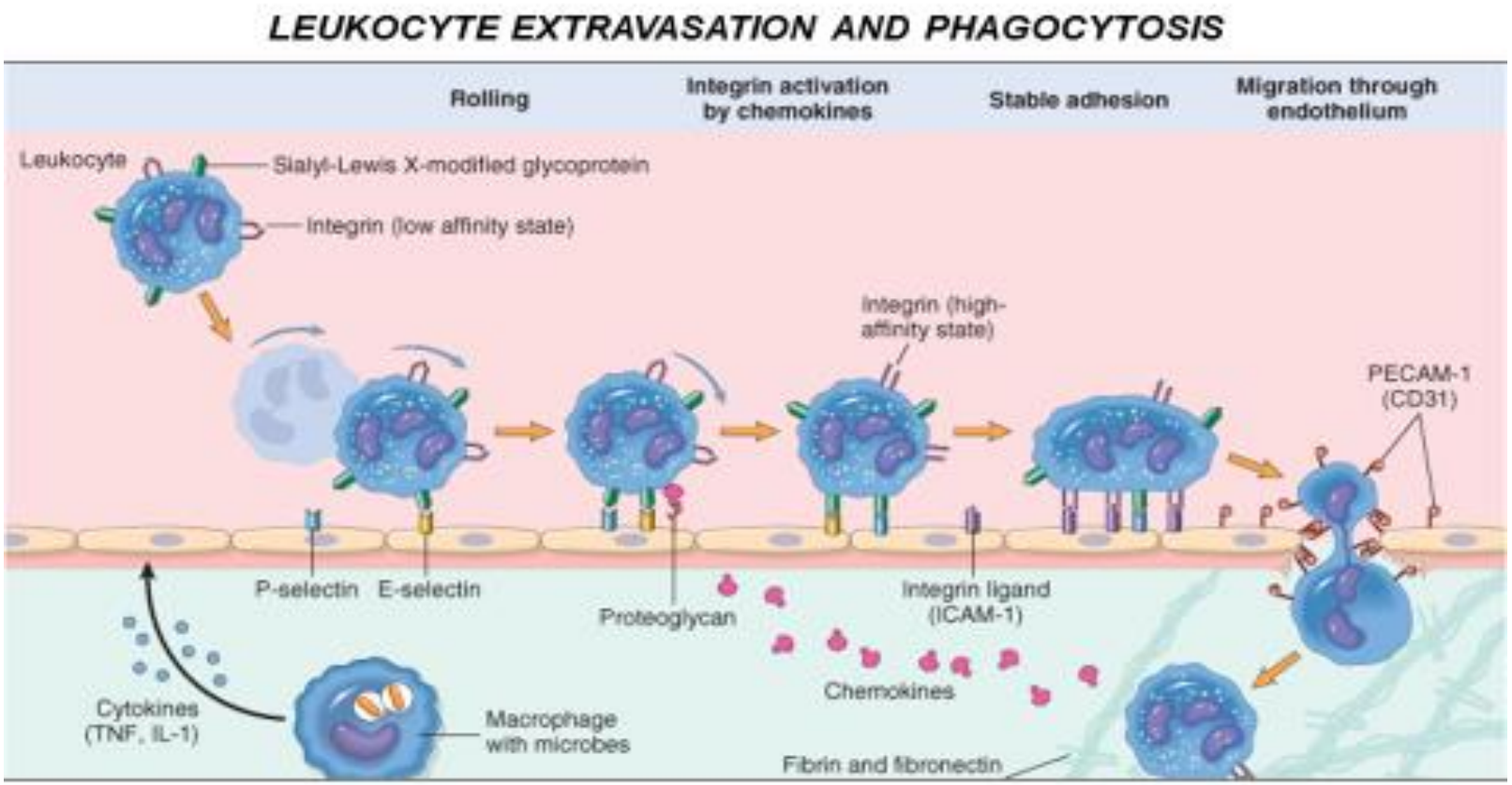
Adhesion Molecules and Receptors

***1. Selectins*, consist of:**

1. E-selectin: confined to endothelium induced by TNF&IL-1
2. P-selectin: present in endothelium and platelets from Weibel-Palade bodies
3. L-selectin: expressed on most leukocyte and endothelium

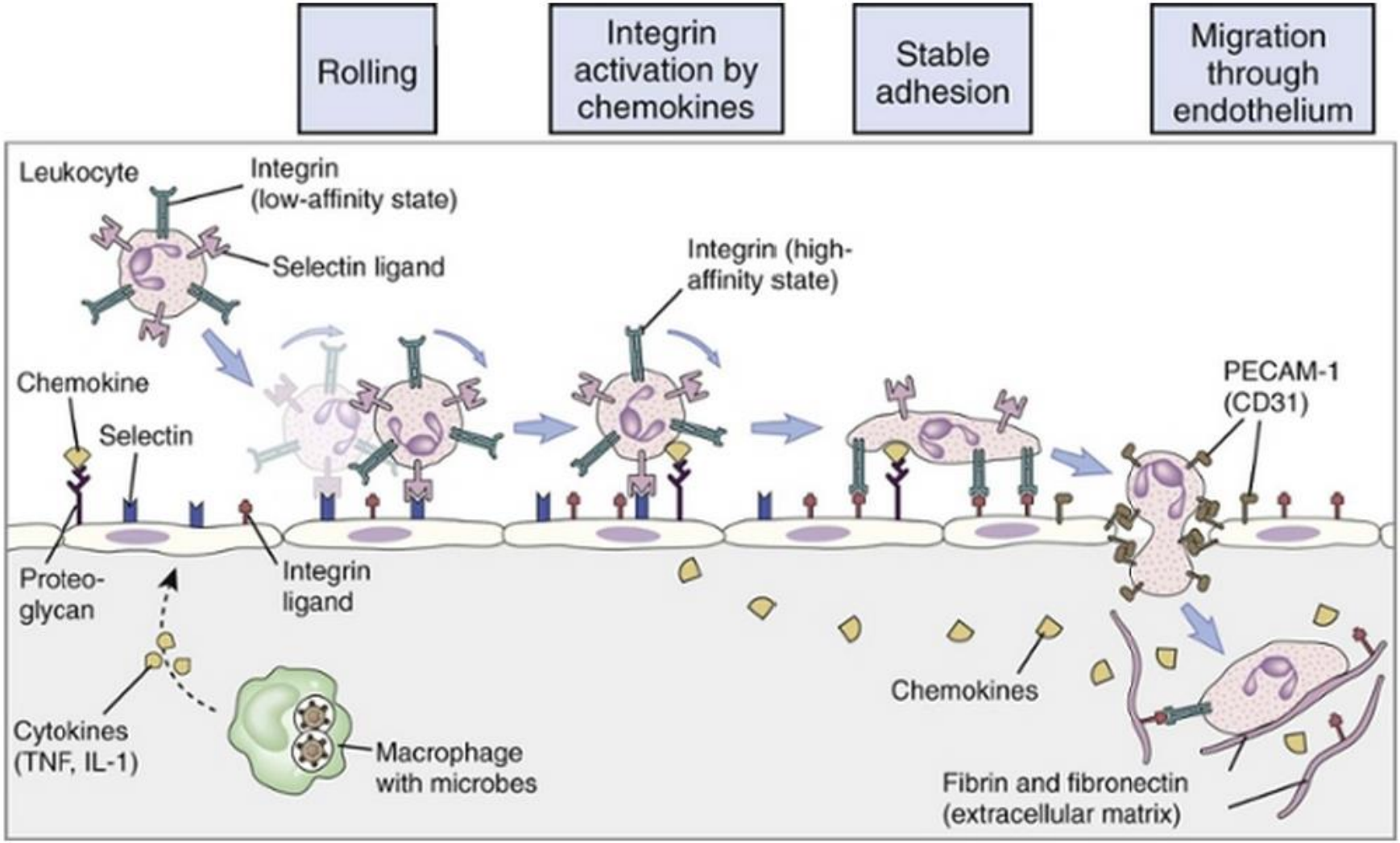


E-selectin & P-selectin bind to Sialyl-Lewis X glycoprotein and slow the leukocytes



Interleukin-1 (IL-1) and tumor necrosis factor (TNF) stimulate the expression of selectin ligands on the surface of neutrophils (L-selectin) and the expression of selectin molecules on the surface of venular endothelial cells (E-selectin, P-selectin)

LEUKOCYTE EXTRAVASATION AND PHAGOCYTOSIS

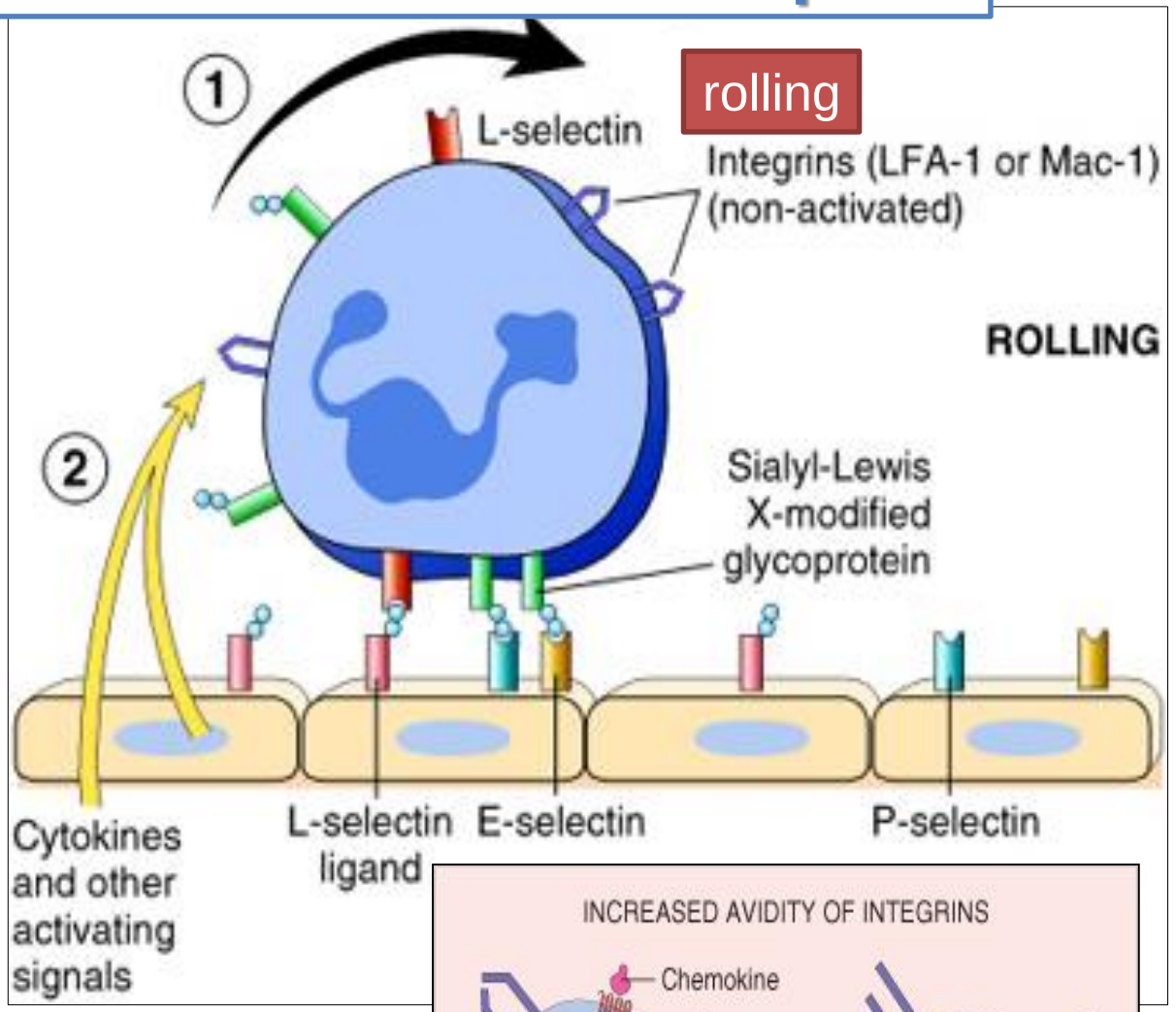


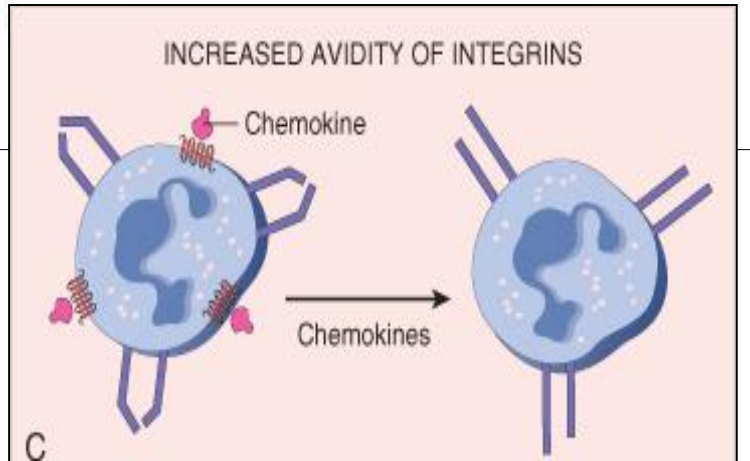
Adhesion Molecules and Receptors

2. Integrins

- are transmembrane heterodimeric glycoproteins, made up of α and β chains expressed on leukocytes and bind to ligands on endothelial cells

- Integrins are up regulated on leukocytes by **C5a & LTB4** resulting in firm adhesion with vessel wall





Leukocyte Adhesion Deficiency

- Autosomal recessive defect of integrins
- Two types:
  - LAD type 1 is a deficiency of β_2-integrin
  - LAD type 2 is a deficiency of an endothelial cell selectin that normally binds neutrophils.
- Clinical findings:
  - Delayed separation of umbilical cord
  - Increased circulating neutrophils (leukocytosis due to loss of the marginating pool)
  - Recurrent bacterial infection that lack pus formation
  - Poor wound healing

Adhesion Molecules and Receptors

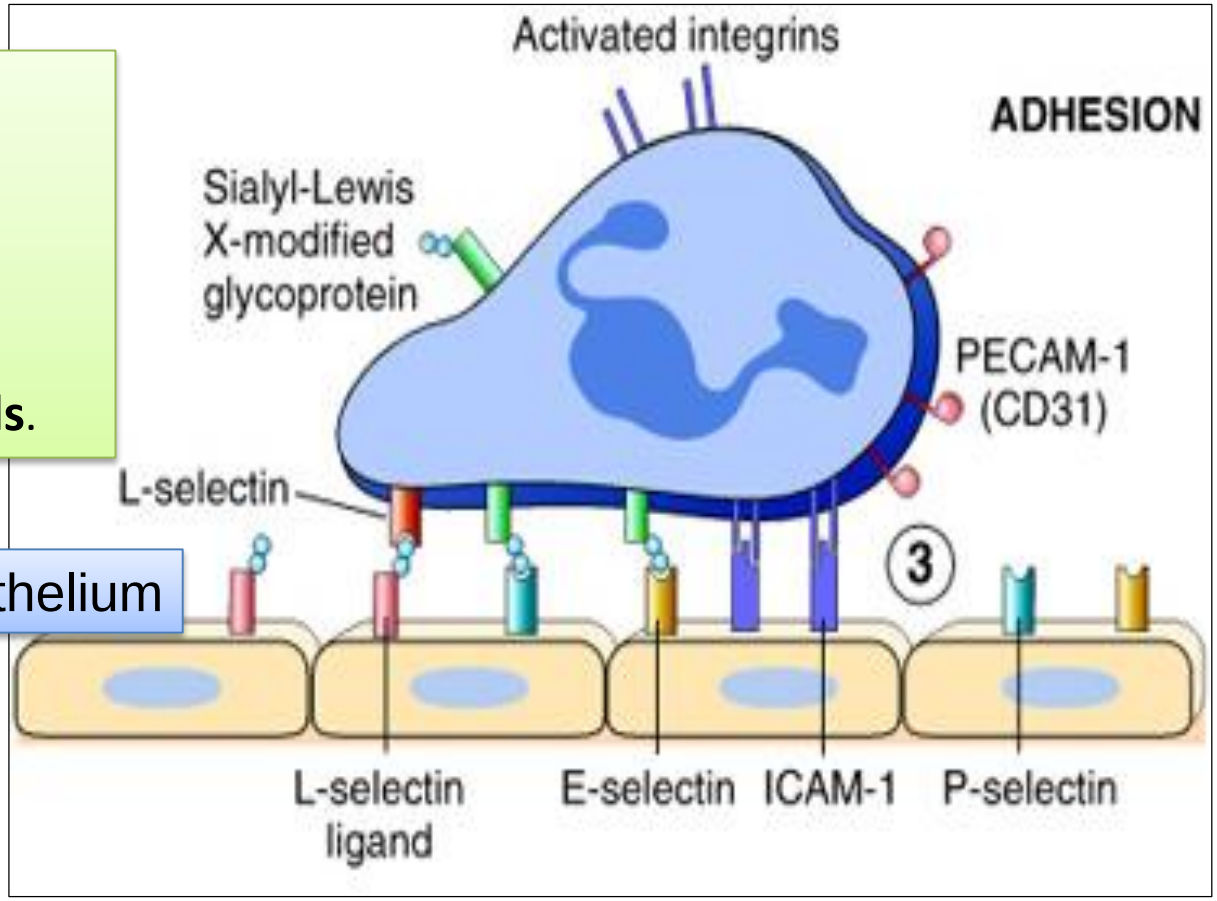
3. The *immunoglobulin family* molecules :

ICAM-1 (intercellular adhesion molecule 1)

VCAM-1 (vascular cell adhesion molecule 1)

IL-1 and TNF activate intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) on venular endothelial cells.

adhesion to endothelium

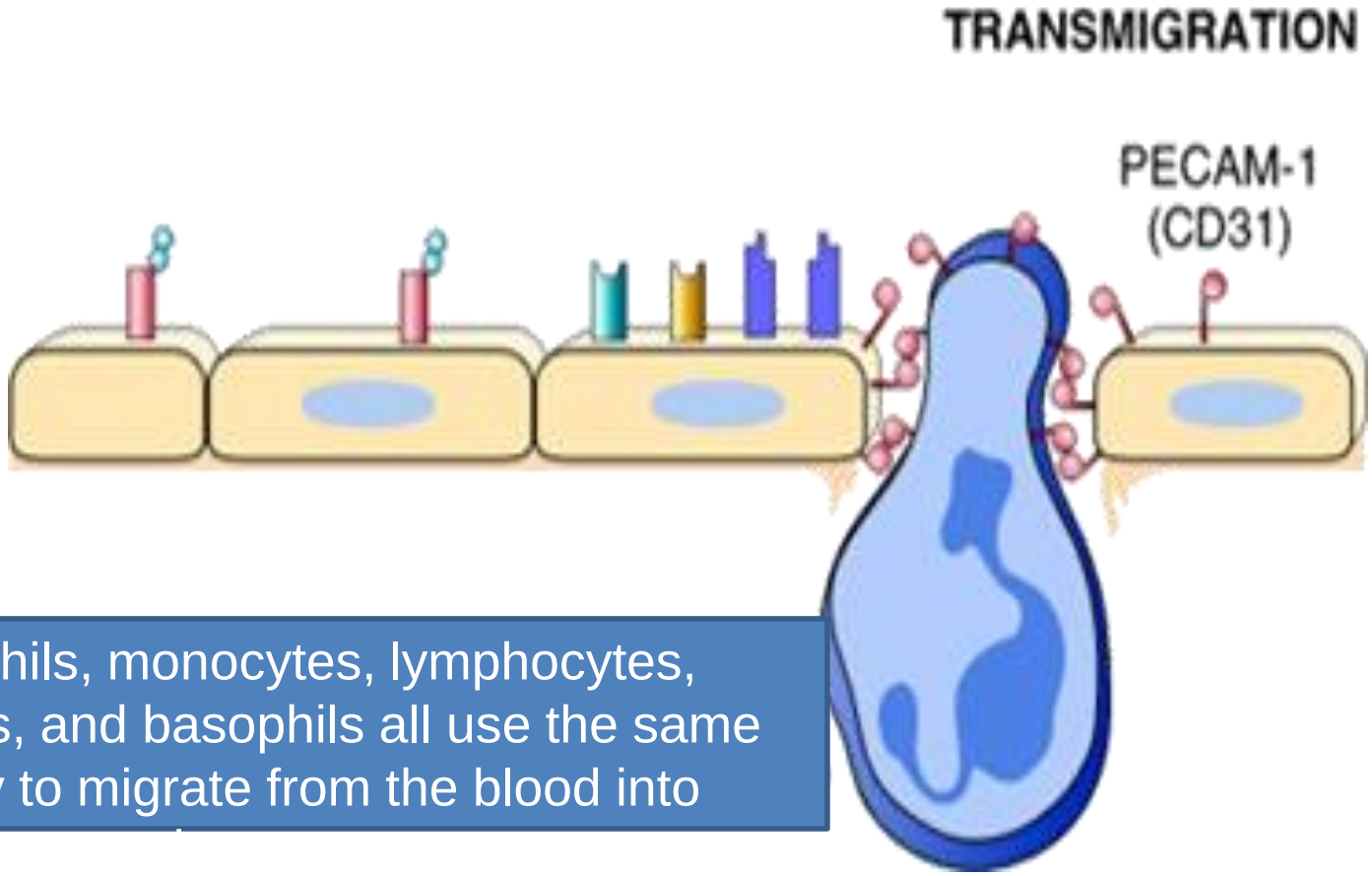


Adhesion Molecules and Receptors

4. Mucin-like glycoproteins: PECAM-1

- these glycoproteins are found in the extracellular matrix and on cell surfaces.

Neutrophils moving along the venular endothelium dissolve the venular basement membrane (release type IV collagenase) exposed by previous histamine-mediated endothelial cell contraction and enter the interstitial tissue.



Neutrophils, monocytes, lymphocytes, eosinophils, and basophils all use the same pathway to migrate from the blood into

Leukocyte Adhesion and Transmigration

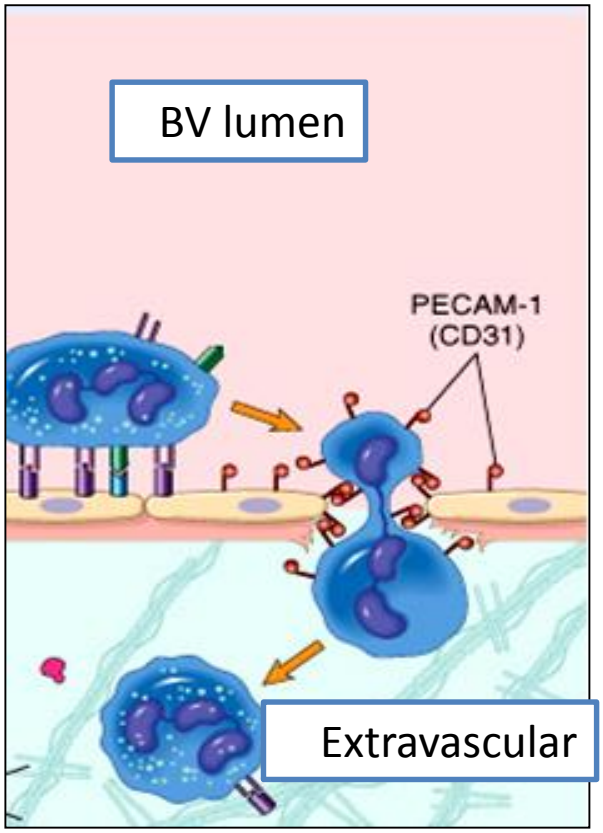
- Migration of the leukocytes through the endothelium is called:

Transmigration

or

Diapedesis

- Diapedesis occurs predominantly in the postcapillary venules



Leukocyte Adhesion and Transmigration

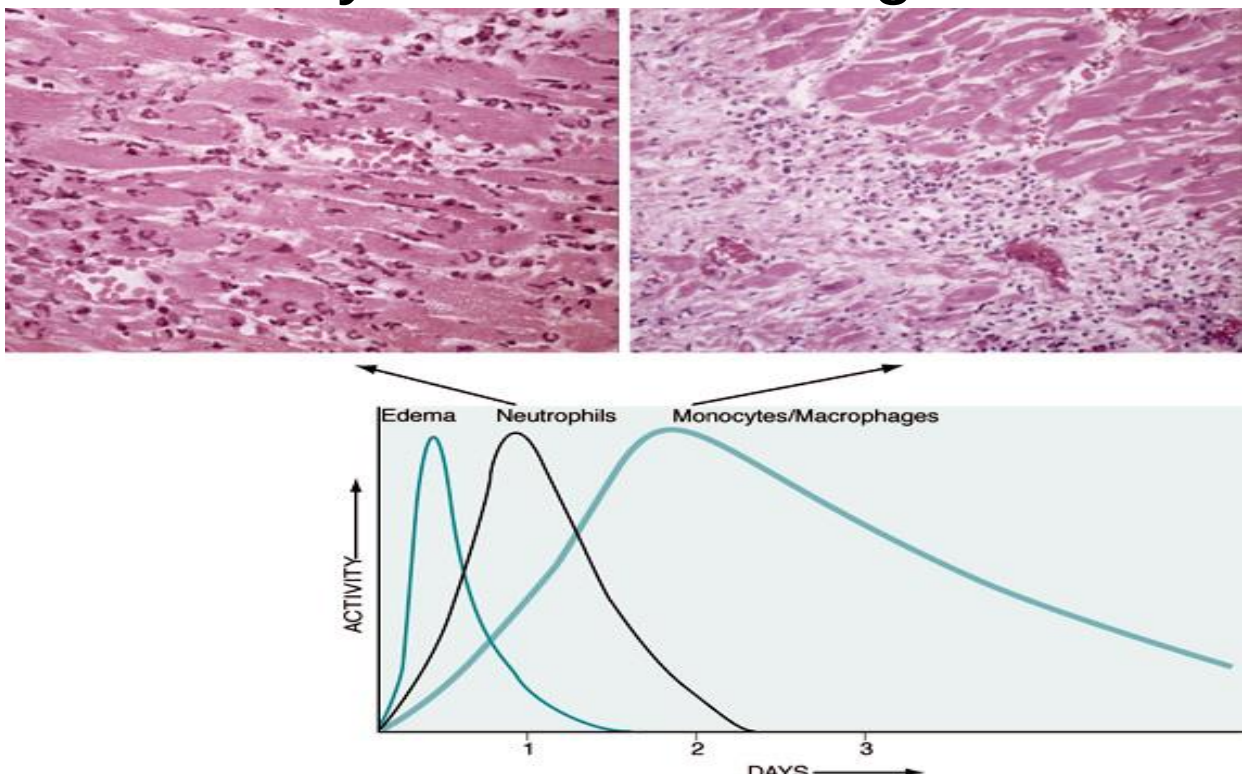
- The type of emigrating leukocyte varies with the age of the inflammatory response
- In most forms of acute inflammation: neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 hours, then are replaced by monocytes in 24 to 48 hours

WHY?

Leukocyte Adhesion and Transmigration

– neutrophils are more numerous in the blood, they respond more rapidly to chemokines,

– but are short-lived; they undergo apoptosis and disappear after 24 to 48 hours, whereas monocytes survive longer.



Leukocyte Adhesion and Transmigration

The type of emigrating leukocyte varies with the type of stimulus:

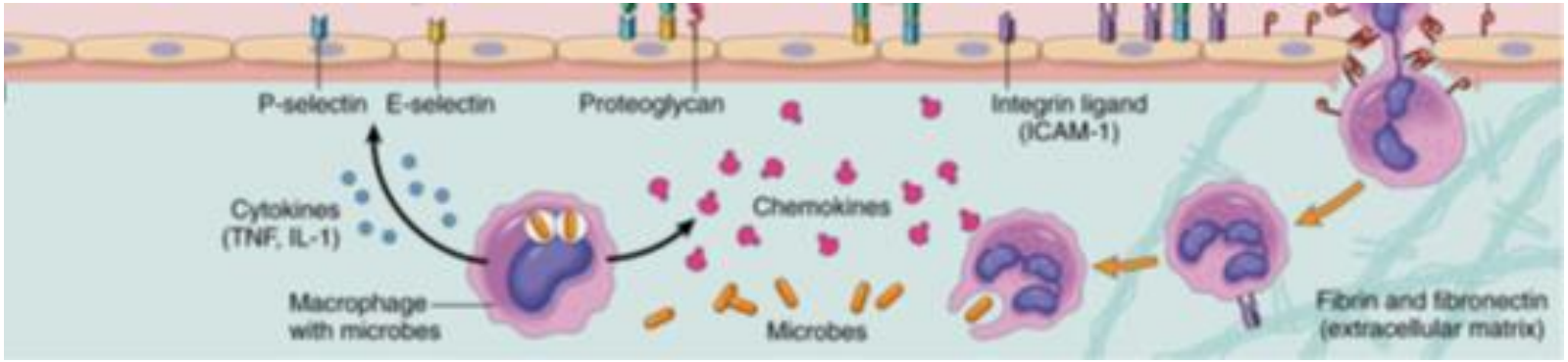
- In viral infections, lymphocytes may be the first cells to arrive
- In some hypersensitivity reactions and parasitic infection, eosinophil may be the main cell type
- Chronic inflammation lymphocytes plasma cells and macrophages are present

Chemotaxis

- After extravasation, leukocytes emigrate in tissues toward the site of injury by a process called *chemotaxis*, defined as locomotion oriented along a chemical gradient !!!!

Chemoattractants

Neutrophils are attracted by bacterial products, IL-8, C5a & LTB4



Chemokines act on the adherent leukocytes and stimulate the cells to migrate toward the site of injury or infection

Chemotaxis

Chemoattractants

Exogenous and endogenous substances

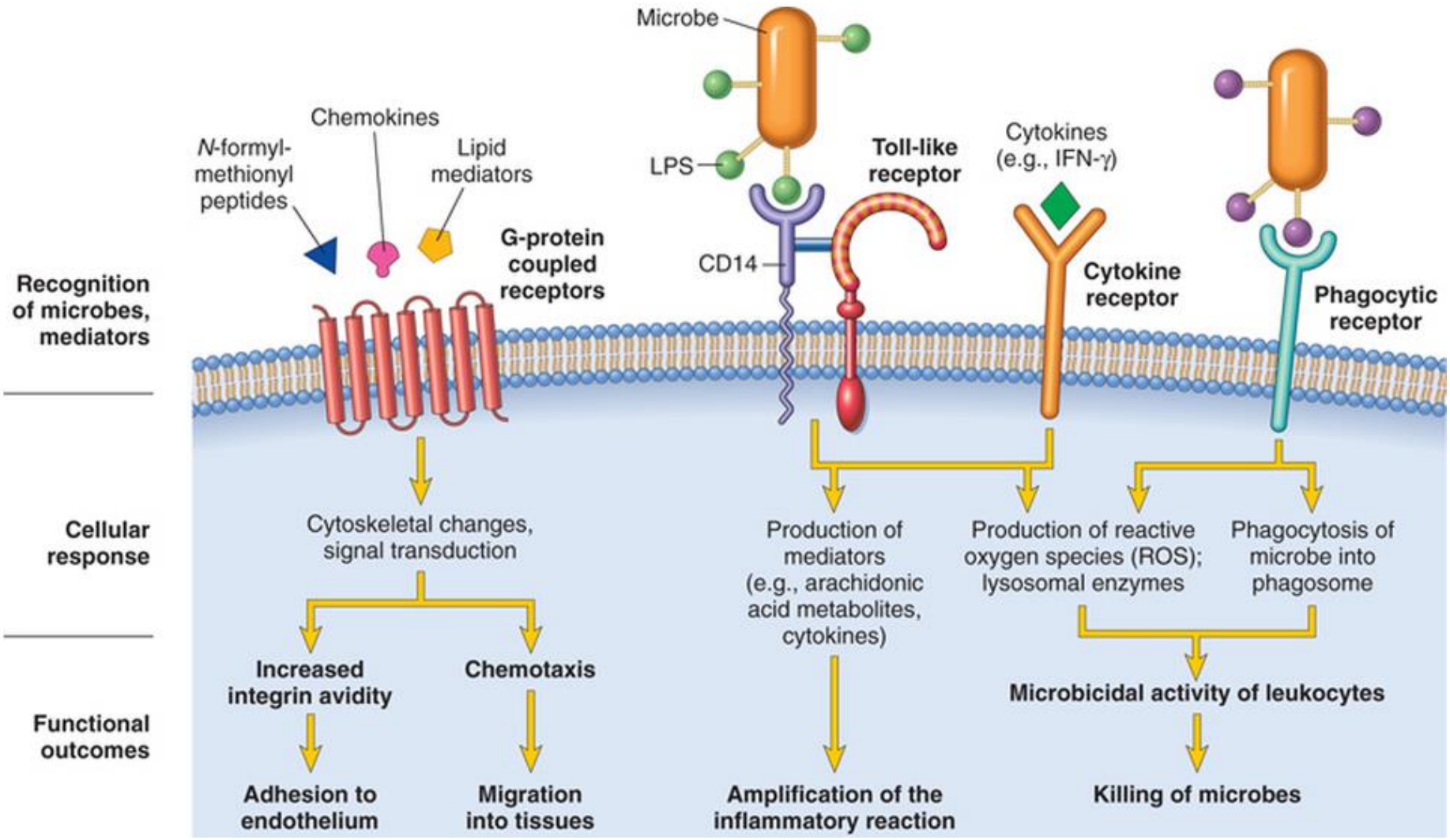
- The most common *exogenous* agents are *bacterial products*.
- *Endogenous* chemoattractants include several chemical mediators:

(1) components of the complement system, particularly C5a

(2) products of the lipoxygenase pathway, mainly leukotriene B_4 (LTB_4)

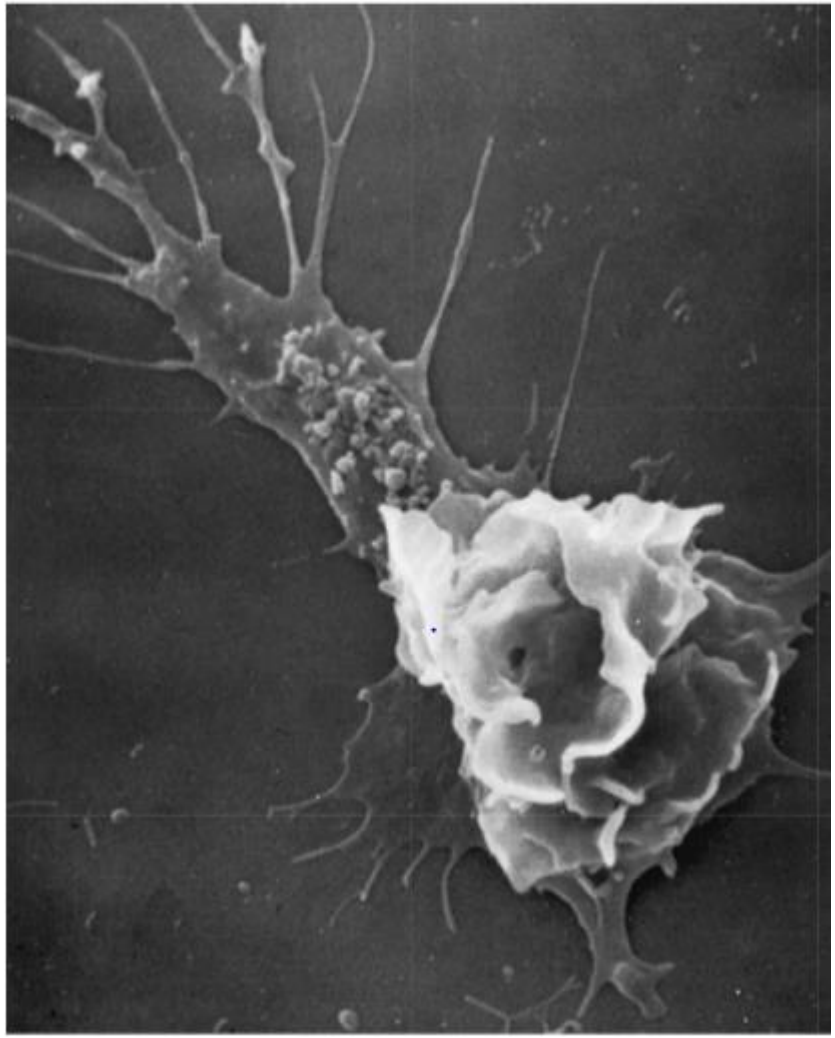
(3) cytokines, particularly those of the chemokine family (e.g., IL-8).

All these chemotactic agents bind to specific seven-transmembrane G-protein-coupled receptors (GPCRs) on the surface of leukocytes.



Scanning electron micrograph of a moving leukocyte in culture showing a filopodium and a trailing tail.

The leukocyte moves by extending filopodia that pull the back of the cell in the direction of extension



Leukocyte Activation

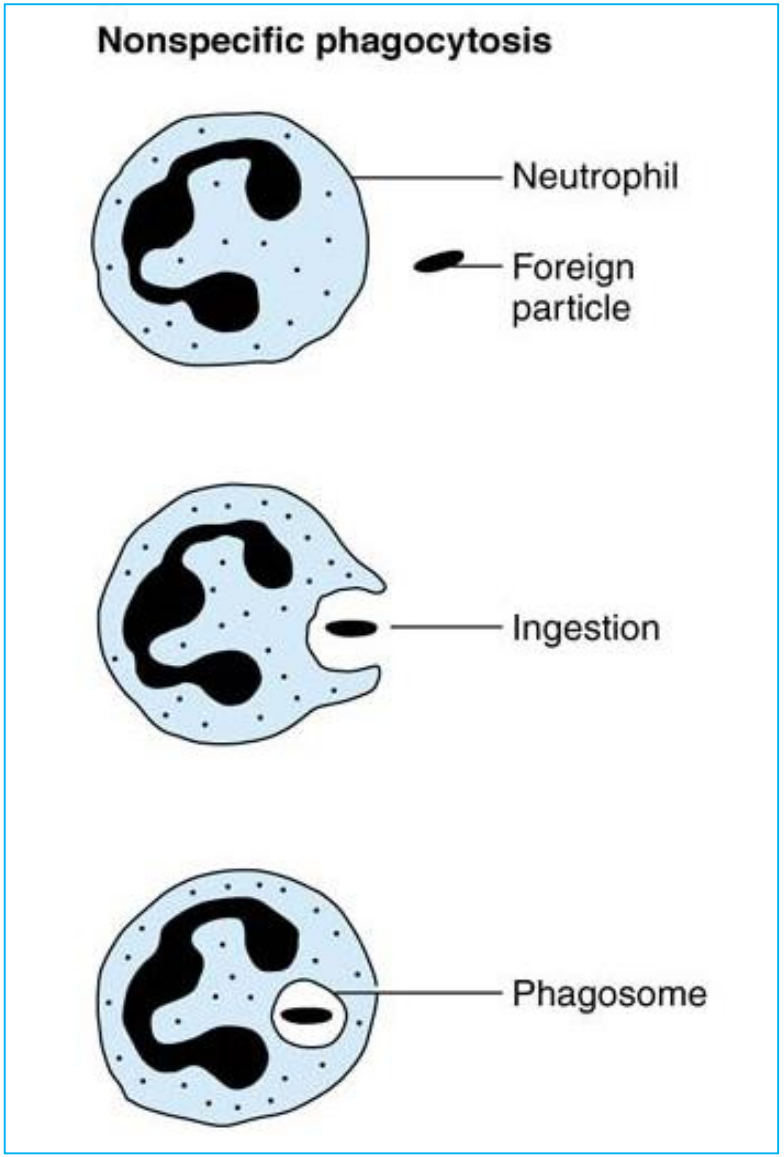
Phagocytosis

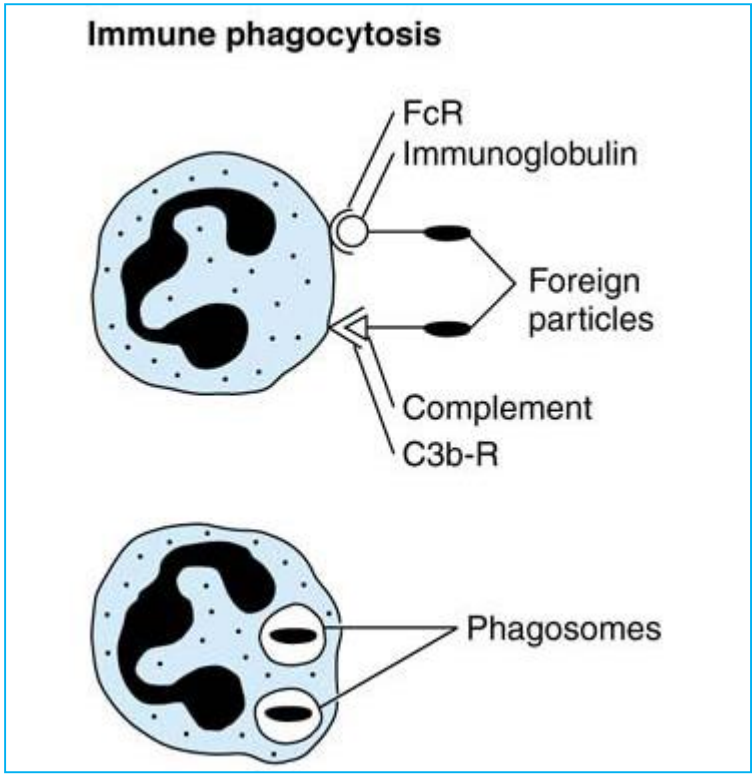
Intracellular destruction

Liberation of substances that destroy extracellular microbes and dead tissues

Production of mediators

Phagocytosis by neutrophils





Immune phagocytosis is much more efficient than nonspecific phagocytosis

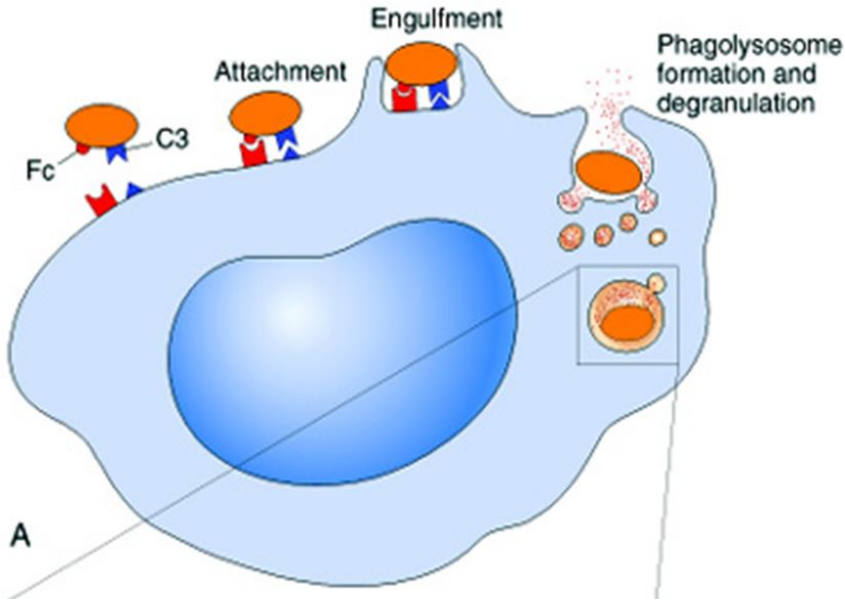
Phagocytosis

- Phagocytosis involves three distinct but interrelated steps

  (1) Recognition and *Attachment* of the particle to be ingested by the leukocyte

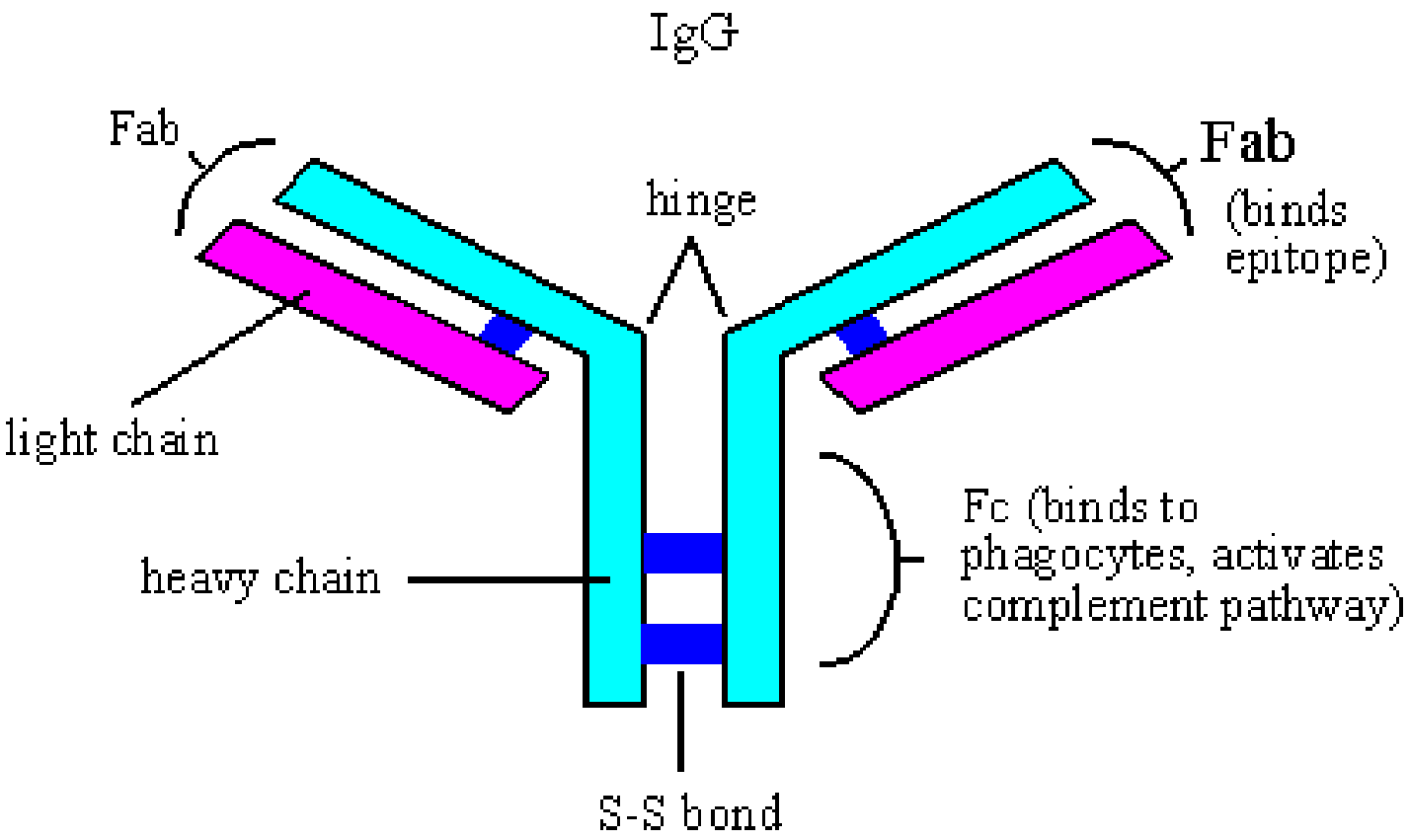
  (2) its *Engulfment*, with subsequent formation of a phagocytic vacuole

  (3) *killing* or *Degradation* of the ingested material.



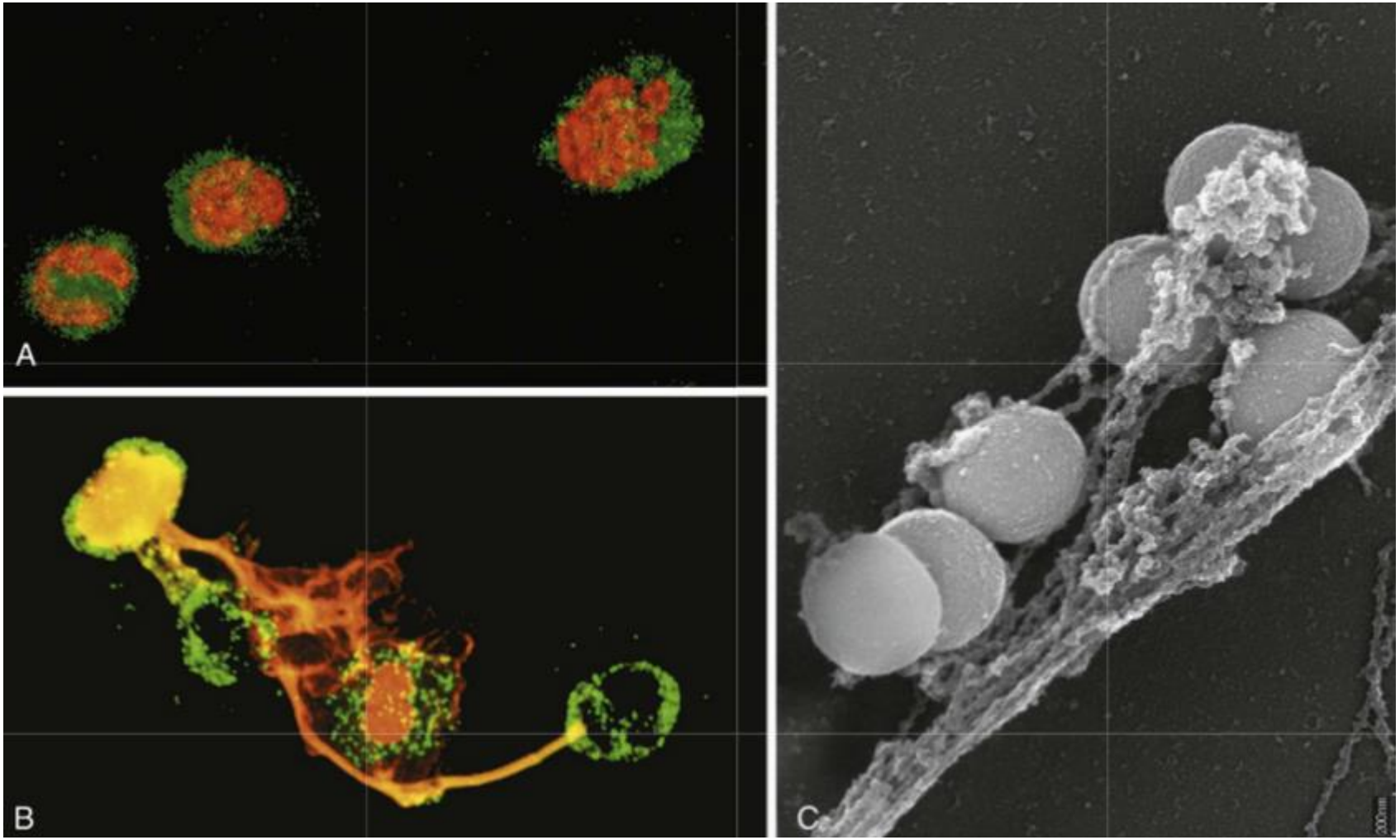
Leukocyte activation
(1) Recognition and Attachment
(Opsonization)

- Is the process of coating a particle, such as a microbe, to target it for phagocytosis
- The substances that do this are *opsonins*.
- These substances include:
  - antibodies (IgG)
  - complement proteins (C3)
  - And others: lectins (mannose-binding lectin (MBL), collectins,fibronectin, fibrinogen, and C-reactive protein
- These can coat microbes and are recognized by receptors on phagocytes (Fc and C3b receptors).


IgG
Fab
hinge
Fab
(binds
epitope)
light chain
heavy chain
Fc (binds to
phagocytes, activates
complement pathway)
S-S bond

Neutrophil extracellular traps (NETs)

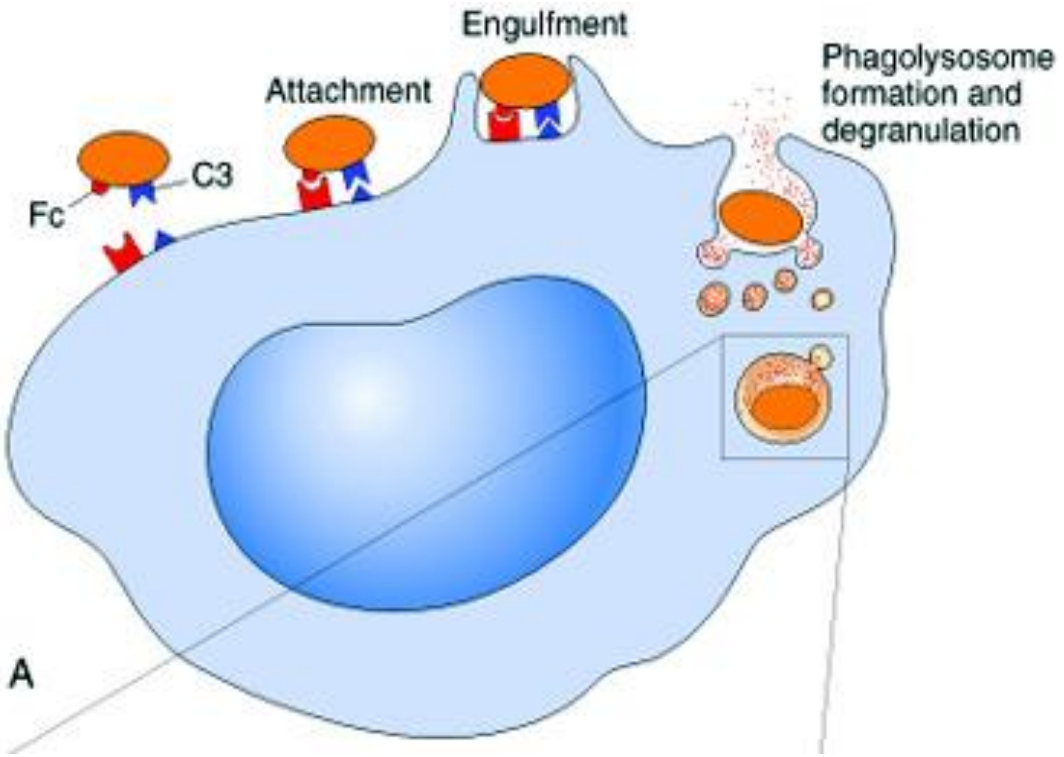
A, Healthy neutrophils with nuclei stained red and cytoplasm green.
B, Release of nuclear material from neutrophils (note that two have lost their nuclei), forming extracellular traps.
C, An electron micrograph of bacteria (staphylococci) trapped in NETs.



Phagocytosis

2. Engulfment

- During engulfment, extensions of the cytoplasm (pseudopods) flow around the particle to be engulfed, eventually resulting in complete enclosure of the particle within a **phagosome**



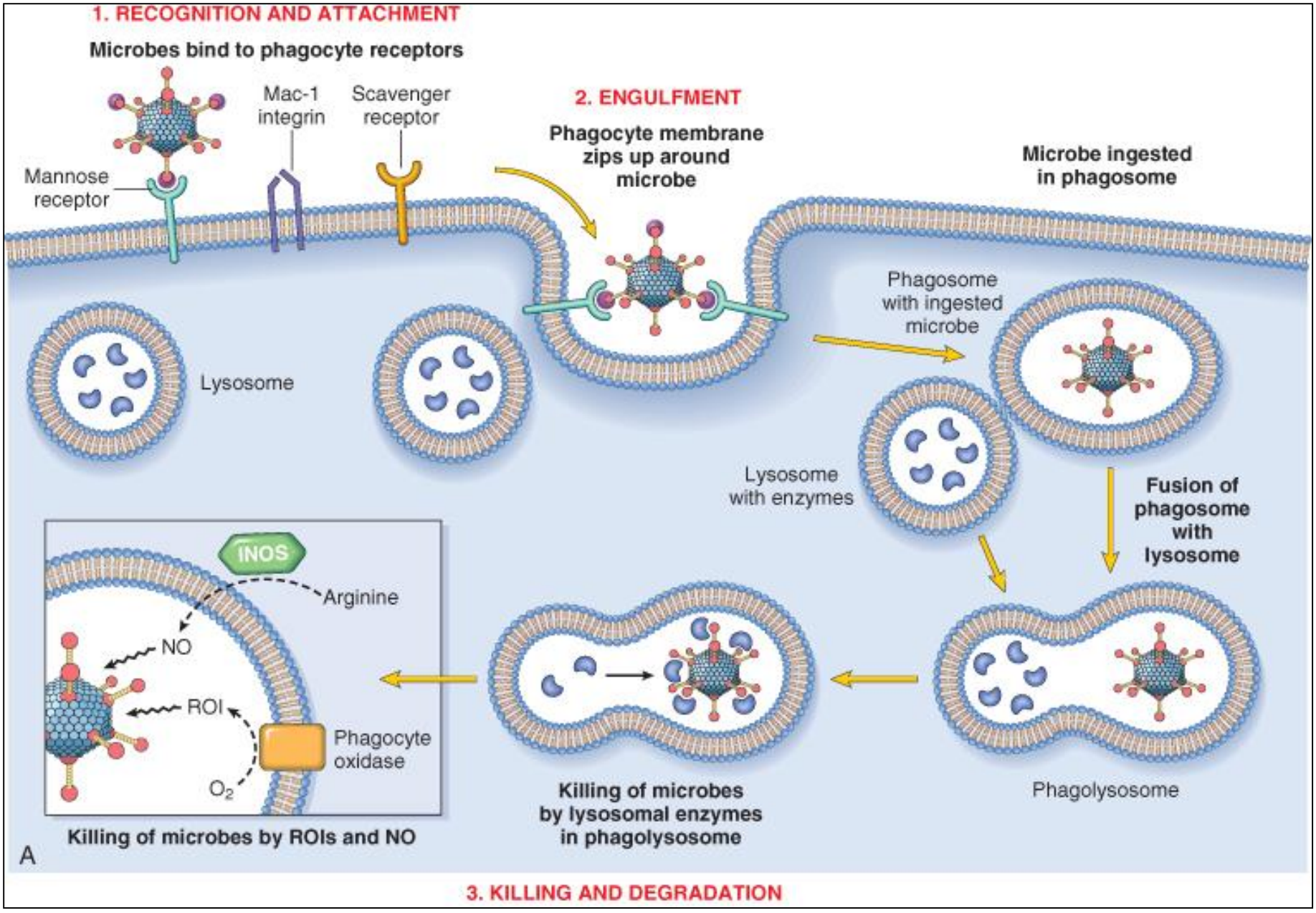
The phagocytic vacuole then fuses with a lysosomal granule, resulting in **phagolysosome**

Defect in **phagolysosome** formation

Chediak-Higashi Syndrome:

- Protein trafficking defect (microtubule defect)
- Autosomal recessive
- Lead to impaired phagolysosome formation

- Clinical feature:
  - Increased risk of pyogenic infection
  - Neutropenia (defect in generation from BM)
  - Giant granule formation (granules formed cannot move in cytoplasm)
  - Defective primary hemostasis (platelet granule are not secreted)
  - Albinism
  - Peripheral neuropathy


1. RECOGNITION AND ATTACHMENT
Microbes bind to phagocyte receptors
Mannose receptor
Mac-1 integrin
Scavenger receptor
2. ENGULFMENT
Phagocyte membrane zips up around microbe
Microbe ingested in phagosome
Phagosome with ingested microbe
Lysosome
Lysosome with enzymes
Fusion of phagosome with lysosome
INOS
Arginine
NO
ROI
Phagocyte oxidase
O_2
Killing of microbes by ROIs and NO
Killing of microbes by lysosomal enzymes in phagolysosome
Phagolysosome
A
3. KILLING AND DEGRADATION

Phagocytosis

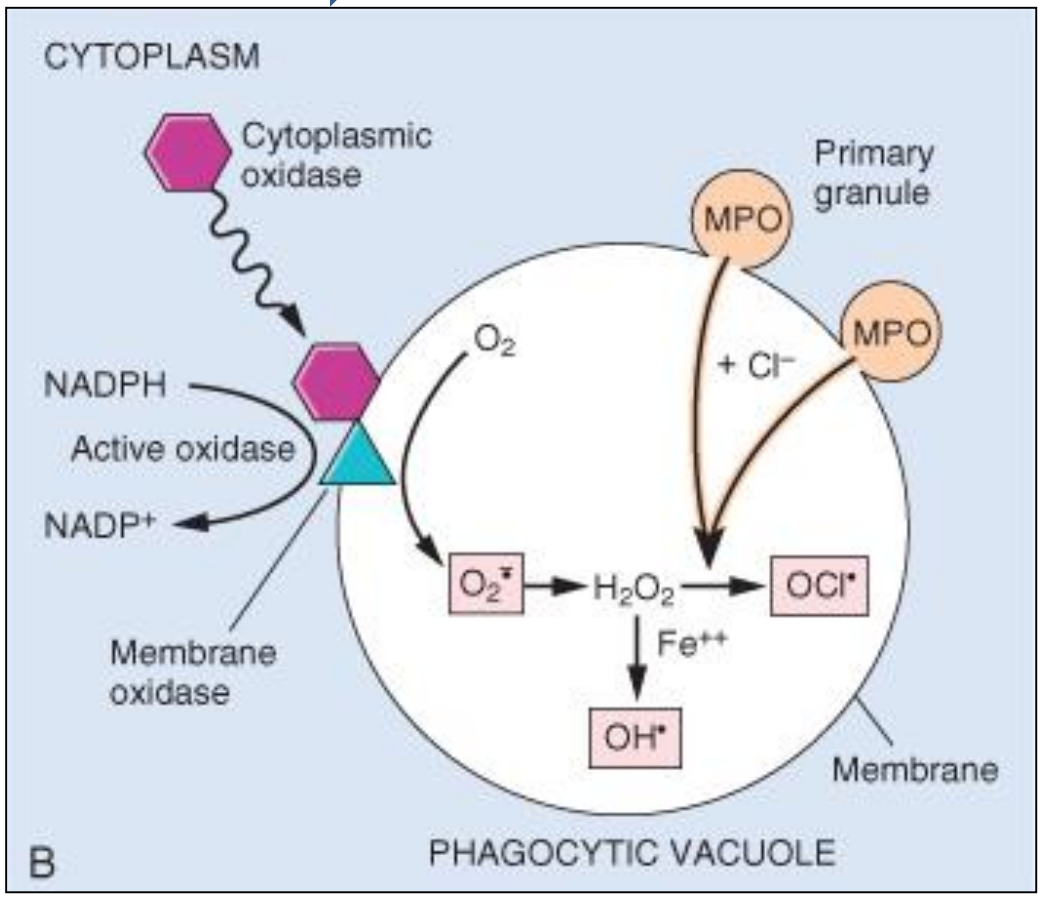
Killing and Degradation

- 2 mechanisms for Microbial killing:
    1. *Oxygen-dependent mechanisms*
    2. *Oxygen-independent mechanisms*

Oxygen-Dependent Mechanisms

The H_2O_2-MPO-halide system is the most efficient bactericidal system in neutrophils

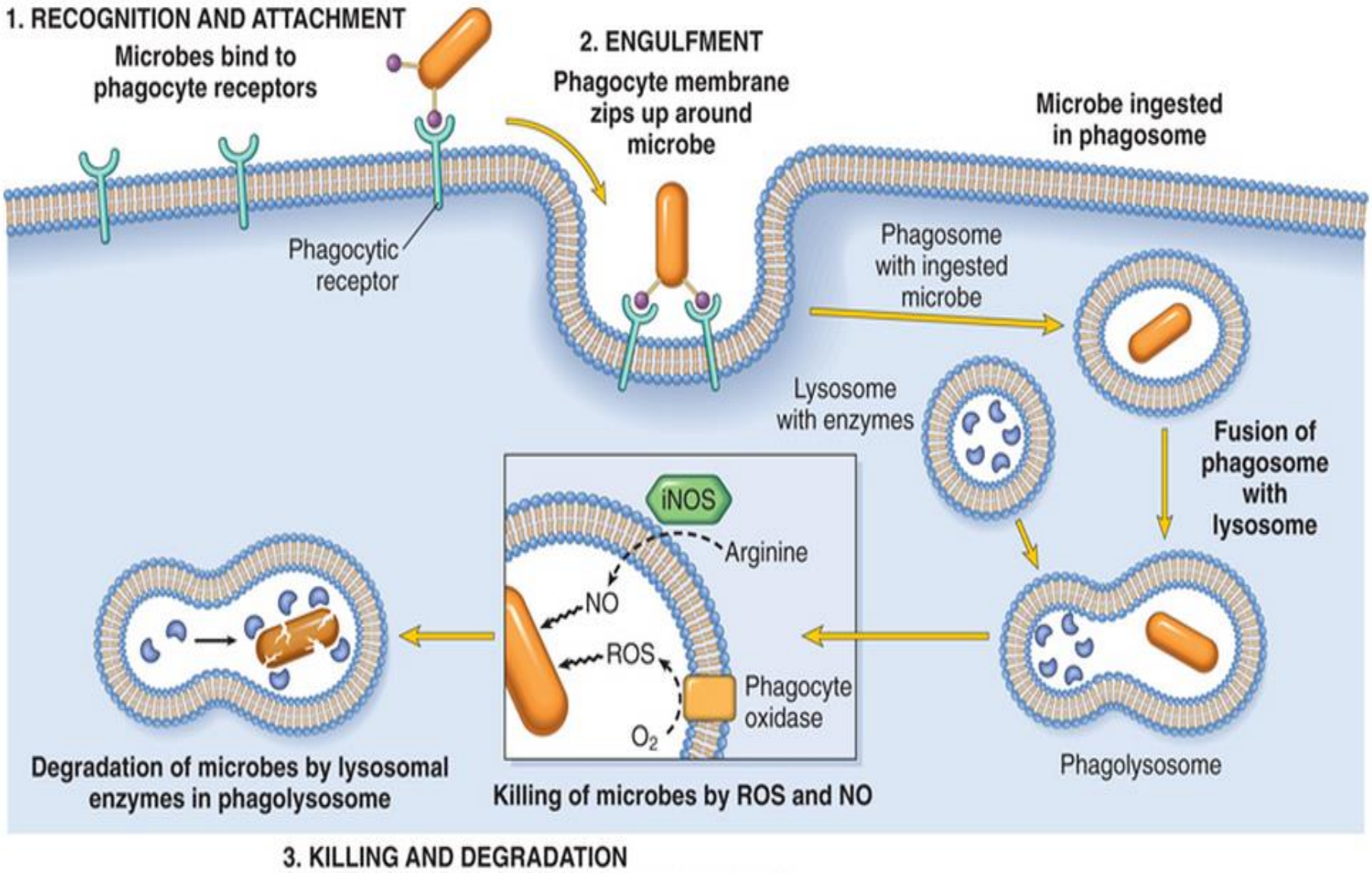
O_2 → oxidase → O_2^- → SOD → H_2O_2 → MPO → HOCL



Oxygen-independent mechanisms

- through the action of substances in leukocyte granules. These include:
  - ***bactericidal permeability increasing protein* (BPI**
  - ***lysozyme***
  - ***lactoferrin***
  - ***major basic protein***
  - ***defensins***
- In addition, neutrophil granules contain many *enzymes*, such as elastase, that also contribute to microbial killing

Summary of Phagocytosis



Defects in Leukocyte Function

- Defects in leukocyte function, both genetic and acquired, lead to increased vulnerability to infections:
  - *Defects in leukocyte adhesion*
  - *Defects in phagolysosome function*
  - *Defects in microbicidal activity*

Defects in Leukocyte Function

Genetic

1. Leukocyte adhesion deficiency 1and 2
2. Chédiak-Higashi syndrome
   - Protein involved in organelle membrane fusion (no phagolysosomes)
3. Chronic granulomatous disease

   Decreased oxidative burst. 2 types:

   A. X-linked: NADPH oxidase (membrane component)

   B. Autosomal recessive:

   a. NADPH oxidase (cytoplasmic components)

   b. Myeloperoxidase deficiency: (absent MPO-H2O2 system) pt. have increased risk of candida infection

Chronic Granulomatous Disease (CGD)

- Infection and granuloma formation with catalase positive organisms e.g. *S aureus, P cepacia, S marcescens, Norcardia* and *aspergillus*
- Nitroblue tetrazolium test (NBT), turn blue if NADPH oxidase can convertO_2 to O_2-
- Remains colorless in CGD (NADPH oxidase)
- NBT test is normal in Myeloperoxidase deficiency type

Comparison of Chronic Granulomatous Disease and Myeloperoxidase Deficiency

	CHRONIC GRANULOMATOUS DISEASE	MYELOPEROXIDASE DEFICIENCY
Inheritance pattern	X-linked recessive	Autosomal recessive
NADPH oxidase	Absent	Present
Myeloperoxidase	Present	Absent
Respiratory burst	Absent	Present
Peroxide (H_2O_2)	Absent	Present
Bleach (HOCl)	Absent	Absent

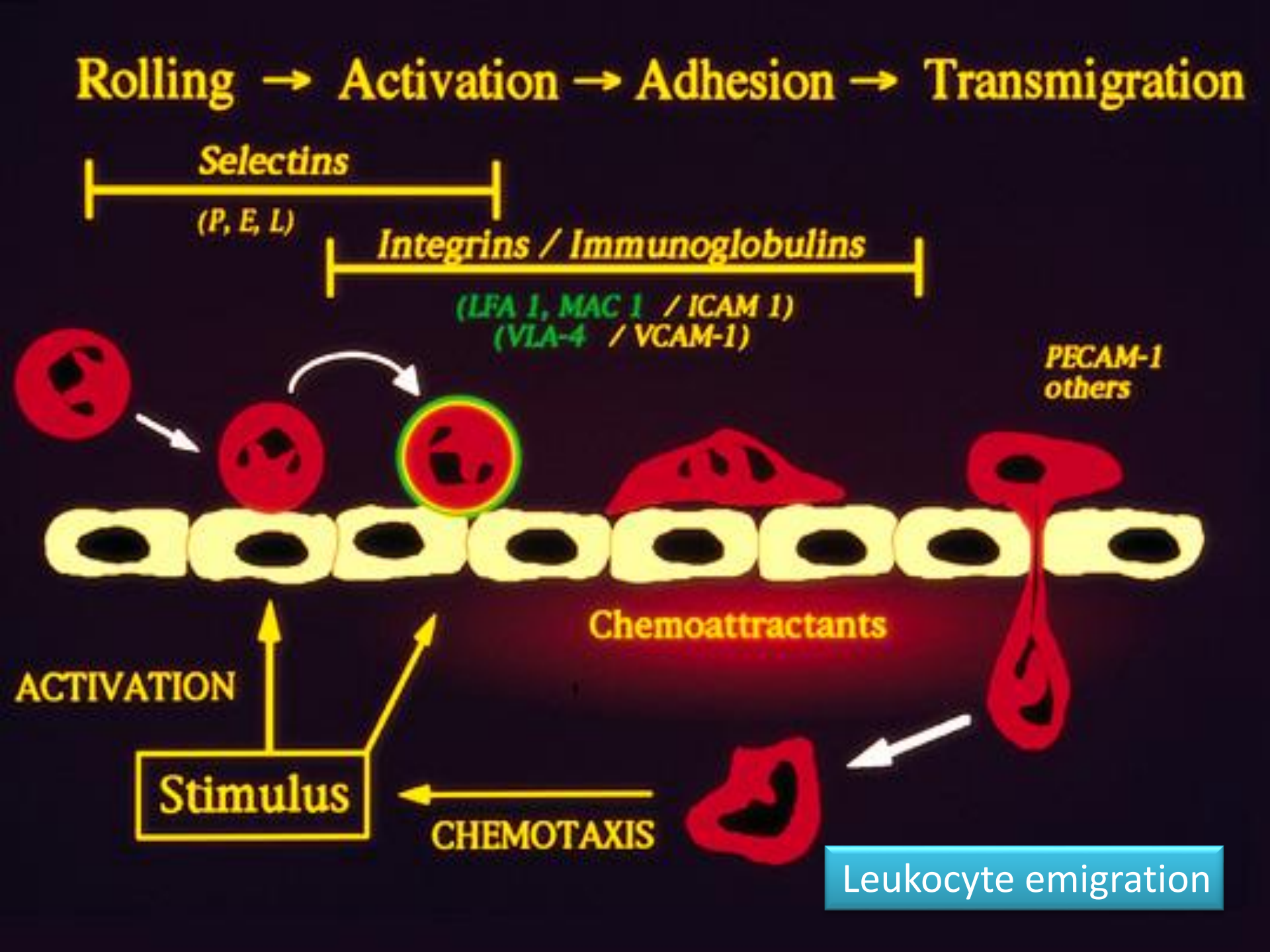
Defects in Leukocyte Function

Acquired

- Thermal injury, diabetes, malignancy, sepsis, immunodeficiencies
  - Chemotaxis
- Hemodialysis, diabetes mellitus
  - Adhesion
- Leukemia, anemia, sepsis, diabetes, neonates, malnutrition
  - Phagocytosis and microbicidal activity

TAKE HOME MESSAGES:

1. Several steps are involved in extravasation of leukocytes from the blood to the tissues.
2. Phagocytosis is important step to get rid of necrotic material and bacteria.
3. Various defects in leukocyte function are present. These could be genetic defects or acquired.



Leukocyte emigration