INFLAMMATION II

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Leukocyte Recruitment to Sites of Inflammation

- Leukocytes that are recruited to sites of inflammation perform the key function of eliminating the offending agents
- The most important leukocytes in typical inflammatory reactions are the ones **capable of phagocytosis**, namely, **neutrophils** and **macrophages**.
- The two cell types share many features, such as phagocytosis, ability to migrate through blood vessels into tissues, and chemotaxis.
- These leukocytes ingest and destroy bacteria and other microbes, as well as necrotic tissue and foreign substances
- Macrophages also produce growth factors that aid in repair
- When strongly activated, they may induce tissue damage and prolong inflammation **"collateral damage"**

Properties of Neutrophils and Macrophages

	Neutrophils	Macrophages
Origin	HSCs in bone marrow	HSCs in bone marrow (in inflammatory reactions) Many tissue-resident macrophages: stem cells in yolk sac or fetal liver (early in development)
Life span in tissues	1-2 days	Inflammatory macrophages: days or weeks Tissue-resident macrophages: years
Responses to activating stimuli	Rapid, short-lived, mostly degranulation and enzymatic activity	More prolonged, slower, often dependent on new gene transcription
Reactive oxygen species	Rapidly induced by assembly of phagocyte oxidase (respiratory burst)	Less prominent
Nitric oxide	Low levels or none	Induced following transcriptional activation of iNOS
Degranulation	Major response; induced by cytoskeletal rearrangement	Not prominent
Cytokine production	Low levels or none	Major functional activity, requires transcriptional activation of cytokine genes
NET formation	Rapidly induced, by extrusion of nuclear	No

HSC: Hematopoietic stem cells
iNOS: inducible nitric oxide synthase
NET: neutrophil extracellular traps

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.

Cellular Events

1.Leukocyte Adhesion to Endothelium

- Stasis,Margination, rolling and adhesion of leukocytes to endothelium at the site of inflammation.

2.Leukocyte Migration through Endothelium

- Transmigration of the leukocytes through the endothelial cells

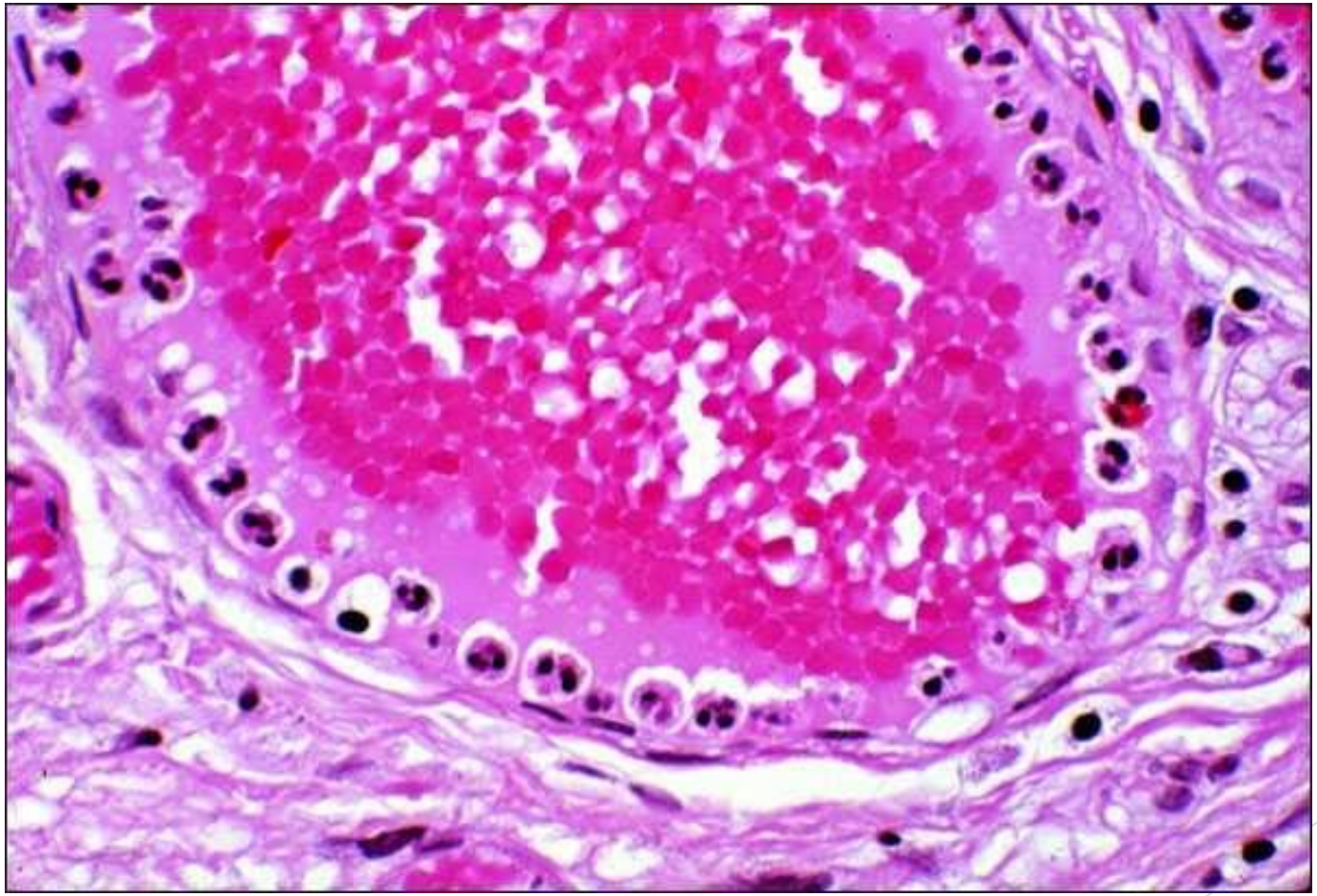
3.Chemotaxis of Leukocytes

- Migration in the interstitium toward the site of stimulus

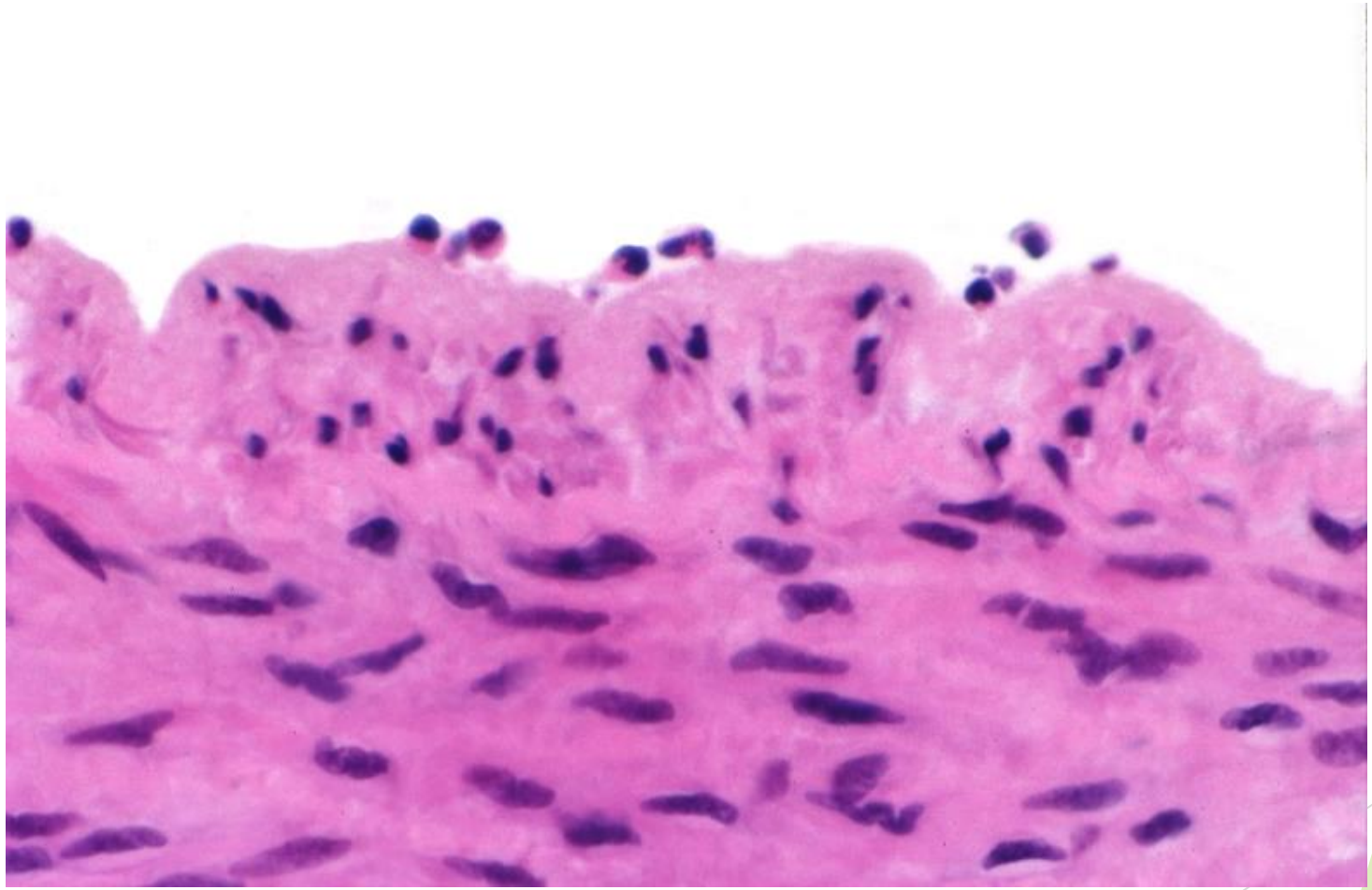
4.Phagocytosis and degranulation and Clearance of the Offending Agent

5.Leukocyte activation and release of products

Neutrophil Margination



Neutrophil Margination



Leukocyte Adhesion to Endothelium

- The attachment of **leukocytes** to **endothelial** cells is mediated by complementary **adhesion molecules** on the two cell types whose expression is enhanced by cytokines

- **Cytokines** are secreted by cells in tissues in response to microbes and other injurious agents

- The two major families of molecules involved in leukocyte adhesion and migration are the **selectins** and **integrins.**

- These molecules are expressed on **leukocytes** and **endothelial** cells, as are their ligands

Leukocyte Adhesion to Endothelium

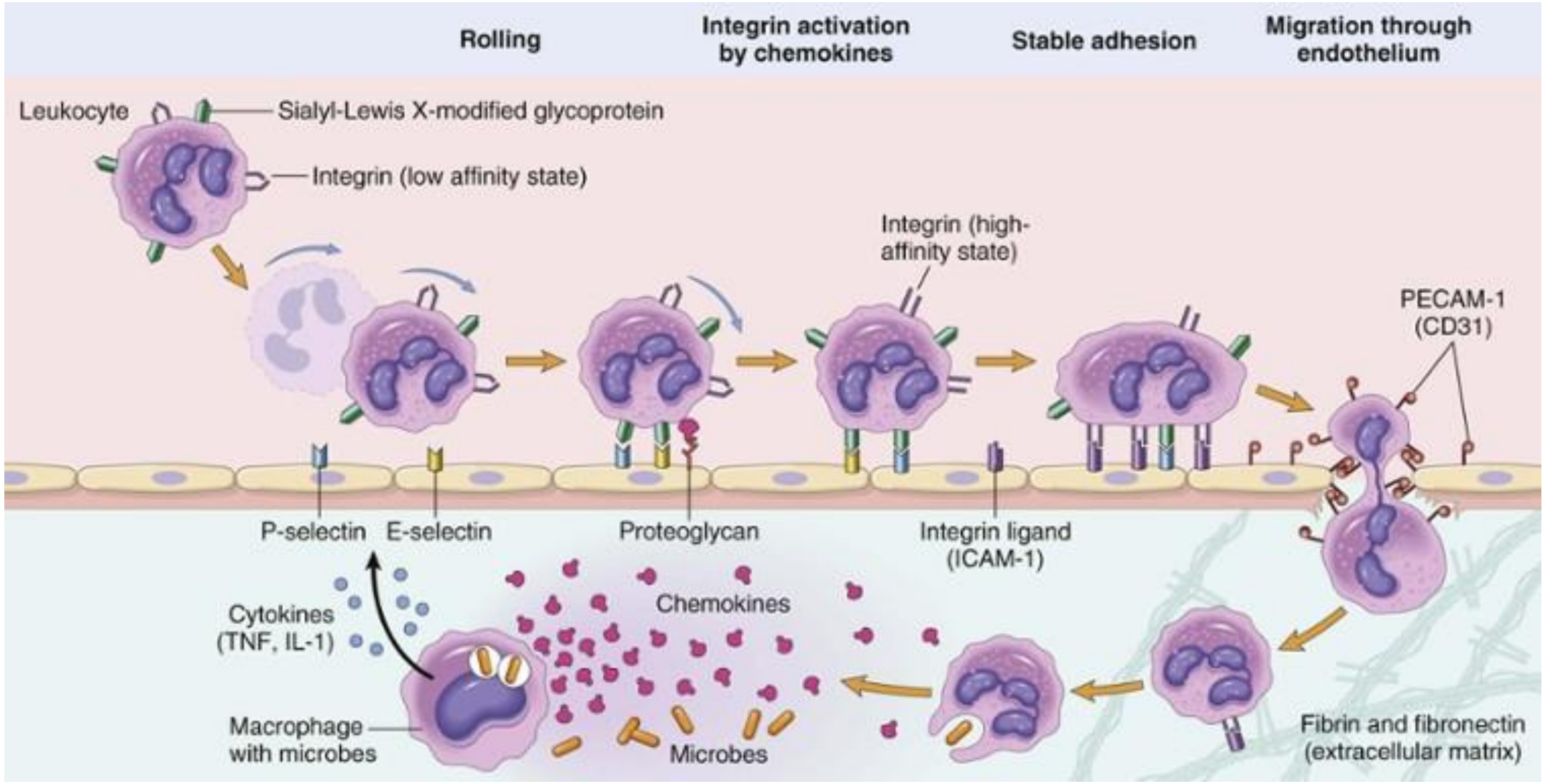
- **Selectins** mediate the **initial weak interactions** between leukocytes and endothelium

- **Firm adhesion** of leukocytes to endothelium is mediated by a family of leukocyte surface proteins called **integrins**

The Process of Extravasation of Leukocytes

1. Selectins and their carbohydrate counterligands mediate leukocyte tethering and rolling.
2. Leukocyte integrins their ligands including immunoglobulin like intercellular adhesion molecules, mediate firm adhesion.
3. Chemokines play a role in firm adhesion by activating integrins on the leukocyte cell surface.
4. The leukocytes are directed by chemoattractant gradients to migrate across the endothelium, and through the extracellular matrix into the tissue to migrate across the endothelium, and through the extracellular matrix into the tissue.

The Multistep Process of Leukocyte Migration



Endothelial and Leukocyte Adhesion Molecules

TABLE 3.4 Endothelial and Leukocyte Adhesion Molecules

Family	Molecule	Distribution	Ligand
Selectin	L-selectin (CD62L)	Neutrophils, monocytes T cells (naïve and central memory) B cells (naïve)	Sialyl-Lewis X/PNAd on GlyCAM-1, CD34, MAdCAM-1, others; expressed on endothelium (HEV)
	E-selectin (CD62E)	Endothelium activated by cytokines (TNF, IL-1)	Sialyl-Lewis X (e.g., CLA) on glycoproteins; expressed on neutrophils, monocytes, T cells (effector, memory)
	P-selectin (CD62P)	Endothelium activated by cytokines (TNF, IL-1), histamine, or thrombin; platelets	Sialyl-Lewis X on PSGL-1 and other glycoproteins; expressed on neutrophils, monocytes, T cells (effector, memory)
Integrin	LFA-1 (CD11aCD18)	Neutrophils, monocytes, T cells (naïve, effector, memory)	ICAM-1 (CD54), ICAM-2 (CD102); expressed on endothelium (upregulated on activated endothelium)
	MAC-1 (CD11bCD18)	Monocytes, DCs	ICAM-1 (CD54), ICAM-2 (CD102); expressed on endothelium (upregulated on activated endothelium)
	VLA-4 (CD49aCD29)	Monocytes T cells (naïve, effector, memory)	VCAM-1 (CD106); expressed on endothelium (upregulated on activated endothelium)
	$\alpha 4\beta 7$ (CD49DCD29)	Monocytes T cells (gut homing naïve effector, memory)	VCAM-1 (CD106), MAdCAM-1; expressed on endothelium in gut and gut-associated lymphoid tissues
Ig	CD31	Endothelial cells, leukocytes	CD31 (homotypic interaction)

CLA, Cutaneous lymphocyte antigen-1
GlyCAM-1, glycan-bearing cell adhesion molecule-1
HEV, high endothelial venule
ICAM, intercellular adhesion molecule
Ig, immunoglobulin
IL-1, interleukin-1
MAdCAM-1, mucosal adhesion cell adhesion molecule-1
PSGL-1, P-selectin glycoprotein ligand-1
TNF, tumor necrosis factor
VCAM, vascular cell adhesion molecule.

Selectins

- Receptors expressed on the surfaces of endothelial cells and leukocytes that bind selected sugars (sialylated oligosaccharides)
- Not expressed on resting endothelial cells, but expressed within 30 minutes of stimulation
- Low affinity binding with a fast-off-rate
- Single chain transmembrane glycoprotein
- Binding to ligand needs Ca
- Distribution:
  - **E-selectin (CD62E): endothelial cells**
  - **P-selectin (CD62P): Platelets & endothelial cells**
  - **L-selectin (CD62L): Leukocytes**

Integrins

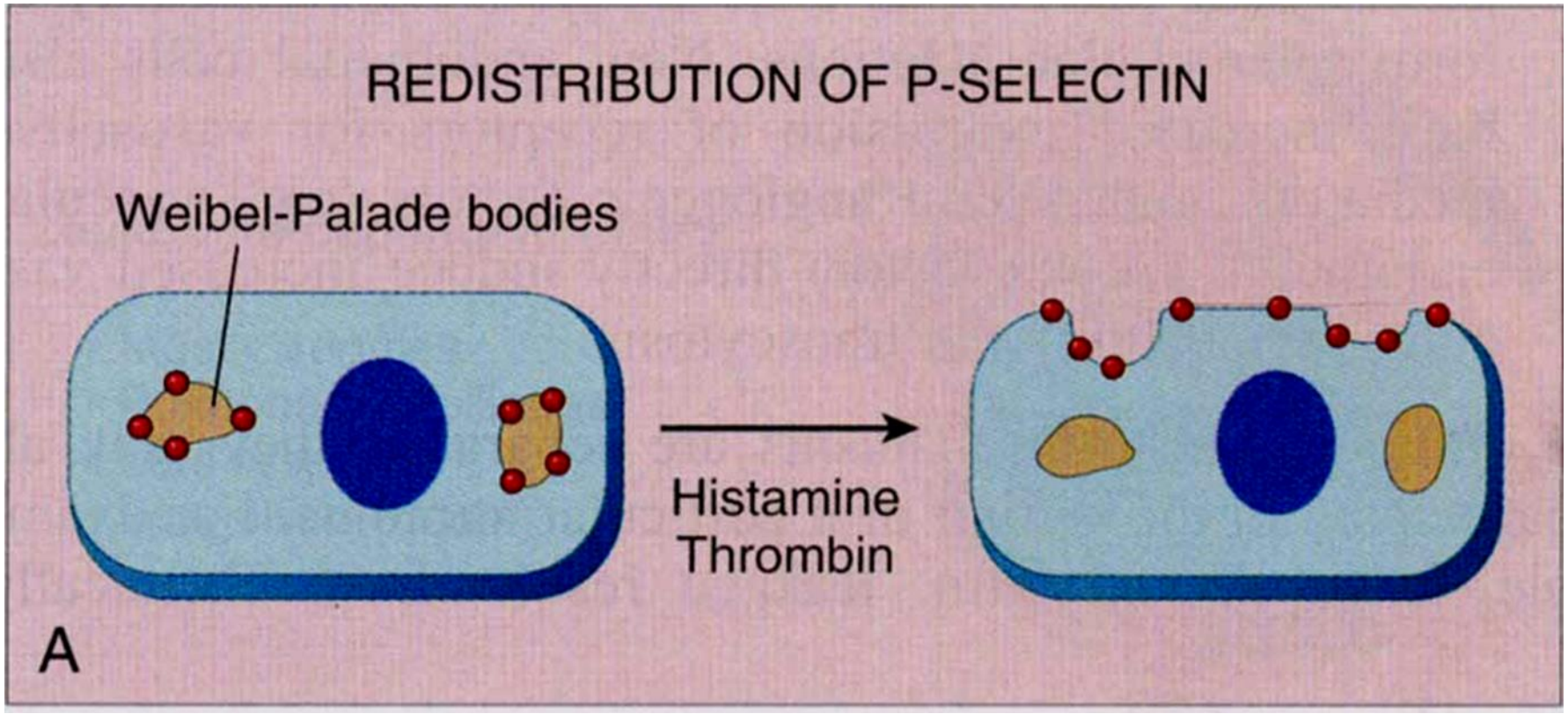
- Heterodimeric cell surface proteins (α & β chains)
- Binds to ligands present in:
  - **Extracellular matrix**
  - **Complement system**
  - **Surface of other cell**
- Cytoplasmic domains bind with cytoskeleton

Adhesion between leukocytes and endothelial cells

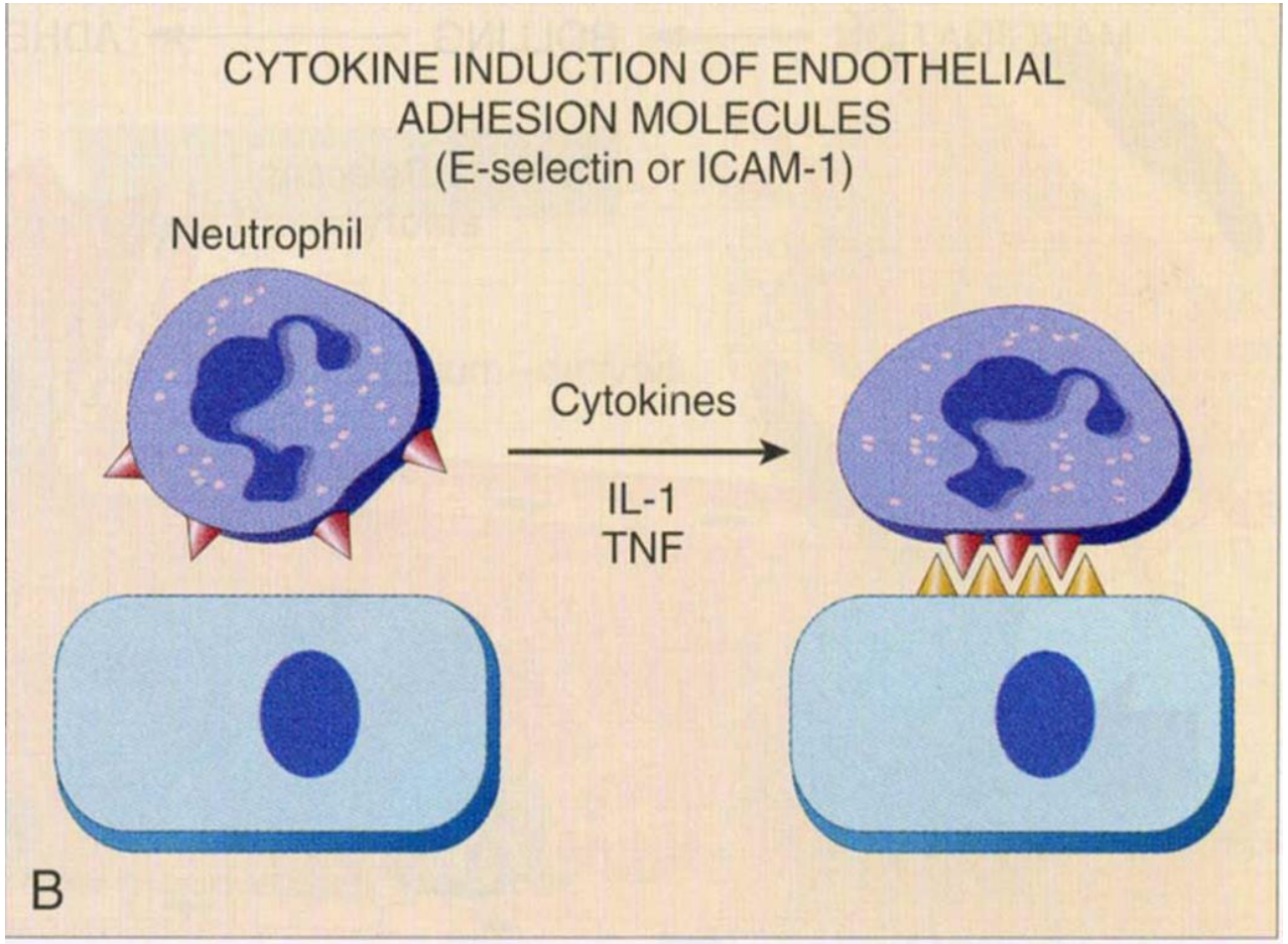
- **Weak adhesion and rolling**
  - Mediated by selectins

- **Firm adhesion**
  - Ig superfamily molecules expressed on endothelial cells such as:
    - ICAM-1
    - VCAM-1
  - Integrins expressed on leukocytes:
    - LFA-1 (CD11a/CD18)
    - Mac-1 (CD11b/CD18)
    - P150,95 (CD11c/CD18)
    - VLA-4

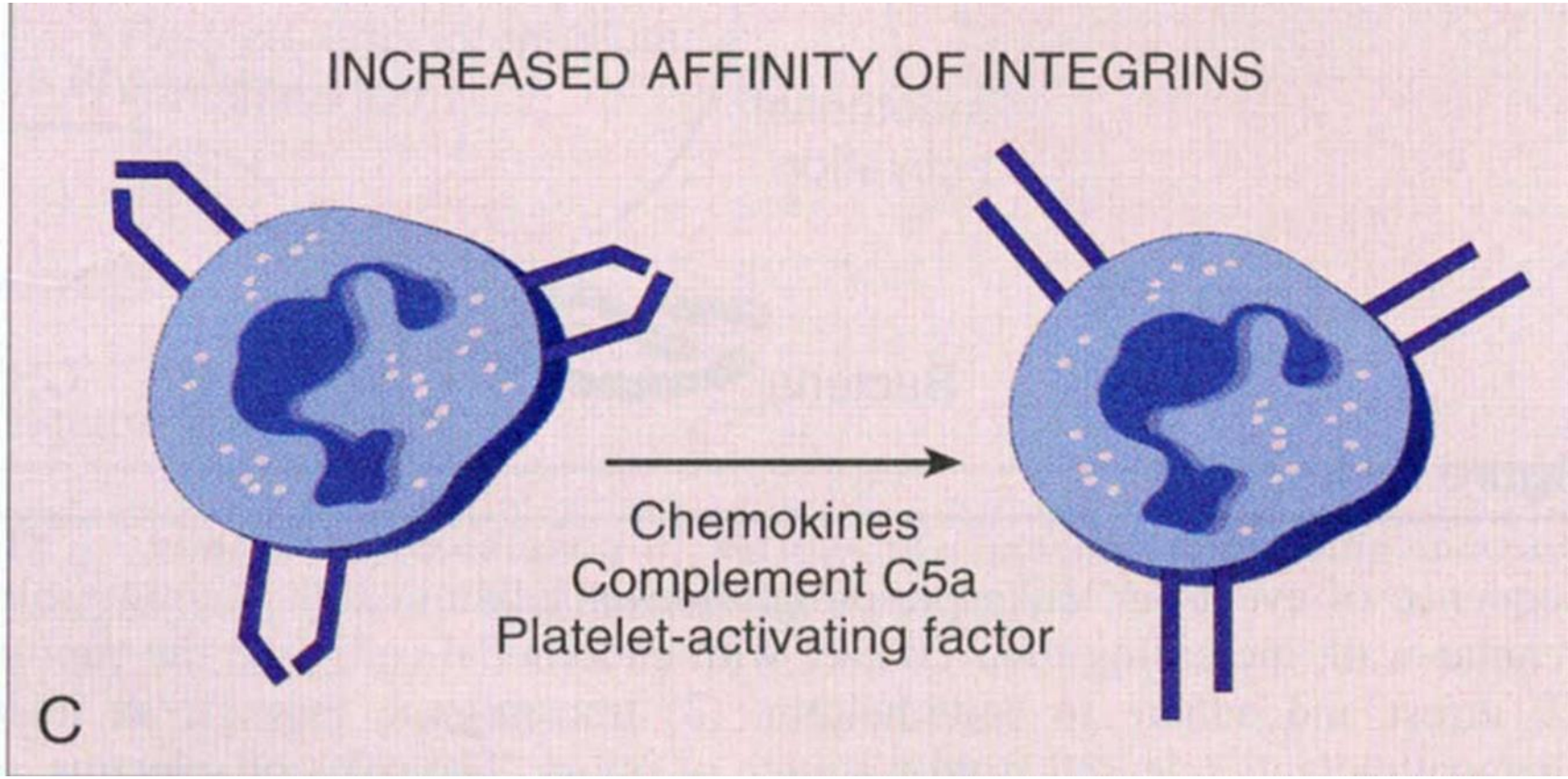
Upregulation of Selectins



Cytokine Induction of Adhesion Molecules



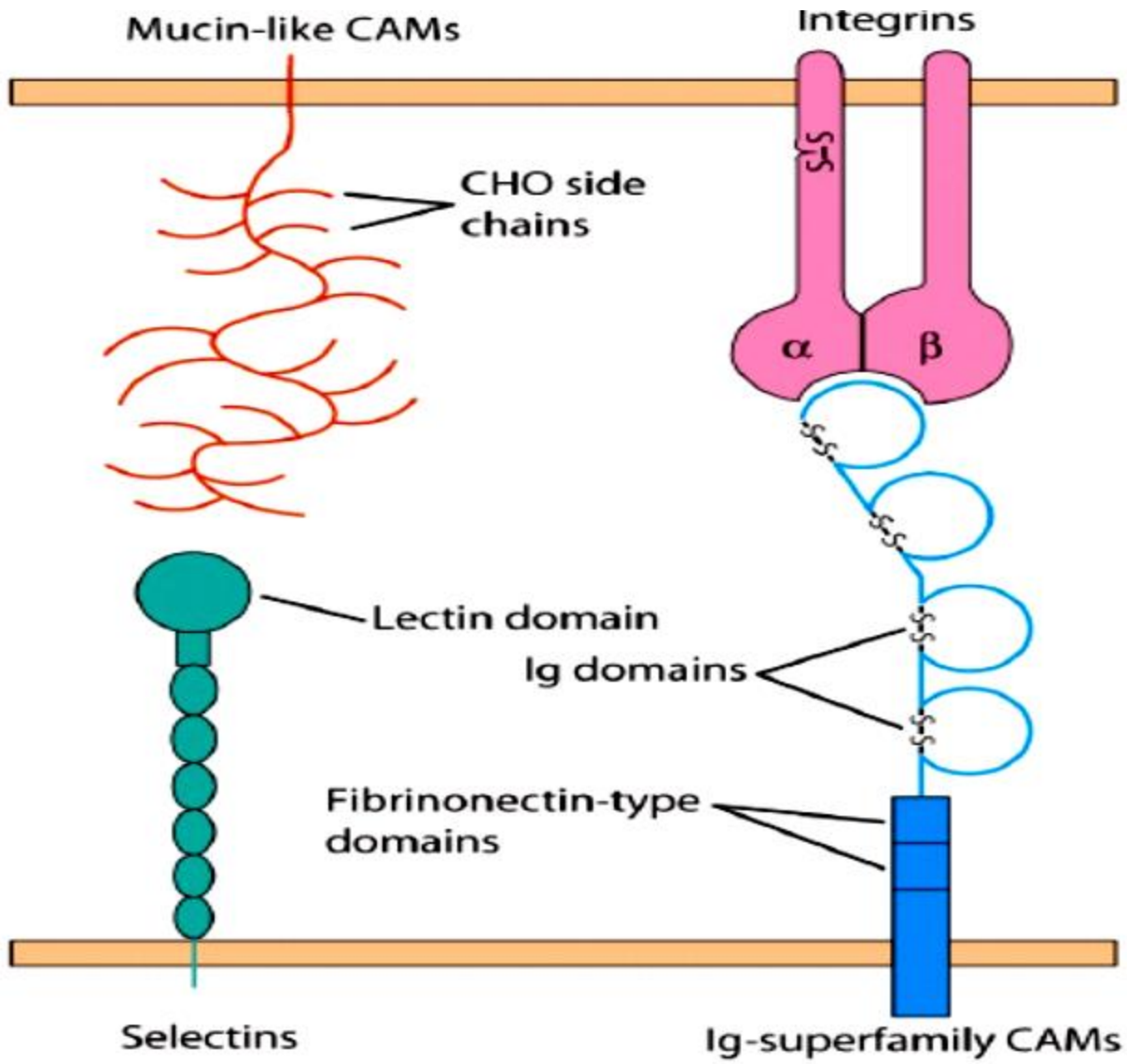
Chemotactics Increase Affinity of Integrins to Adhesion Molecules



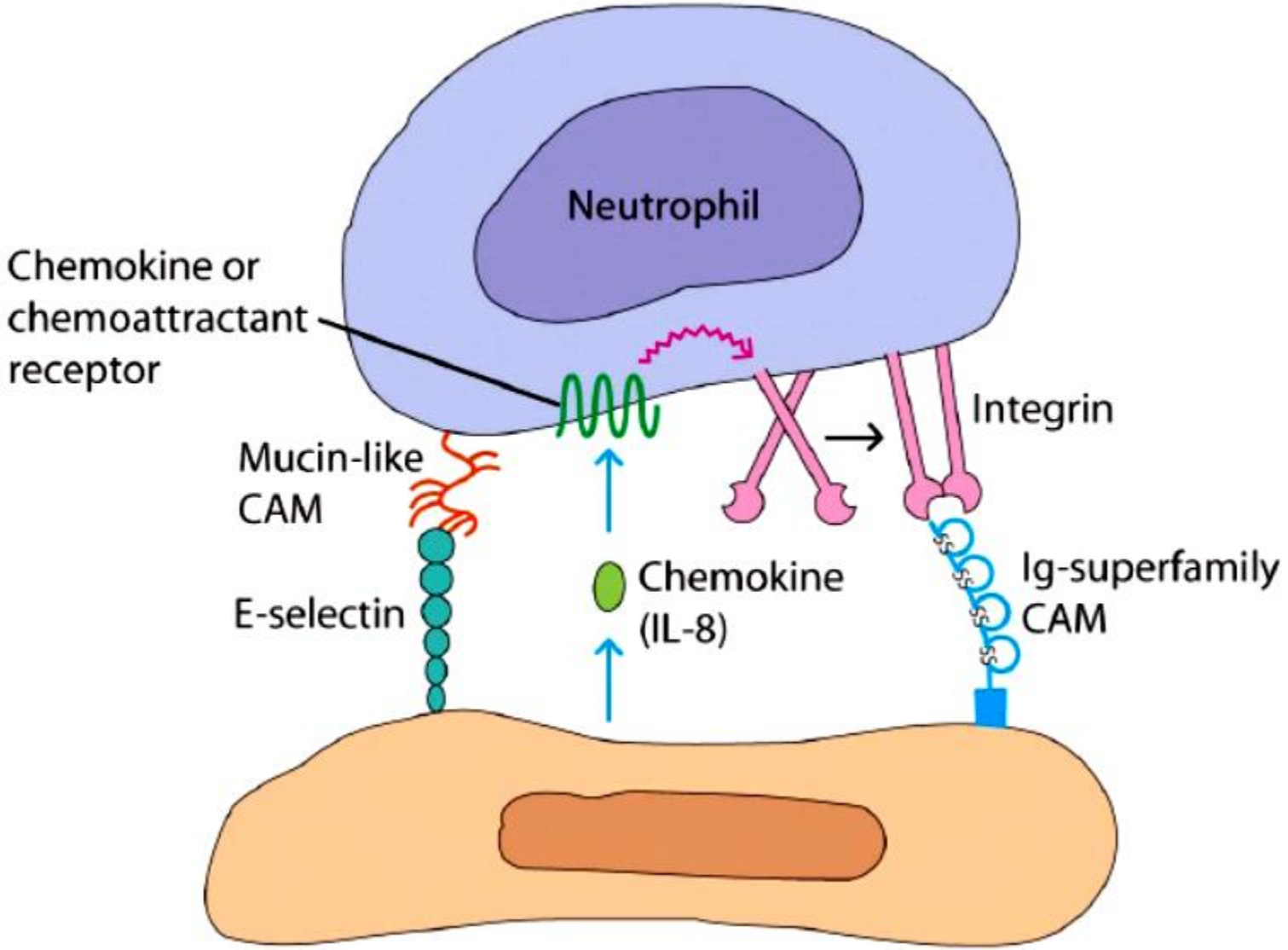
Endothelial and Leukocyte Adhesion Molecule Interactions

ENDOTHELIUM	WBC	FUNCTION
P & E-selectins	X Sialyl-Lewis	Rolling
GlyCAM-1, CD34	L-selectin	Rolling
VCAM-1	VLA-4	Adhesion
ICAM-1	CD11/CD18 (LFA1, MAC1)	Adhesion
CD31 (PECAM-1)	CD31	Transmigration

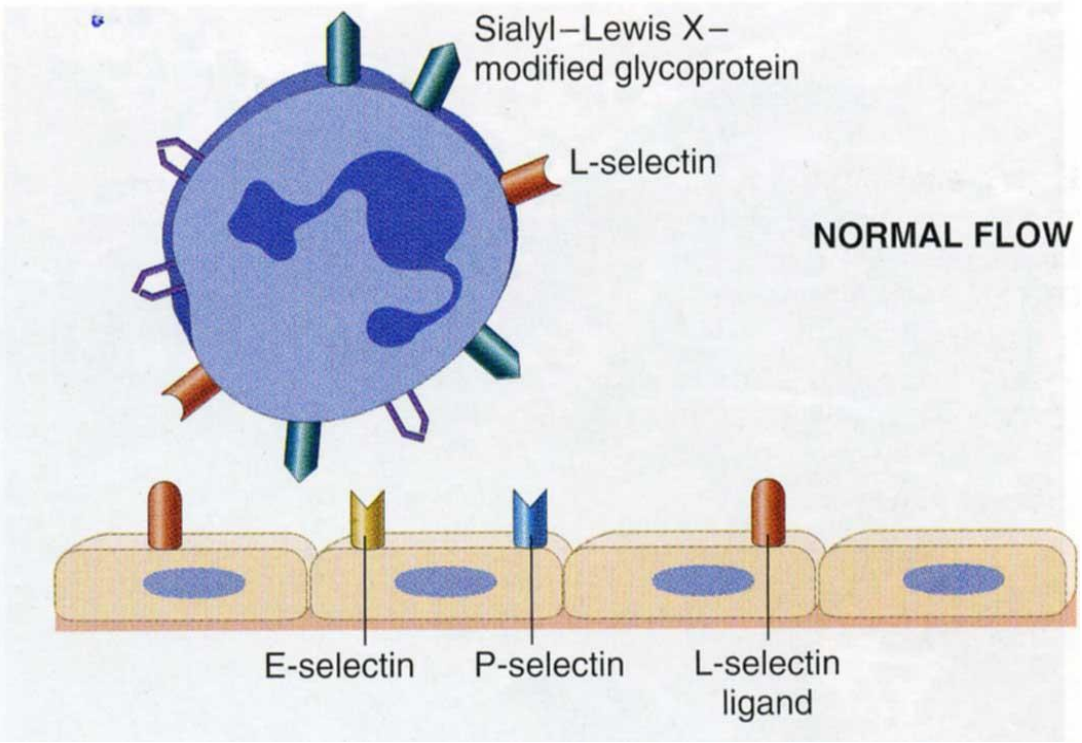
General Structure of CAM Families

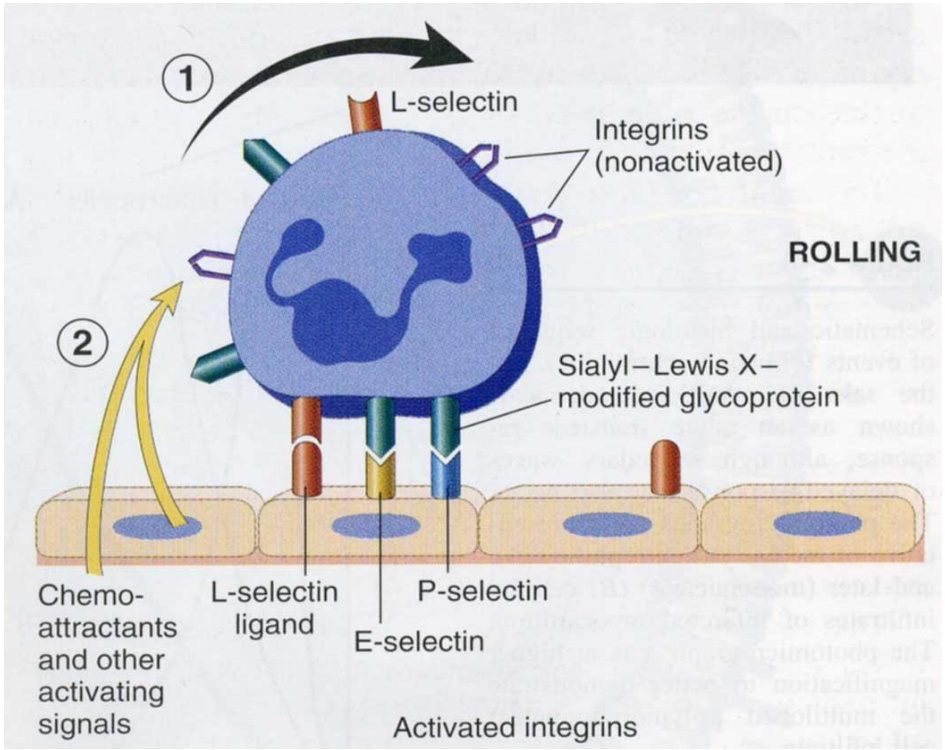


The Process of Rolling, Activation and Firm Adhesion of Leukocytes

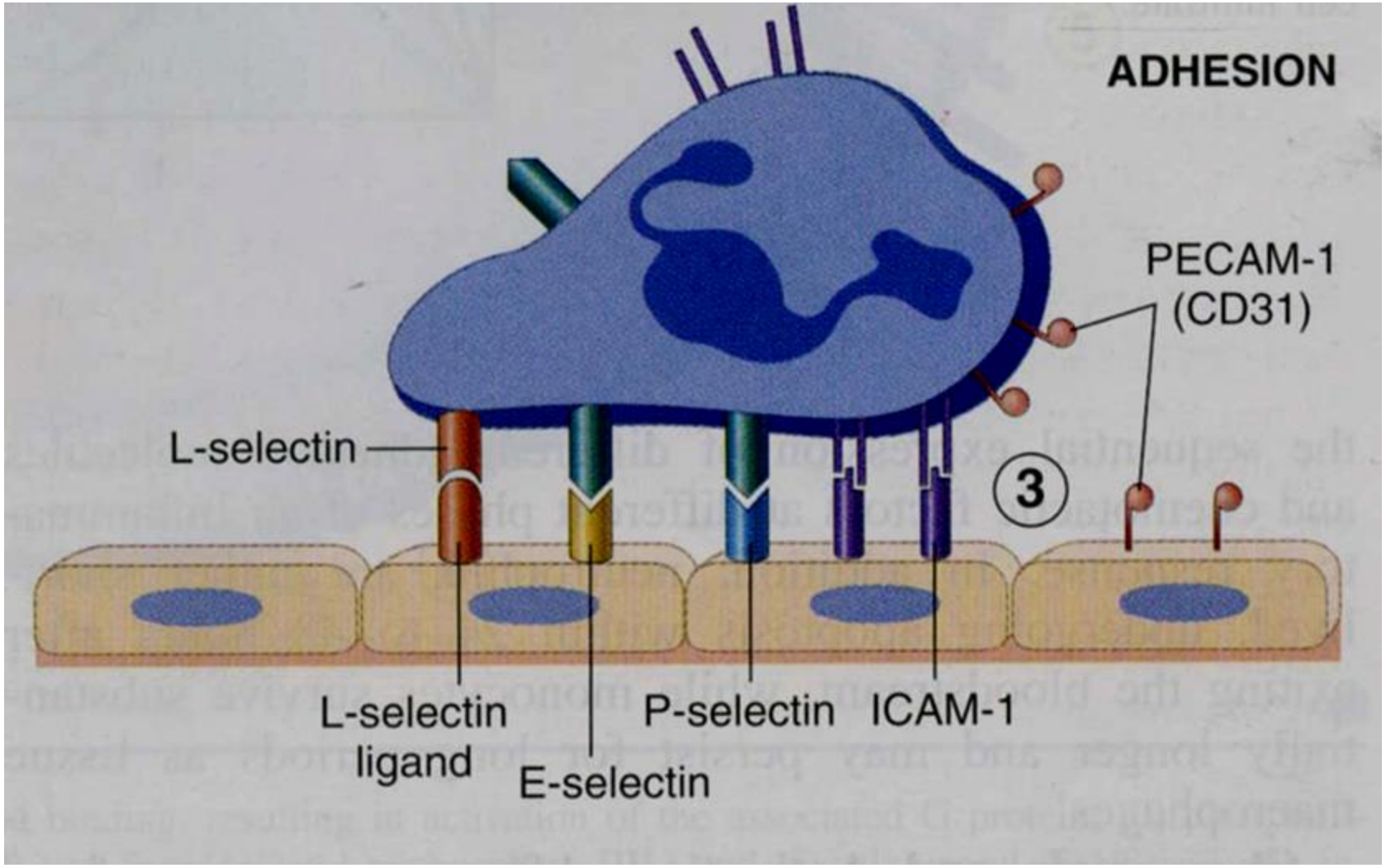


Molecules Mediating Endothelial-Neutrophil Interaction





Firm Adhesion via Integrin ICAM Interactions

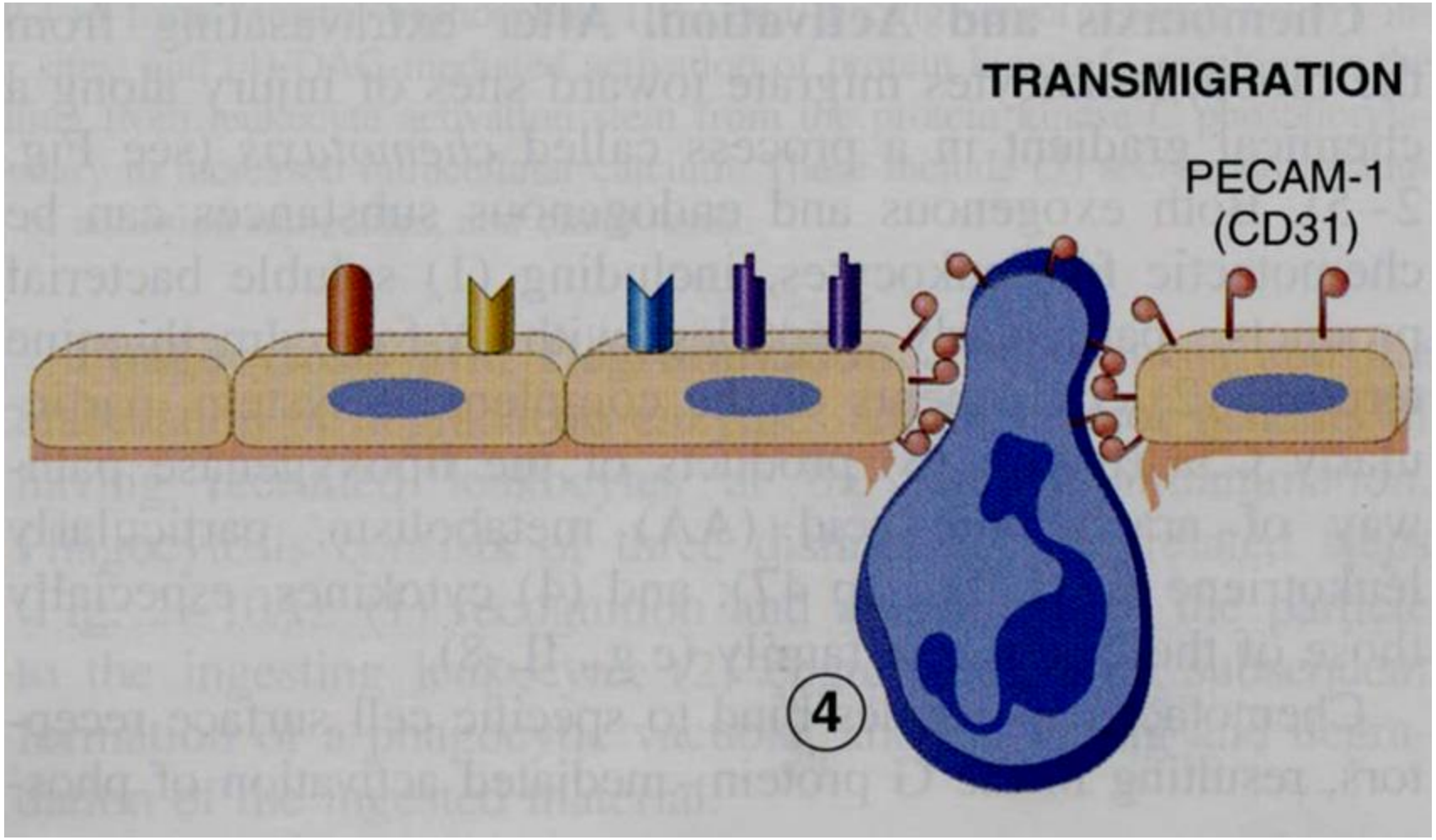


Diapedesis

- Transmigration of leukocytes between endothelial cells at the intercellular junctions

- Facilitated by PECAM-1 (CD31)/PECAM-1 interaction

Transmigration of Neutrophils



Chemotaxis

- Migration of cells along a chemical gradient

- Chemotactic factors:
  - Soluble bacteial products, e.g. N-formyl-methionine termini
  - Complement system products, e.g. C5a
  - Lipooxygenase pathway of arachidonic acid metabolism, e.g. LTB4
  - Cytokines, e.g. IL-8

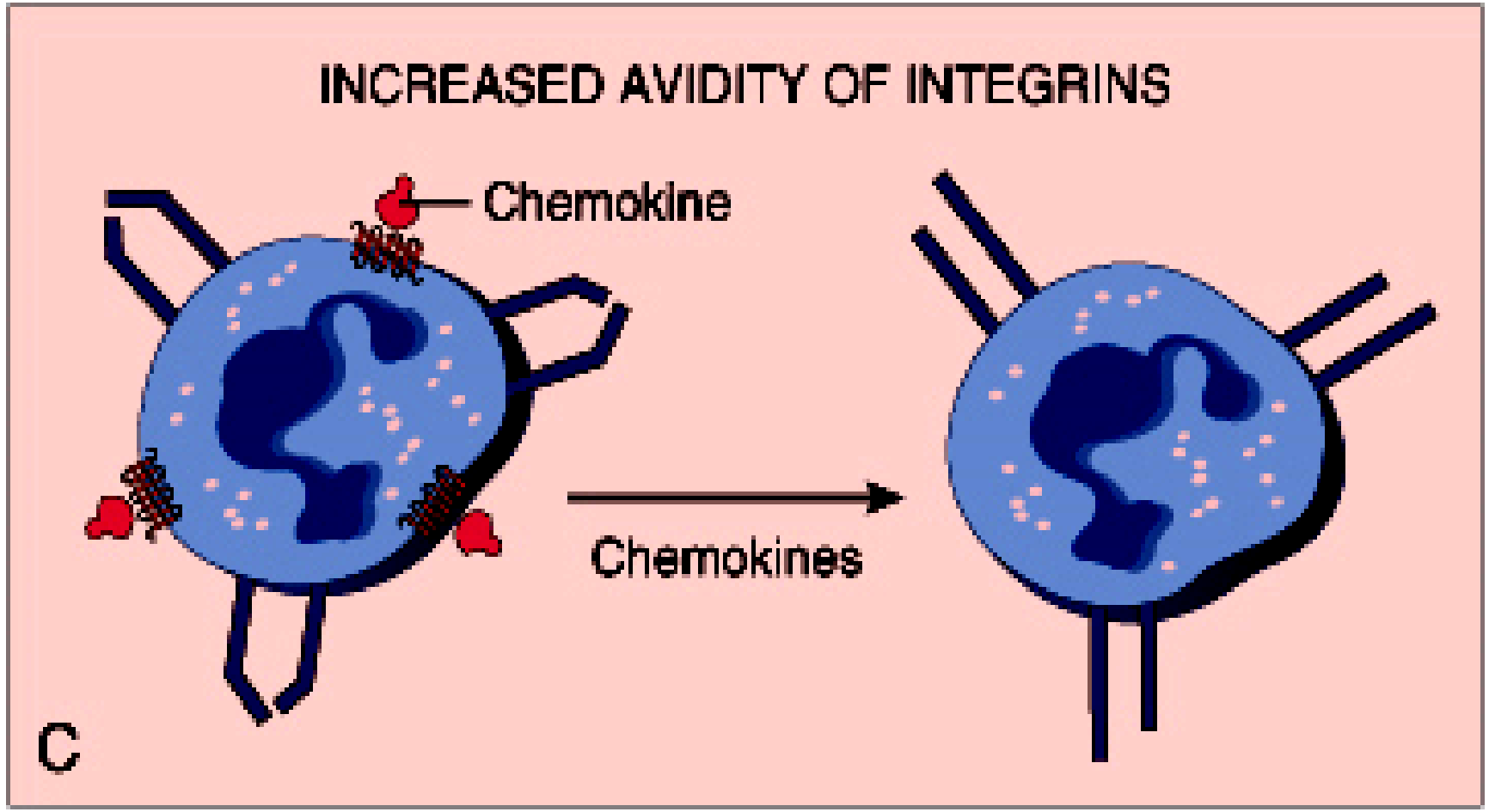
Chemotaxis

- Migration of cells along a chemical gradient
- Both exogenous and endogenous substances can act as chemoattractants.

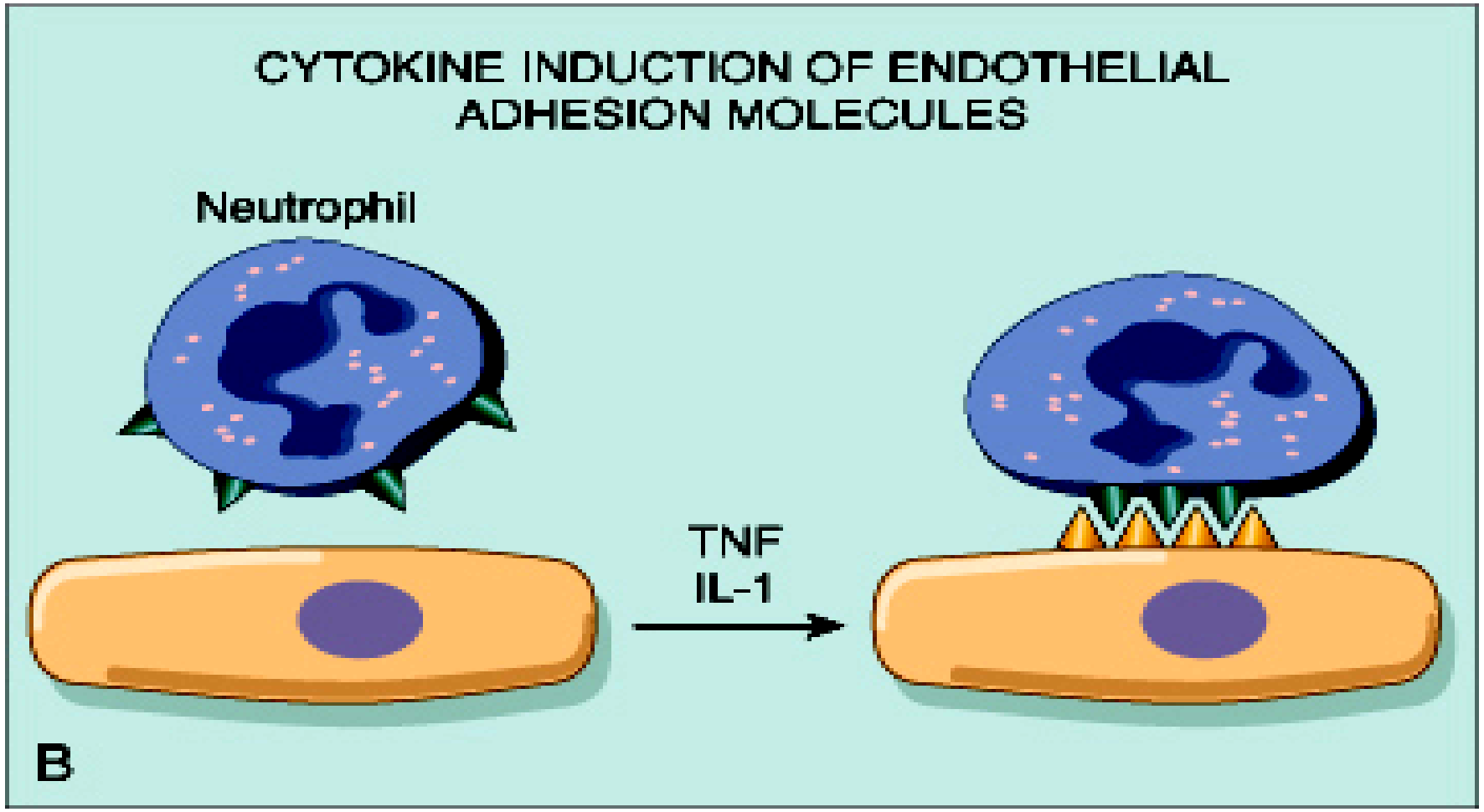
- Chemotactic factors:

  - Bacterial products, particularly peptides with N-formylmethionine termini
  - Cytokines, especially those of the chemokine family (IL-8)
  - Components of the complement system, particularly C5a
  - Products of the lipoxygenase pathway of arachidonic acid (AA) metabolism, particularly leukotriene B4 (LTB4)

Effects of Chemotactic Factors on Leukocytes



Effects of Chemotactic Factors on Endothelial Cells



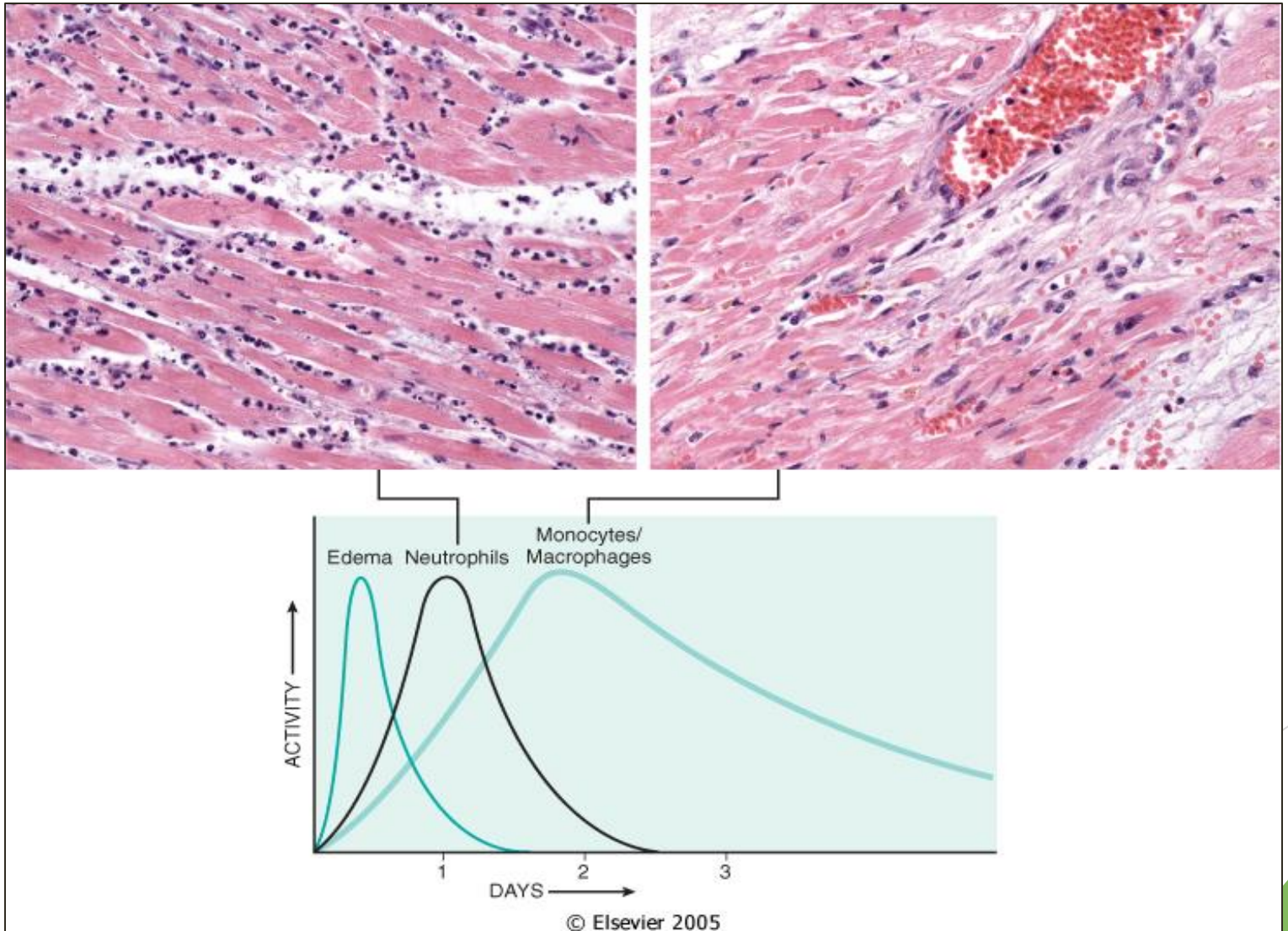
Effects of Chemotactic Factors on Leukocytes

- Stimulate locomotion
- Degranulation of lysosomal enzymes
- Production of AA metabolites
- Modulation of the numbers and affinity of leukocyte adhesion molecules

Nature of Cell Infiltrate

- The nature of the leukocyte infiltrate varies with the age of the inflammatory response and the type of stimulus.
- Bacterial infection—the cellular infiltrate is dominated by neutrophils for several days
- Viral infections- lymphocytes may be the first cells to arrive
- Hypersensitivity reactions are dominated by activated lymphocytes, macrophages, and plasma cells (reflecting the immune response)
- Allergic reactions, eosinophils may be a prominent cell type.

Sequence of Events Following injury

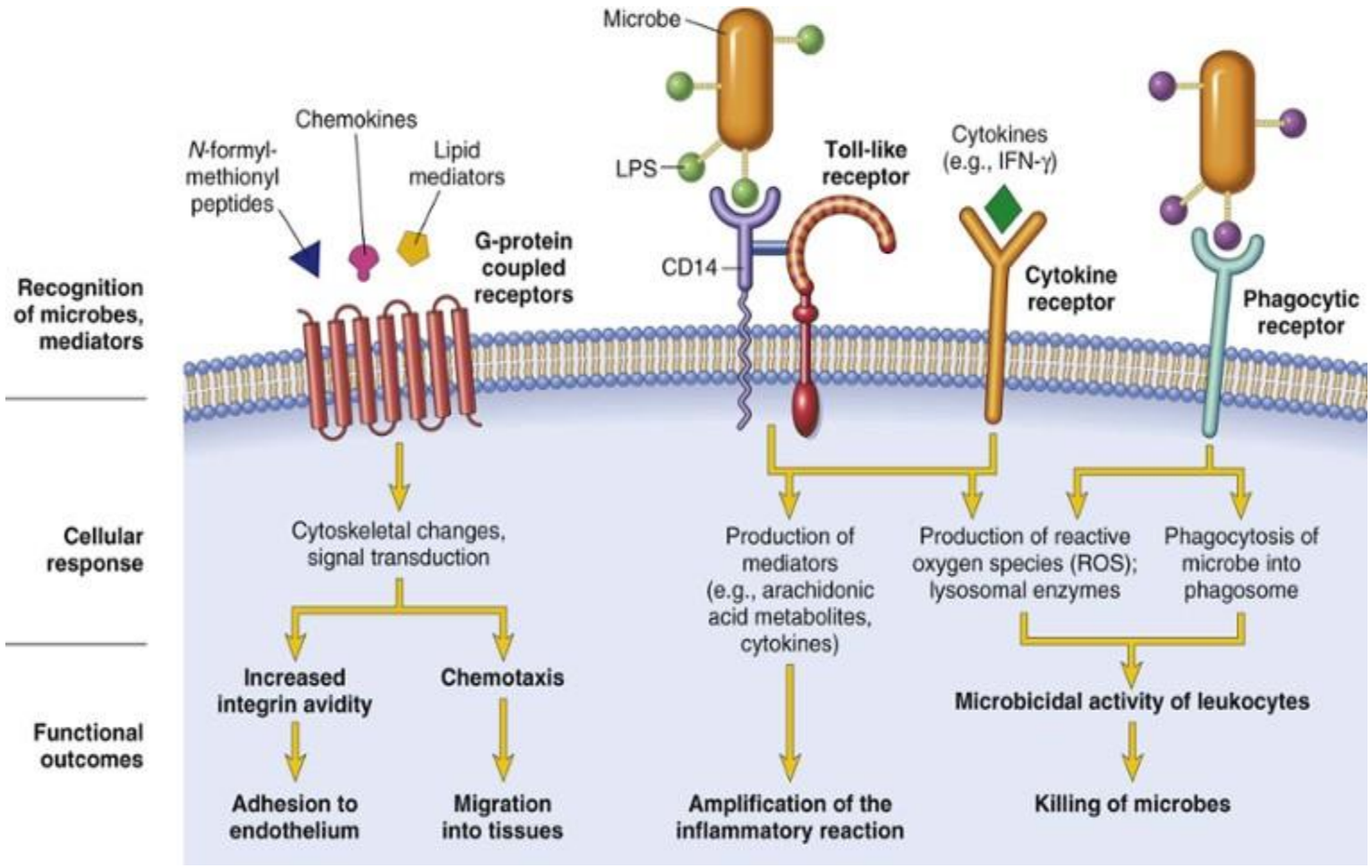


Phagocytosis and Clearance of the Offending Agent

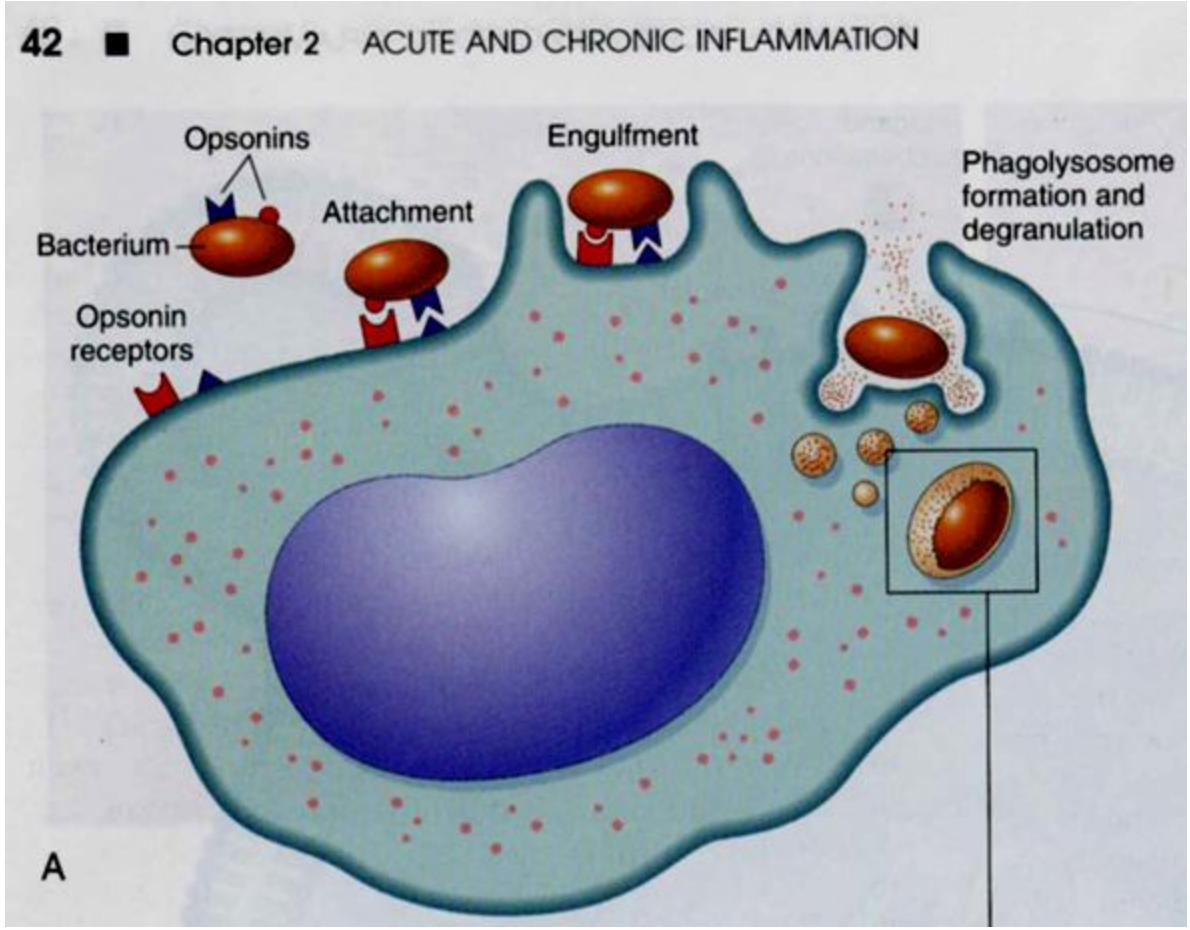
- Recognition of microbes or dead cells induces several responses in leukocytes that are collectively called **leukocyte activation.**
- **Phagocytosis** is the process of ingestion and digestion by cells of solid substances, e.g., other cells, bacteria, necrotic tissue or foreign material
- **Steps of phagocytosis:**

1) Recognition, attachment and binding to cellular receptors
   Opsonins
   IgG, C3b, collectins-
2) Engulfment
3) Fusion of phagocytic vacuoles with lysosomes
4) Killing or degradation of ingested material

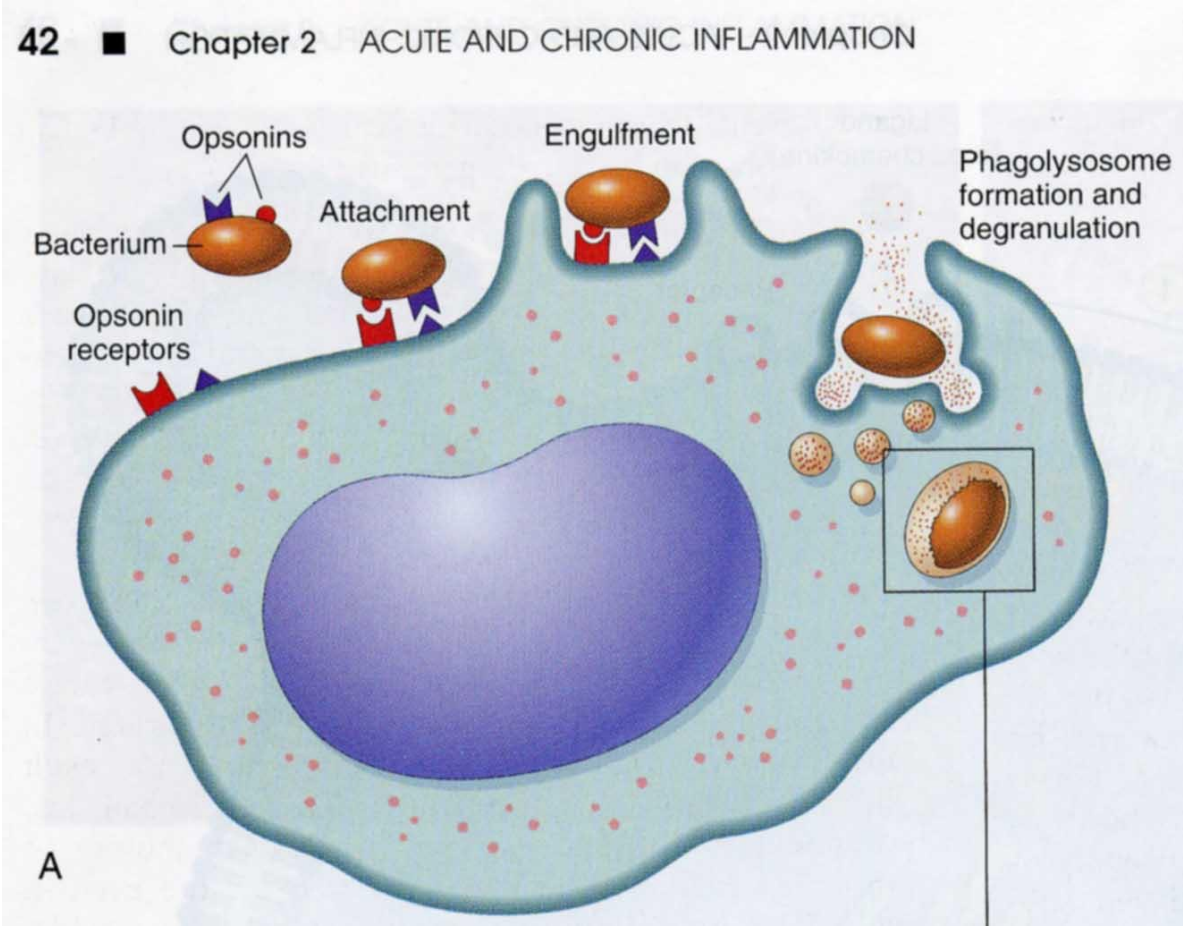
Leukocyte Activation



Phagocytosis



Phagocytosis



Phagocytosis

- **Recognition and attachment by receptors:**

The two most important recognition receptors are:

1- Toll-like receptors are microbial sensors.

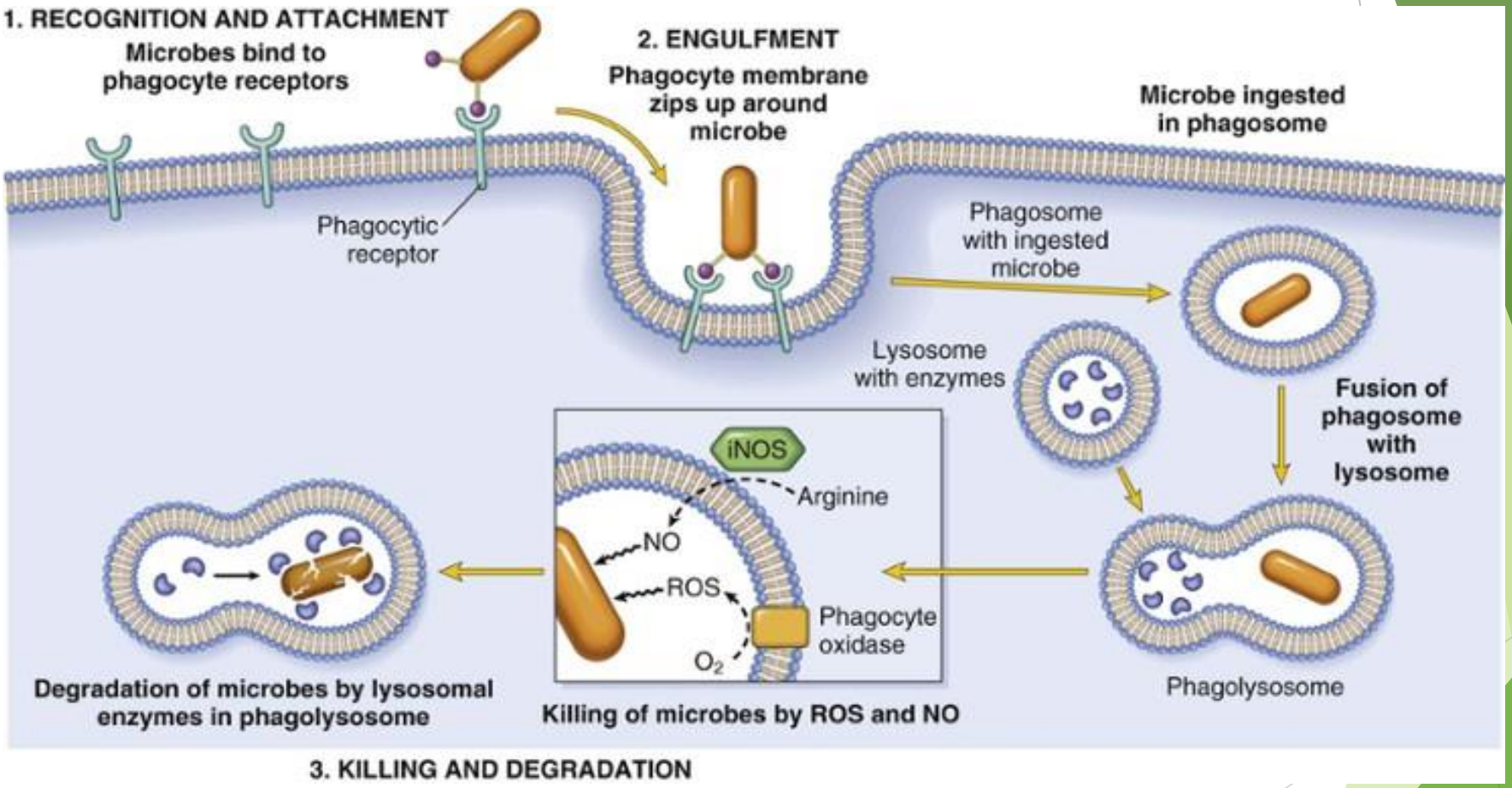
2- Inflammasome is a multiprotein cytoplasmic complex that recognizes products of dead cells such as uric acid---------- activation of caspases-1 ---------- secretion of the biologically active IL-1.

Phagocytosis

- **Recognition and attachment by receptors**:
- Other receptors:
  - Mannose receptors: bind to terminal mannose residues on microbes cell walls.

    Mammalian cells are not recognised by mannose receptors because they contain terminal sialic acid and N-acetyl galactosamine.
  - Scavenger receptors: oxidized LDL, and microbes.
  - Opsonin receptors (high affinity): IgG, C3b, collectins.
  - Cytokine receptors.

Phagocytosis



How Do Leukocytes Kill Infectious Agents? Intracellular Destruction of Microbes and Debris

- Oxygen Burst Products/ Reactive Oxygen Species(ROS)
- Nitric Oxide.
- Neutrophilic Extracellular Traps (NETs)
- 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.

How Do Leukocytes Kill Infectious Agents? Intracellular Destruction of Microbes and Debris

- Granule Enzymes and Other Proteins

1. Specific (or secondary) granules

   lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase.

2. Larger azurophil (or primary) granules

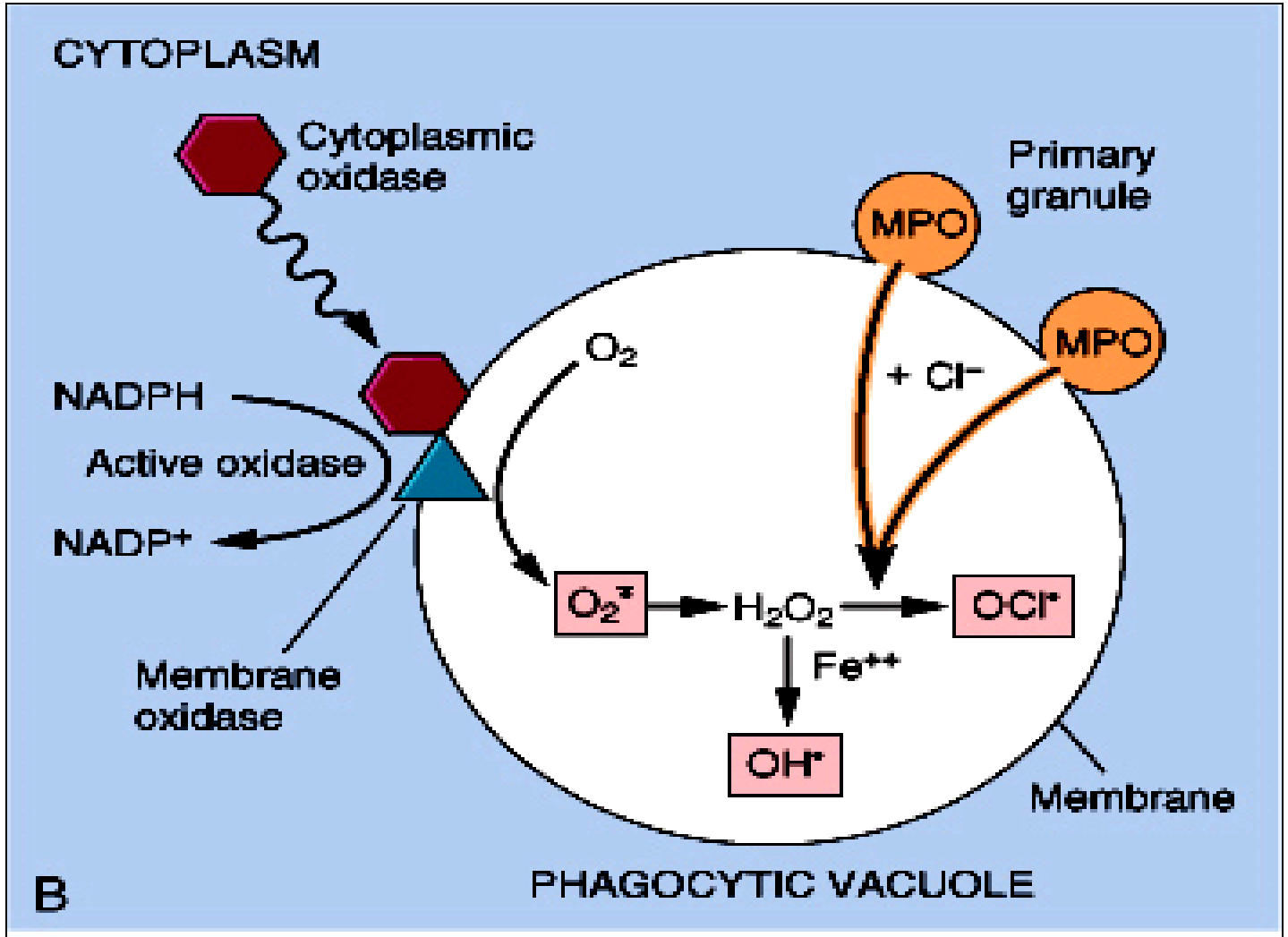
   MPO, bactericidal factors (such as defensins), acid hydrolases, and a variety of neutral proteases (elastase, cathepsin G, nonspecific collagenases, proteinase 3)

Generation of Oxygen Metabolites

- $2O_2 + NADPH \xrightarrow{\text{NADPH oxidase}} 2O_2^- + NADP^+ + H^+$ **(superoxide anion)**
- $O_2^- + 2H^+ \xrightarrow{\text{Dismutase}} H_2O_2$ **(hydrogen peroxide)**
- $H_2O_2 + Cl^- \xrightarrow{\text{Myeloperoxidase}} HOCl^-$ **(hypochlorite)**

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

Oxygen Dependent Bactericidal Mechanisms



Oxygen Derived Free Radicals

At low levels

- Increase:
  - Chemokines
  - Cytokines
  - Adhesion molecules

At high levels

- Endothelial damage & thrombosis
- Protease activation & inhibition of antiproteases
- Direct damage to other cells

Protective mechanisms against free radicals include: transferrin, ceruloplasmin, catalase, superoxide dismutase, and glutathione

Nitric Oxide (NO)

- **Soluble gas produced from arginine by the action of nitric oxide synthase (NOS)**
- **Participates in microbial killing.**
- **Three different types of NOS:**
  endothelial (eNOS)
  neuronal (nNOS)
  inducible (iNOS)

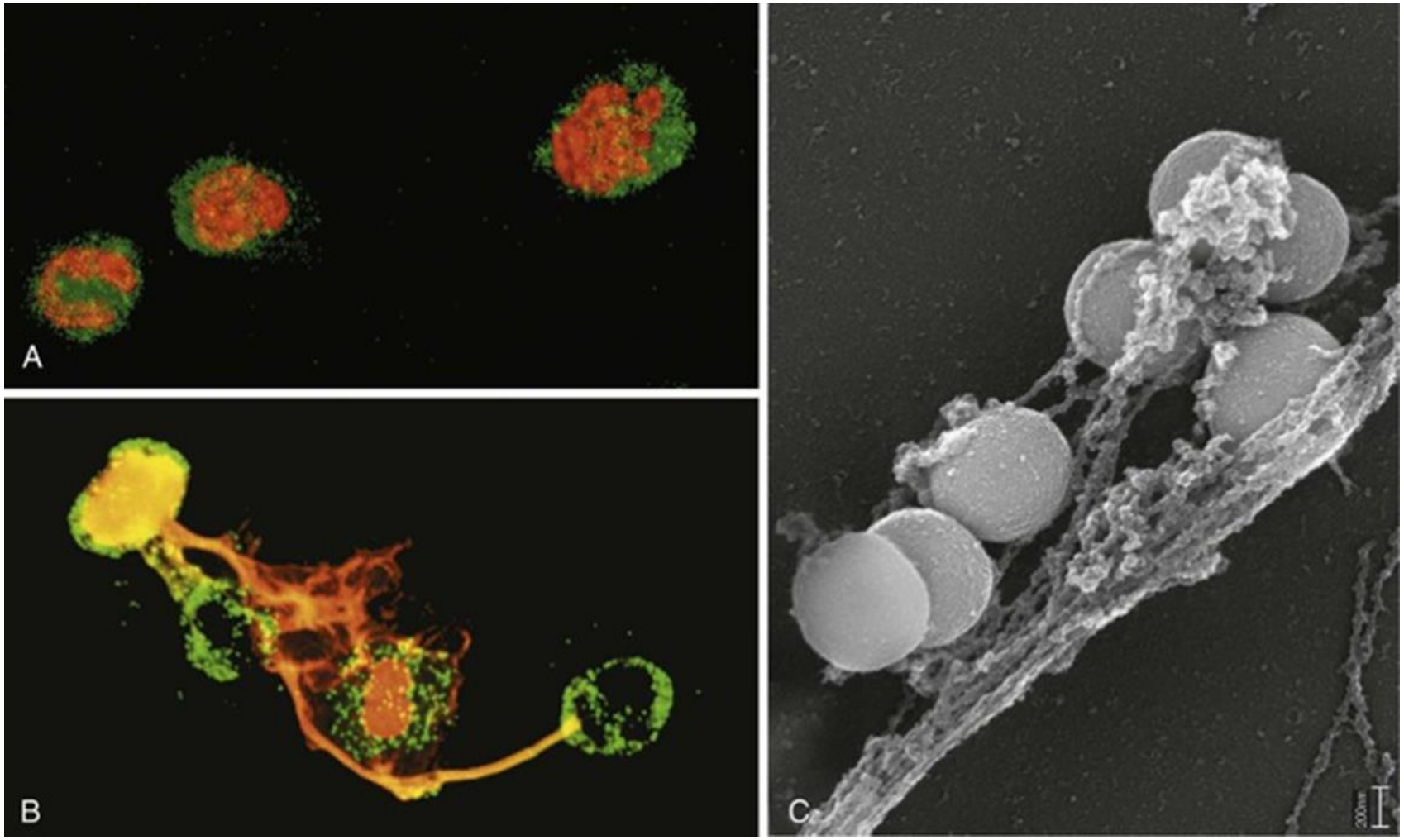
- **eNOS and nNOS are constitutively expressed at low levels, and the NO they generate acts to maintain vascular tone and as a neurotransmitter, respectively.**

- **iNOS, is involved in microbial killing, is expressed when macrophages are activated by cytokines (e.g., IFN-γ) or microbial products, and induces the production of NO**

Neutrophilic Extracellular Traps (NETs)

- Are extracellular fibrillar networks.
- Contains a frame work of nuclear chromatin with granule protein.
- Provides a high concentration of antimicrobial substances.
- In this process the nuclei of the neutrophils are lost.

Neutrophilic Extracellular Traps (NETs)



Lysosomal constituents

- **Released in:**
  - After cell death
  - Leakage upon formation of phagocytic vacuoles
  - Frustrated phagocytosis (fixed on flat surfaces)
  - After phagocytosis of membranolytic substance, e.g. urate..

  Acid proteases: needs low PH as in phagolysomes.
- **Neutral proteases effects:**
  - Elastases, collagenases, and cathepsin
  - Cleave C3 and C5 producing C3a & C5a
  - Generate bradykinin like peptides
- **Minimizing the damaging effects of proteases is accomplished by antiproteases:**
  - Alpha 2 macroglobulin
  - Alpha 1 antitrypsin

The most telling proof of the importance of leukocyte adhesion molecules is the existence of genetic deficiencies in these molecules that result in recurrent bacterial infections as a consequence of impaired leukocyte adhesion and defective inflammation

Genetic defects in leukocyte function

Disease	Defect
Leukocyte adhesion deficiency 1	CD18 unit of integrin
Leukocyte adhesion deficiency 2	Sialyl-Lewis X
Neutrophil-specific granule deficiency	Absent specific granules
Chronic Granulomatous Disease, X-linked	Membrane component of NADPH oxidase
Chronic Granulomatous Disease, autosomal recessive	Cytoplasmic component of NADPH oxidase
Myeloperoxidase (MPO) deficiency	Absent MPO-H2O2 system
Chediak-Higashi disease	Organelle trafficking

Acquired defects in leukocyte function

- Chemotaxis defects
  - burns, diabetes, sepsis, etc.
- Adhesion
  - hemodialysis, diabetes
- Phagocytosis and microbiocidal activity
  - leukemia, sepsis, diabetes, malnutrition, etc