Foundation Block
Pathology
Oct 2019

INFLAMMATION AND REPAIR
Lecture 2
Cellular Events in Inflammation

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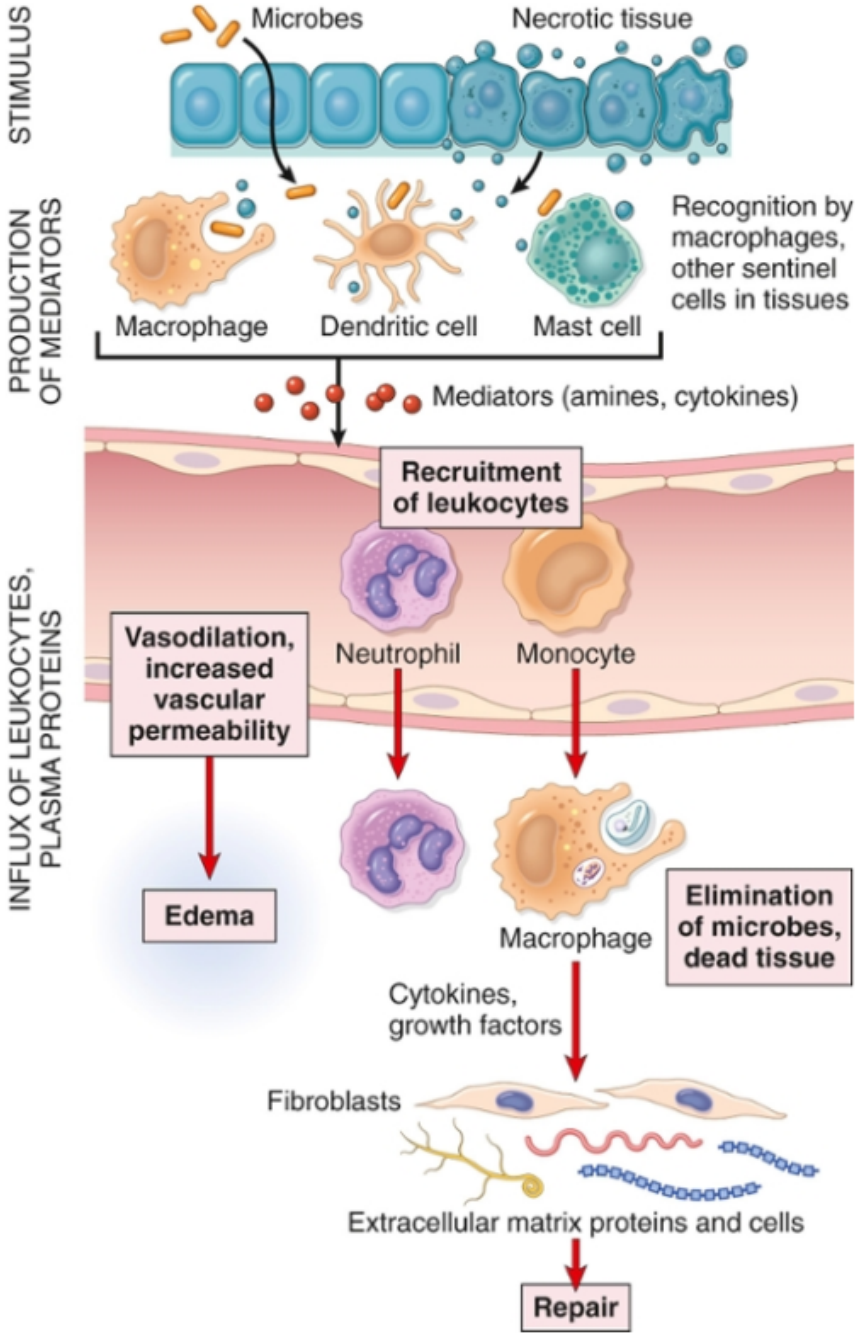

STIMULUS
Microbes
Necrotic tissue
PRODUCTION OF MEDIATORS
Macrophage
Dendritic cell
Mast cell
Recognition by macrophages, other sentinel cells in tissues
Mediators (amines, cytokines)
INFLUX OF LEUKOCYTES, PLASMA PROTEINS
Recruitment of leukocytes
Neutrophil
Monocyte
Vasodilation, increased vascular permeability
Edema
Macrophage
Elimination of microbes, dead tissue
Cytokines, growth factors
Fibroblasts
Extracellular matrix proteins and cells
Repair

Events of acute Inflammation

- Acute inflammation has three main events:

vascular

(1) Hemodynamic changes

(*alterations in vascular caliber that lead to an increase in blood flow)*

(2) Increased vascular permeability

(*structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation)*

cellular

(3) Emigration of the leukocytes from the microcirculation

(their accumulation in the focus of injury, *and their activation* to eliminate the offending agent)

Phases of changes in Vascular Caliber and Flow

1. Transient vasoconstriction of arterioles

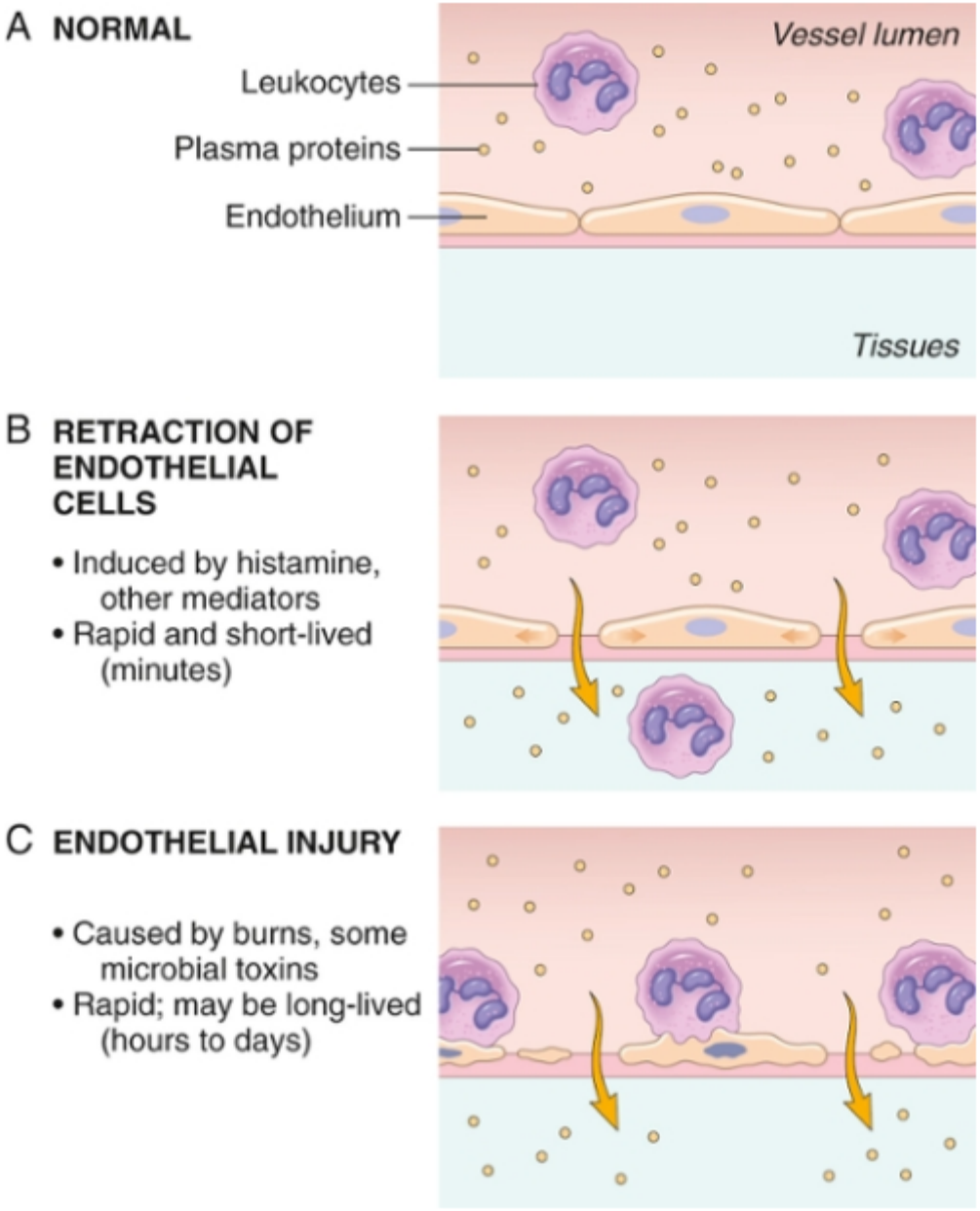
   It disappears within 3-5 seconds in mild injuries

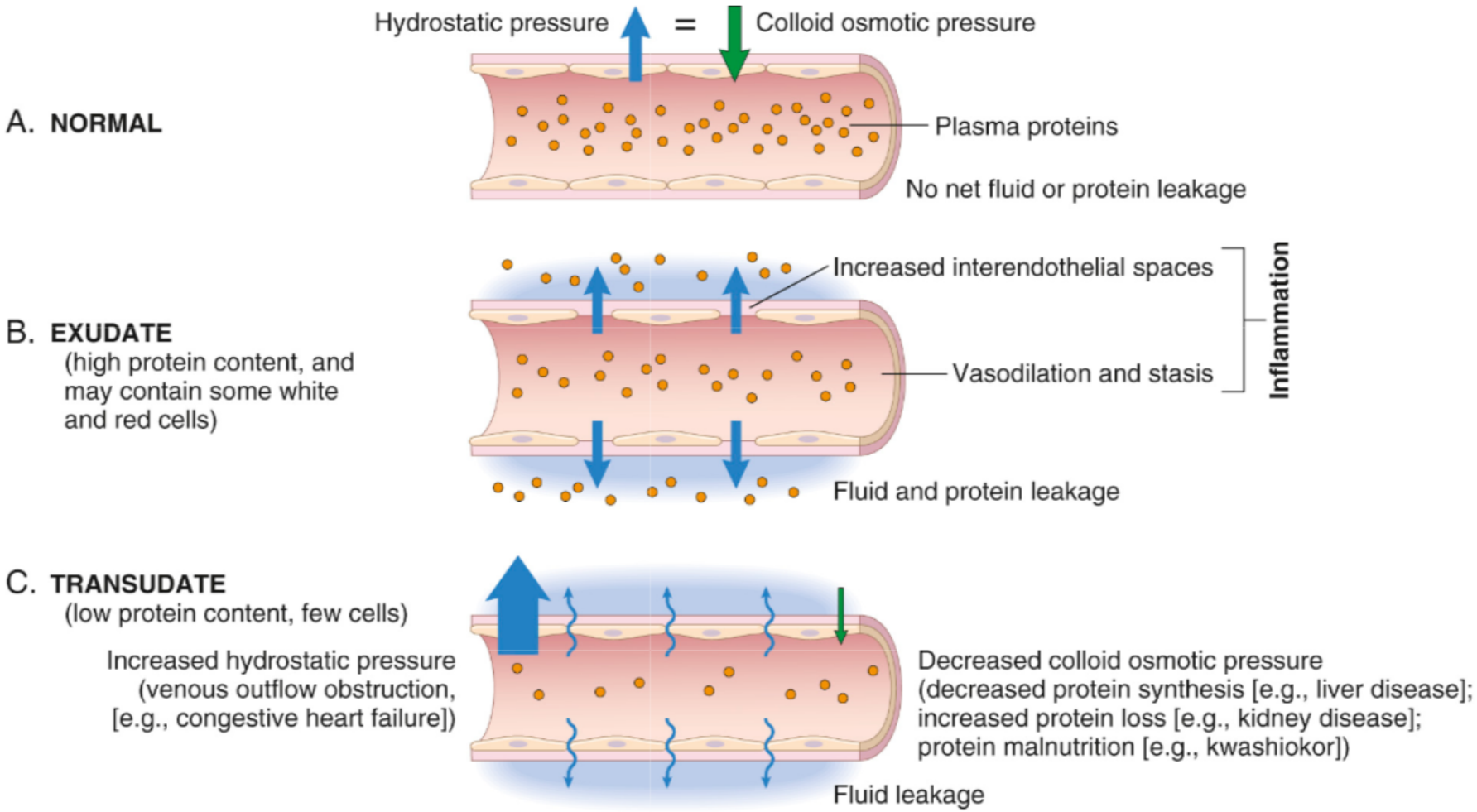
2. Vasodilatation: It involves the **arterioles** results in opening of new microvasculature beds in the area leading to increasing blood flow – Histamine effect

3. Slowing of the circulation

due to increased permeability of the microvasculature, this leads to outpouring of protein-rich fluid in the extravascular tissues.

4. Stasis: slow circulation due to dilated small vessels packed with red cells


A NORMAL
Leukocytes
Plasma proteins
Endothelium
Vessel lumen
Tissues
B RETRACTION OF ENDOTHELIAL CELLS
• Induced by histamine, other mediators
• Rapid and short-lived (minutes)
C ENDOTHELIAL INJURY
• Caused by burns, some microbial toxins
• Rapid; may be long-lived (hours to days)


Hydrostatic pressure
=
Colloid osmotic pressure
A. NORMAL
Plasma proteins
No net fluid or protein leakage
B. EXUDATE
(high protein content, and may contain some white and red cells)
Increased interendothelial spaces
Vasodilation and stasis
Inflammation
Fluid and protein leakage
C. TRANSUDATE
(low protein content, few cells)
Increased hydrostatic pressure (venous outflow obstruction, [e.g., congestive heart failure])
Decreased colloid osmotic pressure (decreased protein synthesis [e.g., liver disease]; increased protein loss [e.g., kidney disease]; protein malnutrition [e.g., kwashiokor])
Fluid leakage

7. Define the terms edema, transudate, and exudate

Edema is defined as an excess of fluid in the interstitial space.

What is the difference between transudates and exudates?

Transudate

is an extravascular fluid with low protein content and a specific gravity of less than 1.012

It is essentially an ultrafiltrate of blood plasma that results from osmotic or hydrostatic imbalance across the vessel wall

No increase in vascular permeability

Exudate

An inflammatory extravascular fluid that has a high protein concentration, cellular debris.
Specific gravity above 1.020

It implies significant alteration in the normal permeability of small blood vessels in the area of injury

Objectives

1. Describe the steps involved in extravasation of leukocytes from the blood to the tissues.
2. Know the steps at which selectins and integrins act.
3. Describe the meaning and utility of chemotaxis. Understand the role that chemokines play in inflammation.
4. Describe the steps involved in phagocytosis and the role of IgG and C3b as opsonins and receptors.
5. List the mechanisms of microbial killing.
6. Know various defects in leukocyte function.

Reference book and the relevant page numbers..

- Robbins Basic Pathology 10th edition, pages 62 - 70

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 will

1) Ingest offending agents
2) Kill bacteria and other microbes
3) Get rid of necrotic tissue and foreign substances.

However

- They may induce tissue damage and prolong inflammation, since the leukocyte products that destroy microbes and necrotic tissues can also injure normal host tissues.

1. Describe the steps involved in extravasation of leukocytes from the blood to the tissues.

1. *Recruitment of leukocytes*
2. *Removal of offending agents*

1. Describe the steps involved in extravasation of leukocytes from the blood to the tissues.

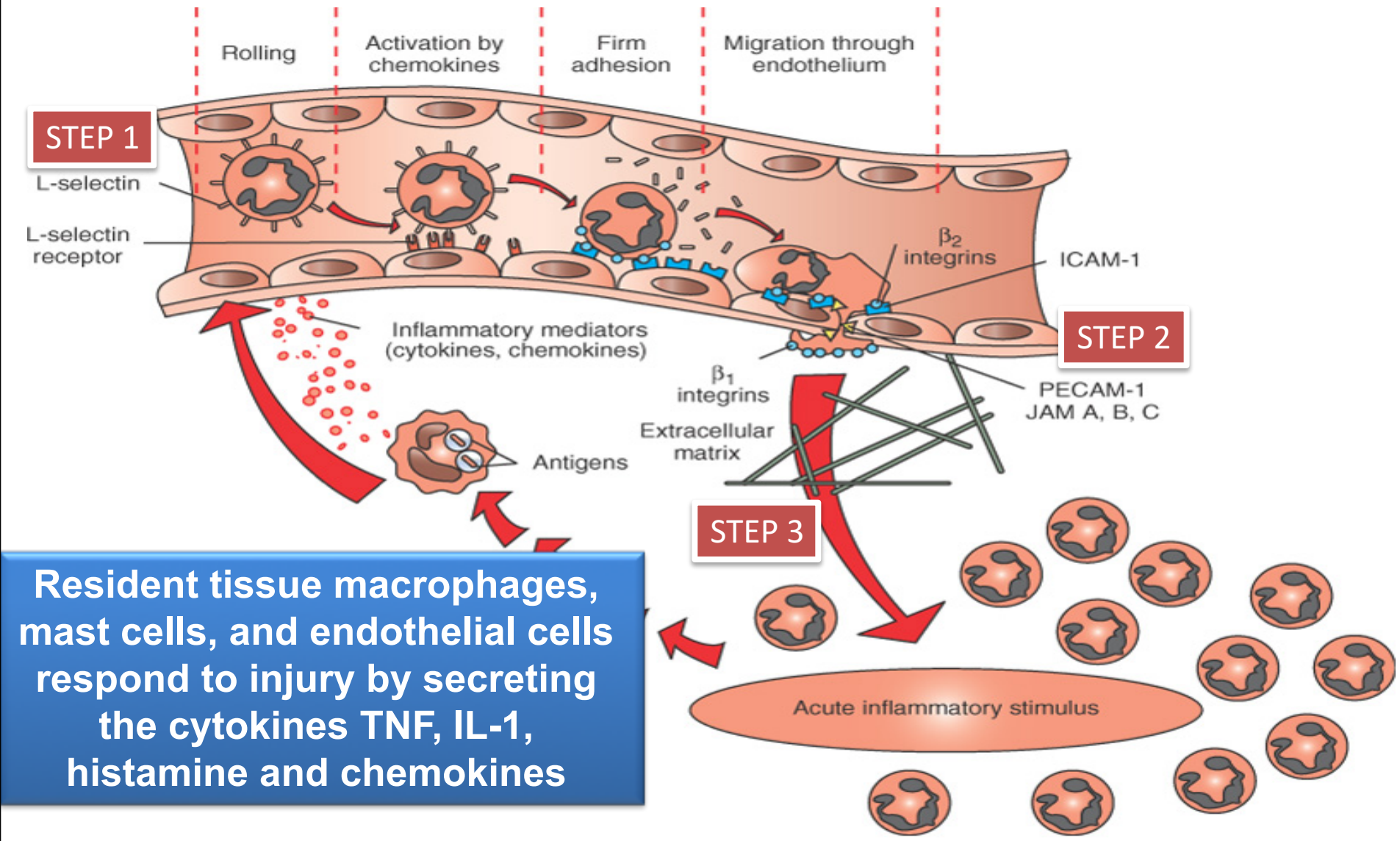
Recruitment of leukocytes

- 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

1. Describe the steps involved in extravasation of leukocytes from the blood to the tissues.



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

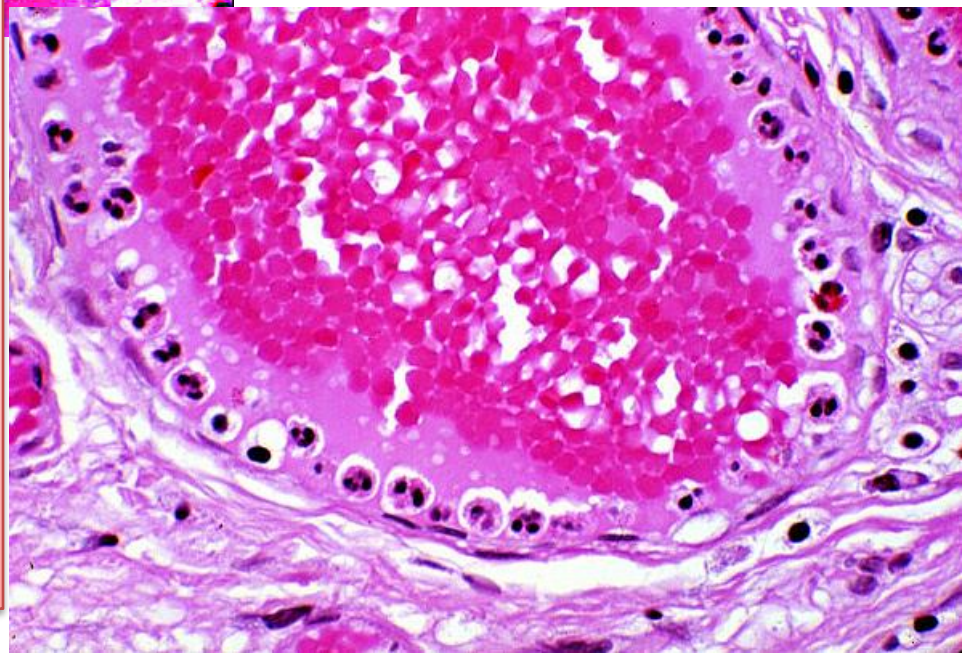
1. Describe the steps involved in extravasation of leukocytes from the blood to the tissues.

Leukocytes Rolling

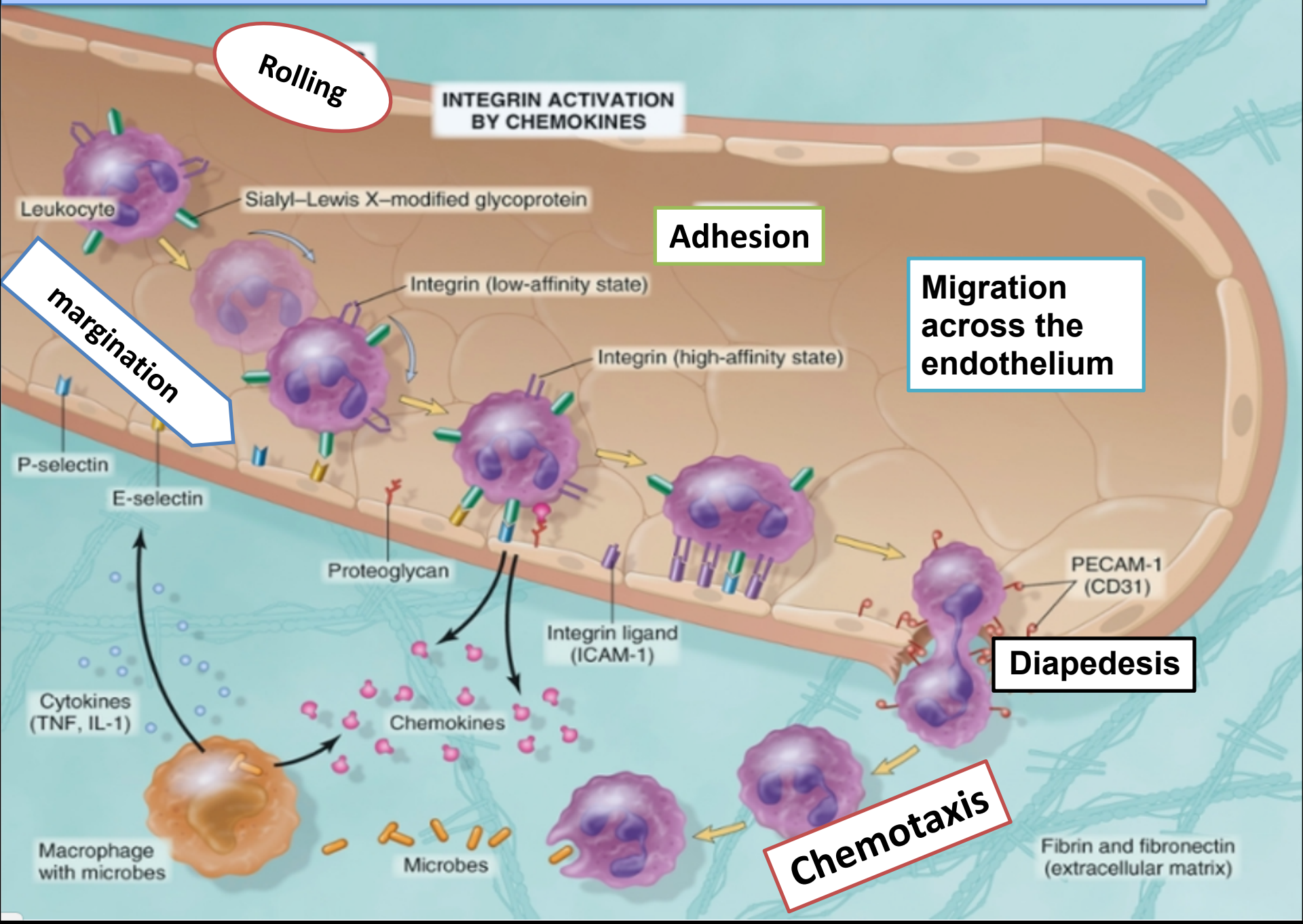
Within a Venule

Margination

- Because blood flow slows early in inflammation (stasis), the endothelium can be lined by neutrophils (pavementation)
- Margination is the first step of leukocytes action during acute inflammation cells



Steps involved in extravasation of leukocytes from the blood to the tissues

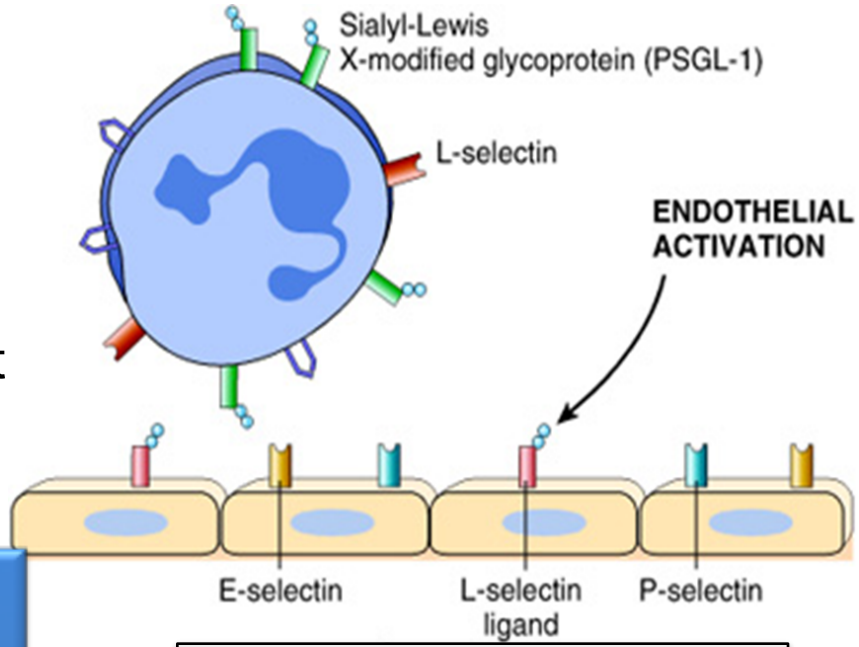


2. Know the steps at which selectins and integrins act.

Adhesion Molecules and Receptors

1. Selectins (carbohydrate-binding adhesion molecules) 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



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

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

Selectin plays a major role for adhesion

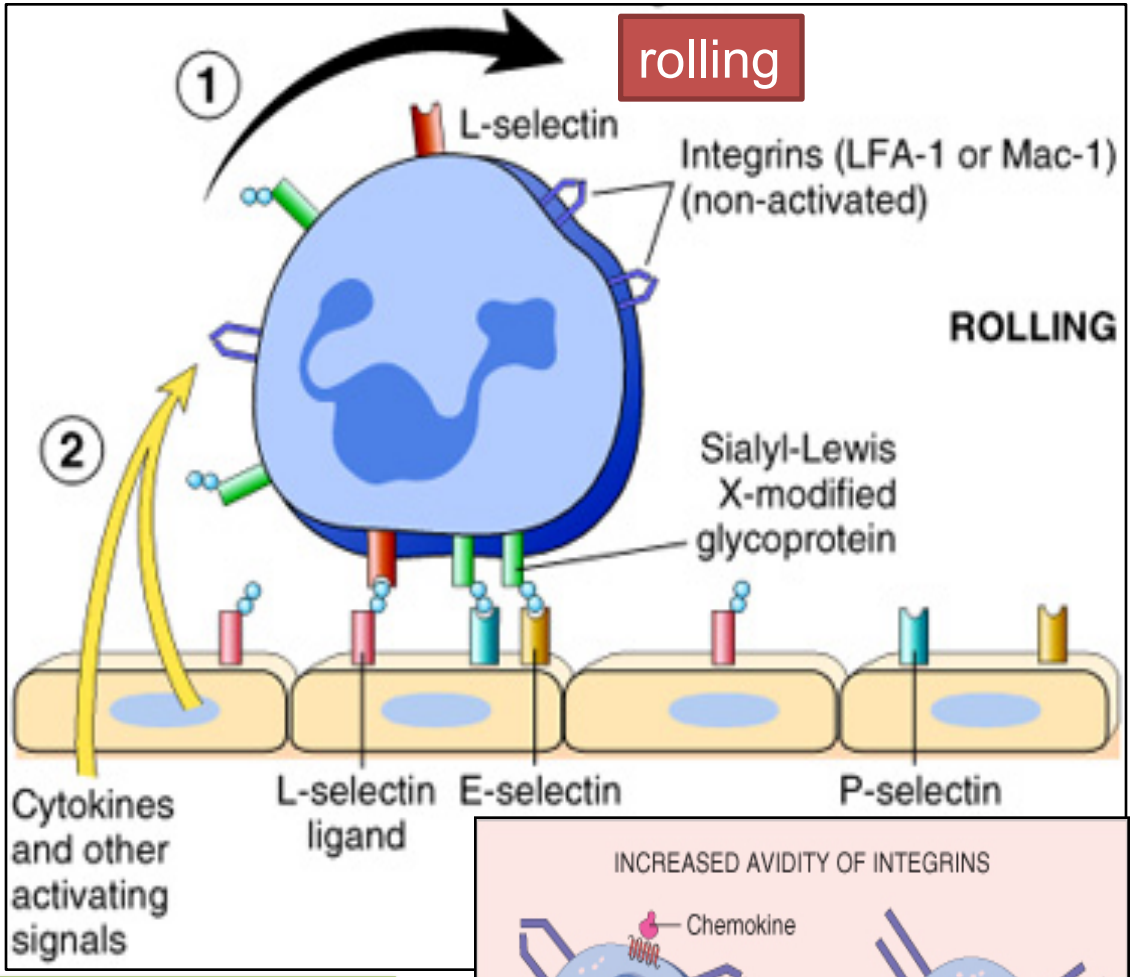
2. Know the steps at which selectins and integrins act.

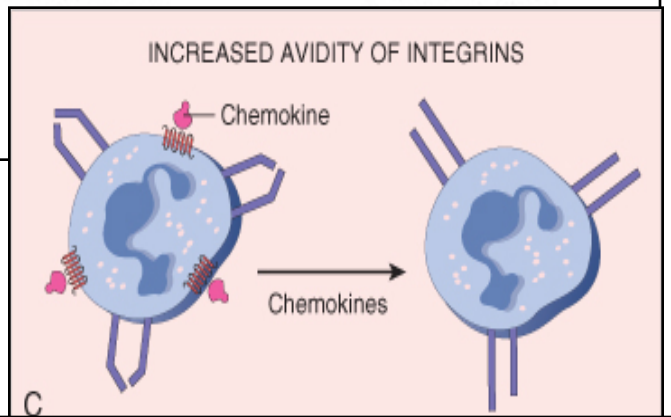
Adhesion Molecules and Receptors

2. Integrins

An adhesion molecule which is seen mainly located on leukocytes and activated during acute inflammation

•made up of α and β glycoproteins chains, expressed on leukocytes and bind to ligands on endothelial cells



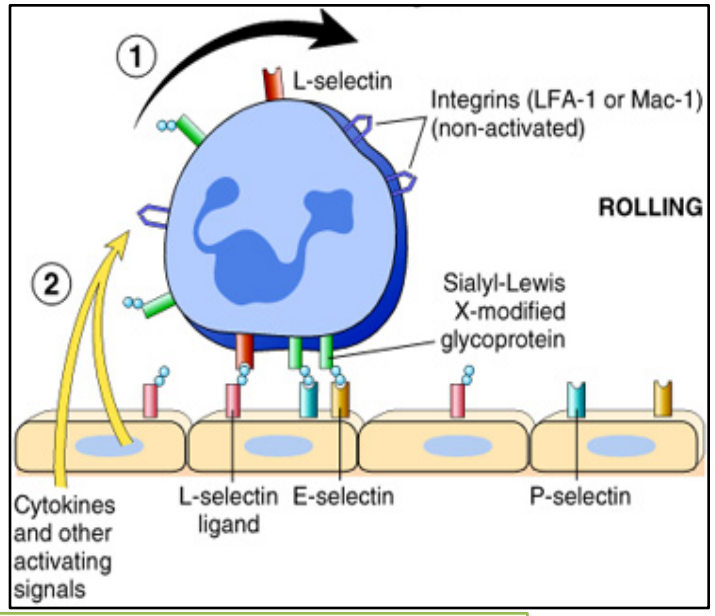


• Integrins are up regulated on leukocytes by **C5a & LTB4** resulting in firm adhesion with vessel wall

2. Know the steps at which selectins and integrins act.

Leukocyte Adhesion Deficiency

- Two types:
  - LAD type 1 is a deficiency of β_2-integrin
  - LAD type 2 is mutations in fucosyl transferase required for synthesis of sialylated oligosaccharide
  - These normally binds selectins.



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

2. Know the steps at which selectins and integrins act.

Adhesion Molecules and Receptors

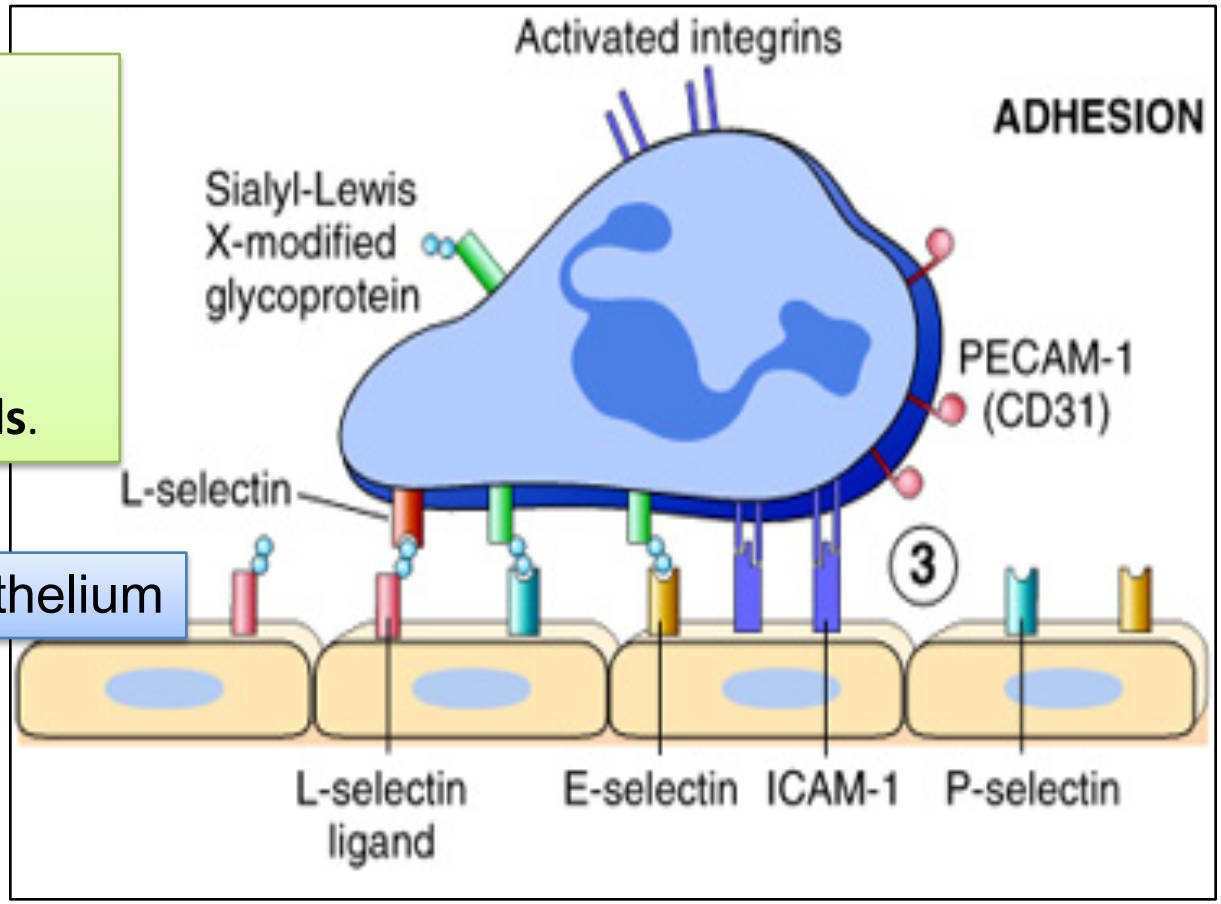
3. The *immunoglobulin family* molecules :

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



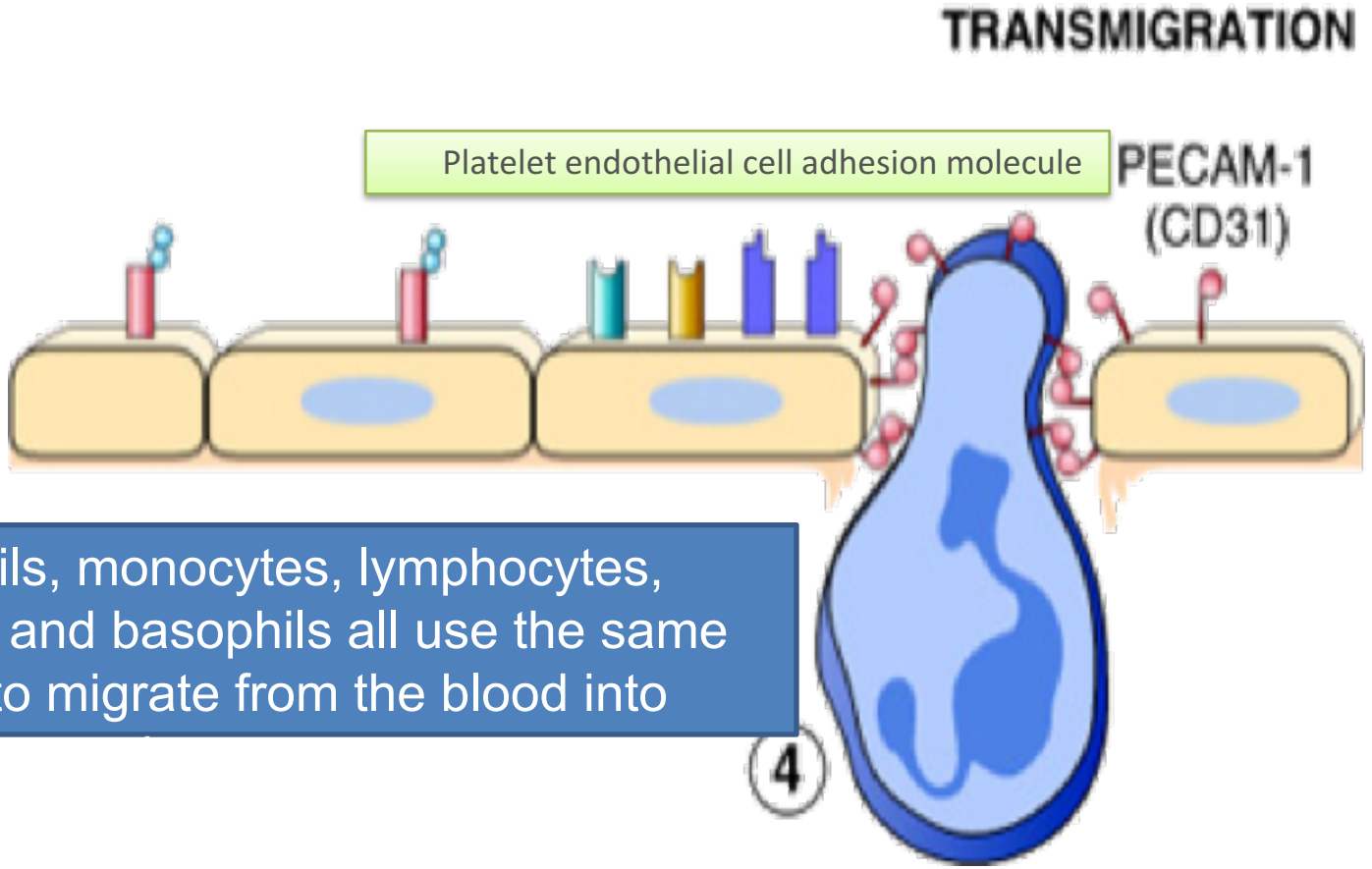
2. Know the steps at which selectins and integrins act.

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

2. Know the steps at which selectins and integrins act.

Leukocyte Adhesion and Transmigration

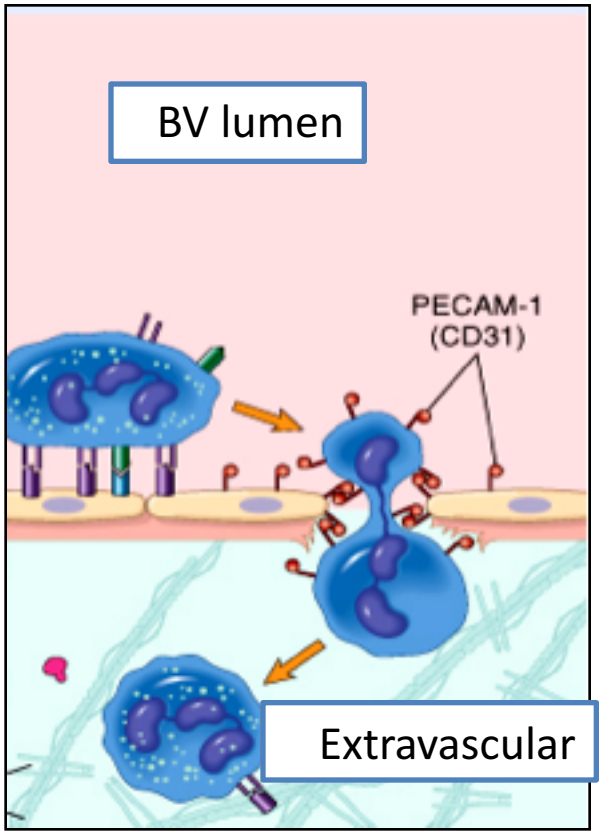
- Migration of the leukocytes through the endothelium is called:

Transmigration

or

Diapedesis

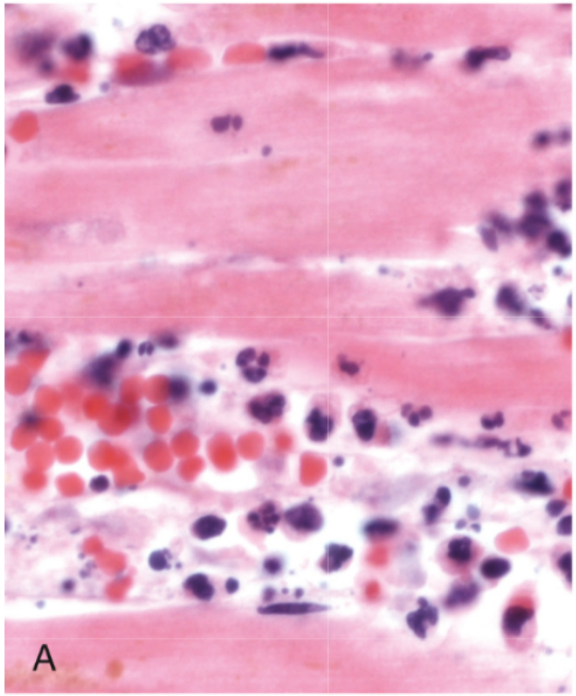
- Diapedesis occurs predominantly in the postcapillary venules

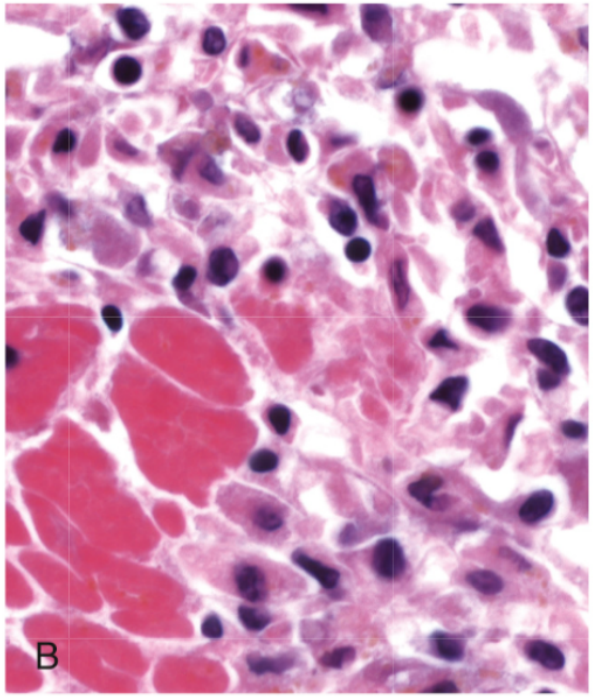


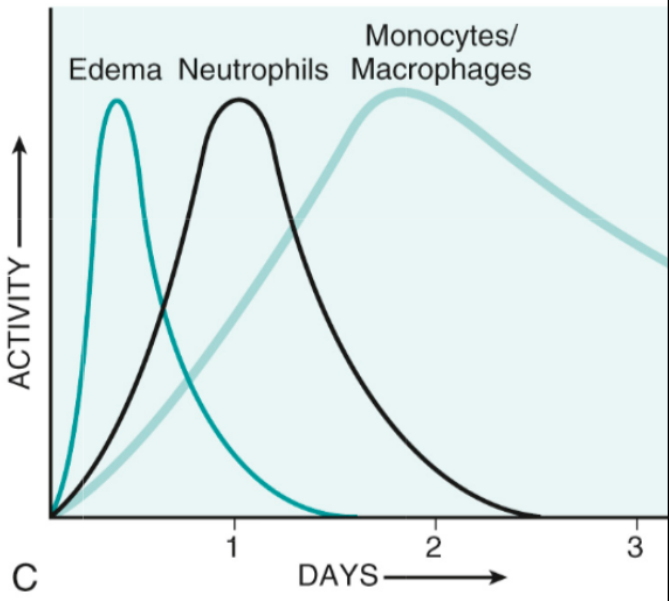
Leukocyte Adhesion and Transmigration

- 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

Extravasation of leukocytes from the blood to the tissues







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.

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

HSC, Hematopoietic stem cells

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

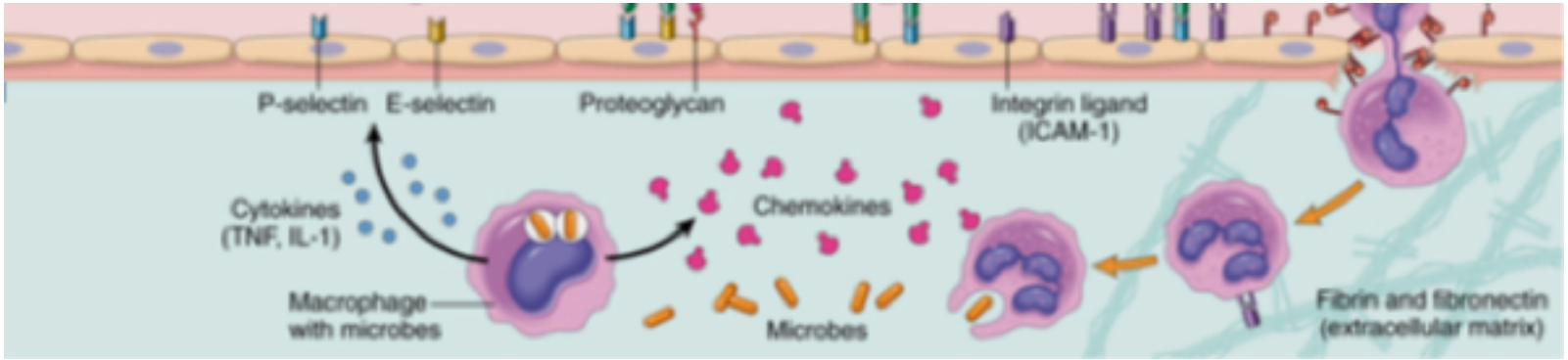
3. Describe the meaning and utility of chemotaxis. Understand the role that chemokines play in inflammation.

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

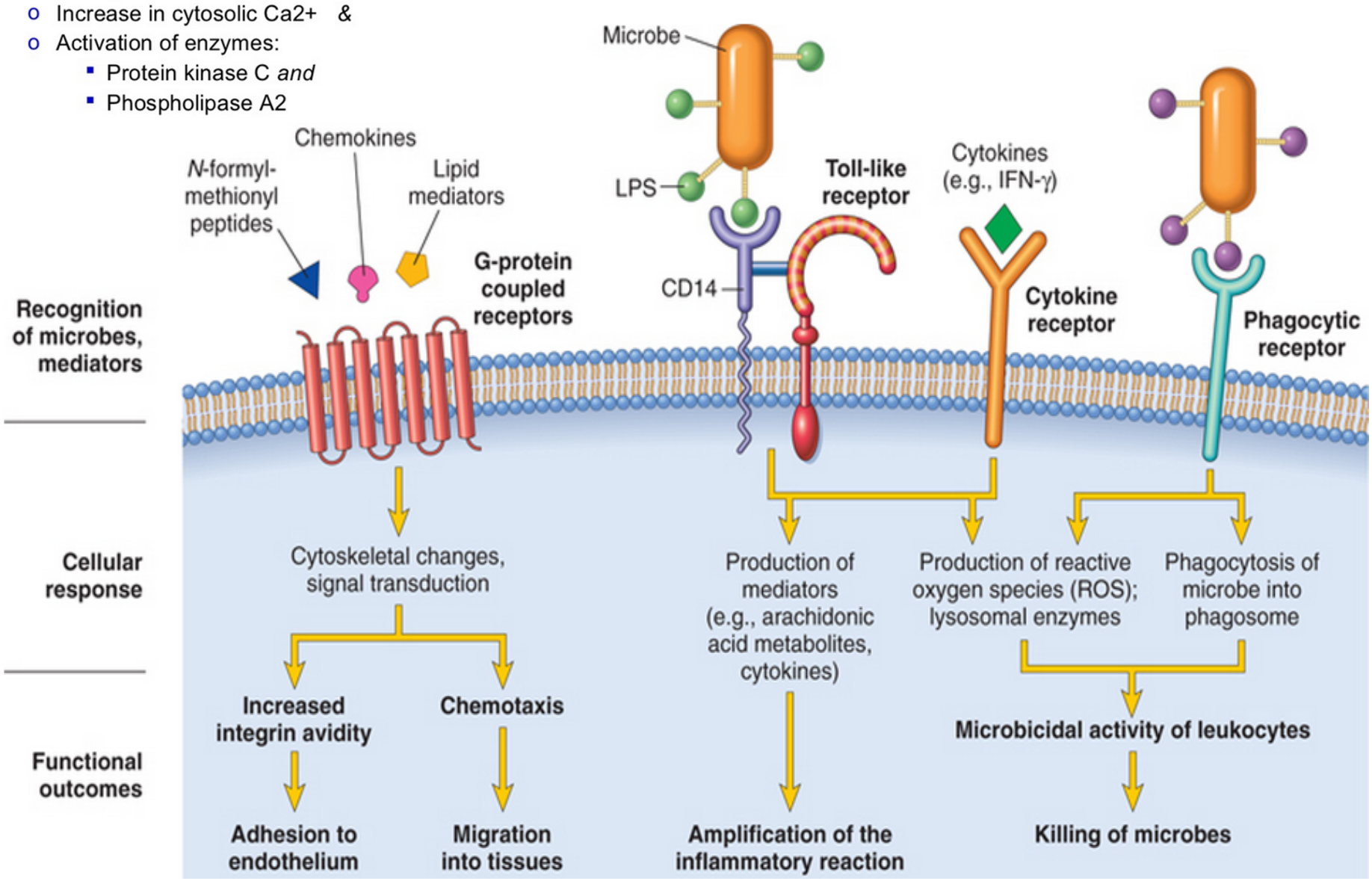
Neutrophils are attracted by bacterial products, IL-8, C5a & LTB4



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

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

- Increase in cytosolic Ca2+ &
- Activation of enzymes:
  - Protein kinase C *and*
  - Phospholipase A2



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

Leukocyte Activation

Phagocytosis

Intracellular destruction

Liberation of substances that destroy extracellular microbes and dead tissues

Production of mediators

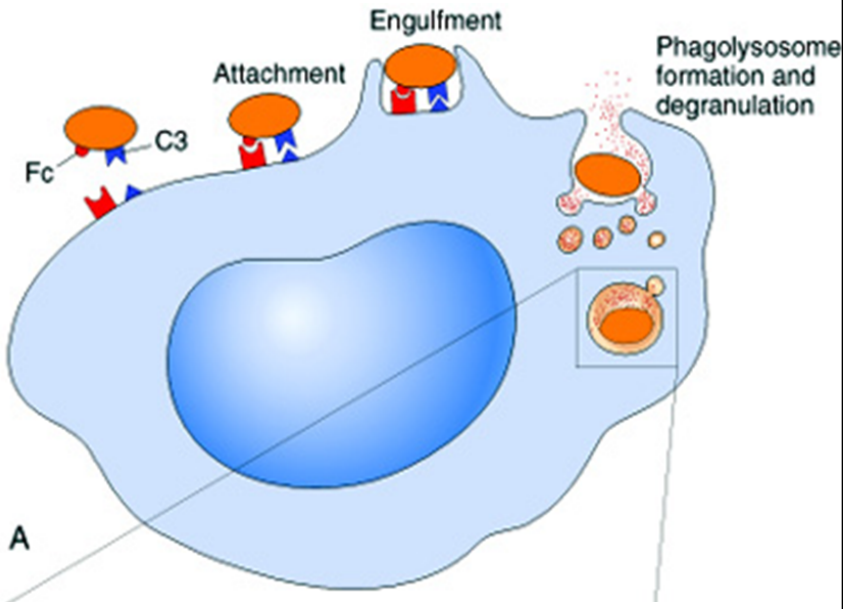
4. Describe the steps involved in phagocytosis and the role of IgG and C3b as opsonins and receptors.

Phagocytosis

- Phagocytosis involves three distinct but interrelated steps
  - *(1) Recognition* and *Attachment* of the particle to be ingested by the leukocyte

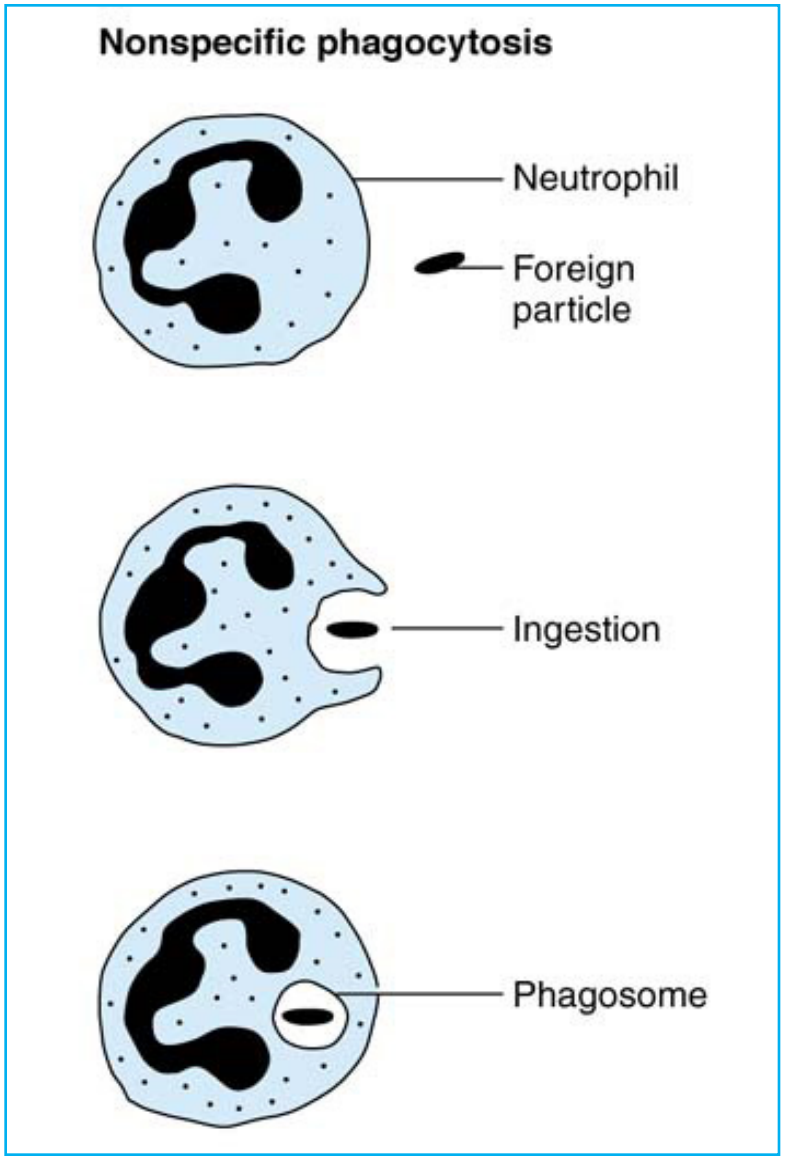
  (2) its *Engulfment*, with subsequent formation of a phagocytic vacuole

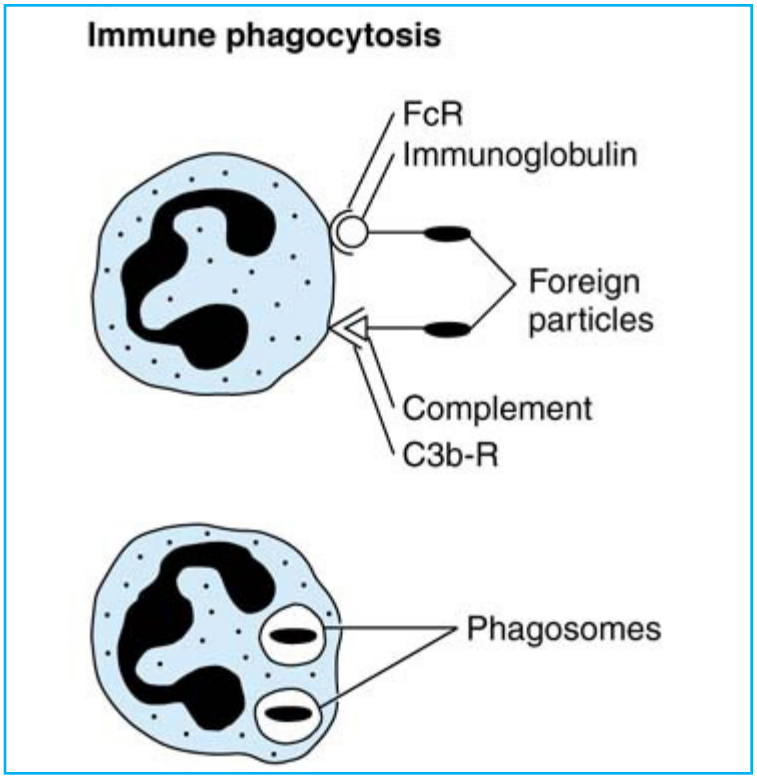
  (3) *killing* or *Degradation* of the ingested material.



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

Phagocytosis by neutrophils





Immune phagocytosis is much more efficient than nonspecific phagocytosis

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

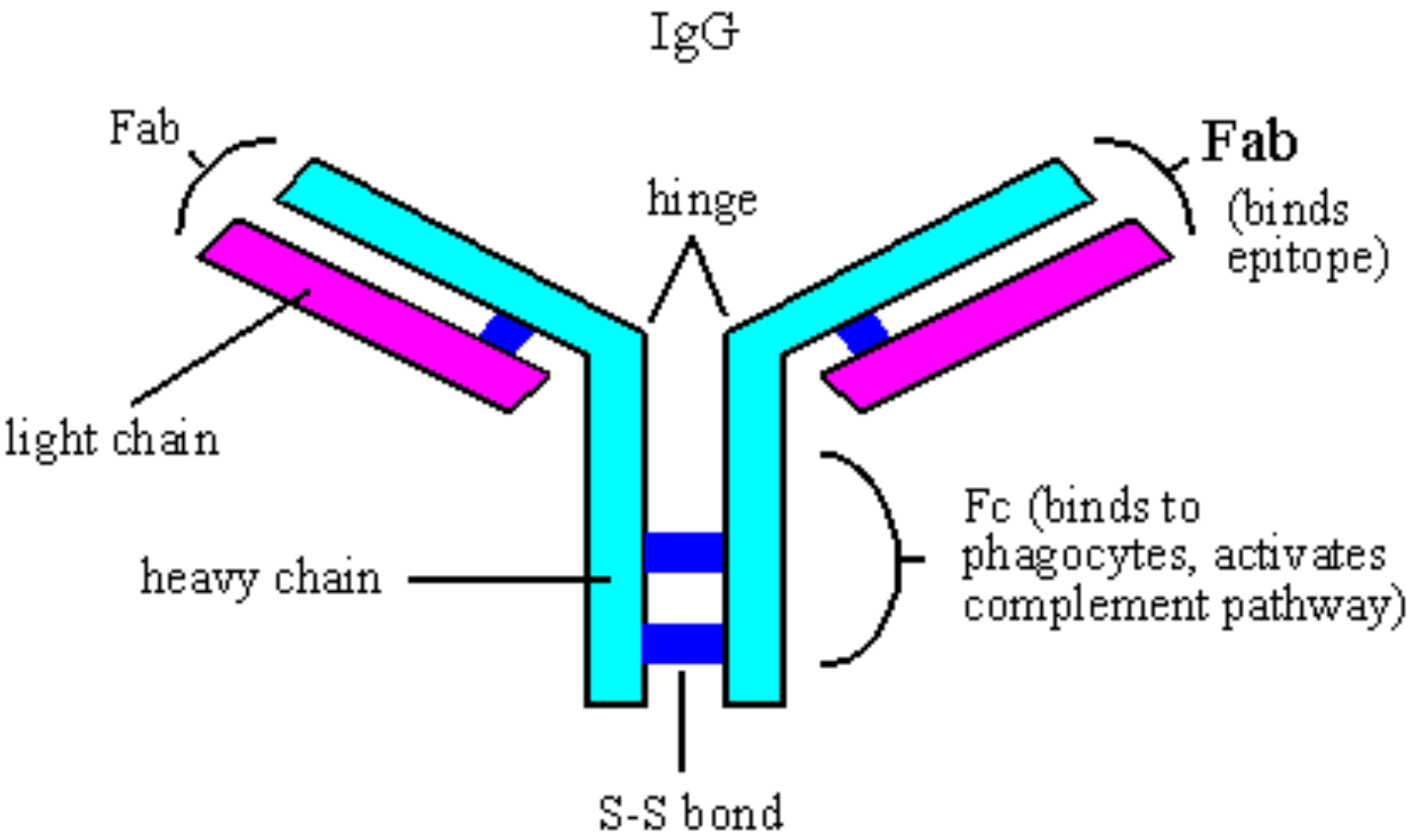
Leukocyte activation

(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).

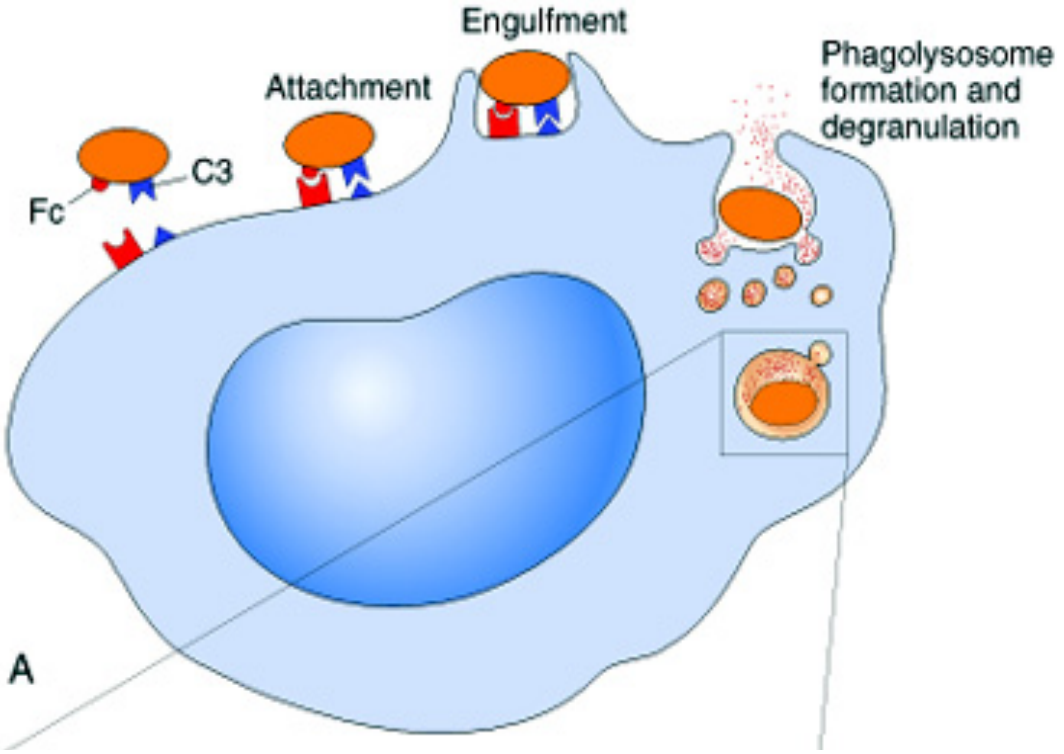
4. Describe the steps involved in phagocytosis and the role of IgG and C3b as opsonins and receptors.



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

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**



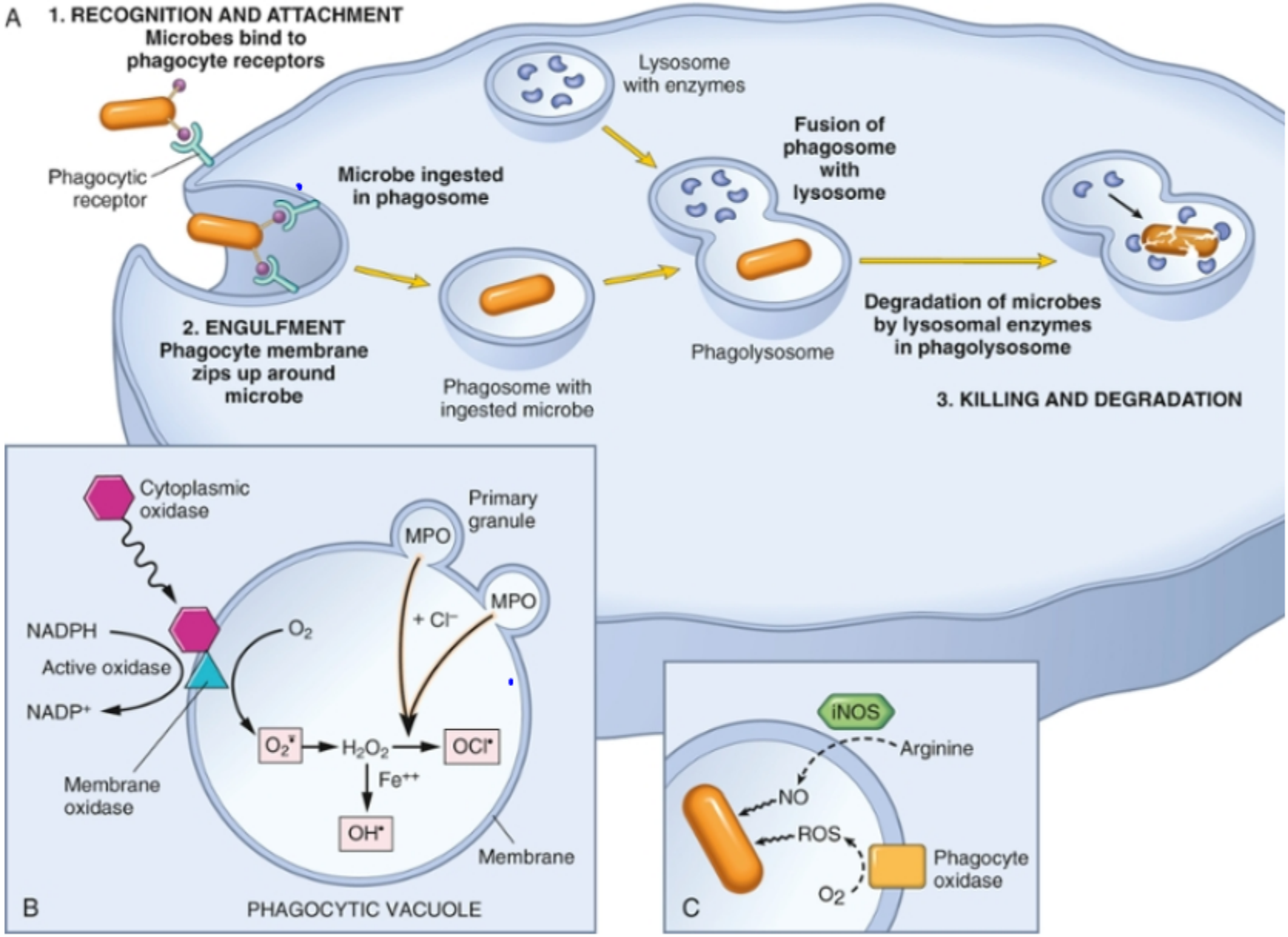
The phagocytic vacuole then fuses with a lysosomal granule, resulting in **phagolysosome**

Defects in Leukocyte Function

Chédiak-Higashi syndrome

- Protein involved in organelle membrane fusion (no phagolysosomes)
  - Protein trafficking defect (microtubule defect)
  - Autosomal recessive
- 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

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



5 . List the mechanisms of microbial killing.

Phagocytosis

Killing and Degradation

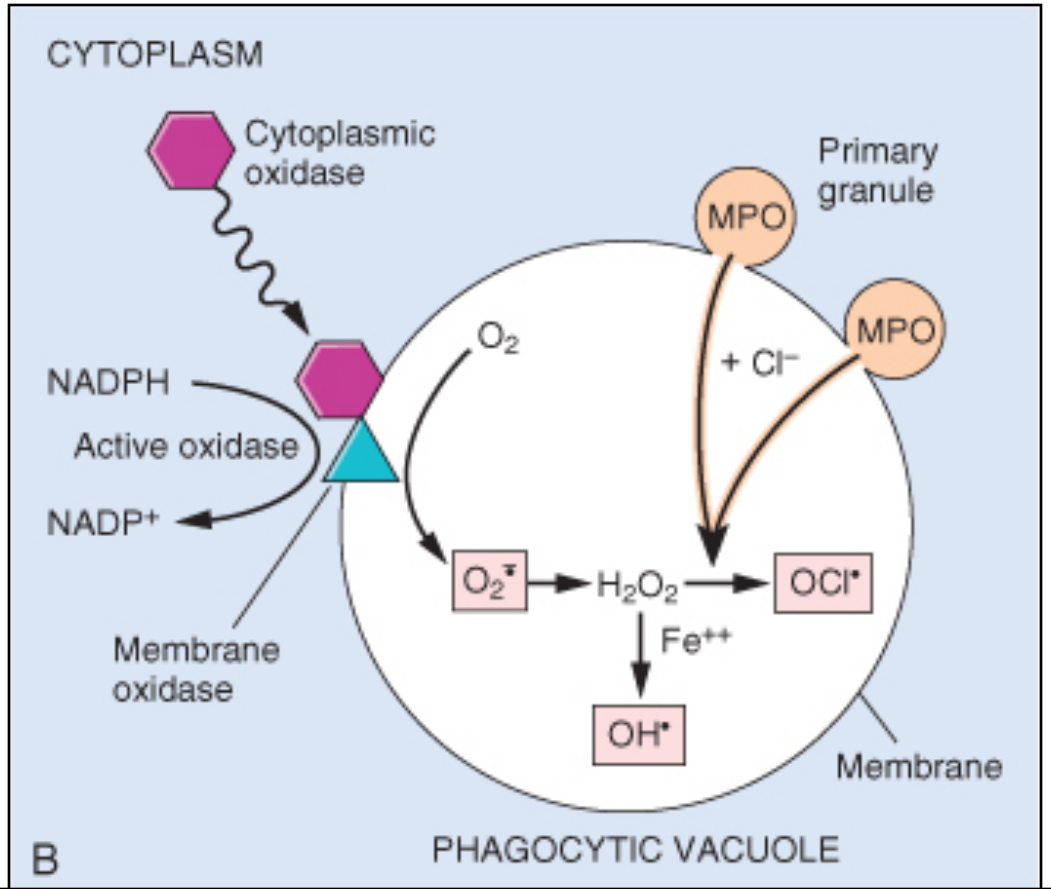
- 2 mechanisms for Microbial killing:
    1. *Oxygen-dependent mechanisms*
    2. *Oxygen-independent mechanisms*

5 . List the mechanisms of microbial killing.

1. 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



Chronic granulomatous disease

Decreased oxidative burst.

Oxygen-Dependent Mechanisms

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

- Infection and granuloma formation with catalase positive organisms e.g. *S aureus, Norcardia* and *Aspergillus*

2. Oxygen-independent mechanisms

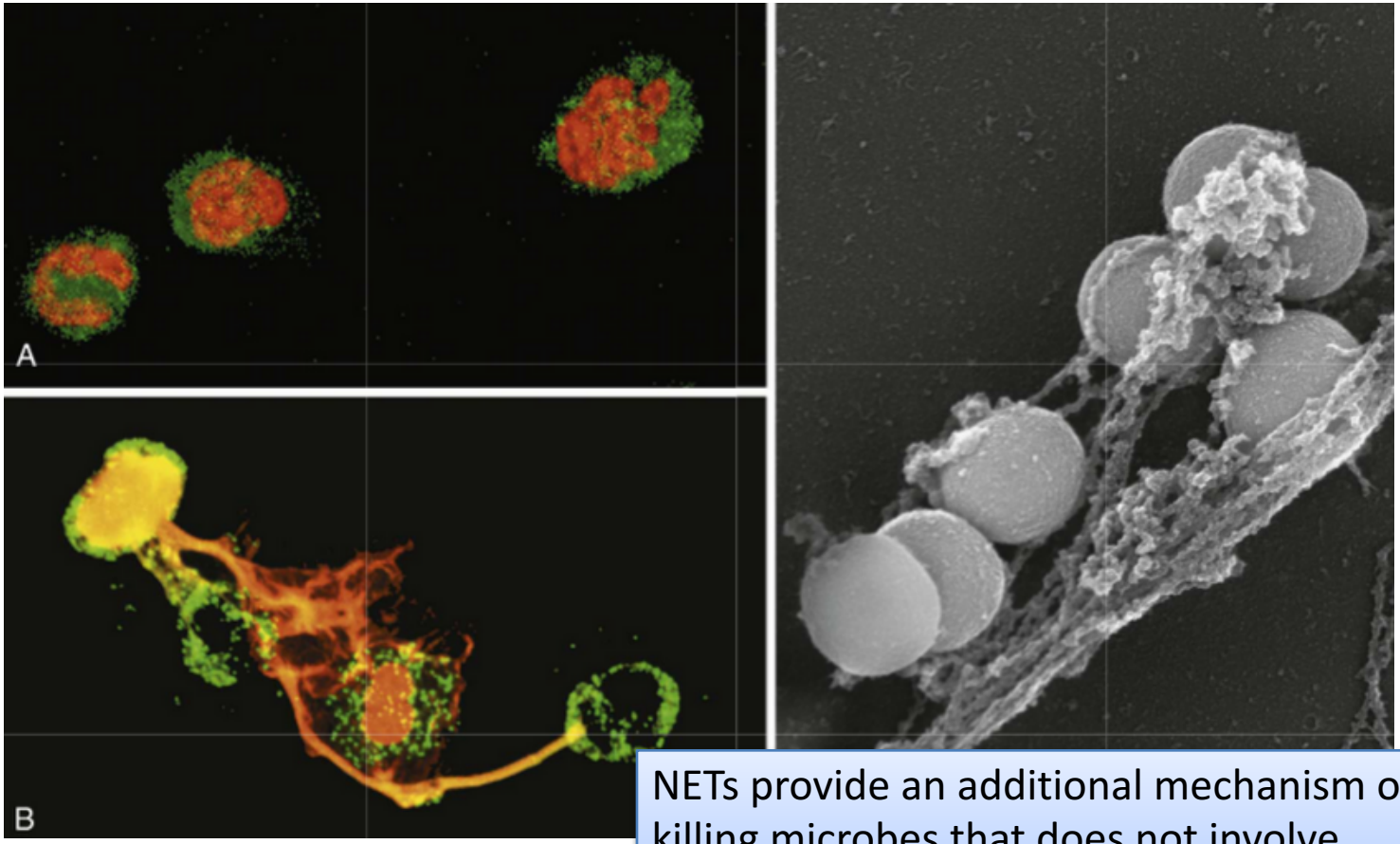
- through the action of substances in leukocyte granules. These include:
  - ***Bactericidal permeability increasing protein* (BPI)**
  - ***Lysozyme***
  - ***Lactoferrin***
  - ***Major basic protein***
  - ***Defensins***

• Can potentiate further inflammation by damaging tissues
•These harmful proteases are controlled by a system of *anti-proteases* in the serum

- Neutrophil granules contain other *enzymes*, such as elastase, that also contribute to microbial killing

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.



NETs provide an additional mechanism of killing microbes that does not involve phagocytosis

Defects in Leukocyte Function

6. Know various defects in leukocyte function.

Defects in Leukocyte Function

Genetic

1. Leukocyte adhesion deficiency 1and 2
2. Chédiak-Higashi syndrome
3. Chronic granulomatous disease:

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

   B. Autosomal recessive:

   a. NADPH oxidase (cytoplasmic components)

   b. Myeloperoxidase deficiency

6. Know various defects in leukocyte function.

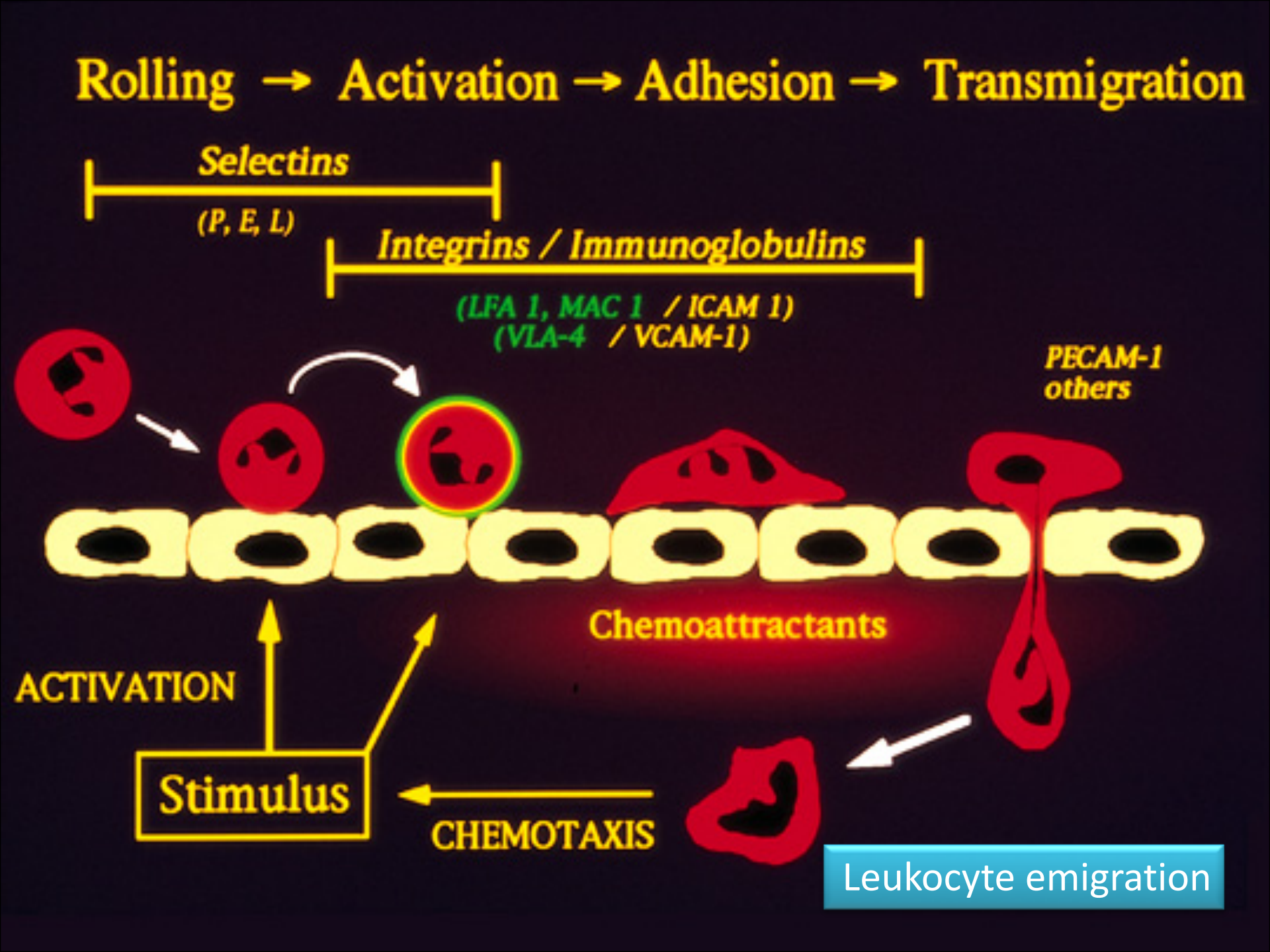
Defects in Leukocyte Function

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