MED444
KING SAUD UNIVERSITY

PATHOLOGY TEAMWORK

MED444

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Vascular and cellular events in inflammation

OBJECTIVES

- Describe the steps involved in extravasation of leukocytes from the blood tissues.
- Know the steps at which selectins and integrins act.
- Describe the meaning and utility of chemotaxis.
- Understand the steps involved in phagocytosis and the role of igG and C3b as opsonins and receptors.
- List the mechanism of microbial killing.
- Know various defects in leukocyte functions.

COLOR INDEX:

MAIN TEXT (BLACK)

FEMALE SLIDES (PINK)

MALE SLIDES (BLUE)

IMPORTANT (RED)

DR'S NOTE (GREEN)

EXTRA INFO (GREY)

Acute Inflammation

CELLULAR EVENTS:

★ A critical function of inflammation is to deliver leukocytes to the site of injury LEUKOCYTE EXTRAVASATION .

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

Functions of Leukocytes

- Ingest offending agents,
- Kill bacteria and other microbes.
- 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 tissue can also injure normal host tissues.

the steps involved in extravasation of leukocytes from the blood to the tissue

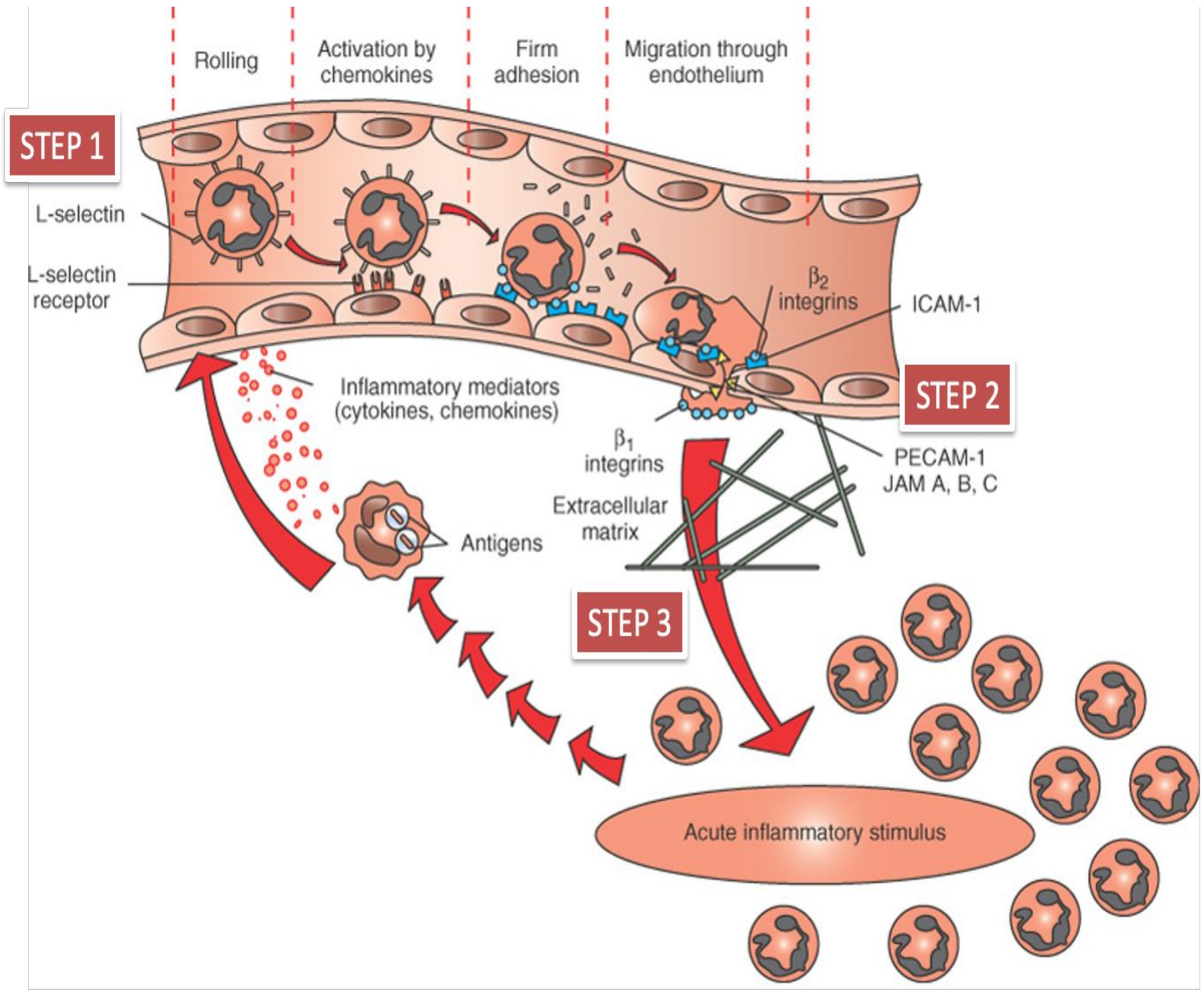
- Recruitment of Leukocytes
- Removal of offending agents

Recruitment of Leukocytes

A multistep process involving attachment of circulating leukocytes to endothelial cells and their migration through the endothelium (**Extravasation**) .

In 3 Steps:

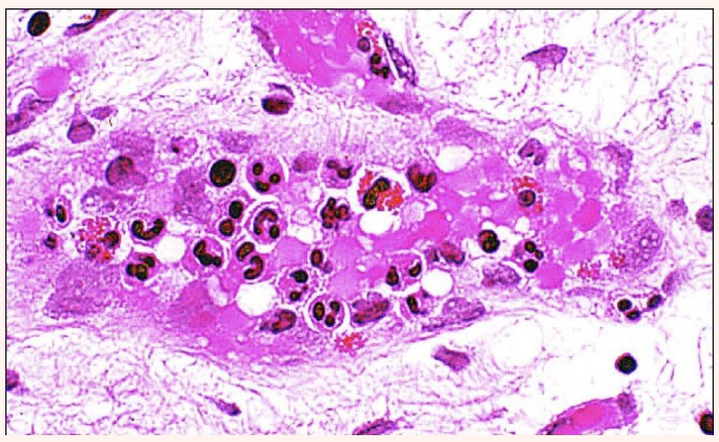
- 1- In the Lumen:
  - 1.1 : Margination
  - 1.2: Rolling
  - 1.3: Adhesion to endothelium
- ★ vascular endothelium normally doesn't bind circulating cells
- 2-Transmission across the endothelium (also called diapedesis)
- 3-Migration in interstitial tissues towards a chemotactic stimulus



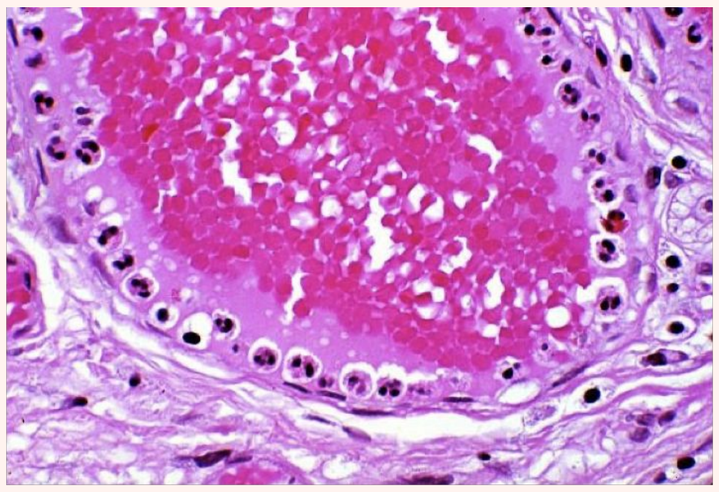
Resident tissue macrophages, mast cells, and endothelial cells respond to injury by secreting the cytokines **TNF, IL-1, histamine** and **chemokines**. Which stimulate selectin

Margination

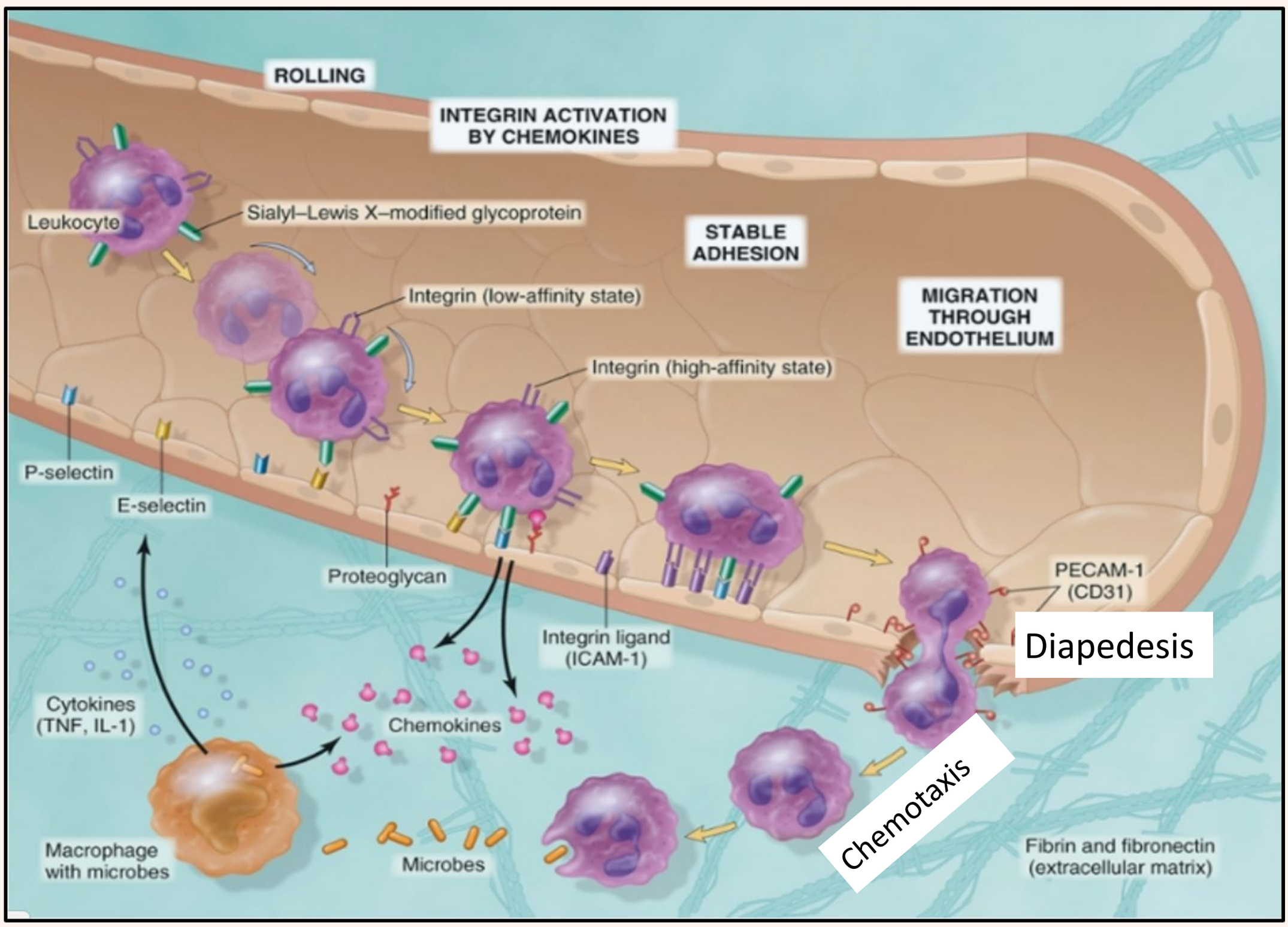
- Because blood flow slows early in inflammation (statis) , the endothelium can be lined by neutrophils (pavementation).
- Margination is the first step of leukocyte action during acute inflammation cells.



Leukocytes Rolling within a Venule



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



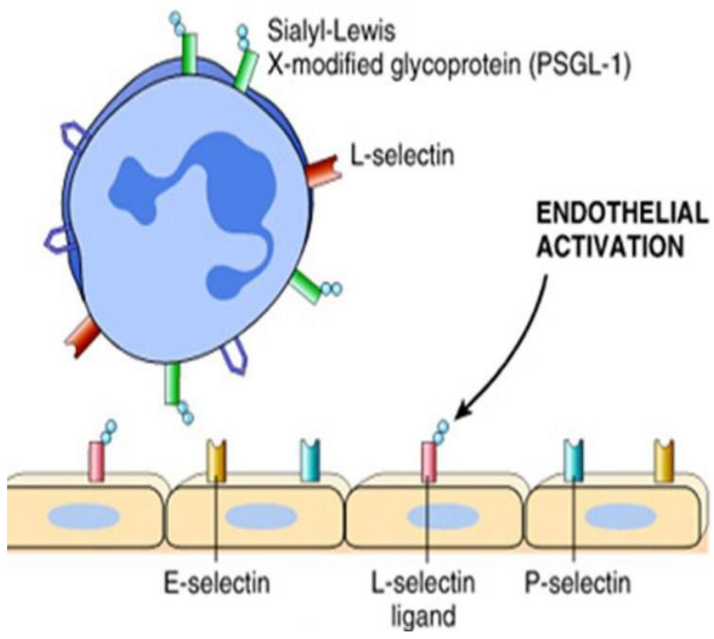
Adhesion Molecules and Receptors

1-Selectins (carbohydrate-binding adhesion molecules) consist of:

E-selectin: confined to endothelium induced by TNF & IL-1.(First letter of endothelium)

P-selectin: present in endothelium and platelets from Weibel-Palade.(First letter of platelets)

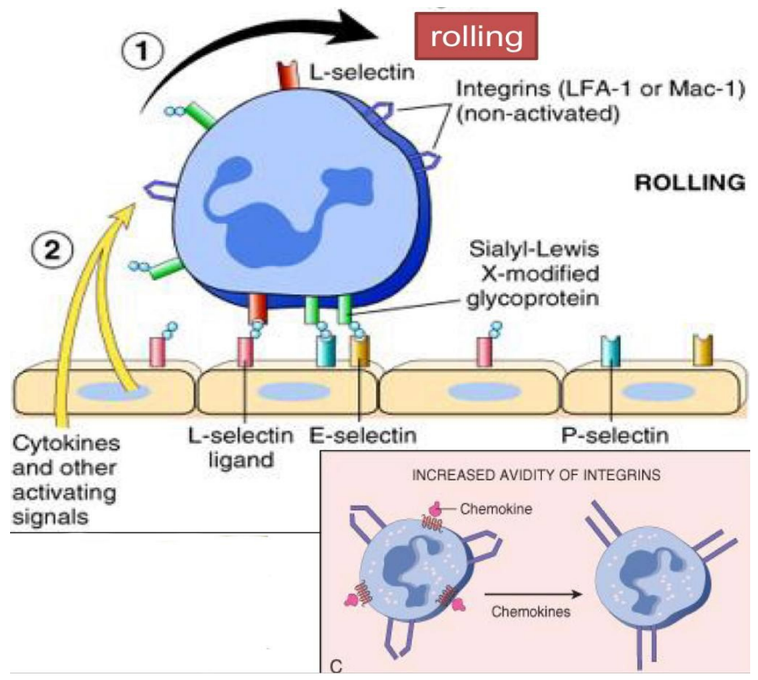
L-selectin: expressed on most leukocyte and endothelium. (First letter of leukocyte)



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

Selectin plays a major role for adhesion

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



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 (chemokine) resulting in firm adhesion with vessel wall

Leukocyte Adhesion Deficiency

Two Types:

LAD type 1 is a deficiency of β2-integrin

LAD type 2 is mutations in fucosyl transferase required for synthesis of sialylated oligosaccharide. (Sialyl Lewis X) these normally

★ these two types 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

Adhesion Molecules and Receptors

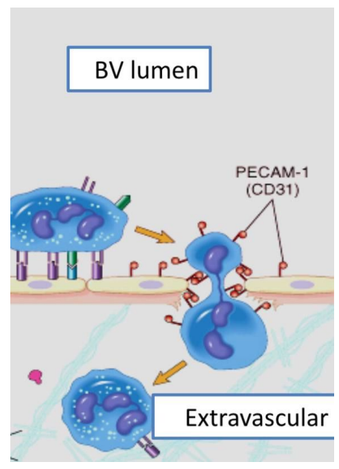
3–The immunoglobulin family molecules:

MED443 Note: PECAM-1 is important for Diapedsis

- ICAM-1 (intercellular adhesion molecule 1)
- VCAM-1 (vascular cell adhesion molecule 1)
- ★ PECAM-1(platelet endothelial cell adhesion molecule)

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

4– Mucin-like glycoproteins:



- PECAM-1 (CD31)

Where?

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 tissue.

Leukocyte Adhesion and Transmigration

- Migration of the leukocytes through the endothelium is called: Transmigration or Diapedesis.

- **Where?**
  It occurs predominantly in the postcapillary venules.

- The type of emigrating leukocyte varies with the age of the inflammatory response and type of the stimulus.

- 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?
  1- neutrophils are more numerous in the blood, they respond more rapidly to chemokines
  2- They are short-lived; they undergo apoptosis and disappear after 24 to 48 hours, whereas monocytes survive longer.

MED443 Notes: hypersensitivity or parasitic diseases related to eosinophils

Properties of Neutrophils and Macrophages:

Note:
HSC= Hematopoietic stem cells

	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

Leukocyte Adhesion and Transmigration

In some expectations:
-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.

Summarize for Adhesion Molecule and Receptors (from MED441)

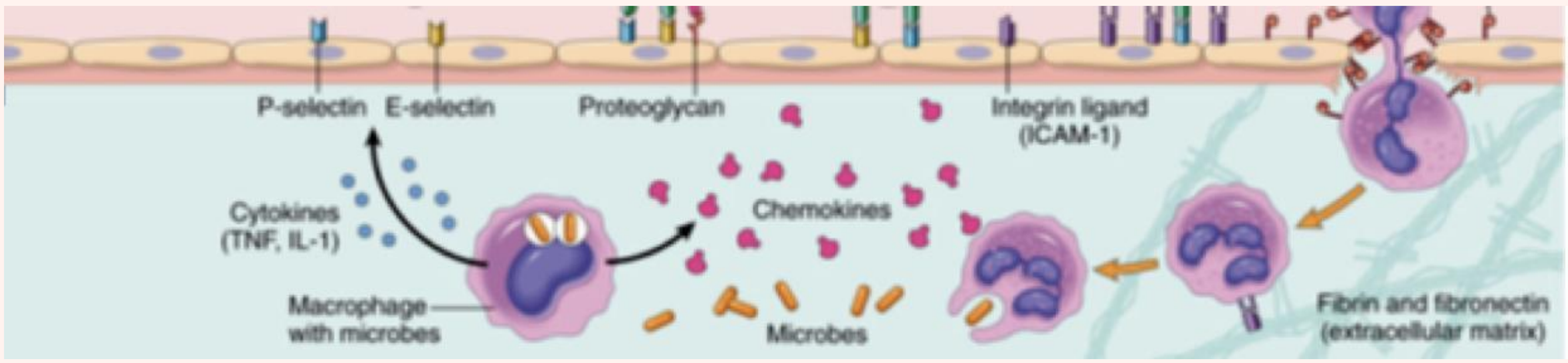
	The Site	Activated by	Bind to
E-selectin	Endothelium	TNF & L-1	Sialyl-Lewis X Glycoprotein PSGL-1 (In leukocytes)
P-selectin	Endothelium & Platelets	Weibel-Palade bodies	Sialyl-Lewis X Glycoprotein PSGL-1
L-selectin	Endothelium & Leukocytes	—------	Integrins
Intergins	Leukocytes	C5a & LTB4	L-selectin (ligands)
ICAM-1 & VCAM-1	Endothelium	TNF & L-1	Integrins
PECAM-1	Extracellular matrix & Cell surface	Histamine	Does not bind “contraction”

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 (of substances produced at site of injury).

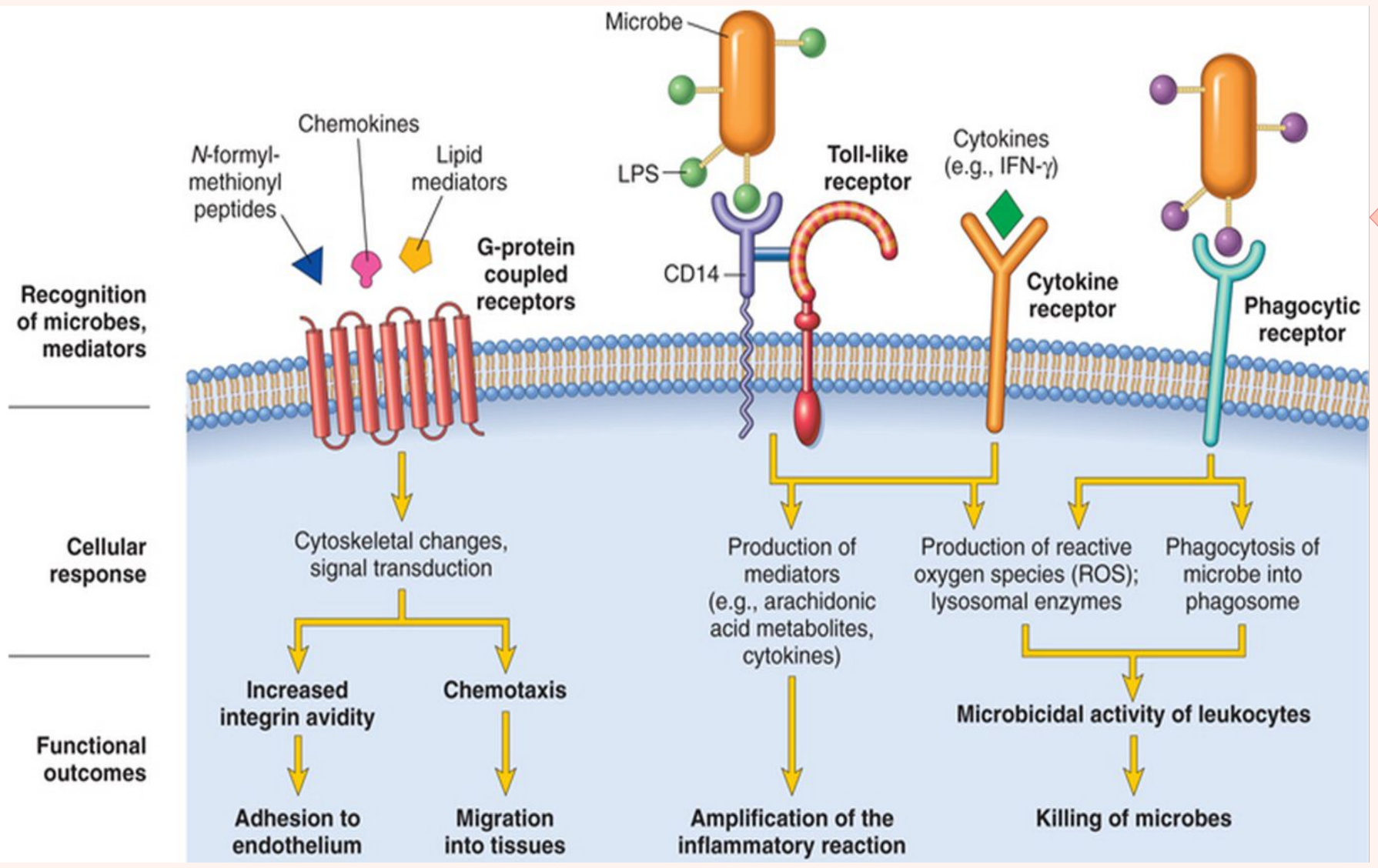
Chemoattractants

- 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
- Phospholipase A2

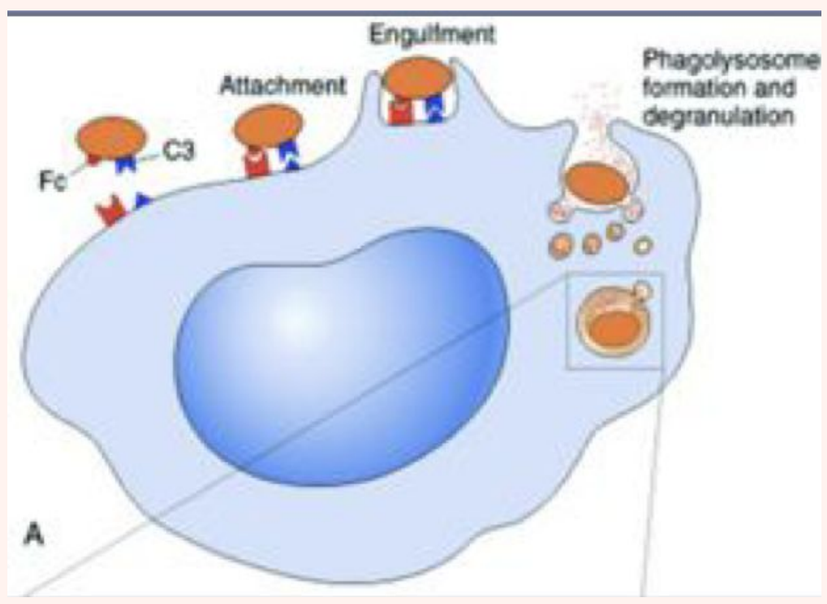
Leukocyte Activation

1. **Phagocytosis**
2. **Intracellular destruction**
3. **Liberation of substances that destroy extracellular microbes and dead tissues**
4. **Production of mediators**

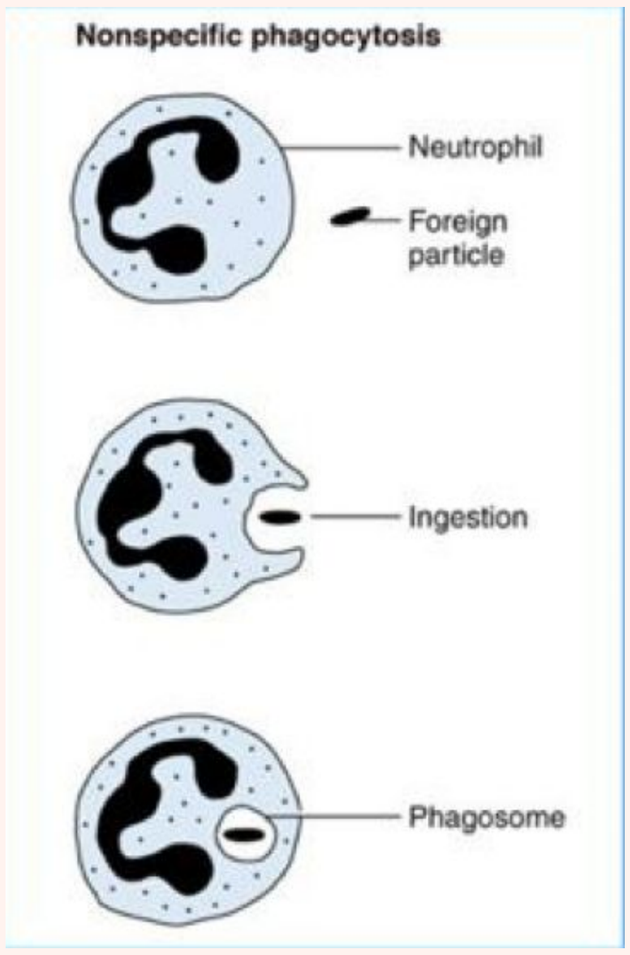
Phagocytosis

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



Phagocytosis by neutrophils



Immune phagocytosis is much more efficient than non specific phagocytosis
(Stronger + rapid at detection & engulfment)

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** (C3b) – And others: **lectins** (mannose-binding lectin (MBL), **collectins**, **fibronectin**, fibrinogen, and **C-reactive protein**

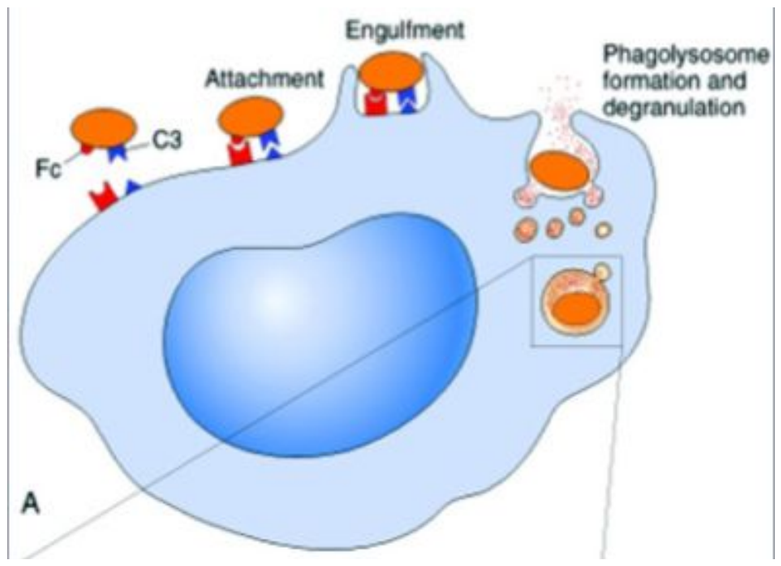
These can coat microbes and are recognized by
receptors on phagocytes (**Fc** and **C3b** receptors).

Leukocyte Activation

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**.

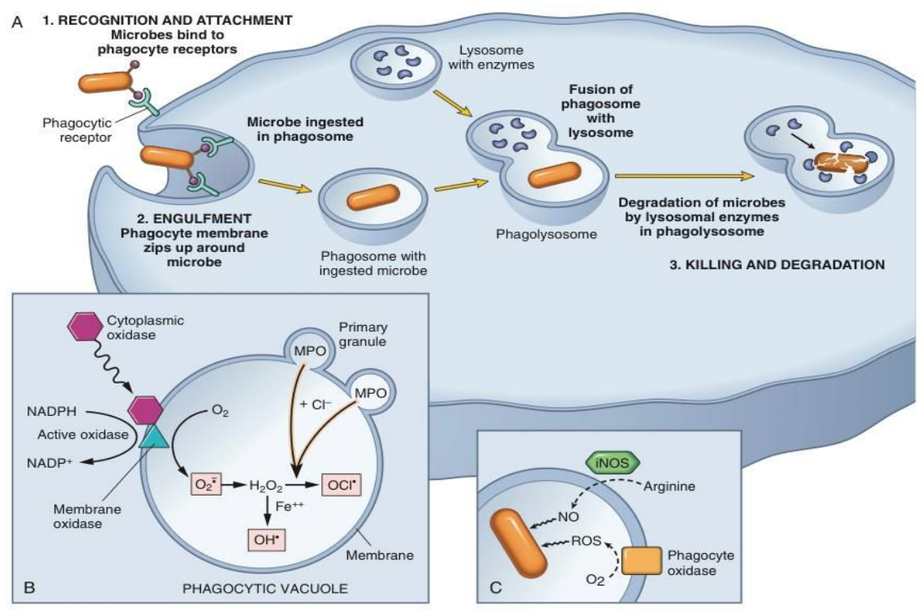
The phagocytic vacuole then fuses with a lysosomal granule, resulting in **phagolysosome**



Defects in leukocyte function:

(Chédiak-Higashi syndrome)

- Protein involved in organelle membrane fusion **(no phagolysosome).**
- **Protein trafficking defect** (microtubule defect).
- **Autosomal recessive.**



Clinical features

1- **increased risk of pyogenic infection** (pus producing bacteria)

2- **Neutropenia** (defect in generation from BM)

3- **Giant granule formation**
-Autosomal recessive (granules formed can't move in cytoplasm)

4- **Defective primary hemostasis** (platelet granules are not secreted)

5- **Albinism**

6- **peripheral neuropathy**

Phagocytosis

Killing and Degradation

There are 2 mechanisms for Microbial killing:

3. Microbial Killing

Oxygen-independent mechanisms

through the action of substances in leukocyte granules.
These include:

Defensins

Major basic protein
These harmful proteases are controlled by a system of anti proteases in the serum

Bactericidal permeability These increasing protein (BPI)
(Superoxide dismutase)

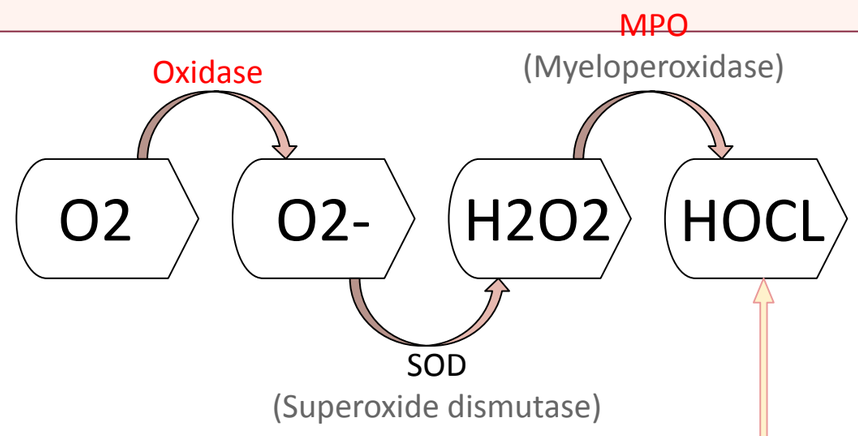
Lactoferrin
Can potentiate further inflammation by damaging tissues.

Lysozyme

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

Oxygen-dependent mechanisms

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



Chronic granulomatous disease

Note: it's a type of oxygen dependent mechanism and a type of genetic defects in leukocytes function

- Autosomal recessive
  - **Myeloperoxidase deficiency:** (absent MPO-H2O2 system) pt. have increased risk of candida infection.
  - **NADPH oxidase** (cytoplasmic components)
- X-linked: **NADPH oxidase** (membrane component)

Infection and granuloma formation with catalase positive organisms e.g. S aureus, Nocardia and Aspergillus.

Defects in Leukocyte Function

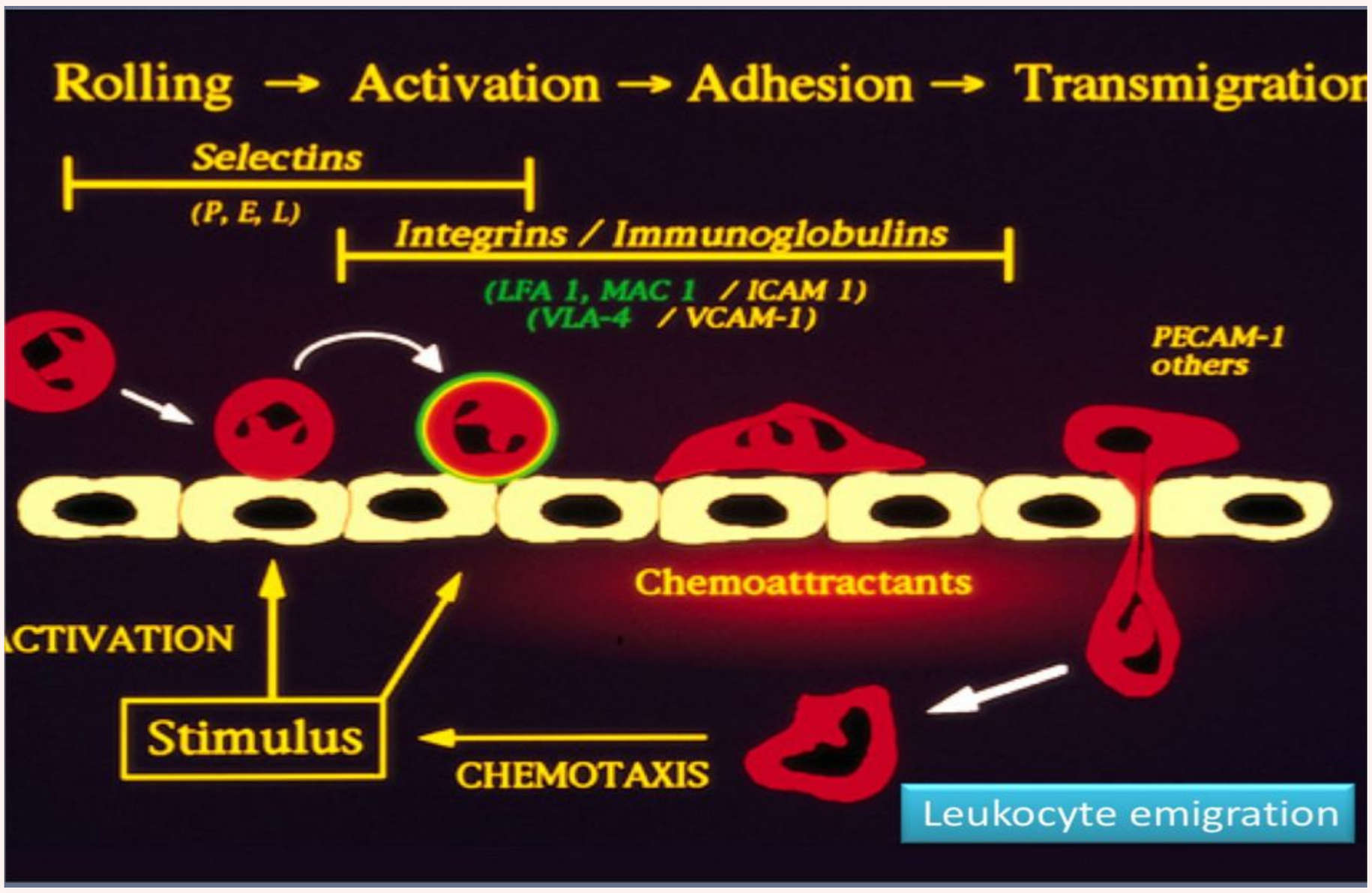
Genetic

(all these cause repeated infection in affected individual)

- Leukocyte adhesion deficiency 1 and 2
- Chédiak-Higashi syndrome
- Chronic granulomatous disease :
  - X-linked: NADPH oxidase (membrane component)
  - Autosomal recessive
    - Myeloperoxidase deficiency
    - NADPH oxidase malnutrition (cytoplasmic components)

Acquired

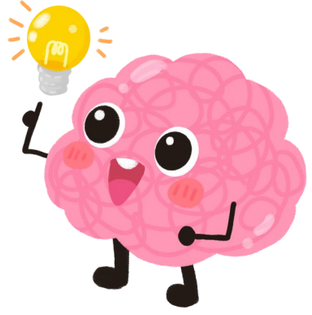
- Chemotaxis: Thermal injury, diabetes, malignancy, sepsis, immunodeficiencies
- Phagocytosis and microbicidal activity: Leukemia, anemia, sepsis, diabetes, neonates, malnutrition
- Adhesion: Hemodialysis, diabetes mellitus



MCQ

1- Where is the first step of leukocyte action?			
A) In tissue	B) In lumen	C) Lymph node	D) Thymus
2- From the following receptors, which one is founded on the surface of the leukocyte?			
A) ICAM-1	B) P-selectin	C) L-selectin	D) VCAM-1
3- What are the neutrophils attracted by in chemotaxis?			
A) Bacterial product	B) TNF	C) IL-1	D) Histamine

Question 1	B
Question 2	C
Question 3	A



KEYWORDS

C3b	Responsible for opsonin
LAD defect leads to	Defect in migration
Rheumatoid arthritis	problem in TNF
MPO	converts H2O2 to HOCL
P-selectin	From Weibel-palade
P-selectin +E-selectin	Binds to SIALYL-LEWIS

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.

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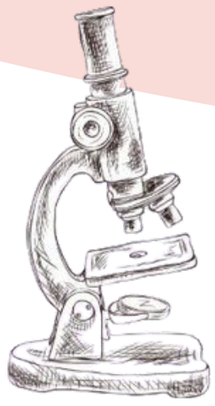

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