INFLAMMATION

Inflammation

- Definition
  - Inflammation is a complex reaction to injury that comprises vascular responses and migration and activation of leucocytes and systemic reaction
  - It starts as body's defense reaction
  - But may be potentially harmful

Inflammation

- Response of **vascularized tissues** to injurious agents and tissue damage
  - It **brings** cells and molecules of host defense from the circulation to **the sites of injury**
  - To **eliminate** the offending agents

Inflammation

- Protective:
  - Serves to **get rid of** initial **cause** of cell injury (microbes, toxins) and **consequences** of injury (necrotic cells/ tissues)
  - Wound would never heal up if there would be no inflammation
- Double edged sword:
  - Some may cause tissue destruction e.g**. immune mediated disorders**

Sequential steps (@five Rs)

- Offending agent **recognized** by host cells and molecules
- **Leukocytes** and **plasma proteins recruited** from circulation to site of offending agent
- Leukocytes and proteins **activated** to eliminate (**re**moval) offending substance
- Reaction is **controlled (regulation)** and **terminated**
- Damaged tissue **repaired (resolution)**

Different stimuli for inflammation

- Physical agents
  - Heat, radiation, mechanical trauma
- Chemical agents
  - Organic and inorganic poisons
- Infectious agents
  - Bacteria, viruses, parasites, fungus, and microbial toxins
- Immunological agents
  - Hypersensitivity reactions
- Deficiency of nutrients
- Tissue necrosis
- Foreign bodies

Recognitions of microbes and damaged cells

- Cellular receptors for microbes:
  - Family of Toll-like receptors (TLRs)
  - Located in plasma membranes and endosomes, & detect extracellular and ingested microbes
  - Recognition stimulates the productions and expression of cytokines
  - Cytokines cause lymphocyte activation and even more potent immune responses

Recognitions of microbes and damaged cells

- **Sensors of cell damage:**
  - All cells have **cytosolic receptors** that recognize **molecules altered** due to cell damage and called **damage-associated molecular patterns (DAMPs)**
  - Uric acid, ATP, reduced intracellular K+ concentration, DNA
  - Receptors with DAMPs activate cytosolic complex; **inflammasome,** which induces production of **IL-1**
  - IL-1 recruits lymphocytes so induces inflammation

Recognitions of microbes and damaged cells

- **Circulating plasma proteins:**
  - Recognize microbes and promote destruction

A. Complement produces mediators of inflammation

B. Circulating protein called **mannose-binding lectin:**

   A. Recognizes **microbial sugars** and

   B. Circulating protein called mannose-binding lectin:
      - Recognizes **microbial sugars** and
      - Promotes microbe ingestion and activation of complement

   C. Collectins bind to microbes and promote phagocytosis

Different types of inflammation

- Acute Inflammation
- Chronic inflammation

Table 3-2 Features of Acute and Chronic Inflammation

Feature	Acute	Chronic
Onset	Fast: minutes or hours	Slow: days
Cellular infiltrate	Mainly neutrophils	Monocytes/macrophages and lymphocytes
Tissue injury, fibrosis	Usually mild and self-limited	Often severe and progressive
Local and systemic signs	Prominent	Less

Acute inflammation

- Transient process
  - Initial, rapid response to infections and tissue damage
- Occurs within minutes of injury (abrupt origin)
- Lasts for hours or days
- Is short duration
- Represents early body reaction
- Usually is followed by repair – a process by which tissue is restored to its original state as far as possible

Acute inflammation

- Hallmarks (main characteristics)
  - Edema and emigration of neutrophils (polymorphonuclear leuckocytes)

- When offenders eliminated it subsides and residual injury repaired.

- It fails to clear the stimulus, progresses to chronic inflammation.

Cardinal signs of inflammation

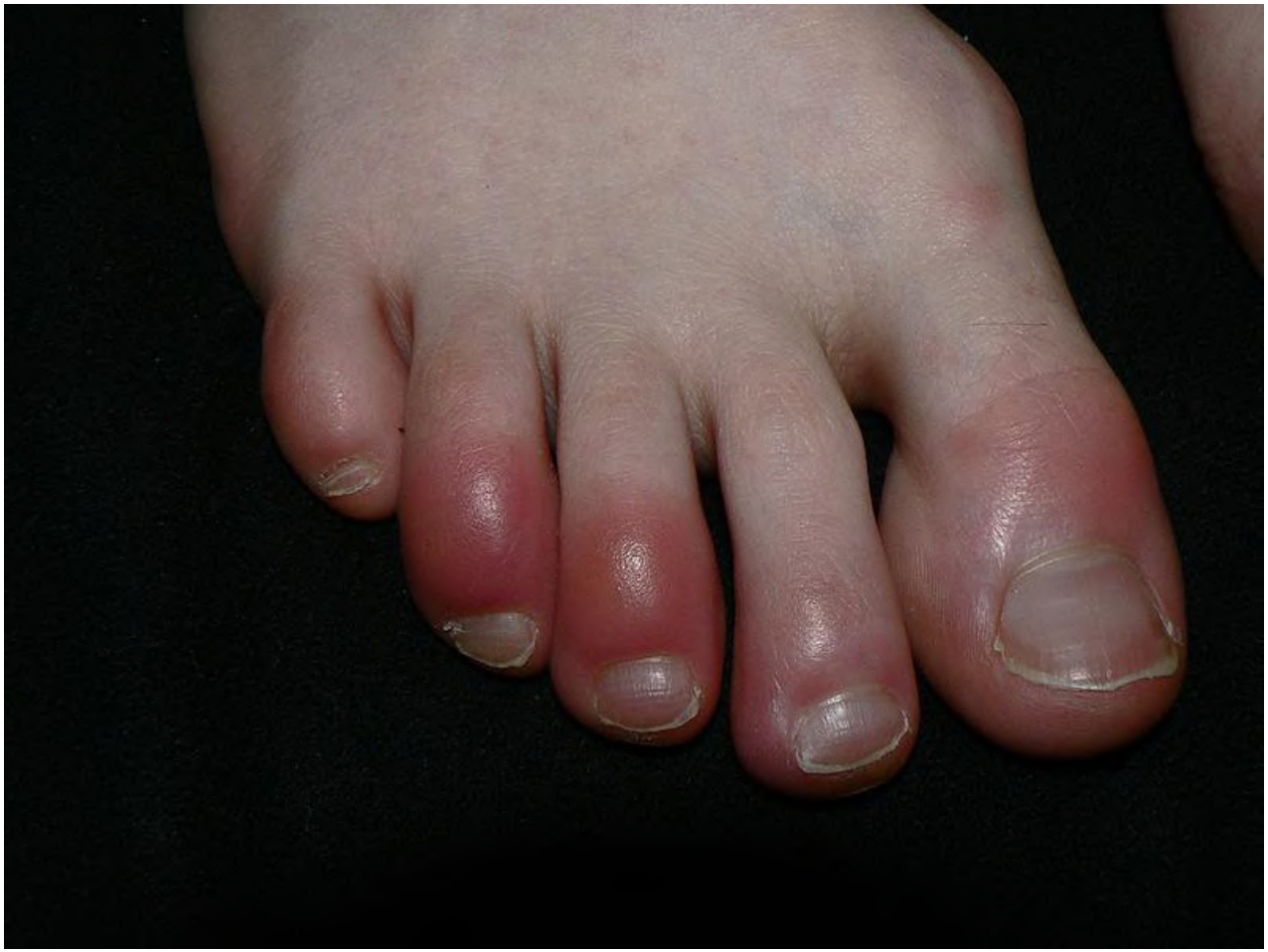
- Rubor (redness)
- Calor (heat)
- Dolor (pain)
- Tumor (swelling)

Proposed by Celsus in 1st century AD

- Function laesa (loss of function)
  - added later by Rudolf Virchow

Cardinal signs of acute inflammation

- **Rubor (redness):**
  - Due to vasodilatation (caused by histamine, NO, etc.)
- **Tumor** (swelling):
  - Due to increased vascularity, edema
- **Calor (heat):**
  - Due to increased blood supply
- **Dolor** (Pain):
  - Due compression of free nerve endings and action of prostaglandin.
- **Functio Laesa** (loss of function):
  - Due to local pain and tissue destruction.



Acute inflammation

- Components of acute inflammation

2 major components

- Vascular events
  i. Changes in vascular flow and caliber
  ii. Increased vascular permeability - permit plasma proteins and leucocytes to leave the circulation
- Cellular events
  i. Leukocyte extravasation
  ii. Phagocytosis

Vascular events in acute inflammation

Immediate transient vasoconstriction of arterioles

↓

Mast cells releases NO and histamine

↓

Persistent progressive vasodilation of arterioles

↓

Opening of new capillary beds

↓

Increased blood volume in microcirculation

(Heat (Calor) and redness (Rubor))

↓

Elevating the hydrostatic pressure

↓

Increased vascular permeability

Leakage of protein rich fluid into the interstitium

(Edema - Tumor)

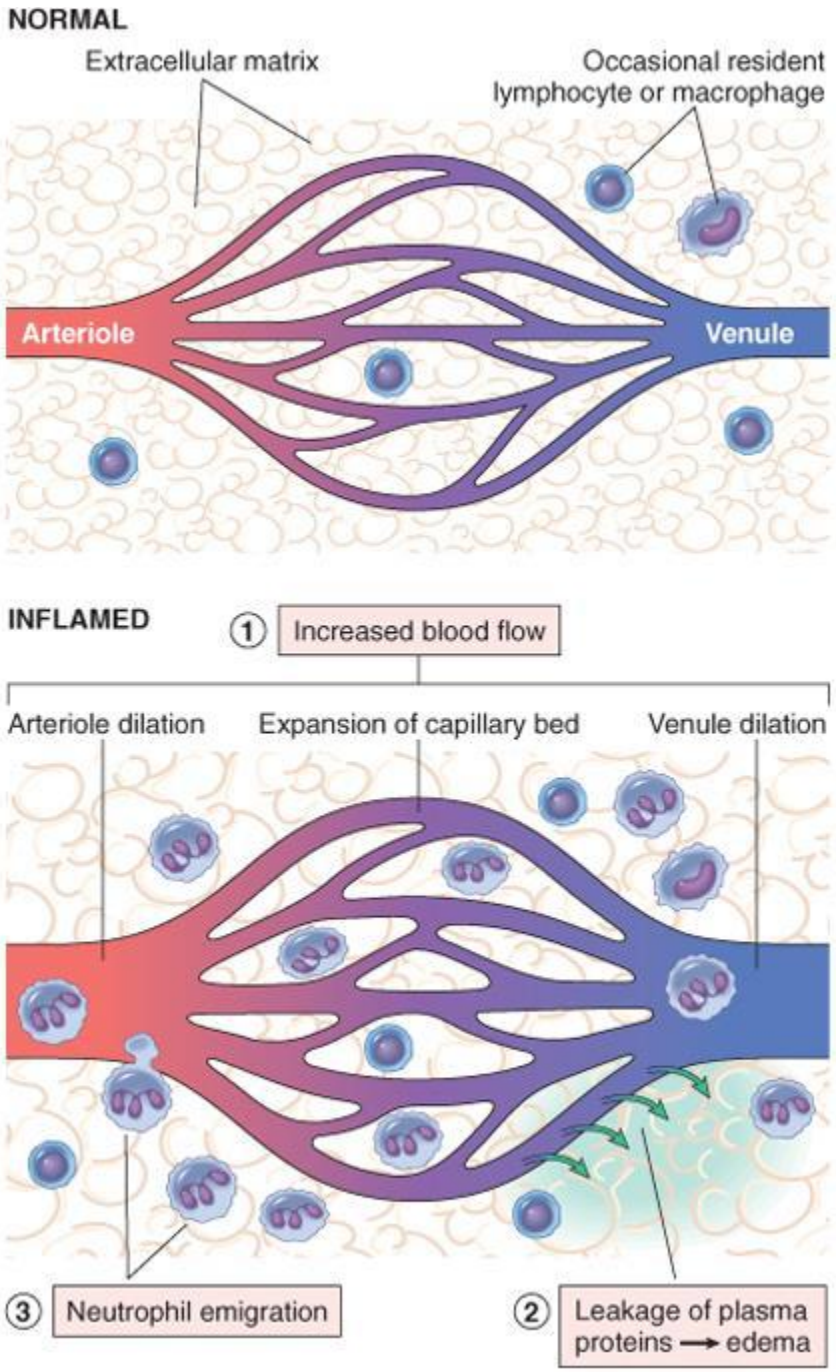
↓

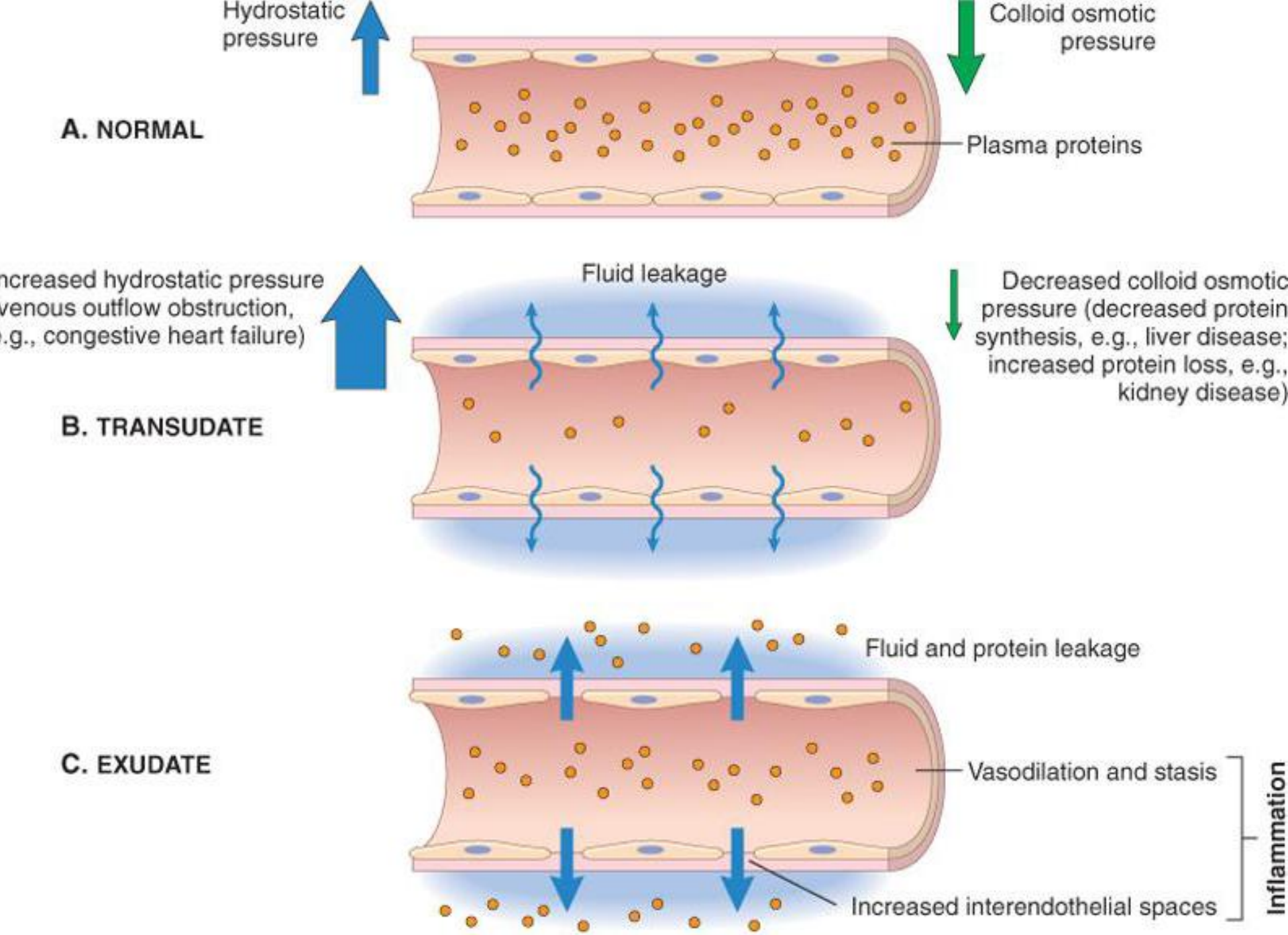
Concentration of RBCs in small blood vessels → increased viscocity of blood

Transudation of fluid into extracellular space

↓

Blood flow slows down →Stasis



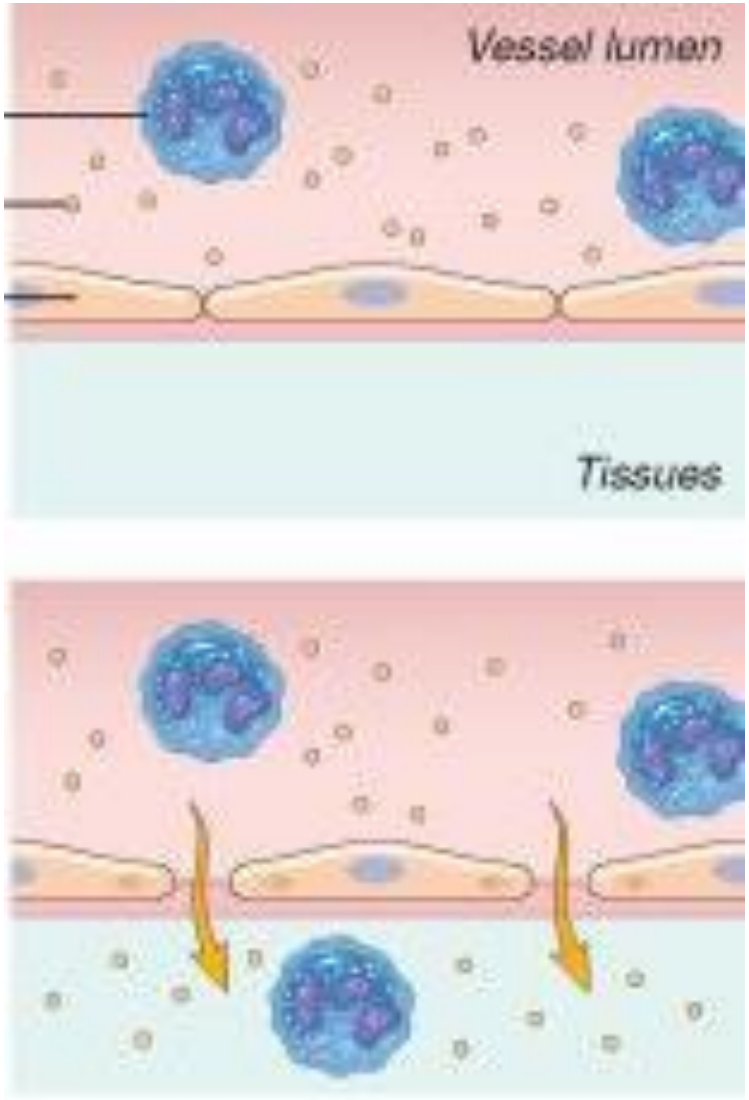


Mechanism of increased vascular permeability

- Retraction of endothelial cells → leading to intercellular gaps
- Direct endothelial injury
- Leukocyte mediated endothelial injury
- Increased transcytosis
- Leakage from new blood vessels

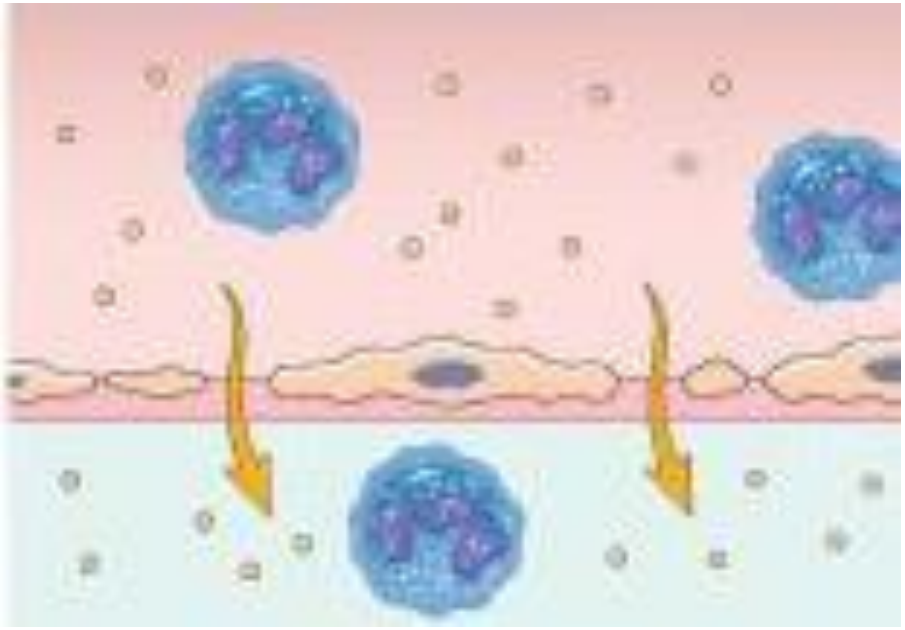
❑Endothelial cell retraction :

- leading to intercellular gaps:
  - Mediator: histamine/ bradykinin → causing contraction of endothelial cells
  - occurs rapidly after exposure to mediators
  - Known as: <u>immediate transient response</u>
  - Short lived response (15 – 30 mins)



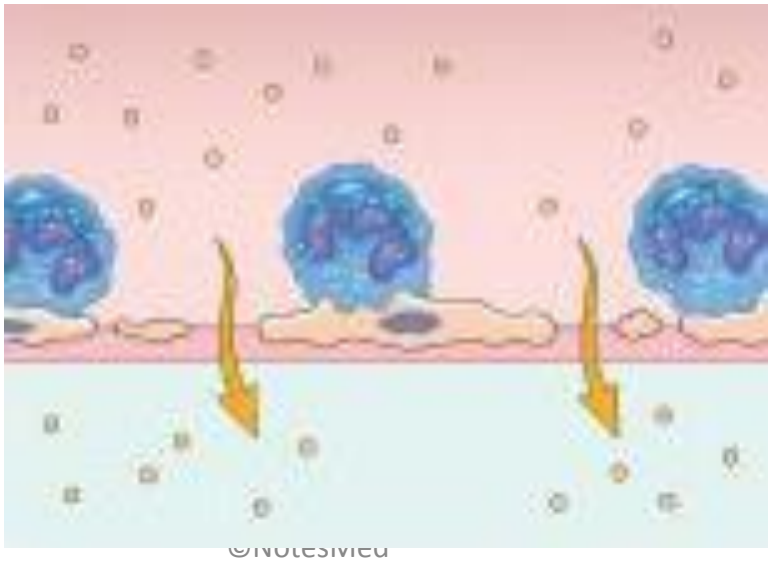
❑ Endothelial injury:

- **Direct damage to endothelium** by severe injuries (burns, bacterial infection)
- Neutrophils that adhere to endothelium - injure the endothelial cells.
- leakage starts immediately after injury
- Is sustained for several hours- known as <u>immediate sustained response</u>



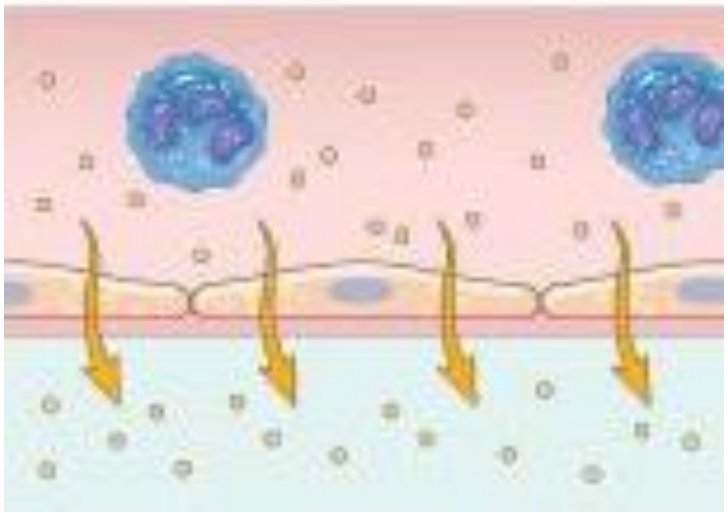
❑ Leukocyte mediated endothelial injury:

- leukocyte adhere to endothelium
- release toxic oxygen species and proteolytic enzymes
- cause endothelial injury or detachment



❑ Increased transcytosis

- Transport of fluids and protein through the endothelial cells is called transcytosis
- Occurs via channels by fusion of intracellular vesicles.
- VEGF(vascular endothelial growth factor)increase the number and size of channels.



Cellular events in acute inflammation

1. Extravasation of leukocytes
   - from vessel lumen to interstitial space

2. Phagocytosis

1. Extravasation of leukocytes

(sequential events)

a. In the lumen
   i. Margination
   ii. Rolling
   iii. Pavementing/adhesion
   iv. Diapedesis

Leukocyte first marginate (Margination)

↓

Then roll (rolling) along the endothelium

↓

These leucocyte then adhere to endothelium (Pavementing)

↓

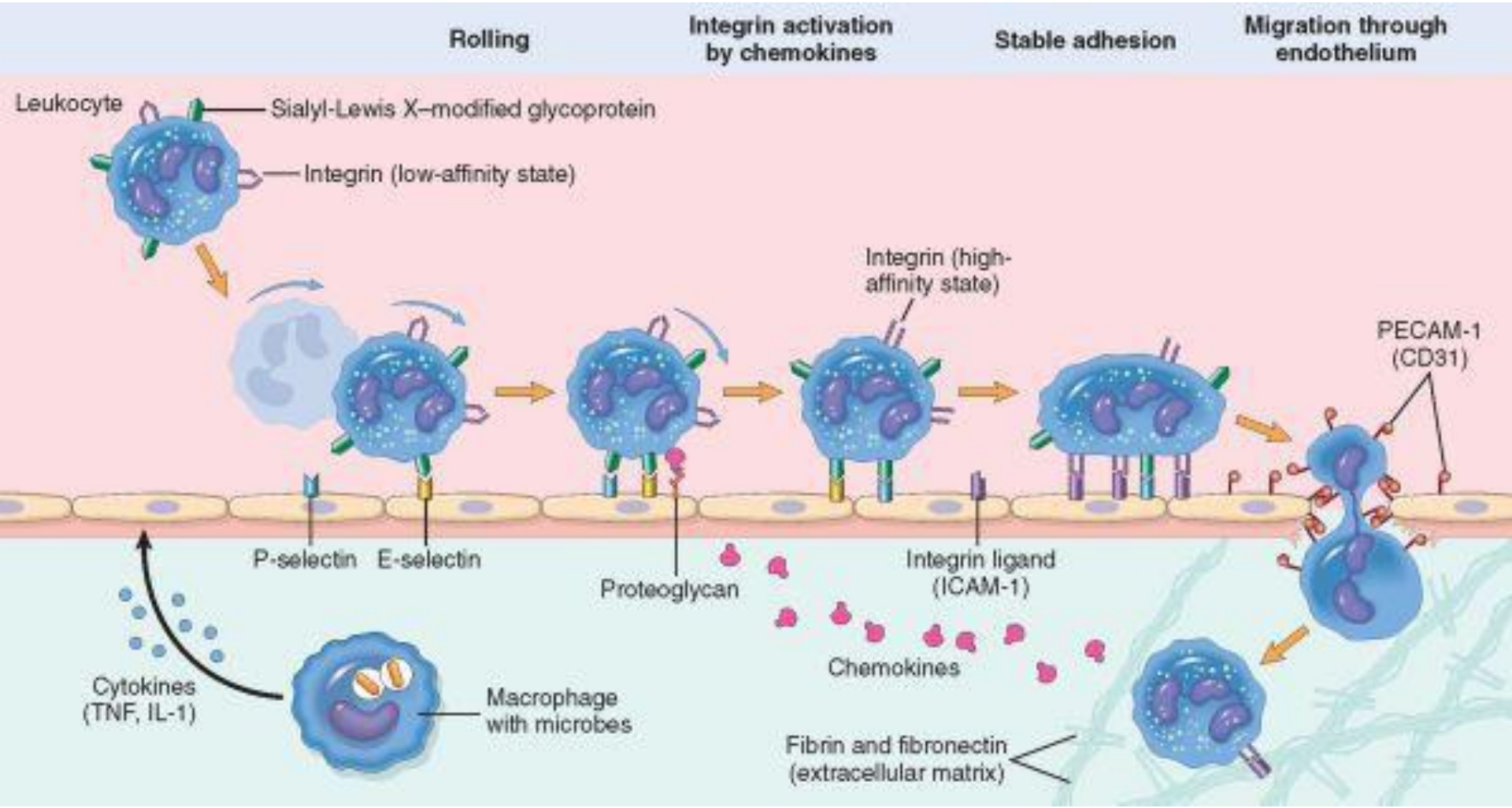
Transmigrate across the endothelium (Diapedesis)

↓

Pierce the basement membrane

↓

Migrate towards chemoattractants emanating from source of injury


Rolling
Integrin activation by chemokines
Stable adhesion
Migration through endothelium
Leukocyte
Sialyl-Lewis X–modified glycoprotein
Integrin (low-affinity state)
Integrin (high-affinity state)
PECAM-1 (CD31)
P-selectin
E-selectin
Proteoglycan
Integrin ligand (ICAM-1)
Chemokines
Cytokines (TNF, IL-1)
Macrophage with microbes
Fibrin and fibronectin (extracellular matrix)

- Adhesion of the leucocytes are regulated by
  - Adhesion molecules on
    - leukocyte and
    - endothelial surfaces
  - Chemical mediators

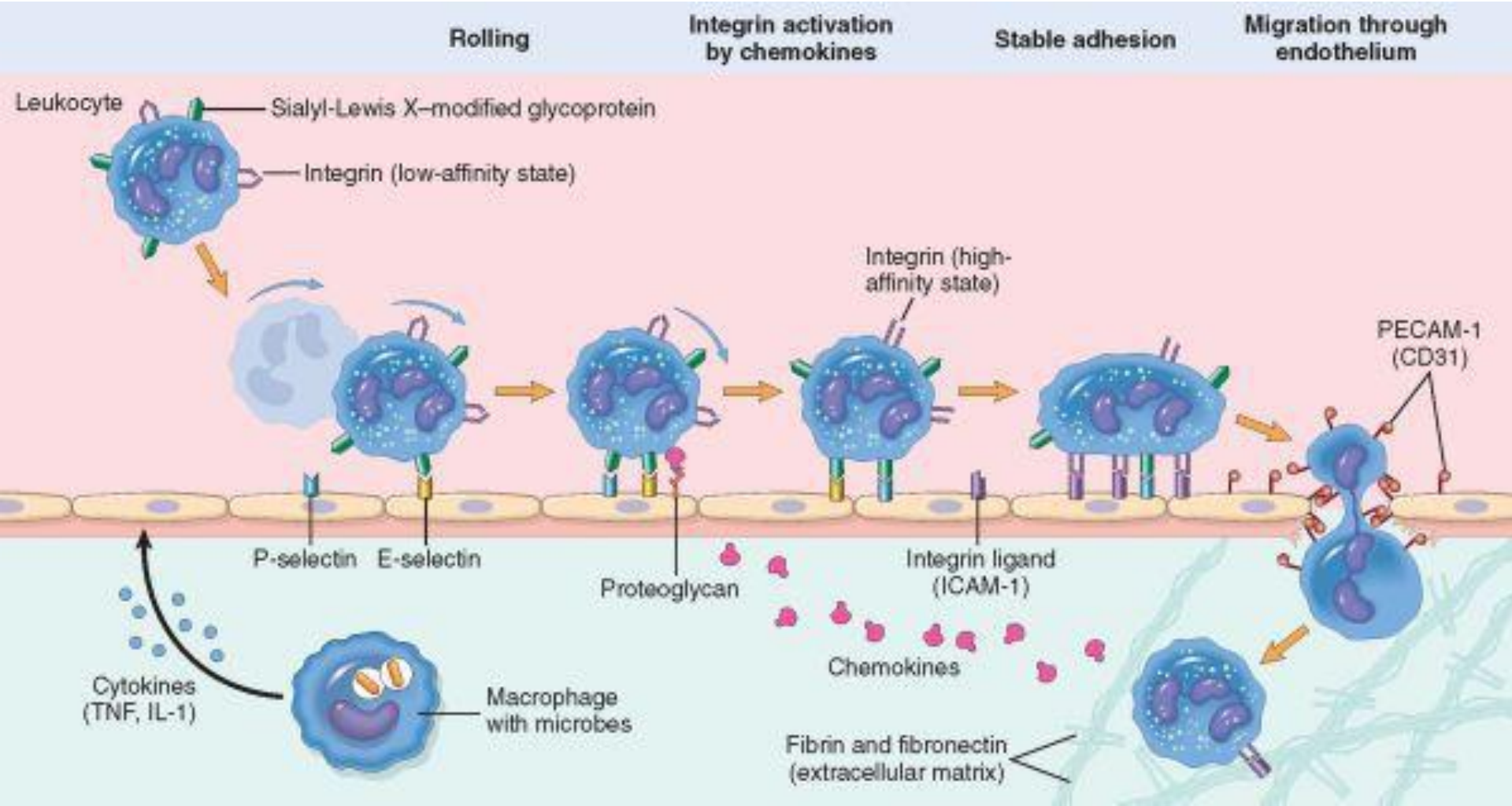
- **Adhesion molecules :**

1. Selectin:
   - Sialyl lewis X modified protein
     – receptors present on leucocyte
   - P-selectin, E-selectin
     - present in endothelial cells

2. Immunoglobulin family:
   - ICAM-1 (intercellular adhesion molecule) and
   - VCAM (vascular cell adhesion molecule)

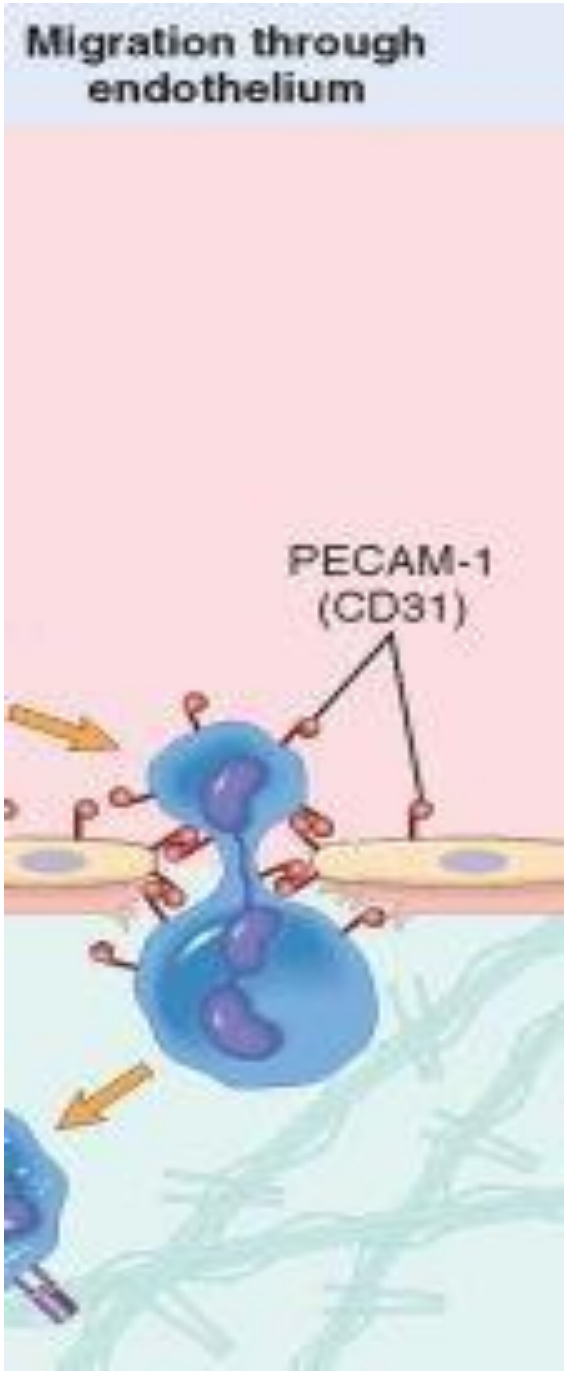
3 Integrins(glycoproteins):
   - Expressed on leucocyte surface interacting with their ligands on endothelial cells.

- **<u>Chemical mediators</u>** :
  - Expression of these adhesion molecules are activated by chemokins (TNF, IL-1)



b. Transmigration across the endothelium (emigration or diapedesis) :

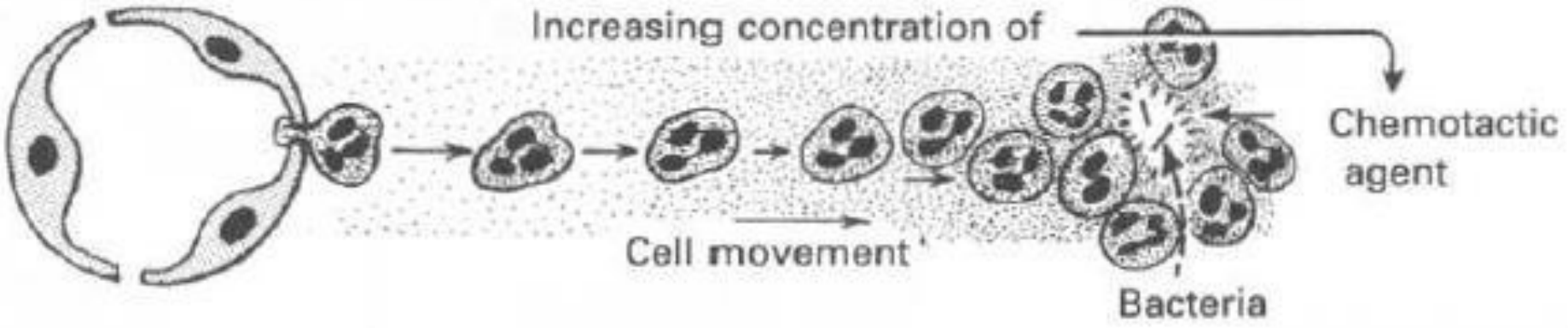
– Emigration is facilitated by focal dissolution of exposed basement membrane by leukocyte derived collagenase



c. Chemotaxis

- After extravasation - Migration of leukocytes in interstitial tissue towards a chemotactic stimulus (chemotaxis)

- Chemotaxis – defined as locomotion oriented along a chemical gradient



- All granulocytes, monocytes exhibit directed movement to the area of injury, which is facilitated by chemotactic agents (chemoattractants)

- Chemoattractants
  - Exogenous – bacterial products
  - Endogenous – C5a, Leukotrienes, cytokines

Acute inflammation

- **Neutrophils predominate in the inflammatory infiltrate during the first 6-24hrs and are replaced by monocytes in 24 to 48 hours.**

2. Phagocytosis

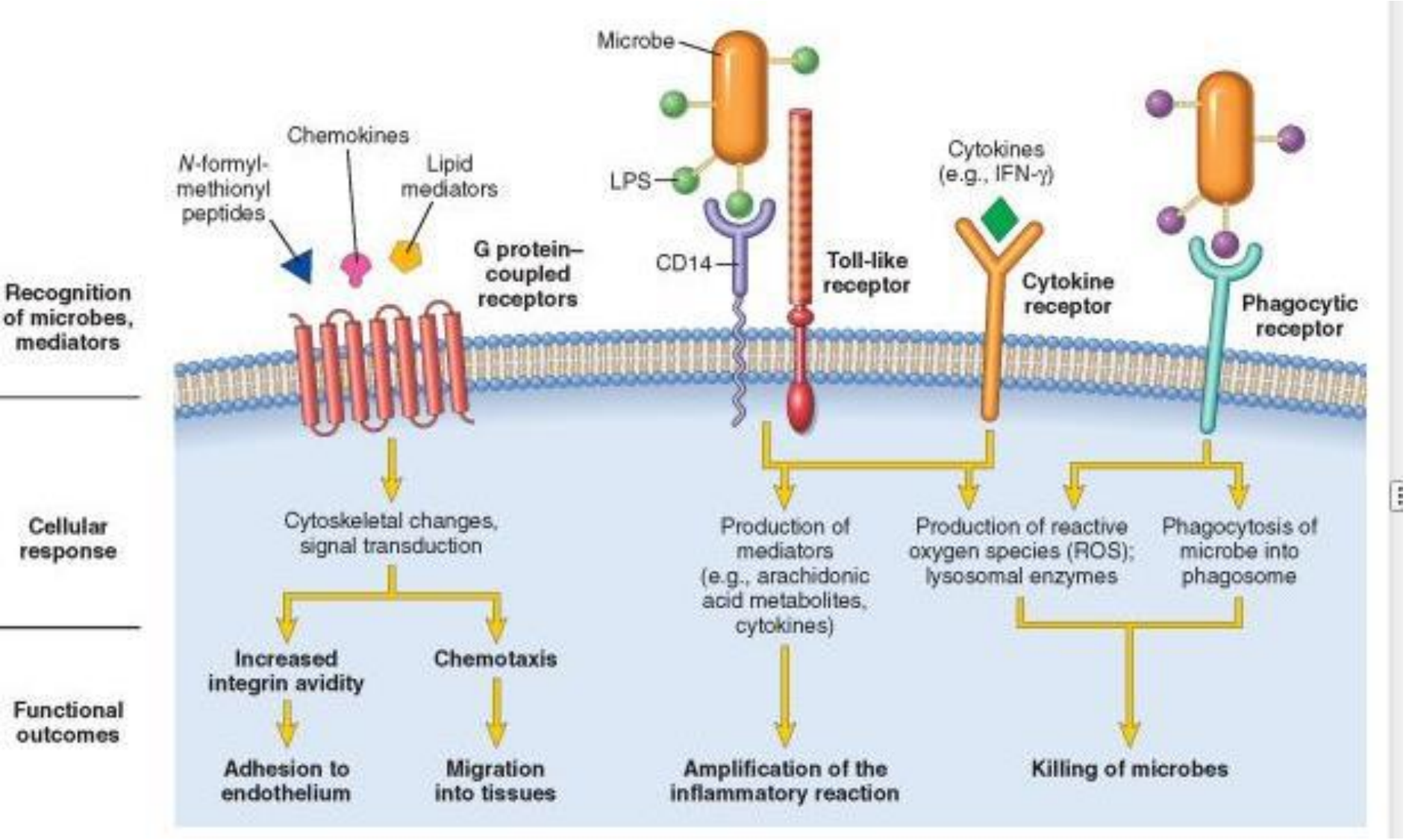
- Definition :
  - The process by which leukocytes recognize, engulf and kill or degrade the ingested materials

- Two main types of phagocytic cells
  - Polymorphs
  - Circulating monocytes/macrophages

- Steps in phagocytosis
  - Recognition and attachment
  - Engulfment
  - Killing and degradation

i. Recognition and attachment

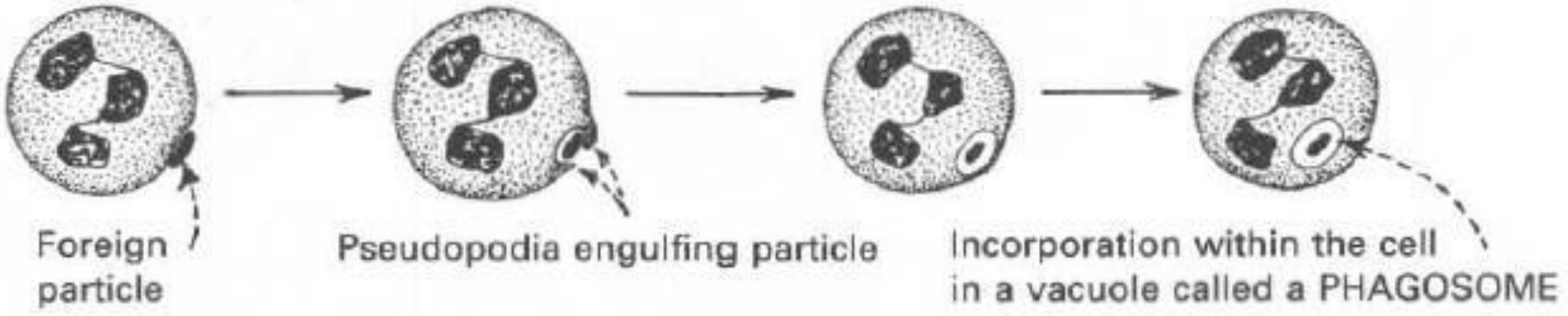
- Opsonins coats microbes
  - IgG,
  - complement protein C3,
  - lectins
- Leucocytes posses receptors for opsonins and thus binds to microbes via specific receptors
  - Fc for IgG,
  - complement receptor 1&3 for complement fragments,
  - C1q for lectins
- Coating by opsonin and targeting them for phagocytosis →Opsonization


Recognition of microbes, mediators
Cellular response
Functional outcomes
Microbe
LPS
CD14
Toll-like receptor
Cytokines (e.g., IFN-γ)
Cytokine receptor
Phagocytic receptor
Chemokines
N-formyl-methionyl peptides
Lipid mediators
G protein–coupled receptors
Cytoskeletal changes, signal transduction
Increased integrin avidity
Chemotaxis
Adhesion to endothelium
Migration into tissues
Production of mediators (e.g., arachidonic acid metabolites, cytokines)
Amplification of the inflammatory reaction
Production of reactive oxygen species (ROS); lysosomal enzymes
Phagocytosis of microbe into phagosome
Killing of microbes

ii. Engulfment

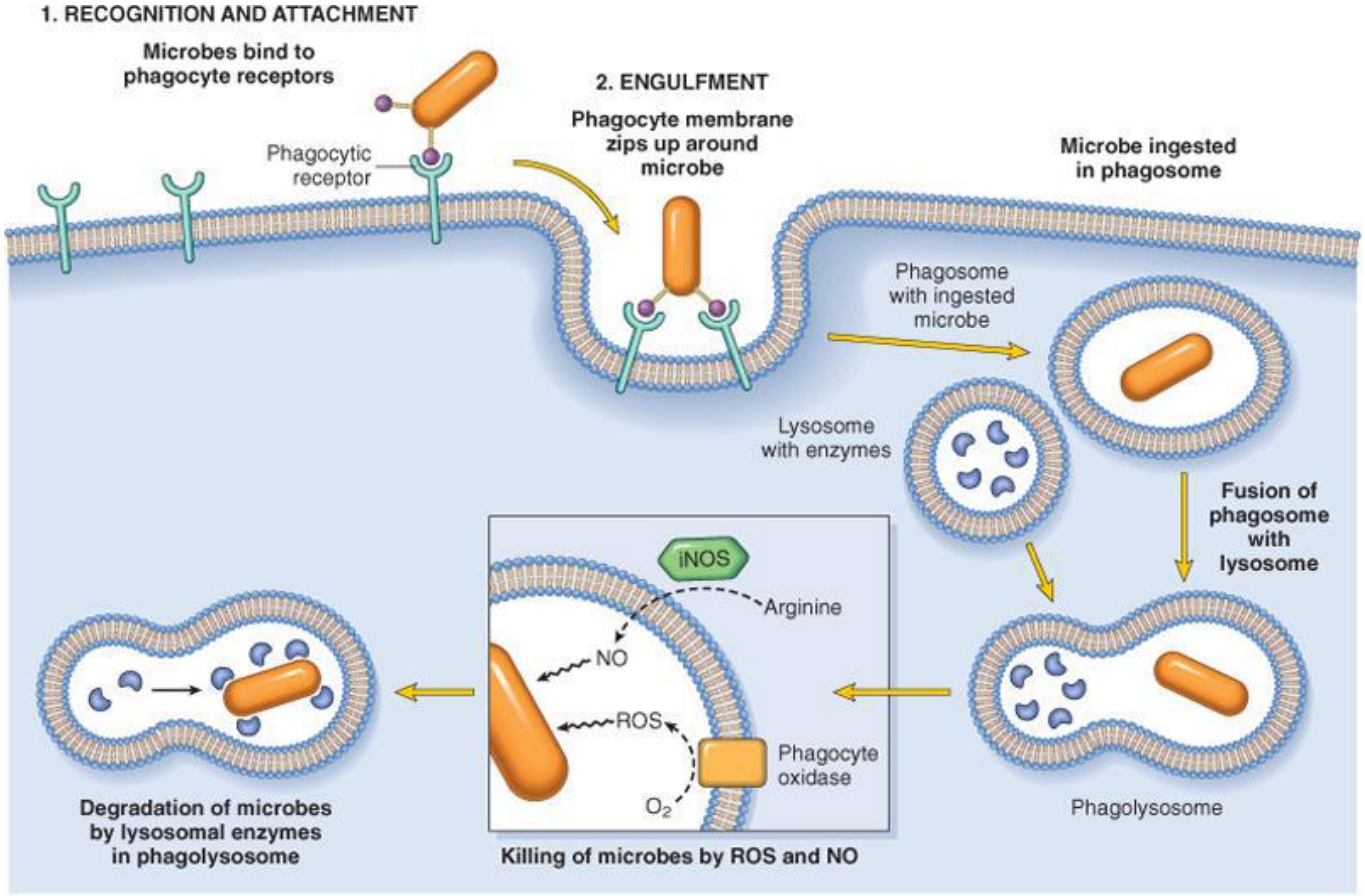
- Bacteria are engulfed by pseudopodia (extension of cytoplasm) and trapped within phagosomes forming phagocytic vacuole

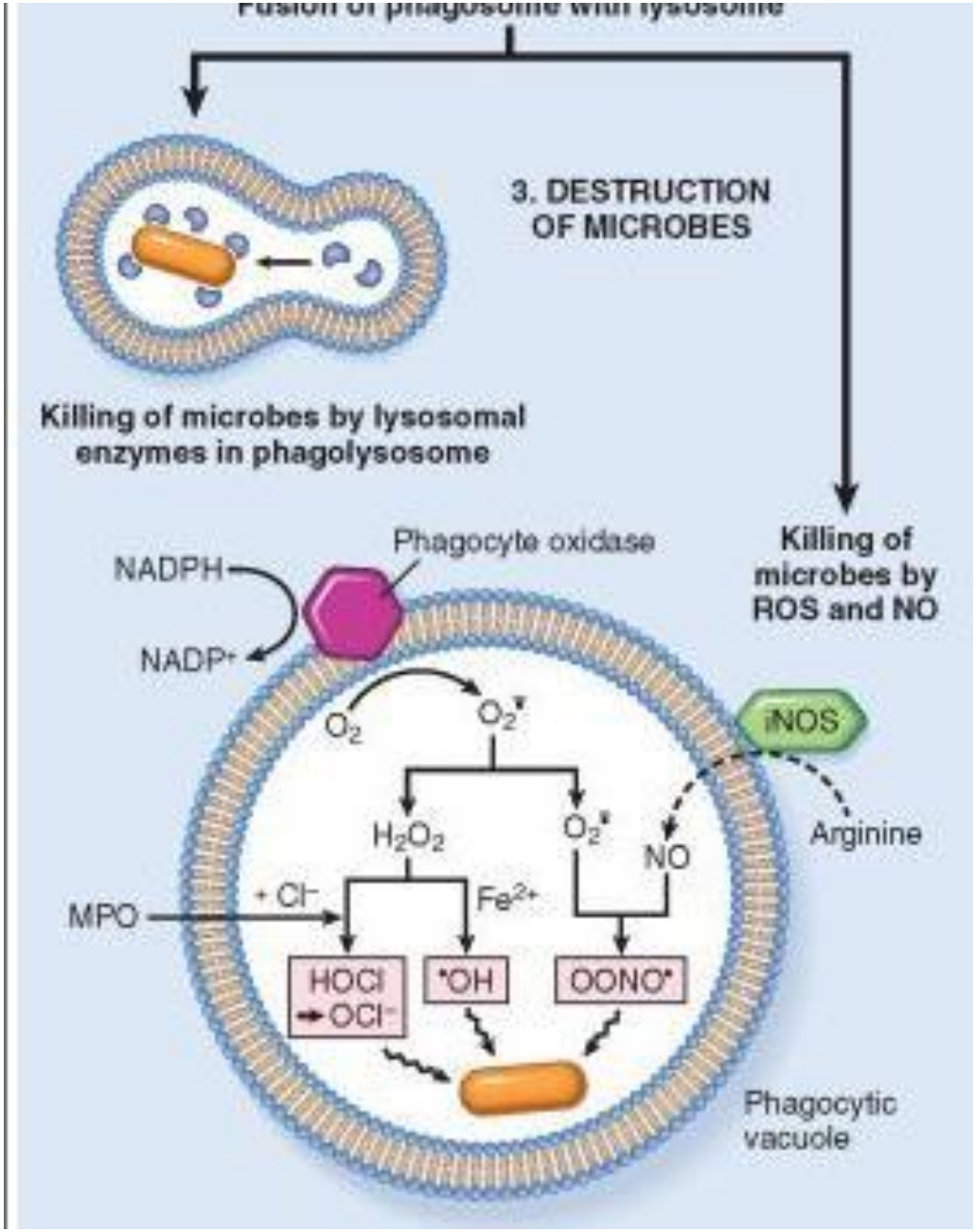
- The phagocytic vacuole fuses with lysosomal granules – resulting in discharge of contents of granule into phagolysosome



iii. Killing and degradation

- Neutrophils and monocytes kills the bacteria by both
  - oxygen dependent (MPO system and O2 derived free radicals) and
  - oxygen-independent (lysosomal enzymes) mechanisms for killing bacteria




Fusion of phagosome with lysosome
3. DESTRUCTION OF MICROBES
Killing of microbes by lysosomal enzymes in phagolysosome
Killing of microbes by ROS and NO
Phagocyte oxidase
NADPH
NADP+
O_2
$O_2^{\bullet}$
iNOS
Arginine
H_2O_2
$O_2^{\bullet}$
NO
MPO
+ Cl^-
Fe^{2+}
HOCl → OCl^-
$^{\bullet}OH$
OONO$^{\bullet}$
Phagocytic vacuole

- **Systemic effects of inflammation:**
  - Systemic changes associated with inflammation are collectively called acute phase response or systemic inflammatory response syndrome (SIRS)
  - Fever
    - Exogenous pyrogens (Bacterial products)
    - Endogenous pyrogens (cytokines IL-1, TNF)
  - Release of acute phase proteins
    - CRP
    - Fibrinogen
    - SAA (Serum amyloid A Protein)
  - Leucocytosis
    - Counts as high as 40,000-100,000 cells/microliter
    - (leukemoid reaction)
  - Increased pulse and blood pressure
  - Decreased sweating
  - Rigors, chills, anorexia, somnolence and malaise

Morphologic patterns of acute inflammation

- Serous inflammation :
  - Characterised by collection of watery relatively protein poor fluid, derived from either the plasma or secretions of mesothelial cells (lining peritoneal, pleural or pericardial cavities)
  - Seen in burns, viral infection etc

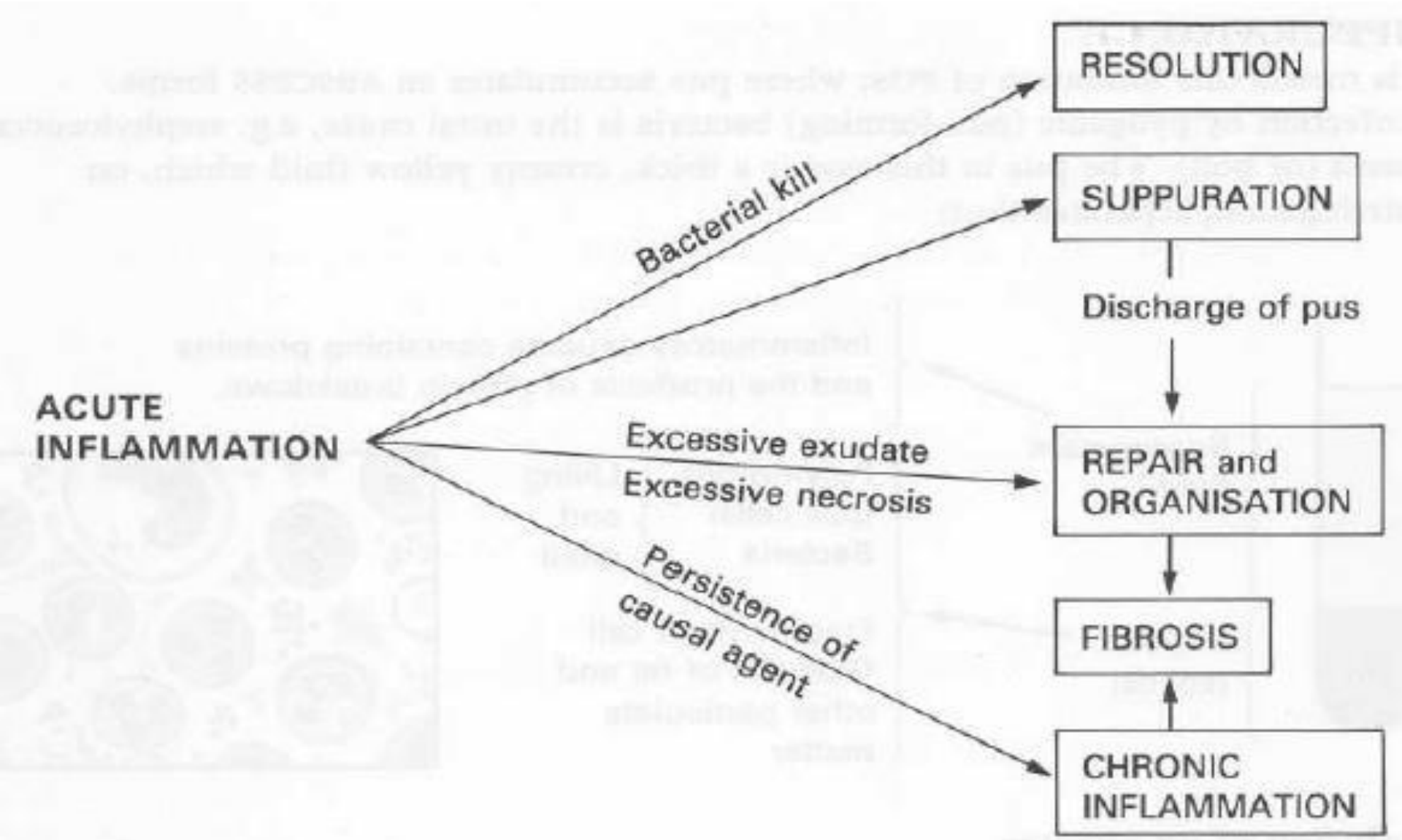
- Fibrinous inflammation :
  - Severe injury → increased vascular permeability → larger molecules like fibrinogen pass the vascular barrier → fibrin is formed and deposited in extracellular space
  - Seen in meninges, pericardium etc

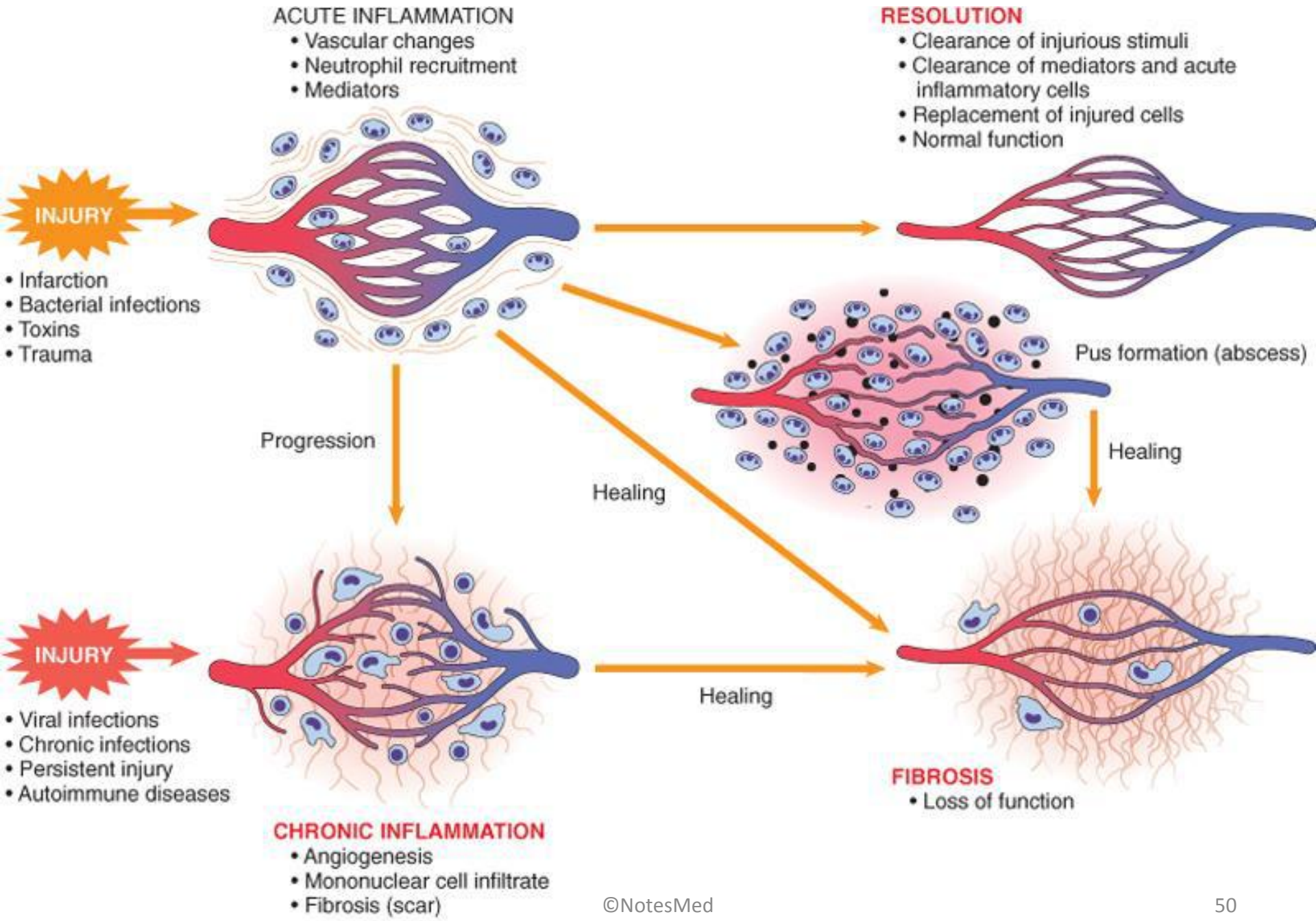
- Suppurative inflammation:
  - Production of large amount of pus or purulent exudate consisting of neutrophils, necrotic cells and edema fluid
  - Seen associated with pus-producing organisms
    - Eg : staphylococci

- Catarrhal inflammation:
  - Excessive production of mucous secretion
    - (Eg : running nose)

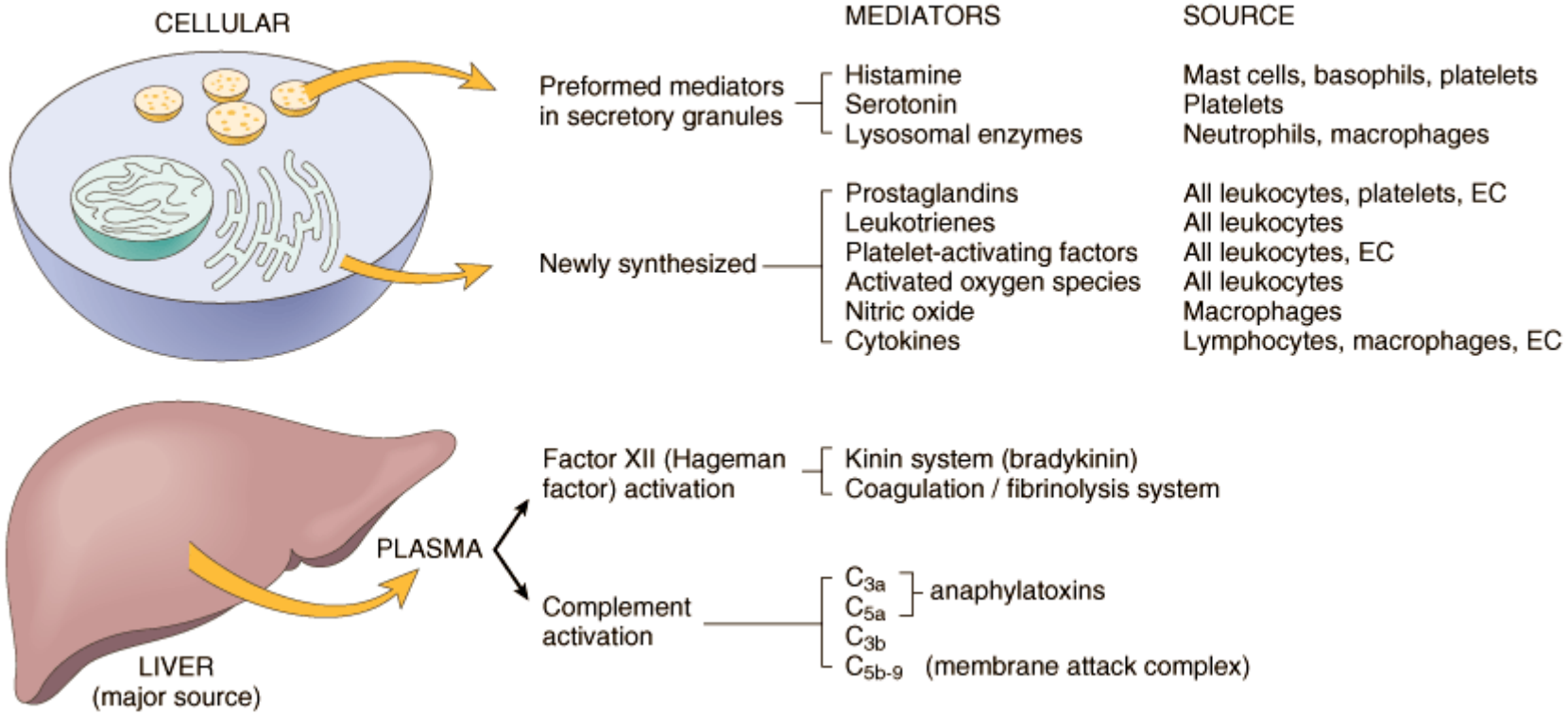
- Abscess
  - Localized collection of pus that is caused by infection with pyogenic organisms
- Cellulitis
  - Ch by thin, watery exudate that spreads throughout subcutaneous tissue
- Ulcer
  - Breach in the epithelium
  - Local defect or excavation on the surface of an organ or tissue that results d.t sloughing of inflammatory necrotic material

Sequels of Acute inflammation





Chemical mediators of inflammation



Major Cell-derived Mediators of Inflammation

- *Vasoactive amines*: histamine, serotonin; main effects are vasodilation and increased vascular permeability
- *Arachidonic acid metabolites: prostaglandins* and *leukotrienes*; several forms exist and are involved in vascular reactions, leukocyte chemotaxis, and other reactions of inflammation
- *Cytokines*: proteins produced by many cell types; mediate multiple effects, mainly in leukocyte recruitment and migration; principal ones in acute inflammation are TNF, IL-1, and chemokines(CXC, CC)

- *Reactive oxygen species*: role in microbial killing, tissue injury
- *Nitric oxide*: vasodilation, microbial killing
- *Lysosomal enzymes*: role in microbial killing, tissue injury

Morphologic patterns of acute inflammation

- Serous inflammation: effusion, protein poor

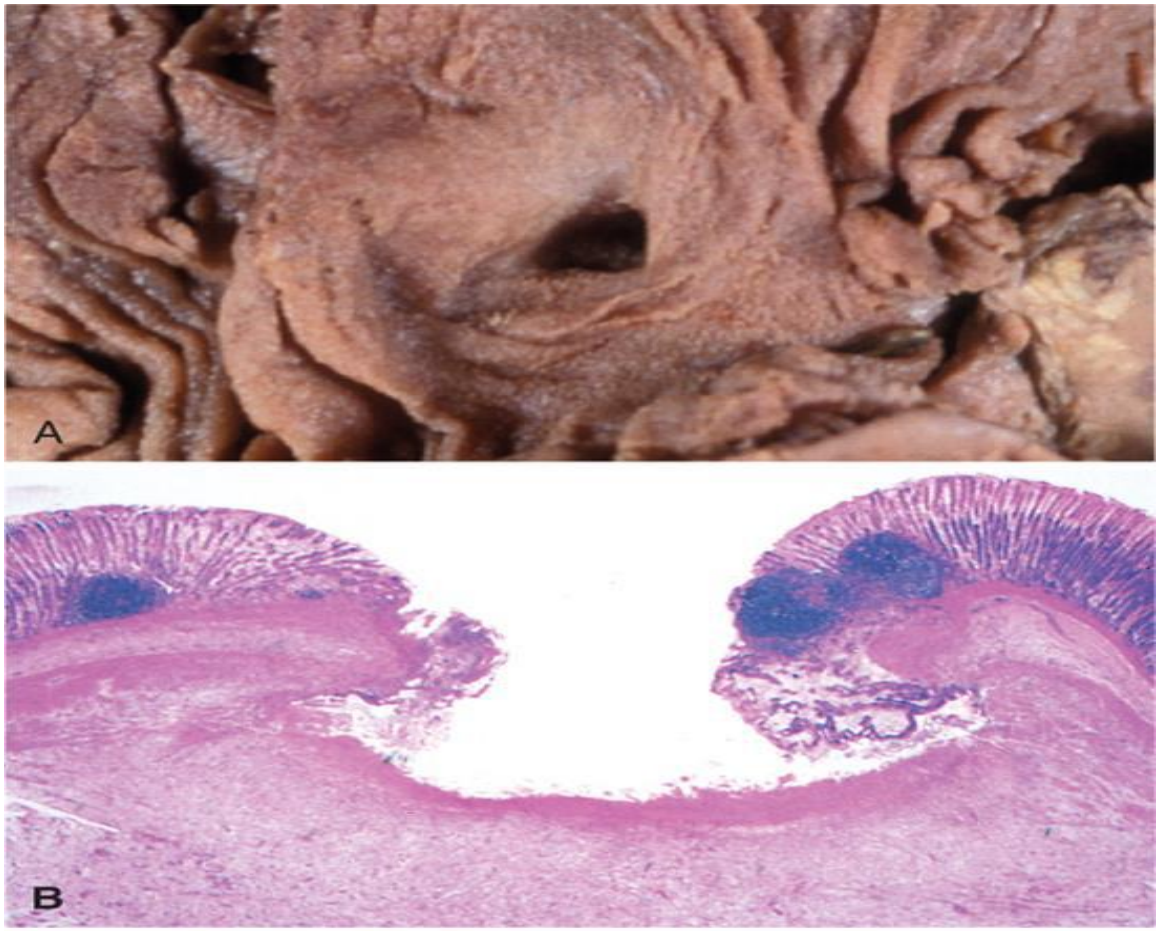
- Pleural effusion, blister on the surface of skin

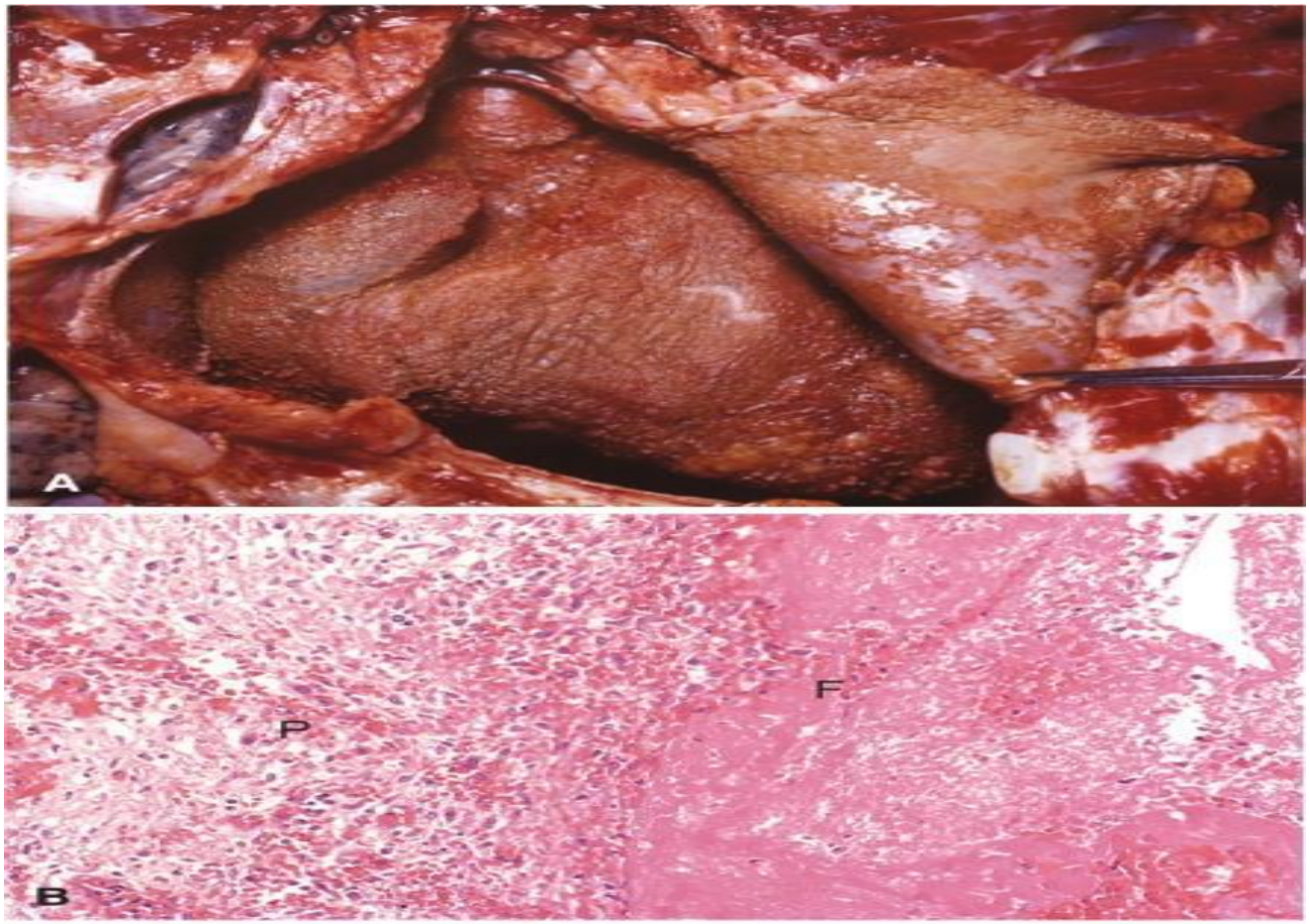
- Fibrinous inflammation: passage of fibrinogen from vessels: fibrinin meshwork, fibrinous pericarditis

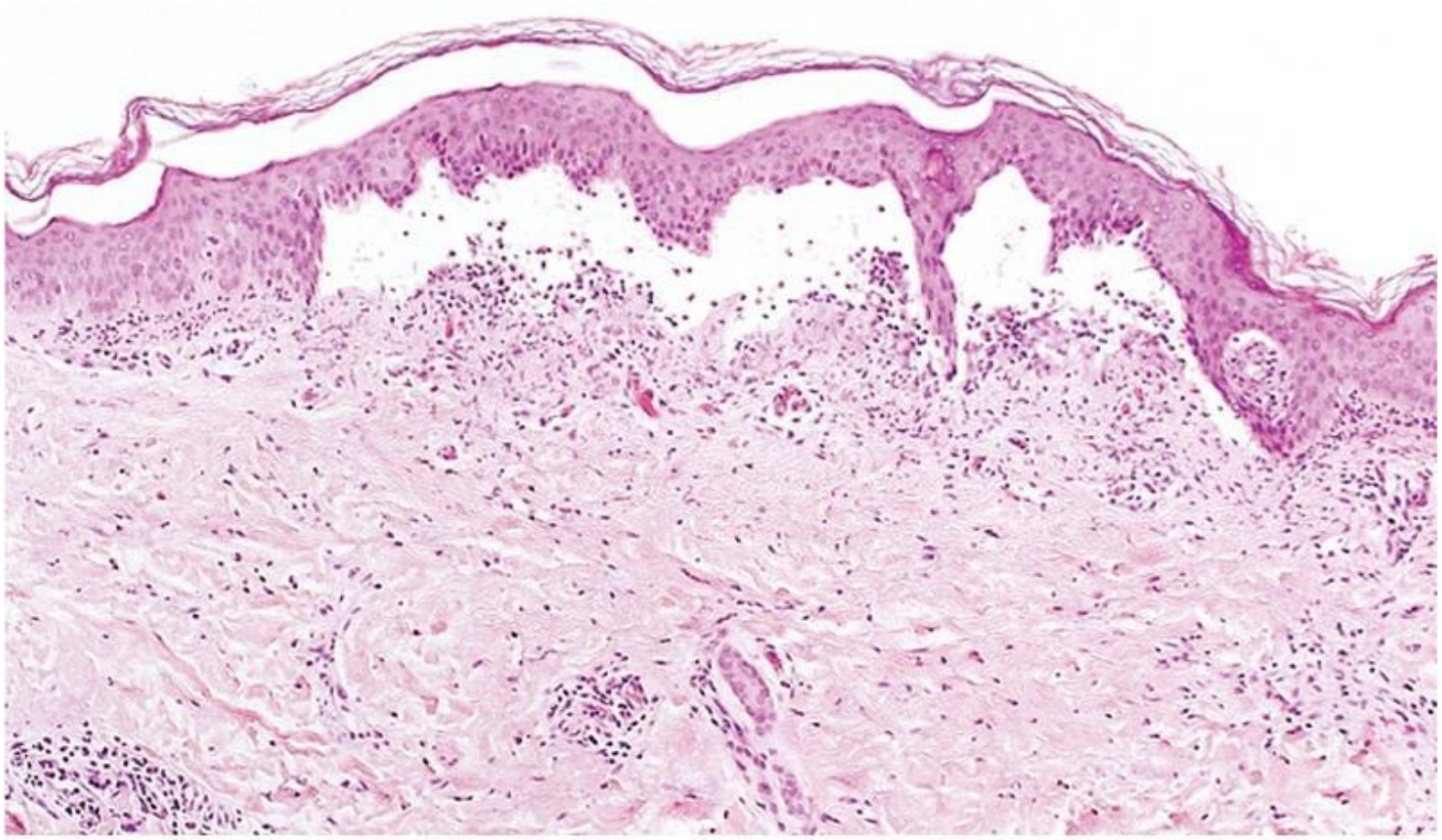
- Suppurative inflammation: abscess

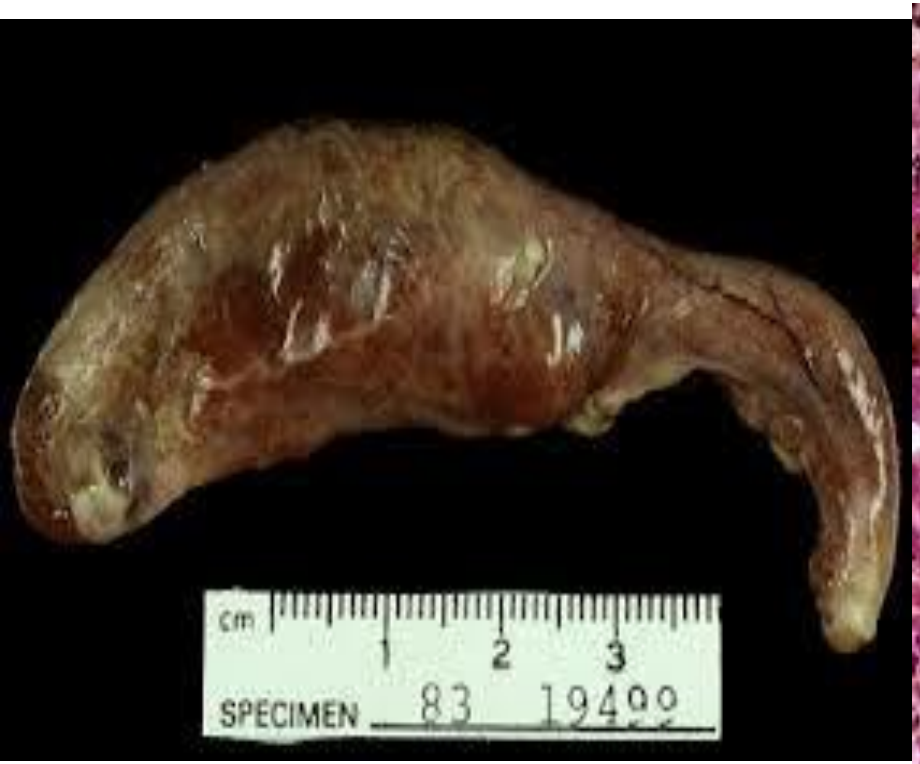
- Ulcer: a local defect of the surface of tissue or organ

necrosis, sloughing of the inflammatory necrotic tissue: peptic ulcer





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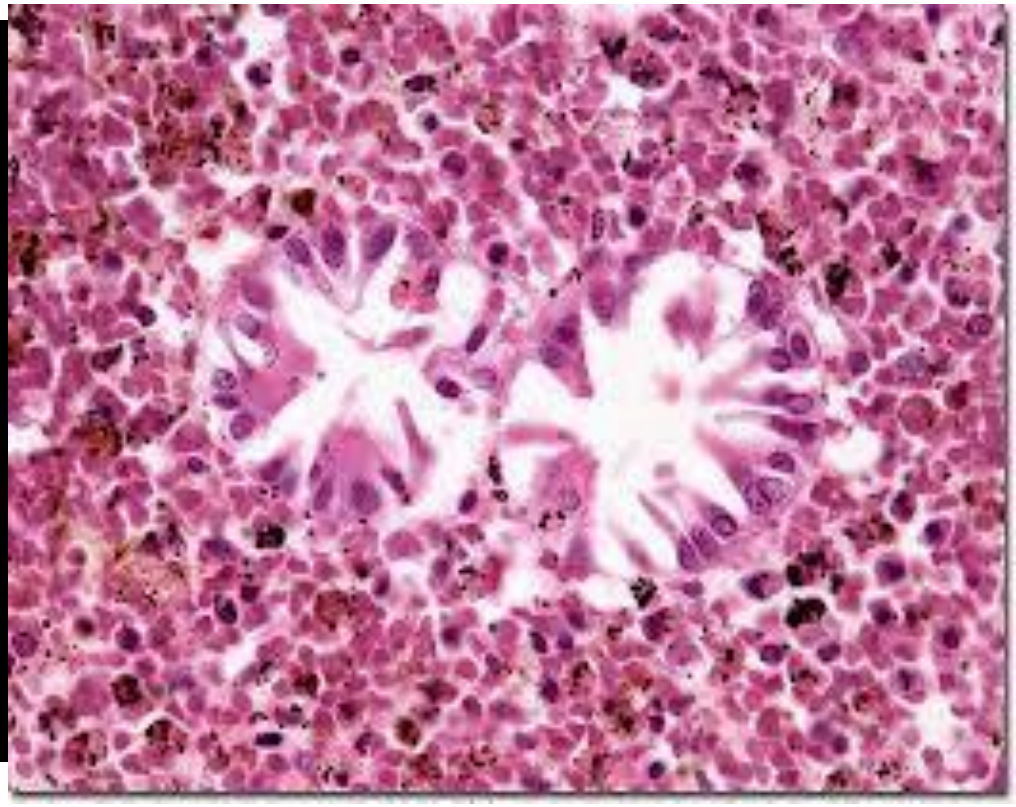


SEQUENCE OF EVENTS

- NORMAL HISTOLOGY →
- VASODILATATION →
- INCREASED VASCULAR PERMEABILITY →
- LEAKAGE OF EXUDATE →
- MARGINATION, ROLLING, ADHESION →
- TRANSMIGRATION (DIAPEDESIS) →
- CHEMOTAXIS →
- PMN ACTIVATION →
- PHAGOCYTOSIS: Recognition, Attachment, Engulfment, Killing (degradation or digestion) →
- TERMINATION →
- 100% RESOLUTION, SCAR, or CHRONIC inflammation

Chronic inflammation

- Inflammation of prolonged durations (weeks or months to years) in which the followings are proceeding simultaneously
  - active inflammation,
  - tissue destruction and
  - healing process
- Eg: chronic cholecystitis, chronic gastritis, Rheumatoid arthritis, tuberculosis

- Active inflammation
  - Infiltration by mononuclear cells: lymphocytes, macrophages, plasma cells
- Tissue destruction
  - induced by products of inflammation
- Repair
  - Involving new vessel proliferation, and fibrosis

Causes of chronic inflammation

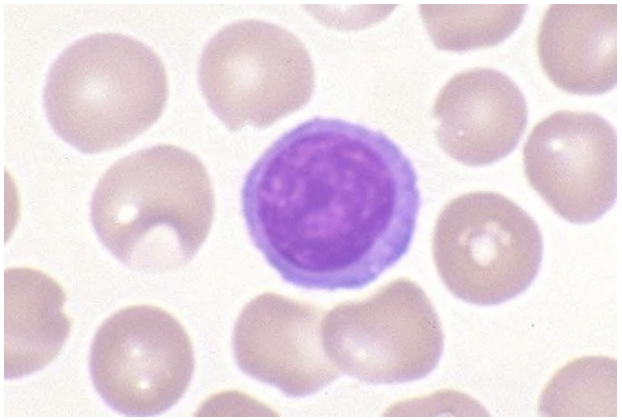
1. Persistent infection by certain microbes which are difficult to irradicate:
   - Eg : Tubercle bacilli, Treponema pallidium, Fungi

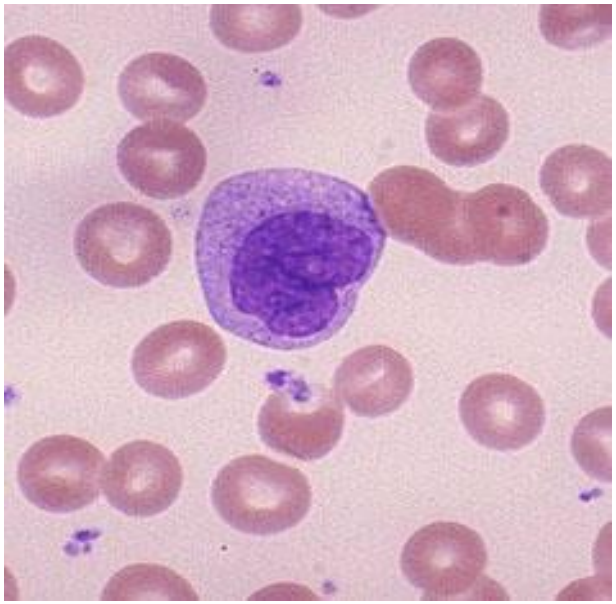
2. Prolong exposure to potentially toxic agents,
   - Either exogenous or endogenous.: silica, asbestos

3. Immume mediated inflammatory diseases:
   - Autoimmunity: immune reaction developing against individuals own tissue: chronic tissue damage
   - Rheumatoid arthritis

Chronic inflammatory cells

1. **Macrophages**
2. **Lymphocytes**
3. Mast cells
4. Plasma cells

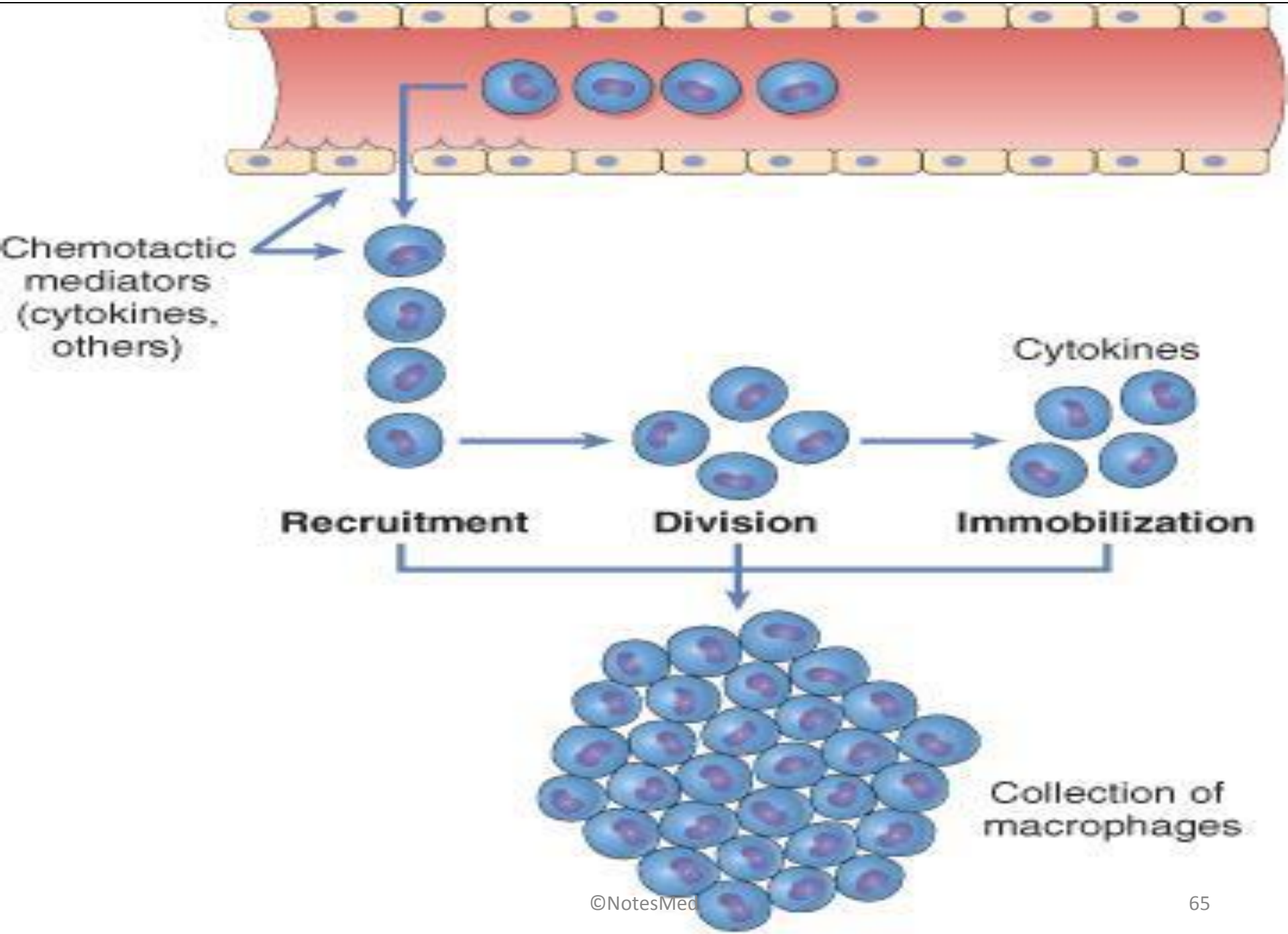




MACROPHAGES

- play central role in chronic inflammatory infiltrate- macrophages are the most effective phagocytic cells in acute and chronic inflammatory response
- enzymatic degradation and phagocytic activity

- Following activation-macrophages produce biologically active products, such as:
  - Enzymes - neutral and acid proteases
  - Chemotactic factors for leukocytes
  - Growth factors and promoting factors for fibroblasts and blood vessels- thus macrophages may modulate a formation of non-specific granulation tissue
  - Cytokines, such as interleukin I , TNF,etc.


Chemotactic mediators (cytokines, others)
Cytokines
Recruitment
Division
Immobilization
Collection of macrophages

- **PLASMA CELLS**
  - Produce antibodies directed against persistent antigens or against altered tissue components

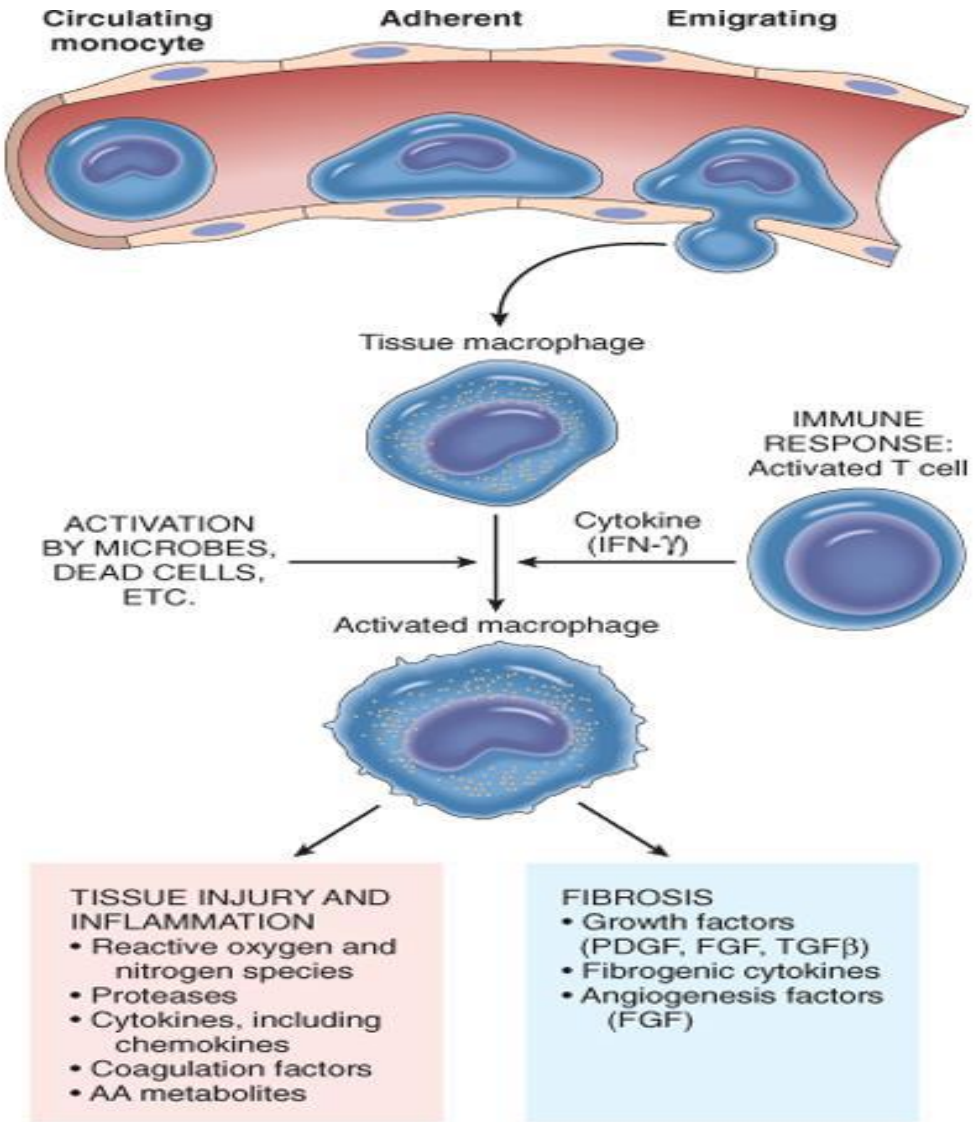
- **LYMPHOCYTES**
  - When activated by the contact with antigen, lymphocytes release cytokines- many of them stimulate macrophages

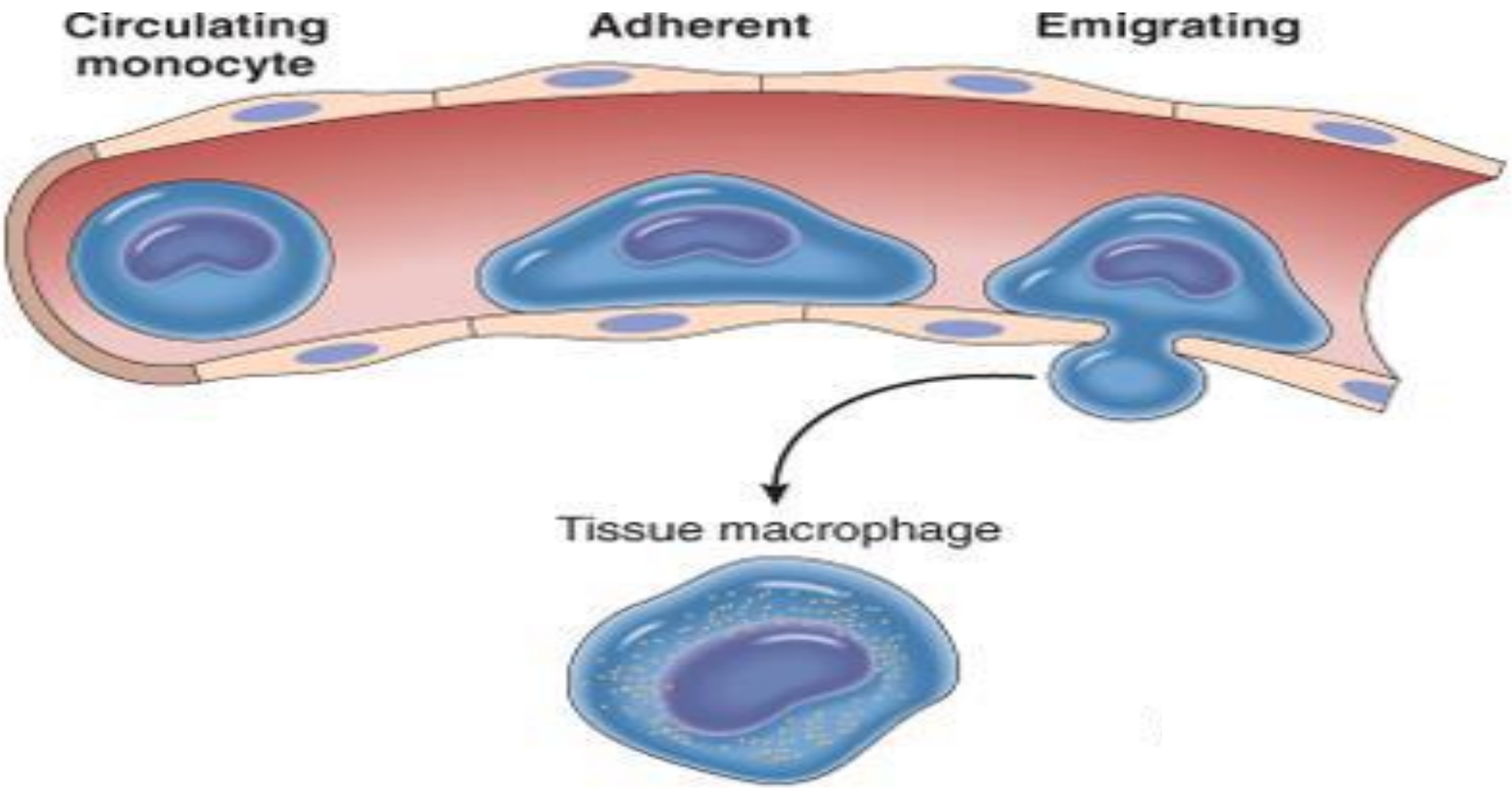
- **EOSINOPHILS**
  - characteristic of immunologic reaction mediated by IgE and of parasitic infections

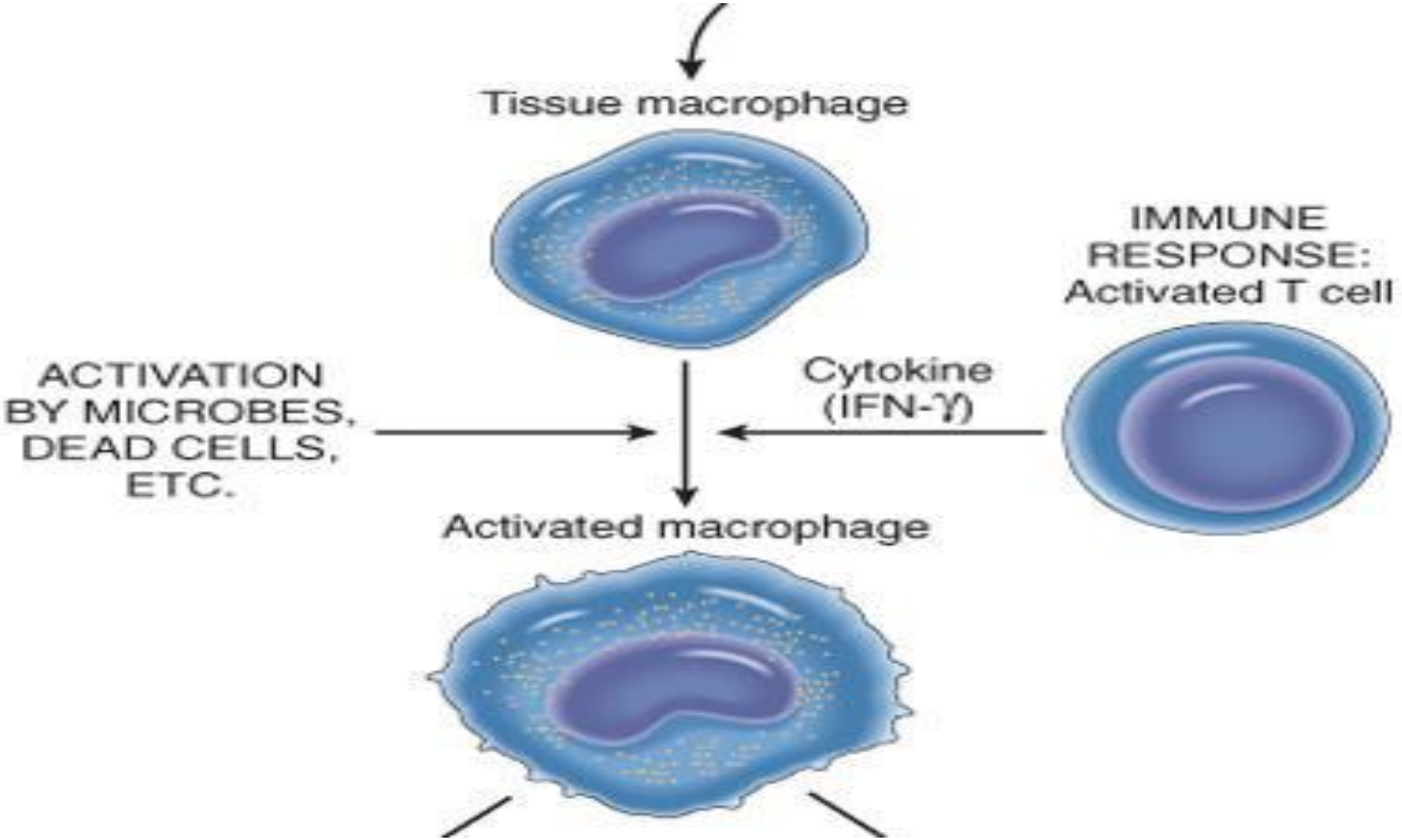
- **NEUTROPHILIC LEUKOCYTES**
  - In chronic inflammation of bone marrow (osteomyelitis)- large numbers of neutrophils may persists for months
  - chronic inflammation of fallopian tube may have the pattern of chronic suppuration with large numbers of neutrophils

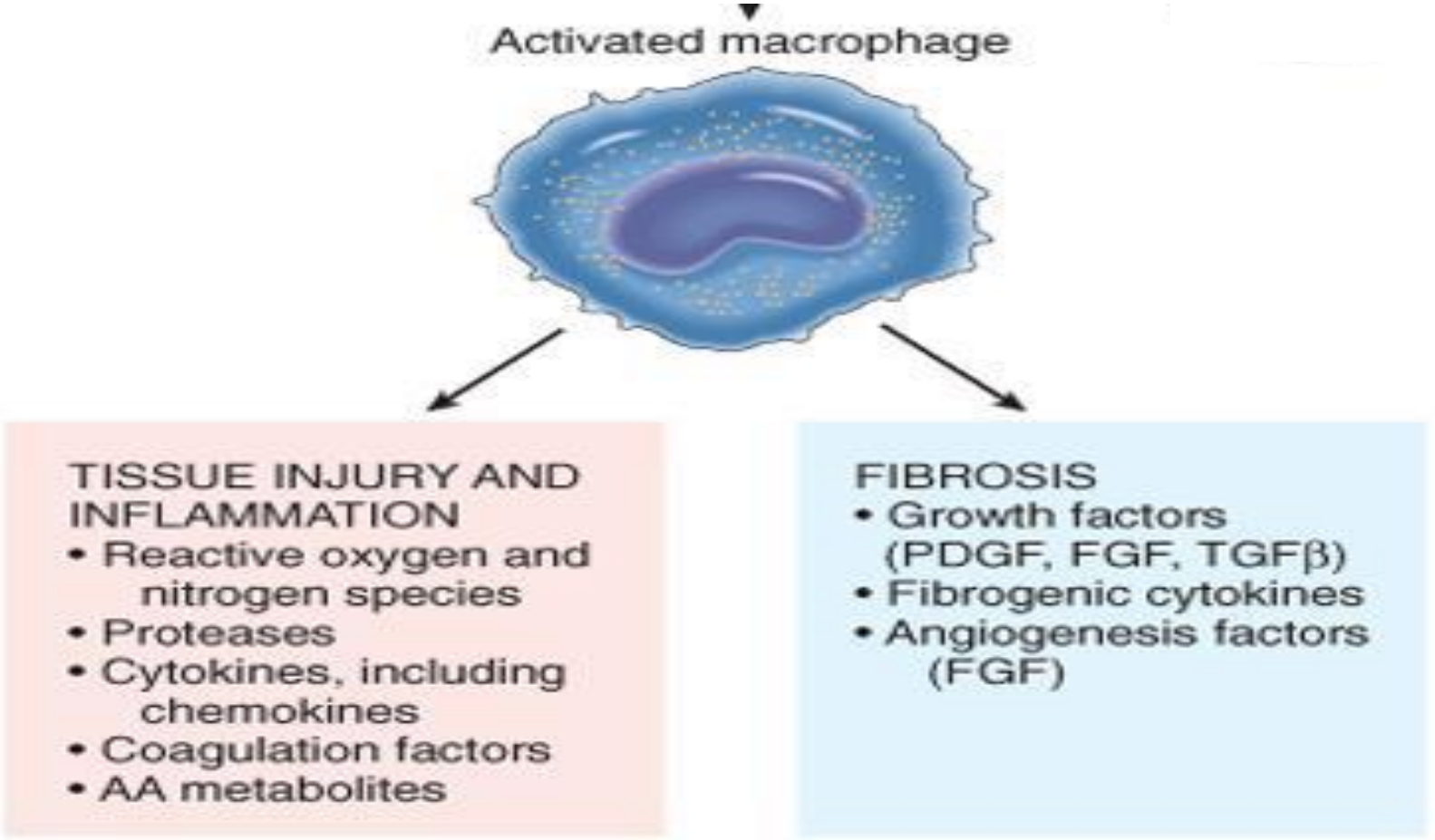
- **FIBROBLASTS**
  - Fibroproduction and accumulation of extracellular proteins- characteristic features of chronic inflammatory response

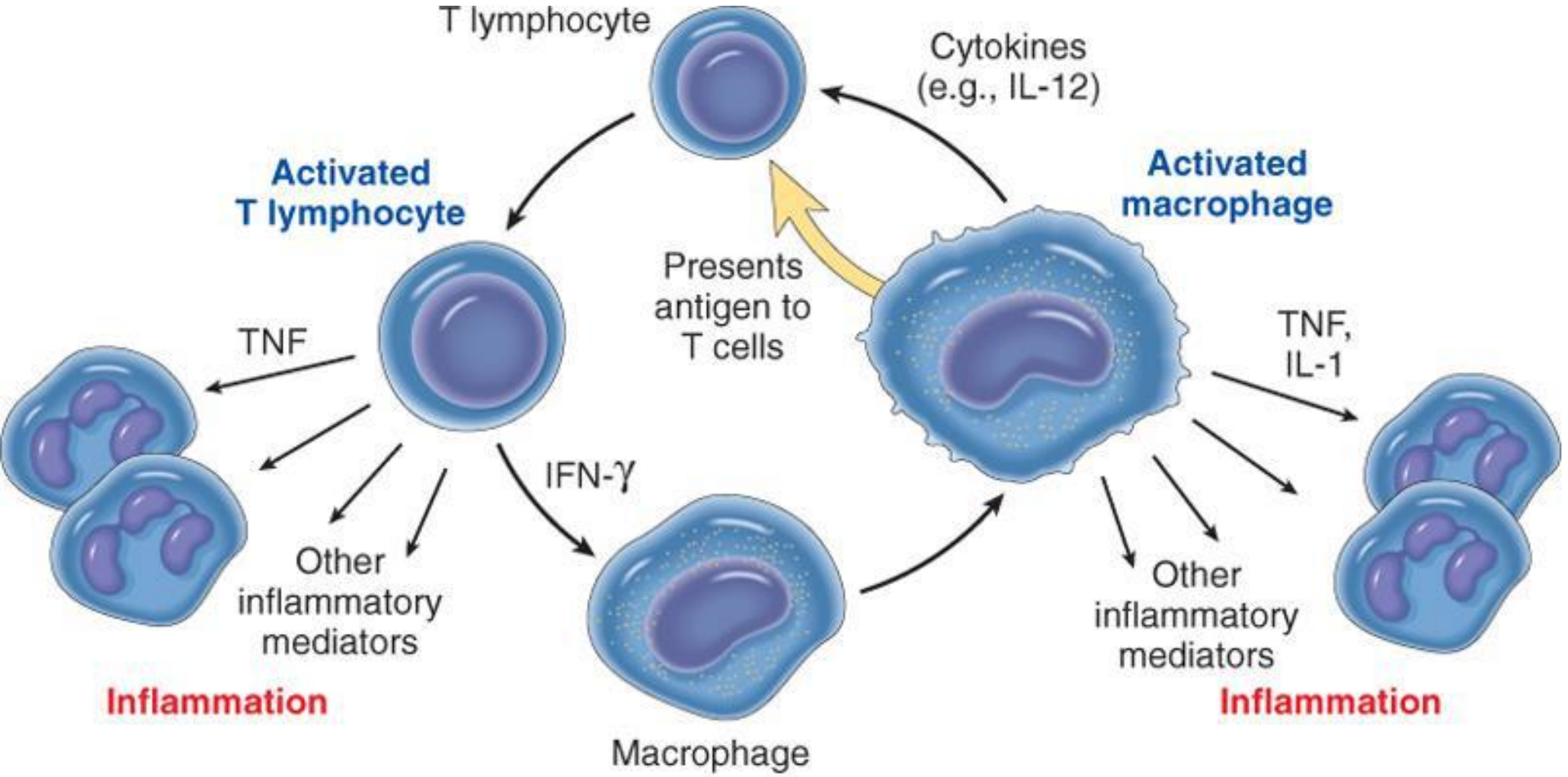
Pathogenesis




Circulating monocyte
Adherent
Emigrating
Tissue macrophage


Tissue macrophage
IMMUNE RESPONSE: Activated T cell
ACTIVATION BY MICROBES, DEAD CELLS, ETC.
Cytokine (IFN-γ)
Activated macrophage





Granulomatous inflammation

- Distinctive pattern of chronic inflammatory reaction
- Characterized by focal accumulation of activated macrophages
- which often develop an epithelial – like (**epithelioid**) appearance

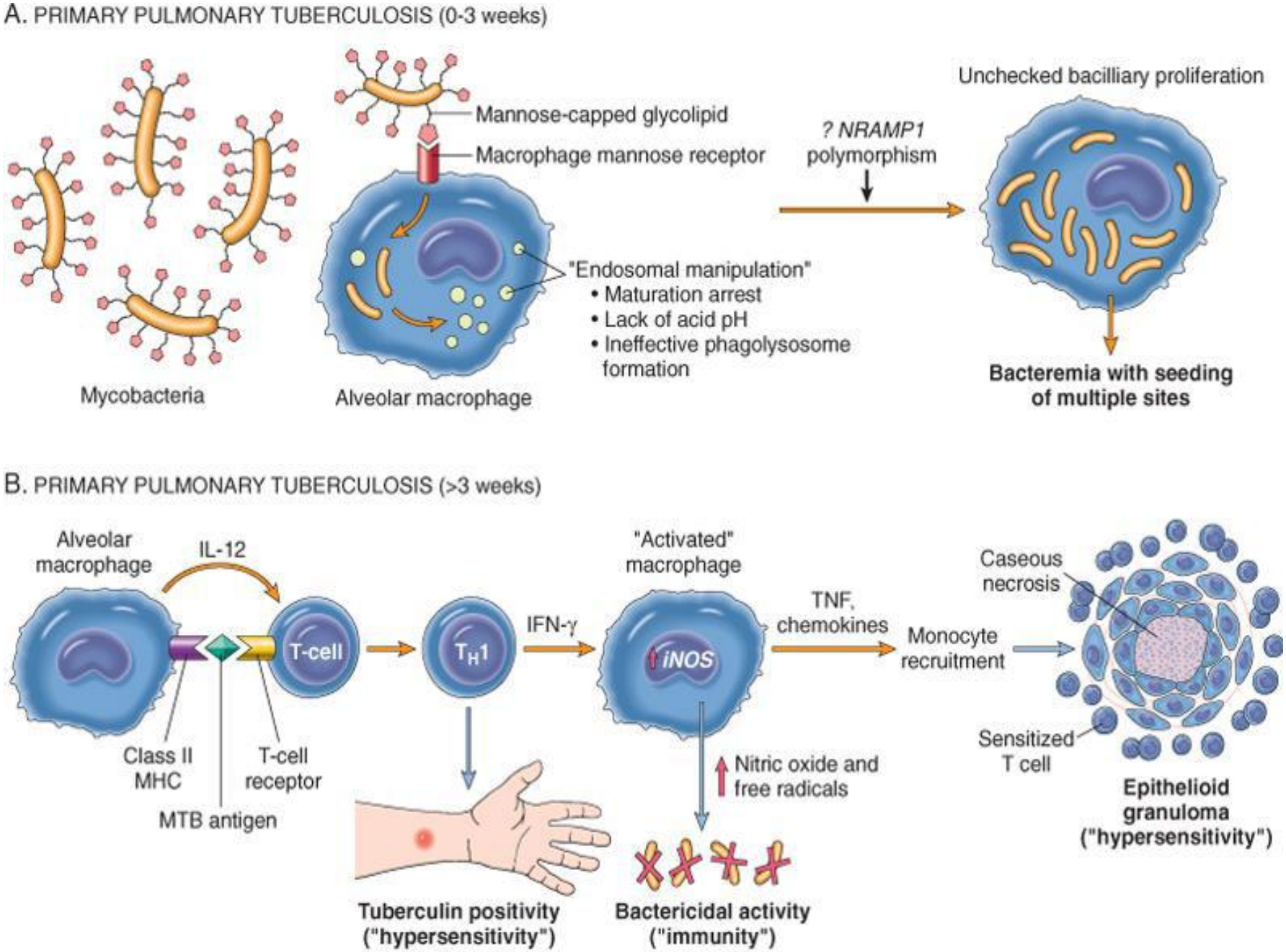
Causes of granulomatous inflammation

Bacterial	: Tuberculosis, leprosy
Fungal	: Rhinosporidiosis
Parasitic	: Schistosomiasis
Foreign bodies	: Suture material
Autoimmune	: Wegener's granulomatous
Unknown	: Sarcoidosis

Commonest cause : Tuberculosis, Leprosy, Syphilis, Cat scratch disease

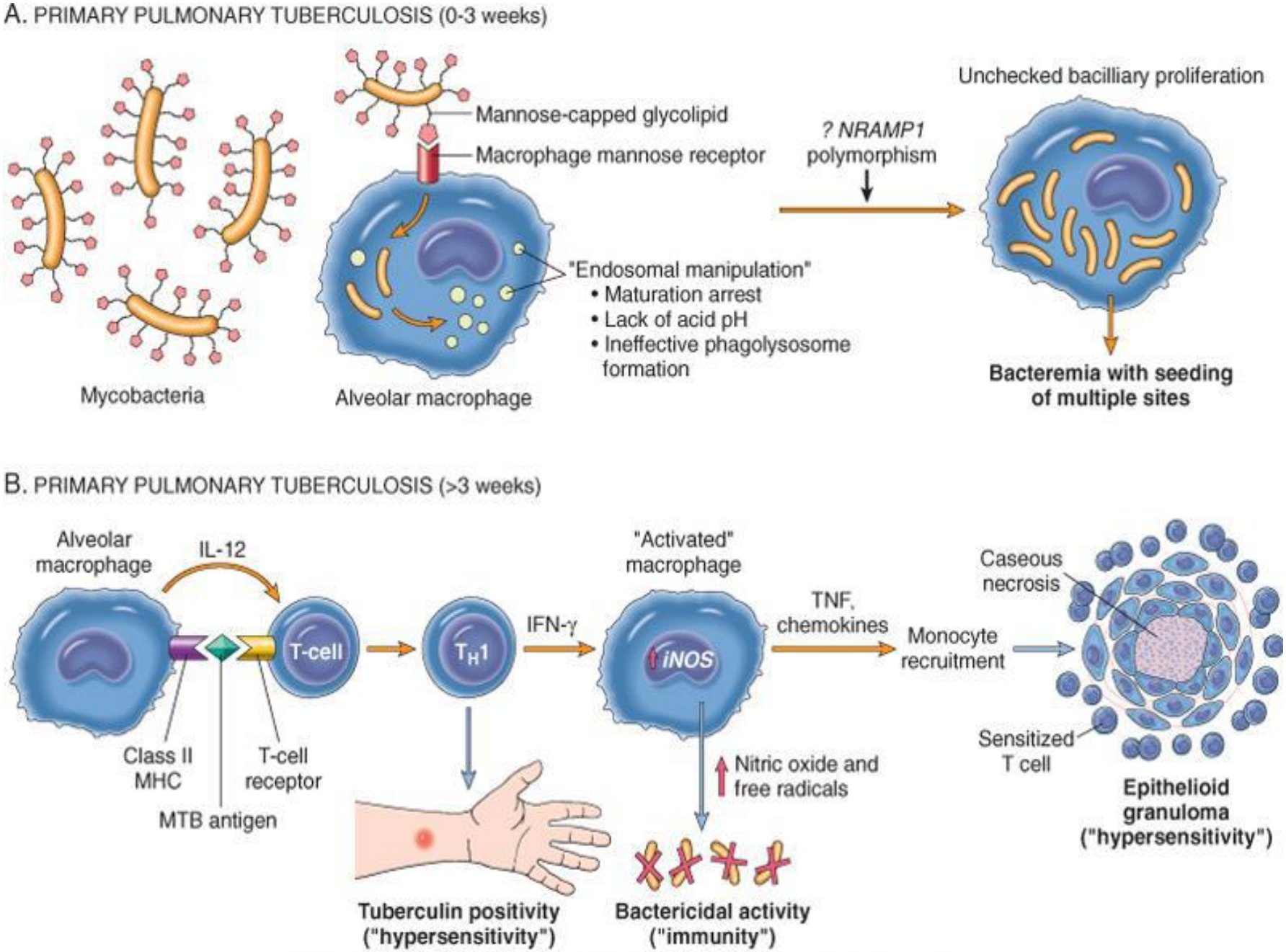
Pathogenesis

- Prototypic disease : Tuberculosis
- Mycobacterum tuberculosis enters macrophages by endocytosis
- Mediated through several macrophage receptors such as
  - Mannose receptors that bind to lipoarabinomannan (LAM) and
  - Complement receptors that bind to opsonised organisms



- Mycobacteria replicate within the macrophage by blocking
  - the formation of phagolysosome by inhibition of calcium signal
  - The recruitment and assembly of proteins which cause the formation of phagolysosome
- Proliferation of bacteria within the macrophages

  (Patients at this stage are asymptomatic or have flu like illness)



2. Proliferation of bacteria within macrophages

↓

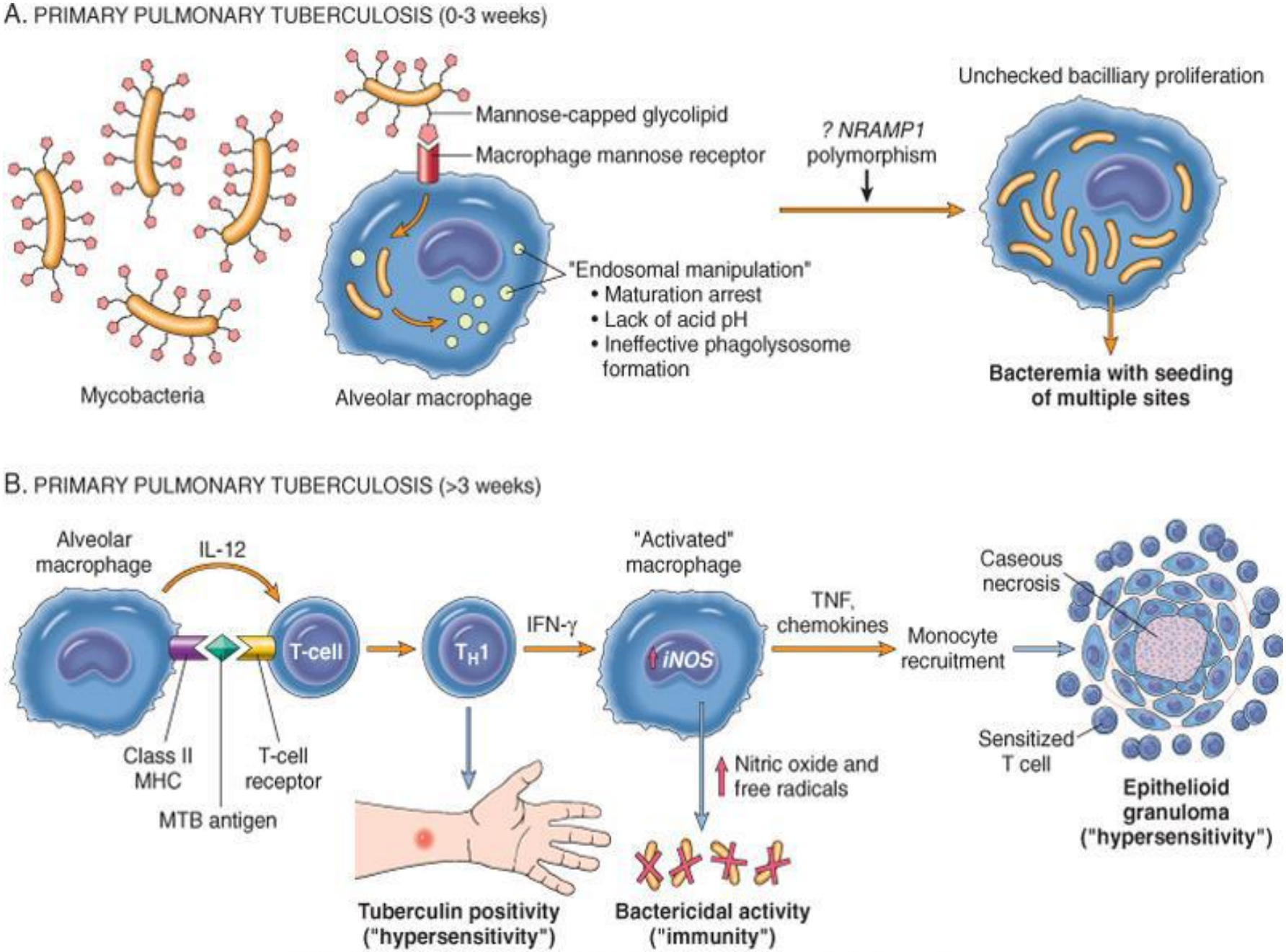
Differentiation of T_H1 cells under the influence of IL-12

↓

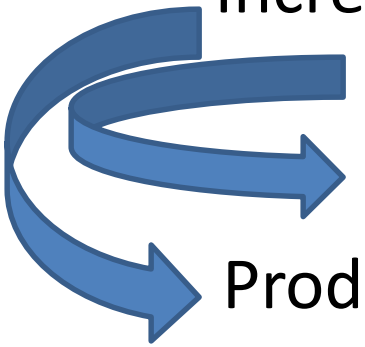
T_H1 cells activates macrophages to become bactericidal by producing ϒ-IFN

↓

Macrophages ⟶ Activated macrophages



- Activation of macrophages associated with
  - Formation of phagolysosome
  - Increased NO
  - Increased free radicals



Contribute to killing of mycobacteria

Production of TNF

↓

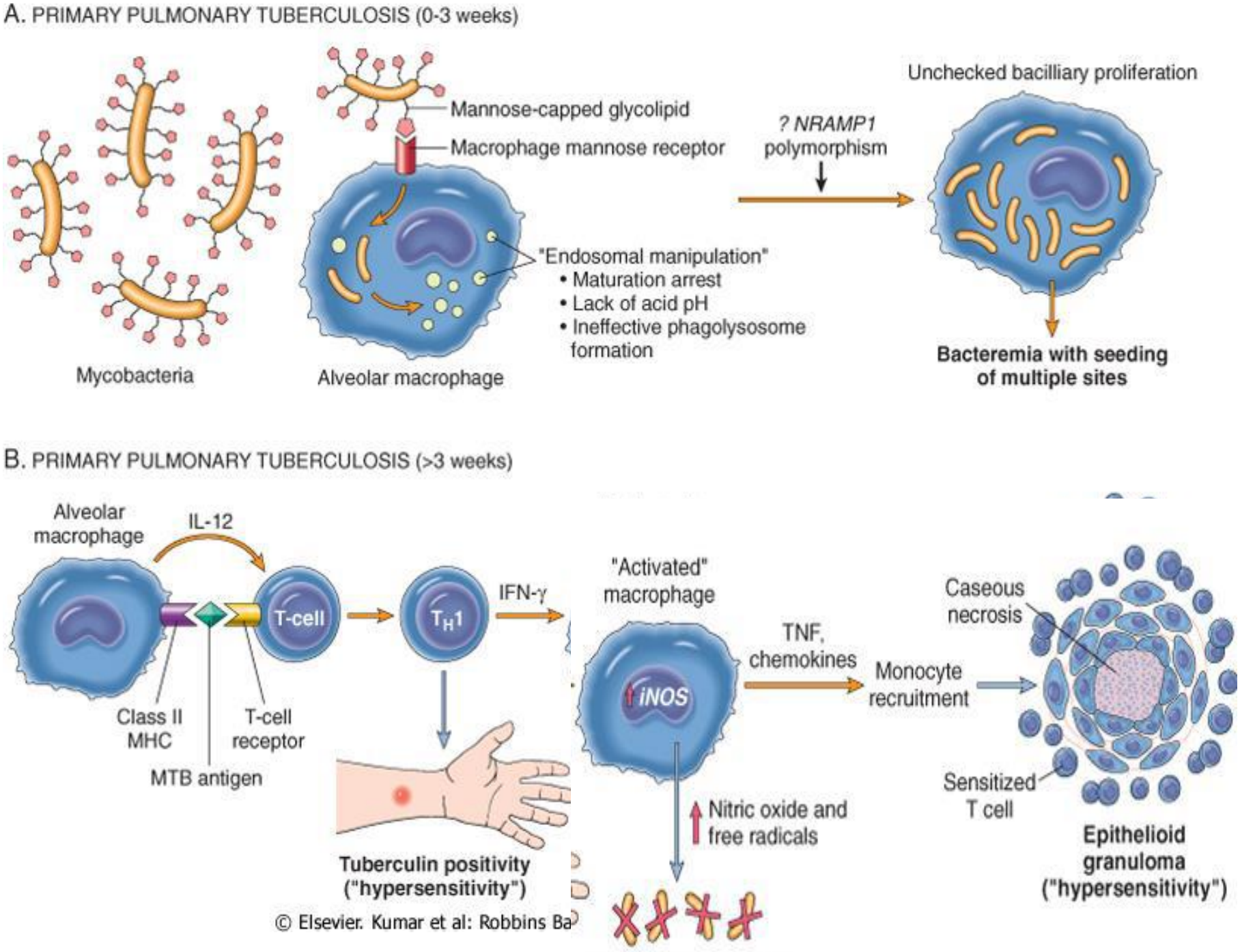
Monocyte recruitment

↓

Differentiation of monocytes into epitheloid cells

↓

Granulomatous response (Hypersensitivity)


A. PRIMARY PULMONARY TUBERCULOSIS (0-3 weeks)
Mycobacteria
Mannose-capped glycolipid
Macrophage mannose receptor
Alveolar macrophage
"Endosomal manipulation"
• Maturation arrest
• Lack of acid pH
• Ineffective phagolysosome formation
? NRAMP1 polymorphism
Unchecked bacilliary proliferation
Bacteremia with seeding of multiple sites
B. PRIMARY PULMONARY TUBERCULOSIS (>3 weeks)
Alveolar macrophage
IL-12
T-cell
T_H1
IFN-γ
Class II MHC
T-cell receptor
MTB antigen
Tuberculin positivity ("hypersensitivity")
"Activated" macrophage
iNOS
TNF, chemokines
Monocyte recruitment
Nitric oxide and free radicals
Caseous necrosis
Sensitized T cell
Epithelioid granuloma ("hypersensitivity")
© Elsevier. Kumar et al: Robbins Ba

Granuloma

- Focus of chronic inflammation consisting of microscopic aggregation of epitheloid cells surrounded by collar of mononuclear leukocytes and occasionally plasma cells

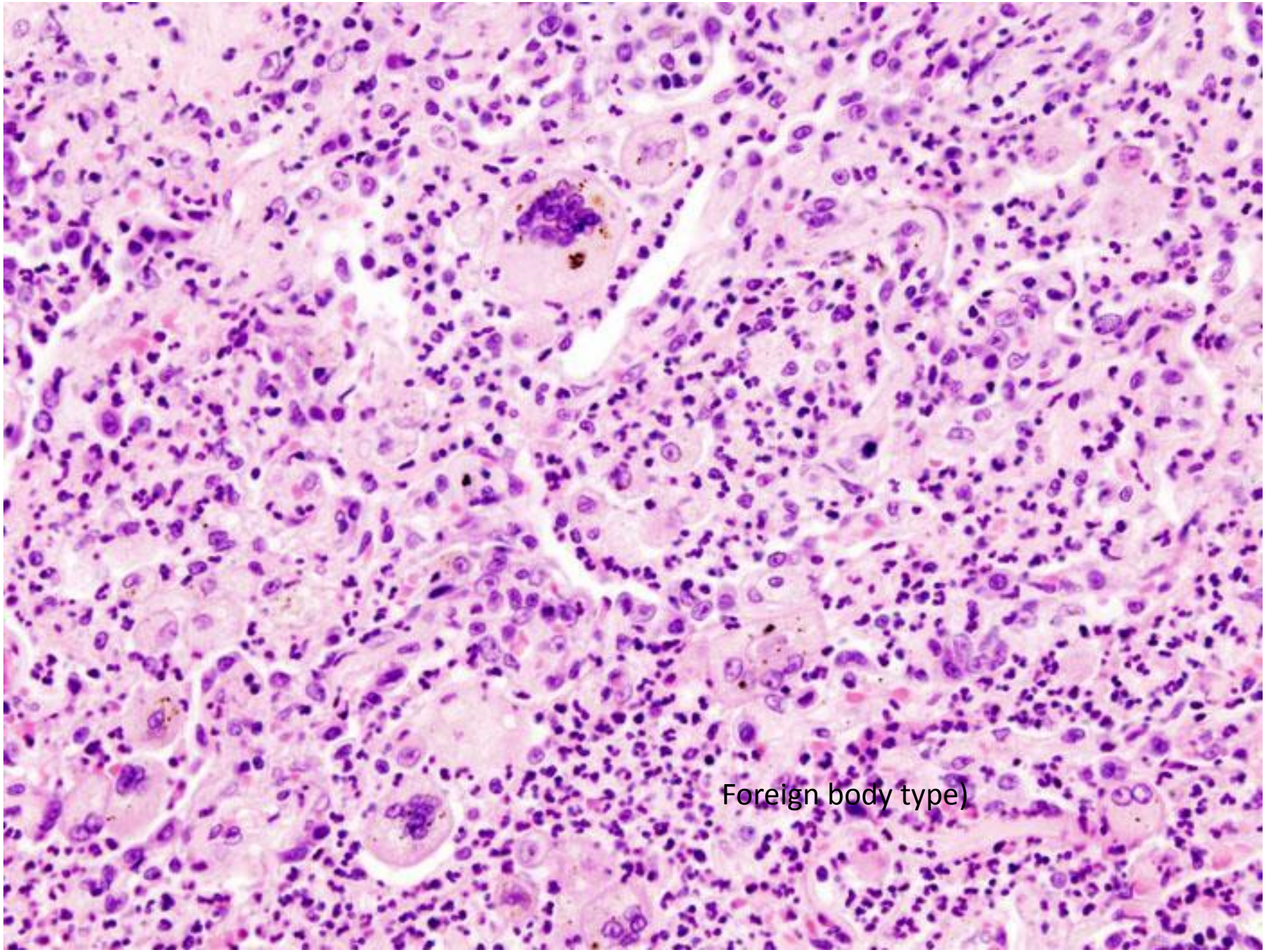
Histological features of granuloma

- Epithelioid cells:
  - Activated macrophages
  - Pale pink granular cytoplasm with indistinct cell boundaries, often appearing to merge into another
  - The nucleus is less dense, oval or elongated and may show folding of nuclear membrane. (slipper shaped)

- Epithelioid cells fuse to form giant cells

  – The giant cells may attain diameter of 40-50 micrometer

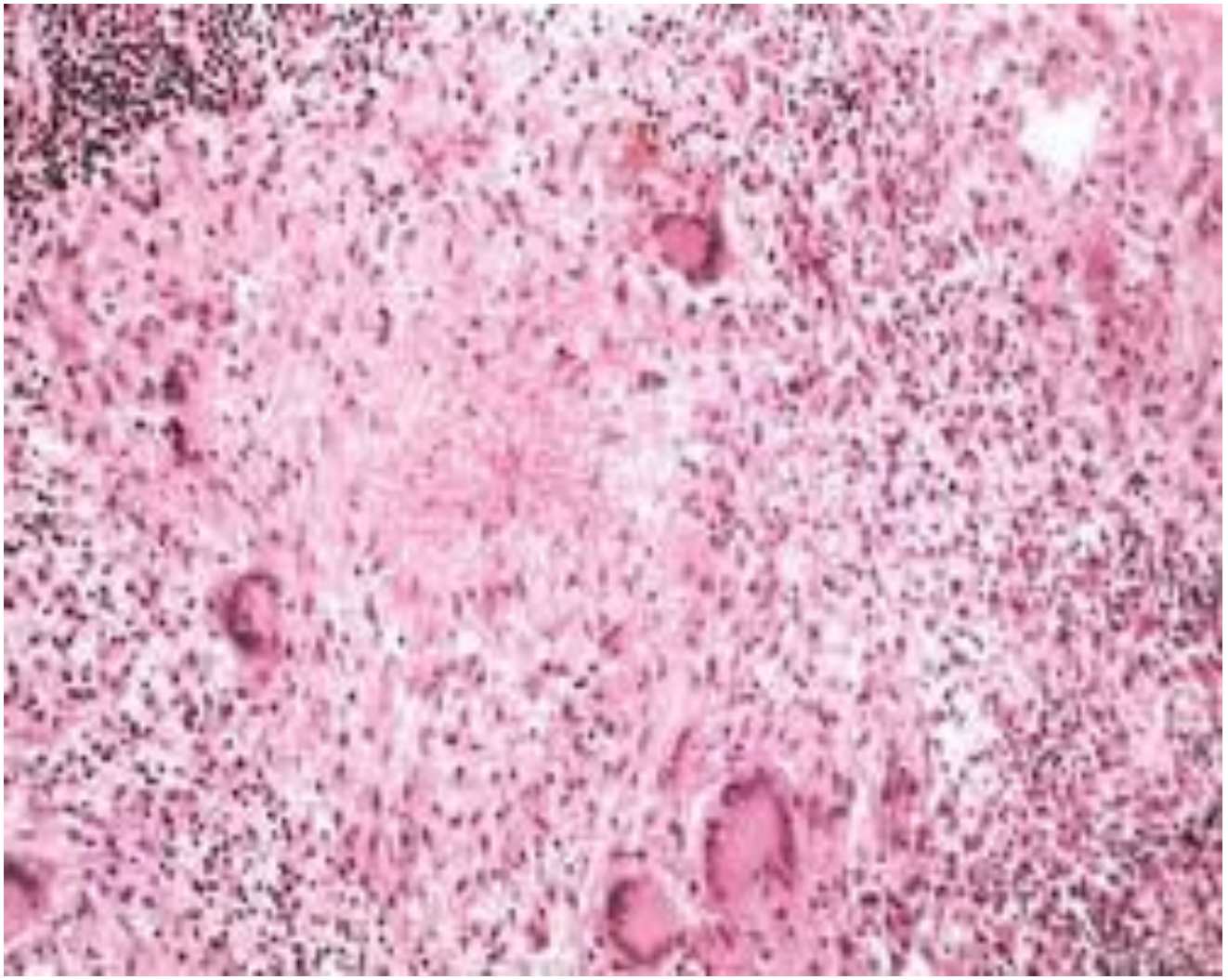
  – They contain abundant cytoplasm containing 20 or more small nuclei arranged either
    - Peripherally (Langhans-type giant cells) or
    - Haphazardly (foreign body type- giant cell)


Foreign body type)

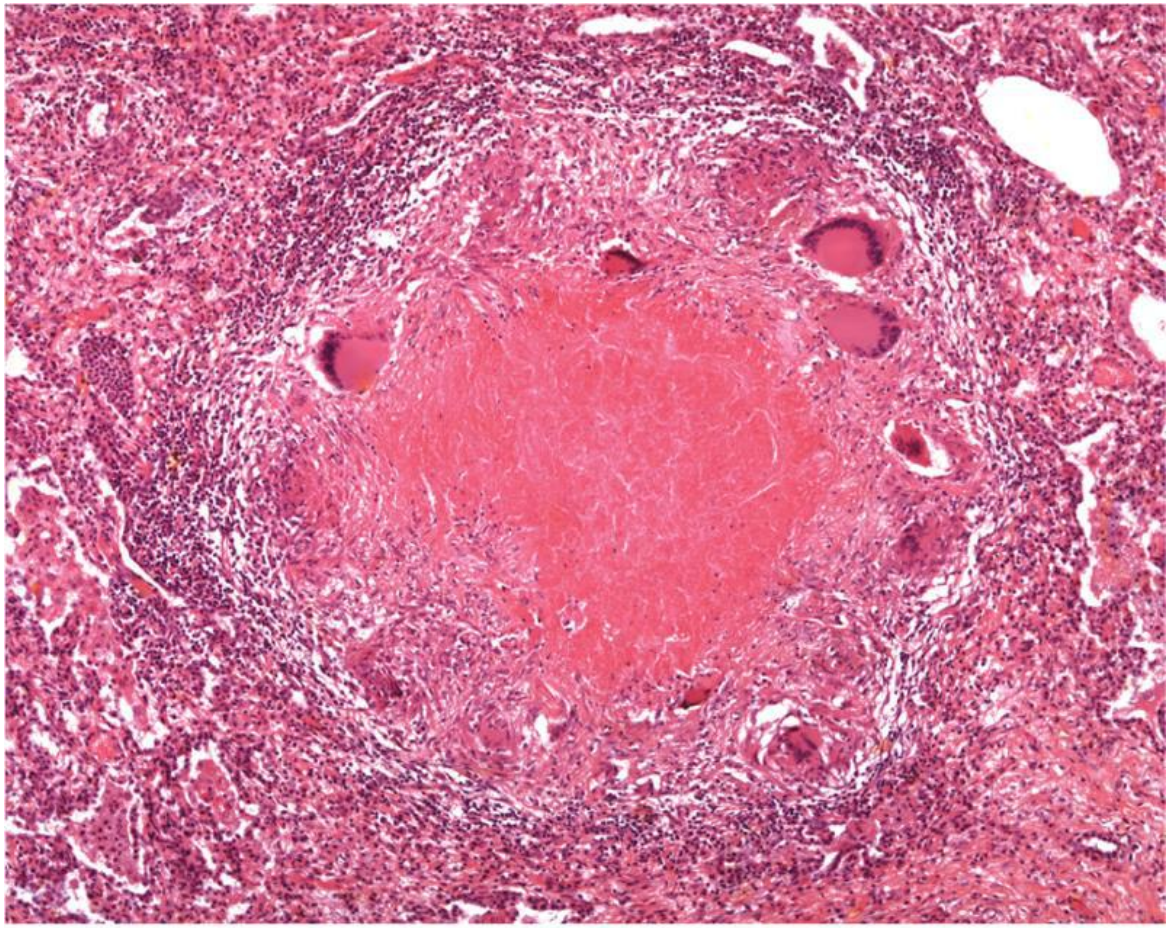
Granuloma

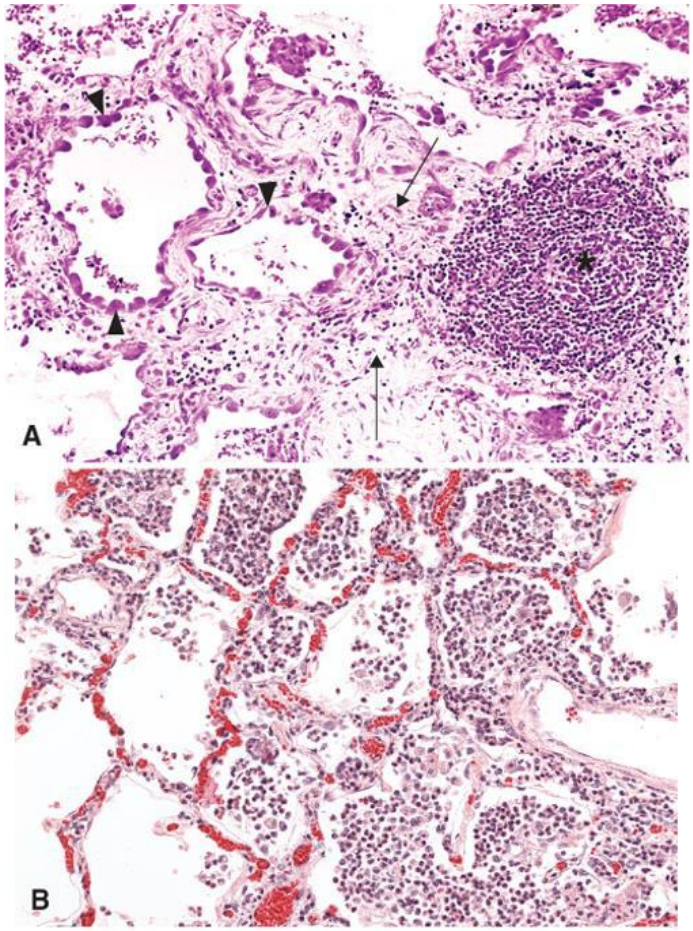
CASEATING (TB)

NON-CASEATING (Sarcoidosis)



Typical tuberculous granuloma showing an area of central necrosis, epithelioid cells, multiple Langhans-type giant cells, and lymphocytes.





SYSTEMIC MANIFESTATIONS OF CHRONIC INFLAMMATION

- **FEVER, CHILLS:**
  - **cytokines(TNF,IL-1) → stimulate Prostaglandin in hypothalamus**
- **C-Reactive Protein (CRP) synthesis**
  - **stimulated by cytokines**
- **Erythrocyte Sedimentation Rate (ESR) increases**
- **Leukocytosis**
  - **colony stimulating factor acting on bone marrow**
- **Pulse, Blood Pressure, low in septic shock**
  - **due to high level of TNF**

The-end