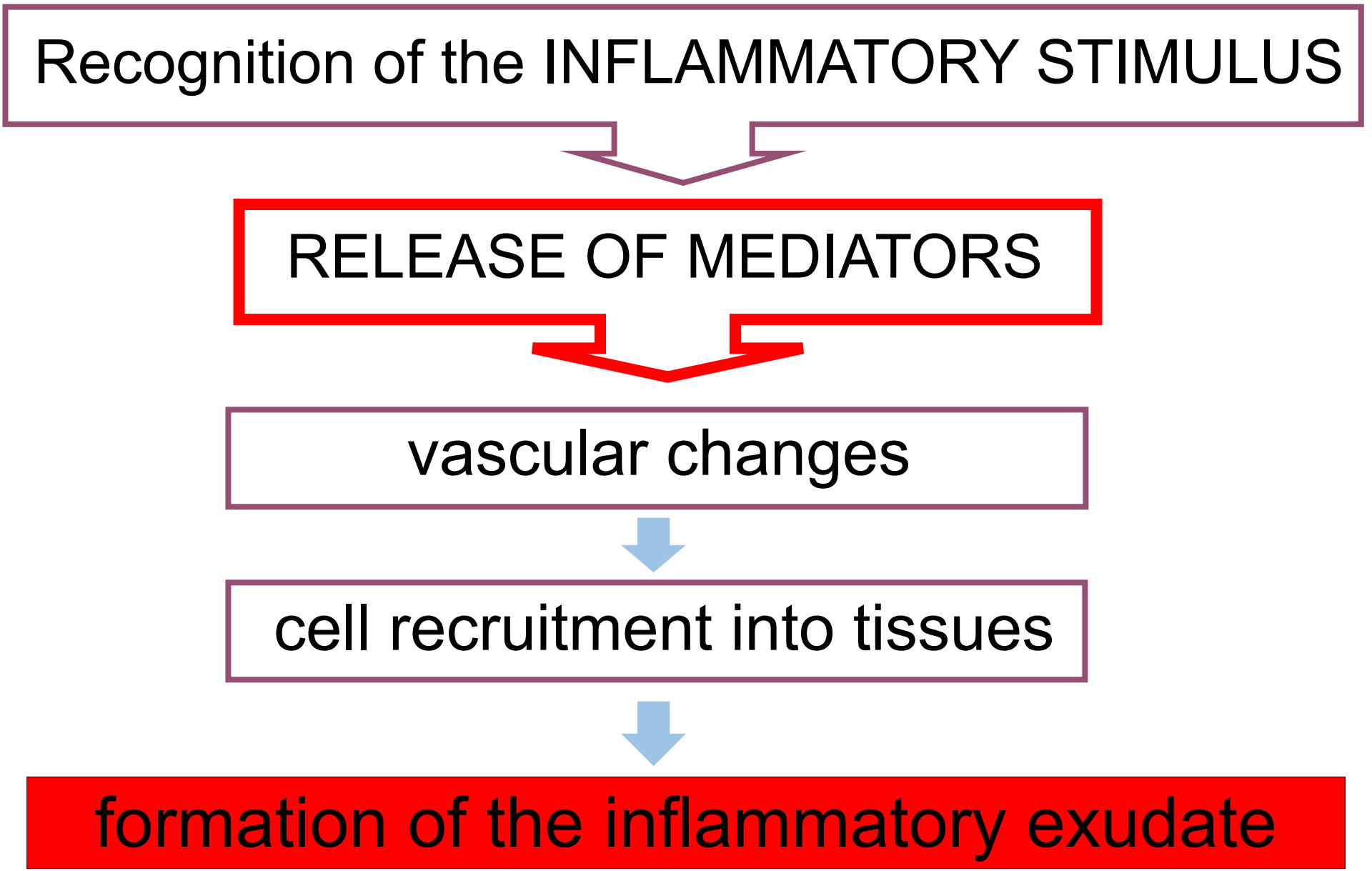
Main events in acute inflammation



Phase 2: release of **inflammatory mediators**

- Mediators are **essentials** for the development of inflammation
- Represent the "chemical language" of inflammation, as they **timely regulate all the flogistic events**
- May be **produced locally** (by **cells** at the site of inflammation), or may be **derived from circulating inactive precursors** (typically synthesized by the liver) that are activated in inflamed area
- **Most** mediators act by **binding to specific receptors** on different target cells; others (e.g., lysosomal proteases, ROS) have **direct enzymatic and/or toxic activities**
- Their actions are **tightly regulated** and usually **shortlived**
- Knowing **how** they are generated and act, has helped to design many **anti-inflammatory drugs**

Principal mediators of inflammation

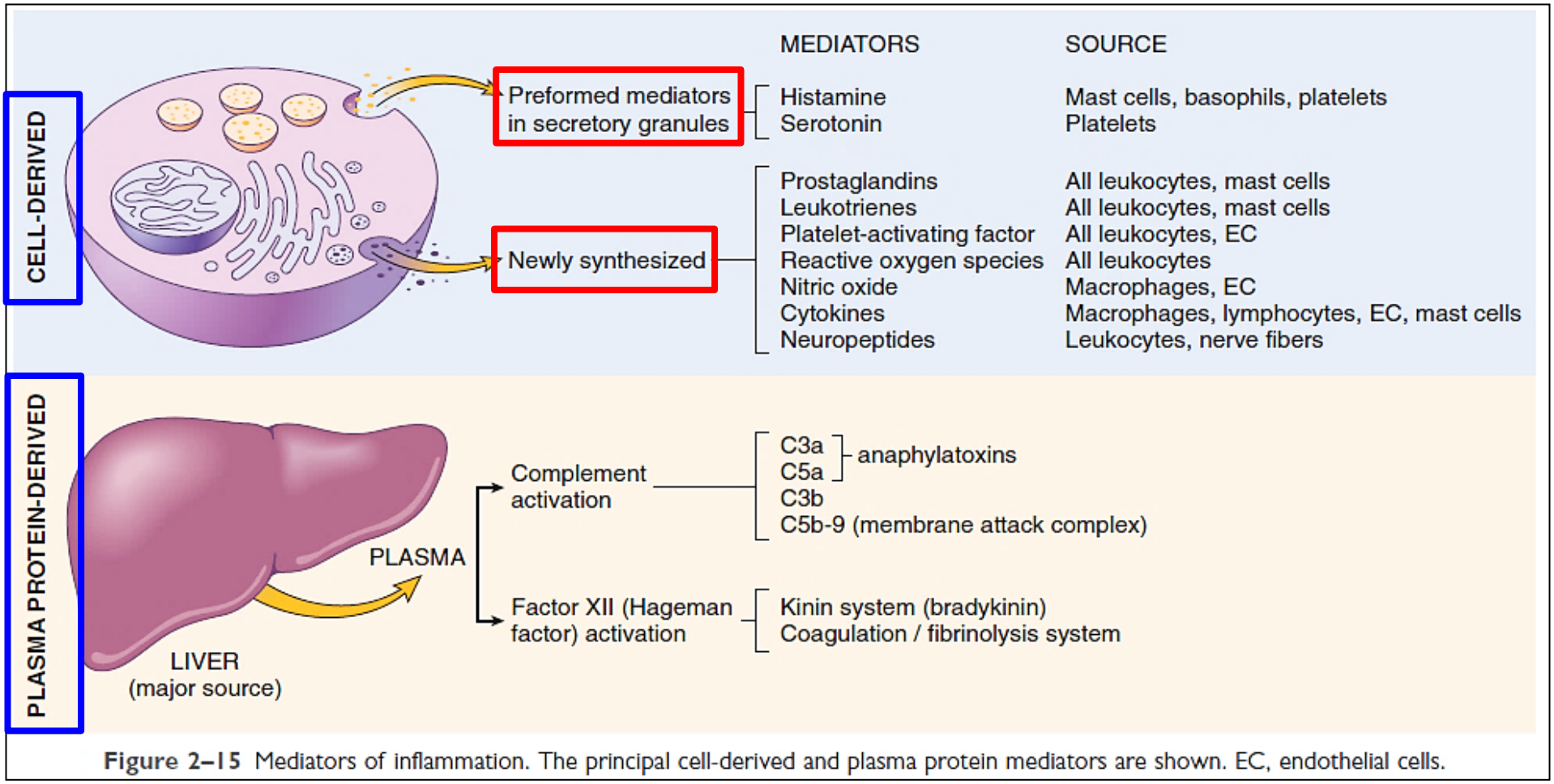


Figure 2–15 Mediators of inflammation. The principal cell-derived and plasma protein mediators are shown. EC, endothelial cells.

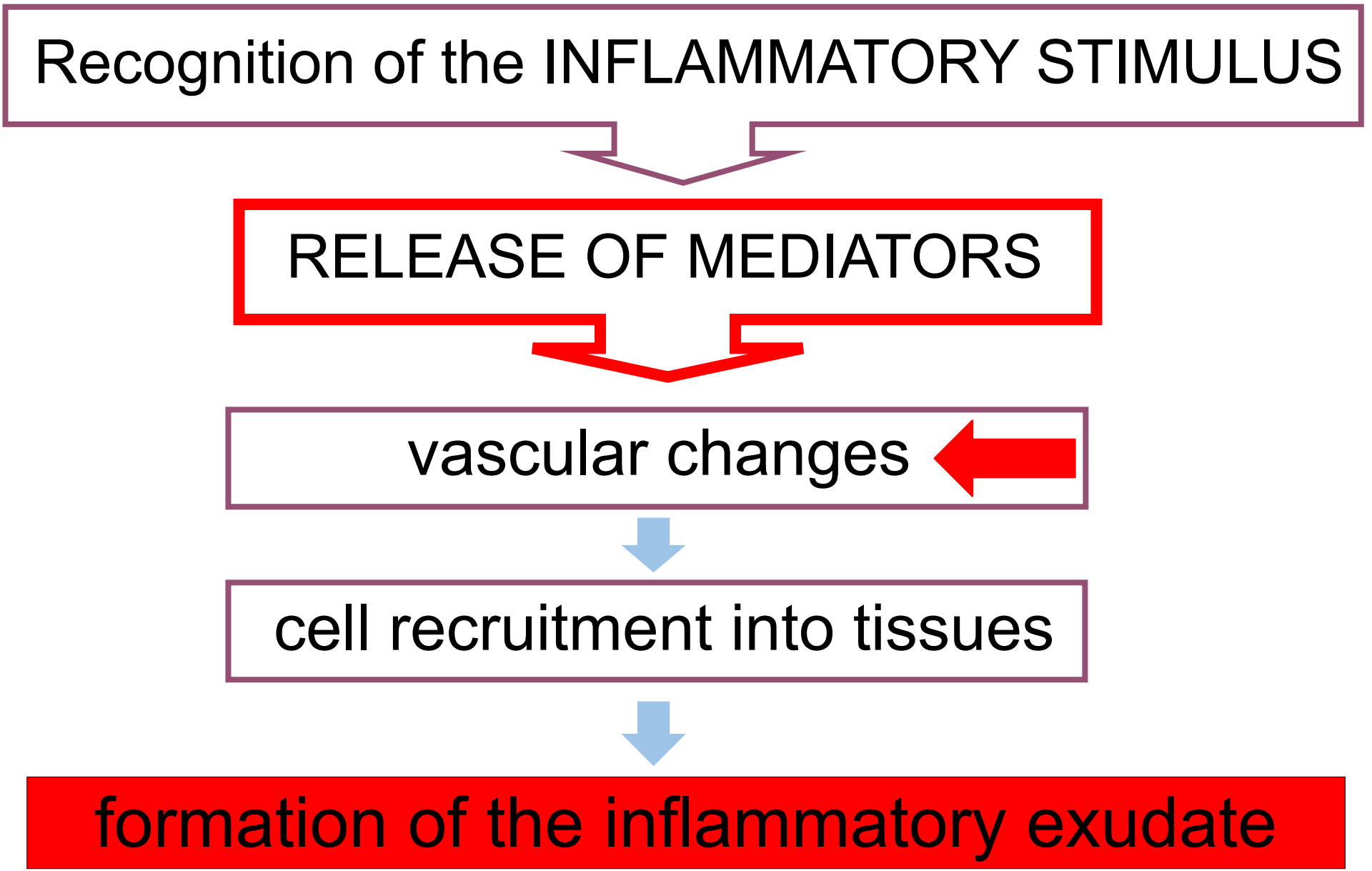
Sources and **actions** of the principal inflammatory mediators

Table 2–5 Actions of the Principal Mediators of Inflammation

Mediator	Source(s)	Actions
Cell-Derived		
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Serotonin	Platelets	Vasoconstriction
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion and activation
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Reactive oxygen species	Leukocytes	Killing of microbes, tissue damage
Nitric oxide	Endothelium, macrophages	Vascular smooth muscle relaxation; killing of microbes
Cytokines (TNF, IL-1, IL-6)	Macrophages, endothelial cells, mast cells	*Local*: endothelial activation (expression of adhesion molecules). *Systemic*: fever, metabolic abnormalities, hypotension (shock)
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation
Plasma Protein–Derived		
Complement	Plasma (produced in liver)	Leukocyte chemotaxis and activation, direct target killing (MAC), vasodilation (mast cell stimulation)
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain
Proteases activated during coagulation	Plasma (produced in liver)	Endothelial activation, leukocyte recruitment

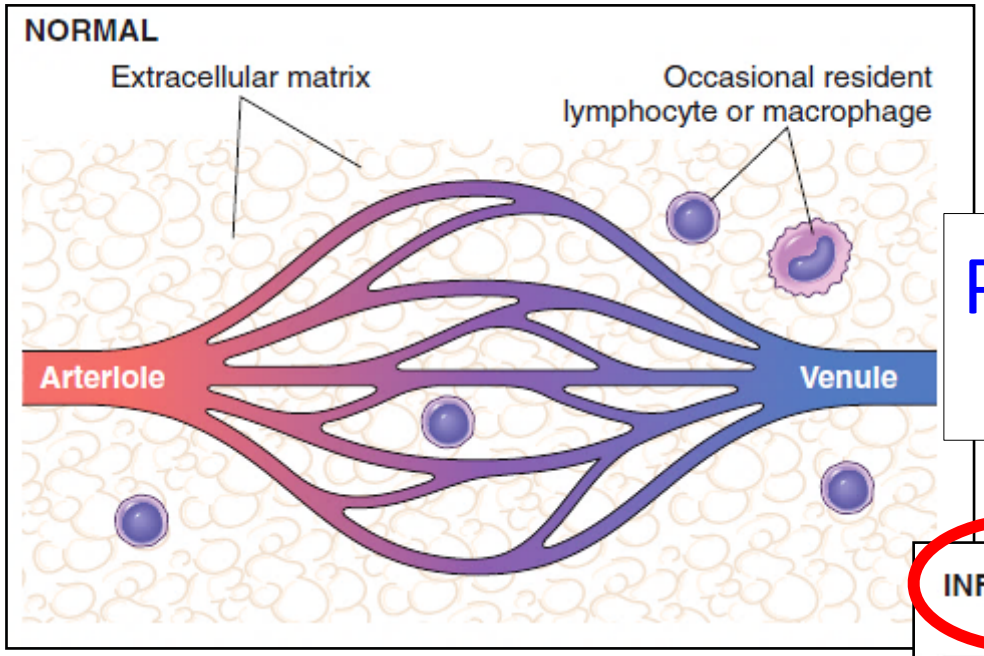
IL-1, IL-6, interleukin-1 and -6; MAC, membrane attack complex; TNF, tumor necrosis factor.

Main events in acute inflammation



Phase 3: **vascular reactions**

- transient **vasoconstriction** (**if** occurs, lasts only few seconds)
- **arteriolar vasodilation**: locally increased blood flow and hydrostatic pressure; engorgement of the down-stream capillary beds [**erythema** (*rubor*), **warmth** (*calor*)]
- **increased vascular permeability**: microvasculature becomes more permeable; protein-rich fluid moves into the extravascular tissues [**edema** (*tumor*)]
- the flowing blood becomes more concentrated, **blood viscosity increases**, slowing of the circulation [**stasis**]
- Vascular walls become **pro-adhesive** and leukocytes (principally **neutrophils**) begin to accumulate along the vascular endothelial surface [**margination**]



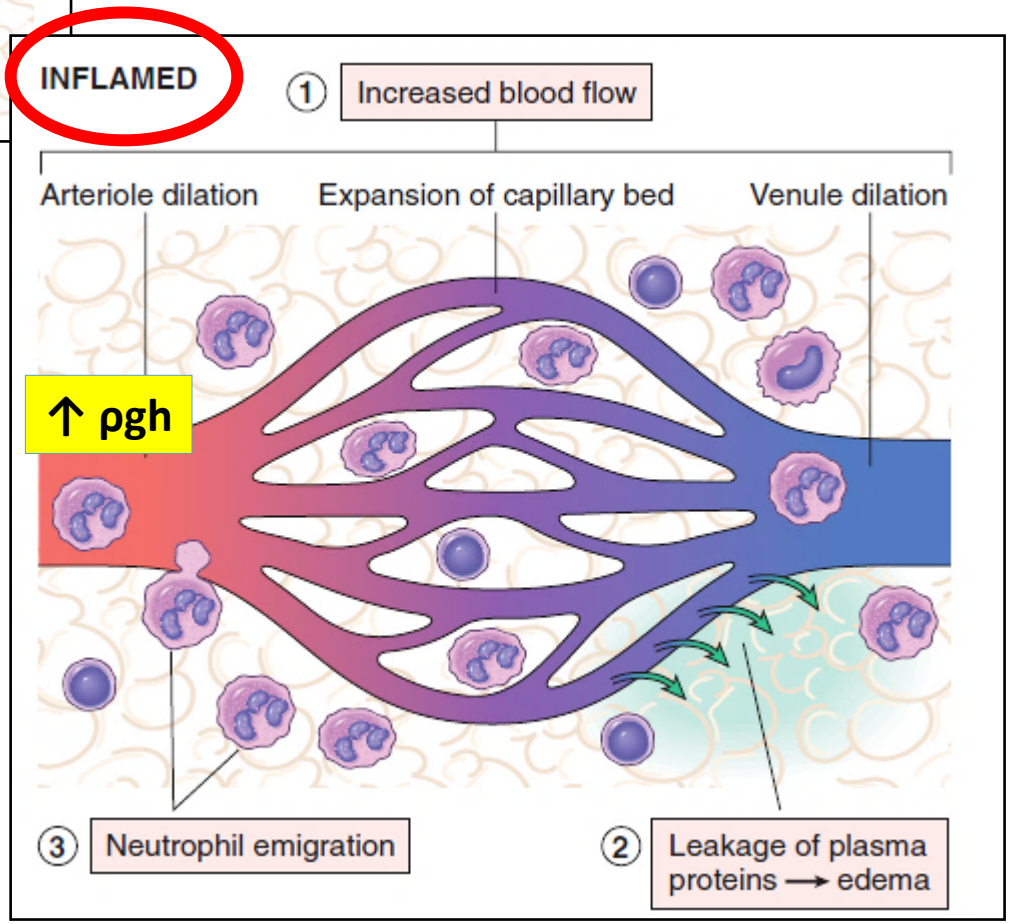
Phase 3: vascular reactions in acute inflammation

Major local manifestations of acute inflammation

(1) vascular dilation and increased blood flow (**erythema** and **warmth**); **hydrostatic pressure increases**

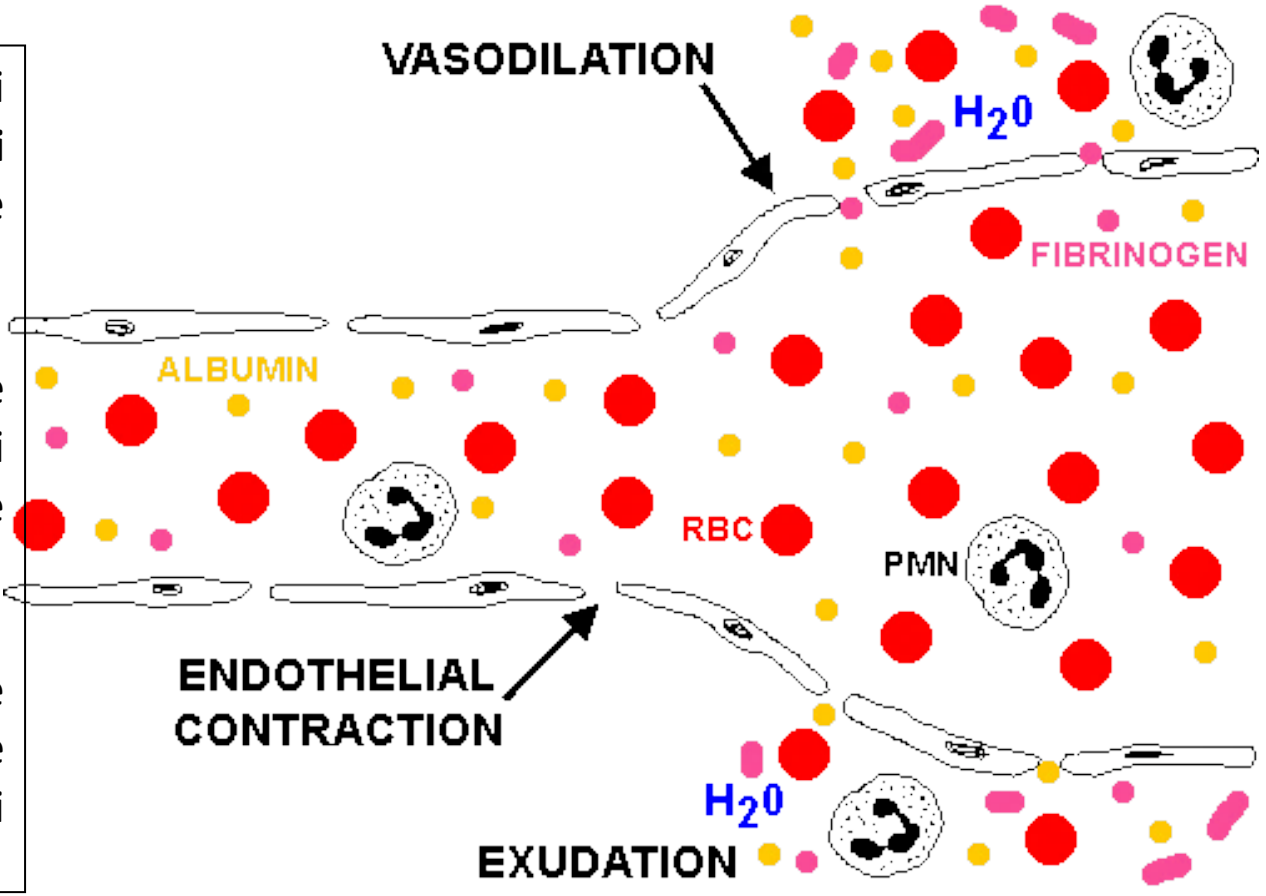
(2) extravasation of plasma fluid and proteins (**edema**); blood slows down

(3) **neutrophil** emigration and accumulation in the interstitium



Formation of **exudate** in acute inflammation: plasma proteins and neutrophils

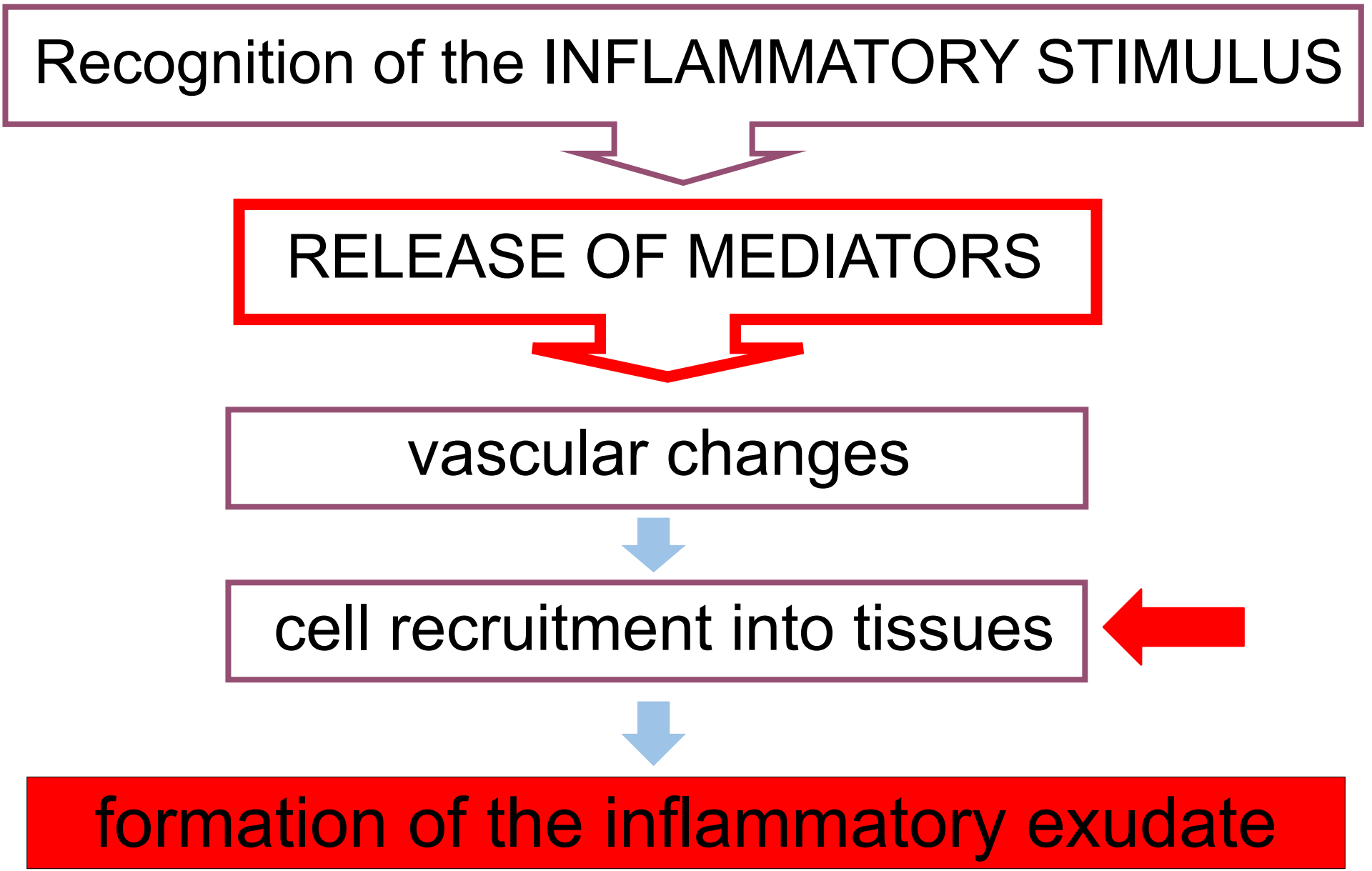
- Alcuni mediatori infiammatori interagiscono con **recettori** specifici presenti sulle cellule endoteliali
- Innesco del signaling che promuove la **fosforilazione** di proteine citoscheletriche e contrattili
- **Contrazione** delle cellule endoteliali con separazione delle giunzioni intercellulari (da 5-10 nm a 100-300 nm)



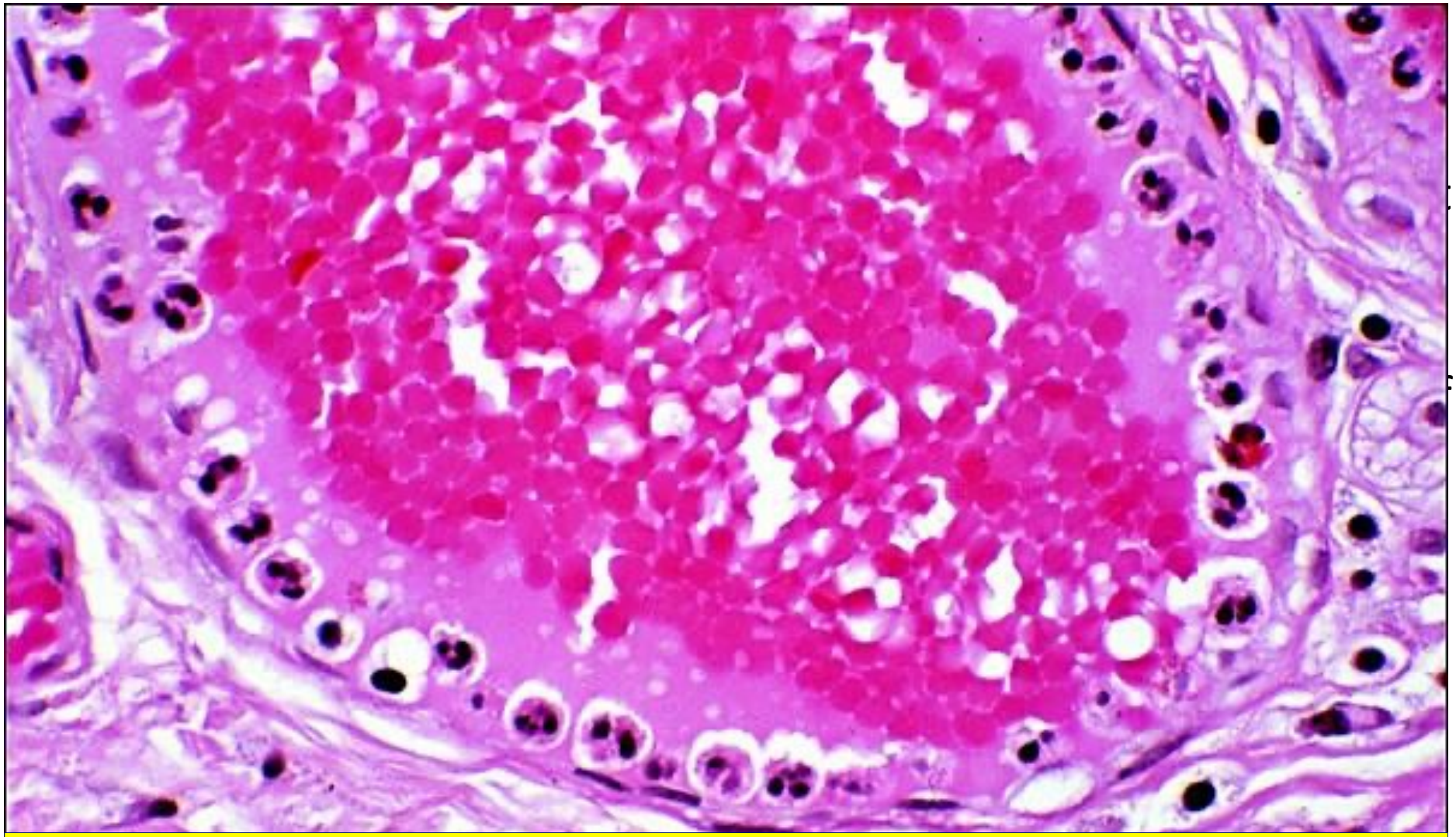
Soluble **defensive factors** move from the blood to the extra-vascular space:

- **antibodies** - **complement** fragments - **drugs**

Main events in acute inflammation



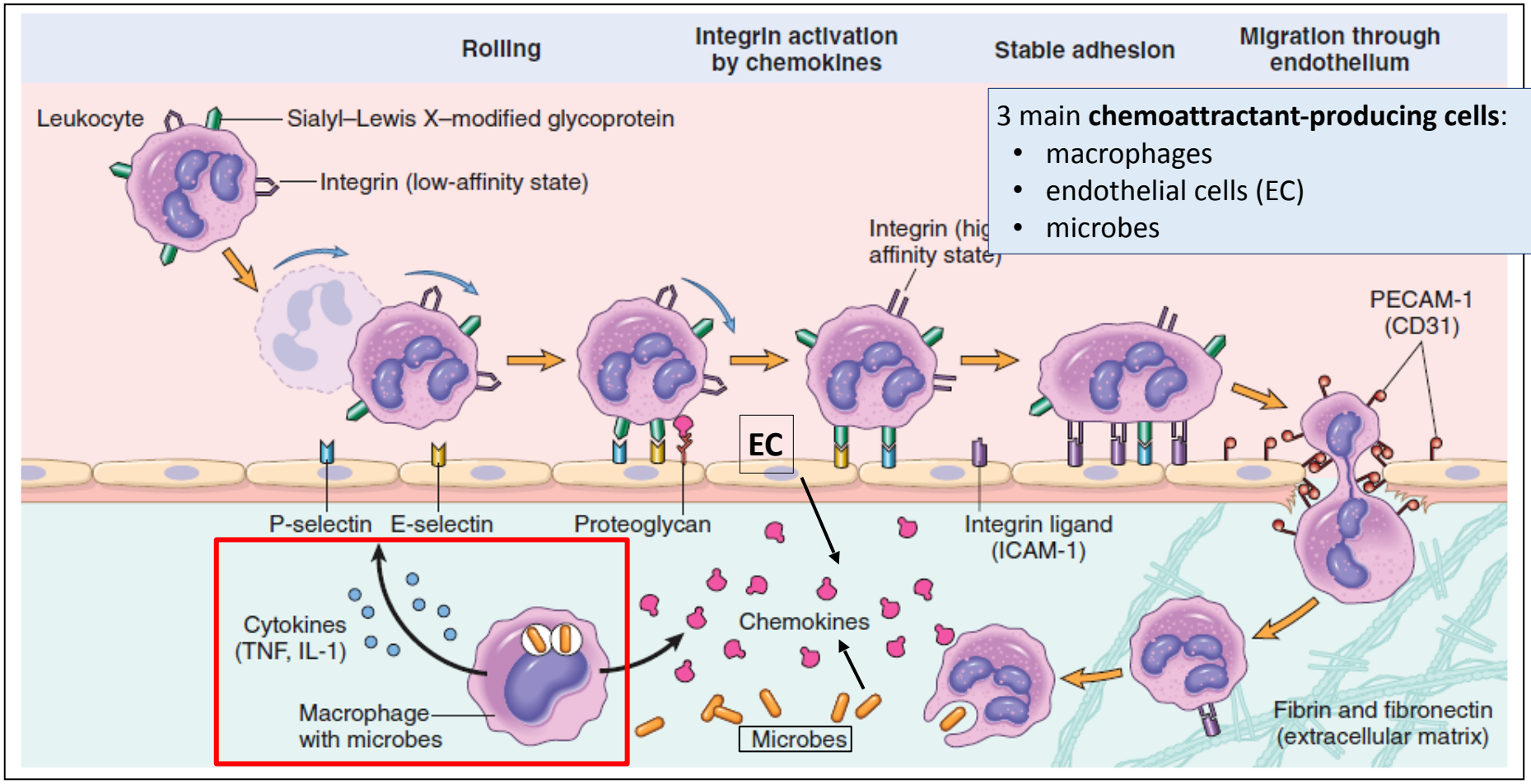
Phase 4: **leukocyte recruitment** into tissue



Post-capillary venule section in an inflamed lung:

hyperemia ⟶ **stasis** ⟶ **leukocyte margination**

Neutrophil recruitment into infected tissue: the prototype model of leukocyte migration



3 main **chemoattractant-producing cells**:

- macrophages
- endothelial cells (EC)
- microbes

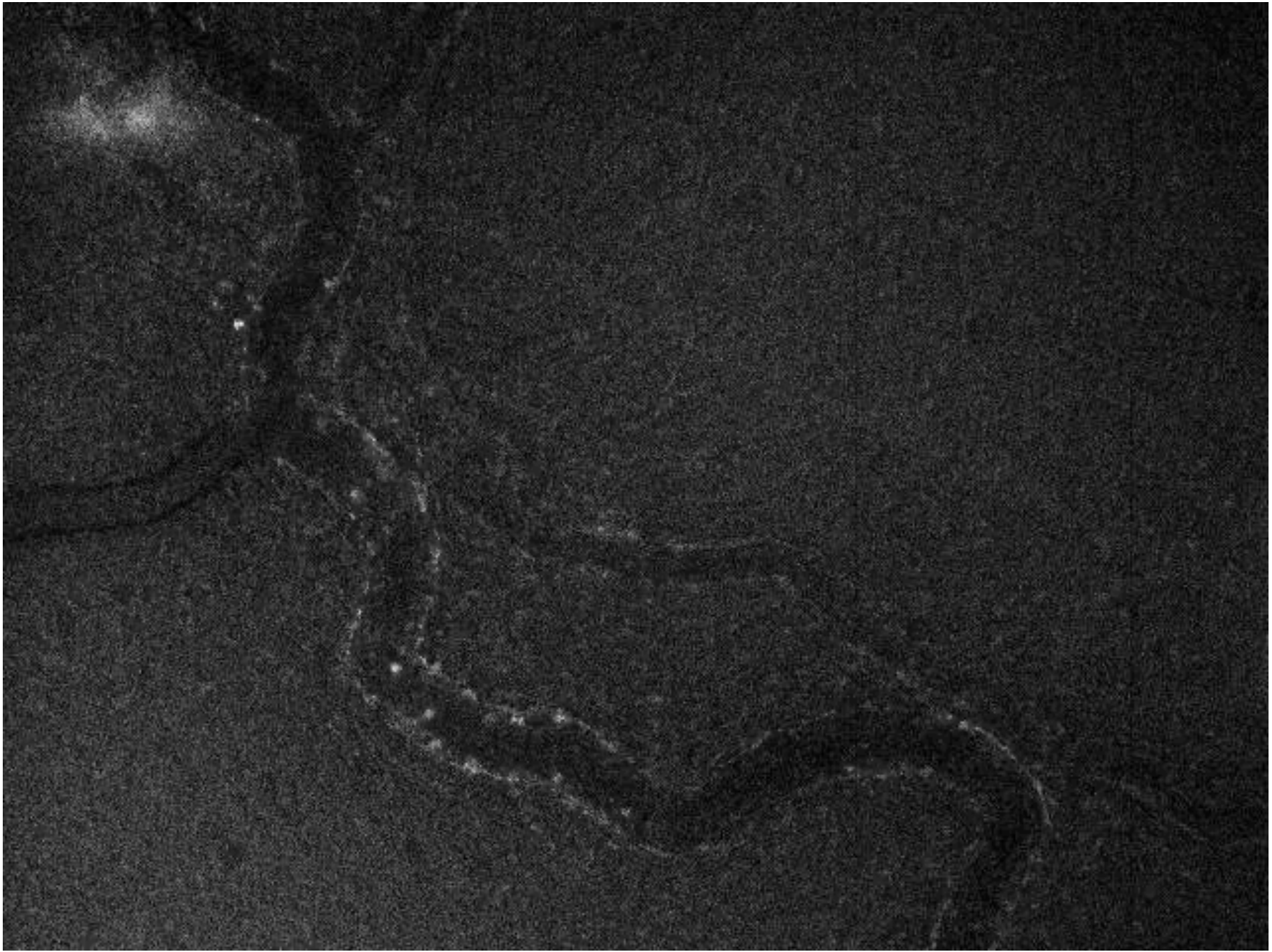
Neutrophils first **roll**, then become activated and **adhere** to endothelium, then **transmigrate** across the endothelium, pierce the basement membrane, and **migrate toward chemoattractants** emanating from the source of injury

Specific molecular interactions with endothelial cells regulate leukocyte migration into the extra-vascular microenvironment

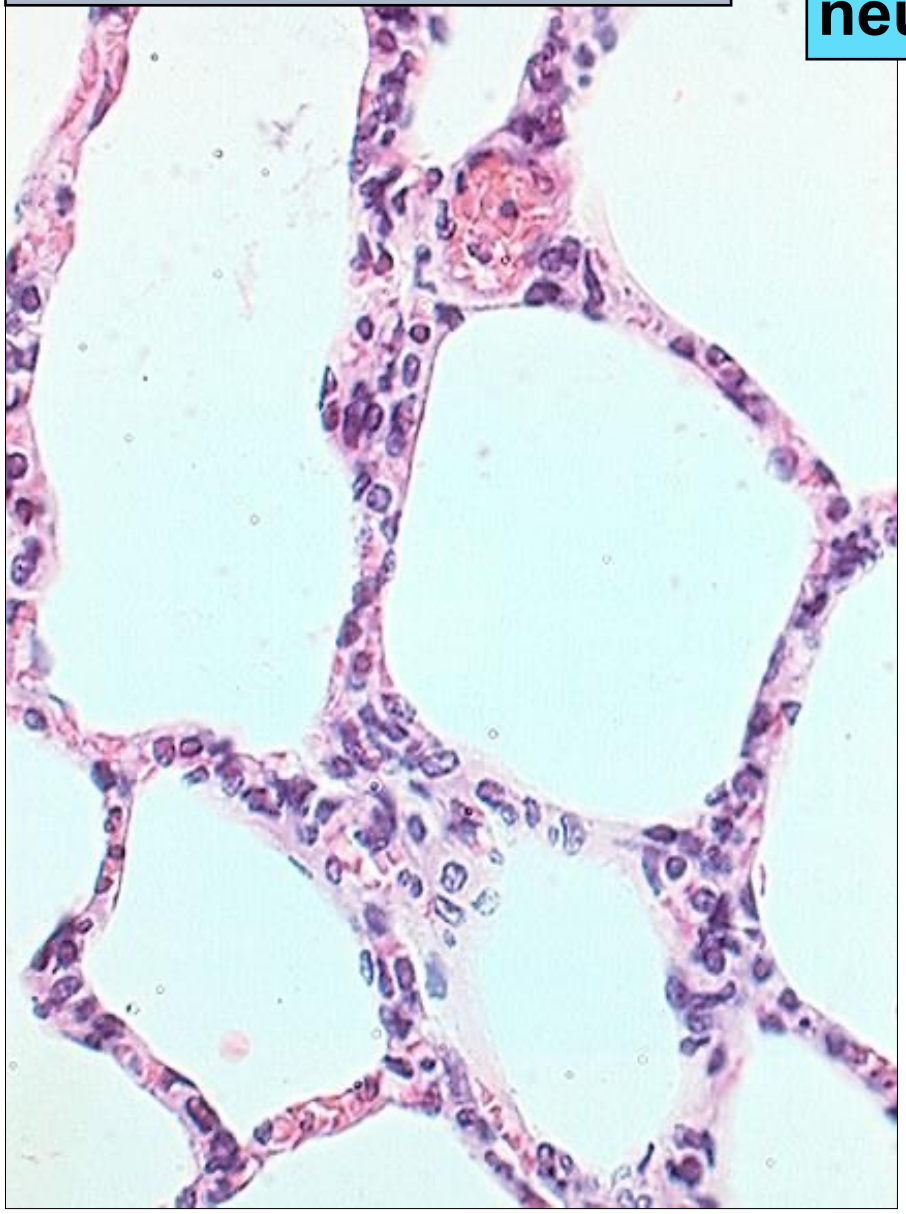
Table 2–2 Endothelial and Leukocyte Adhesion Molecules

Endothelial Molecule	Leukocyte Molecule	Major Role(s)
Selectins and Selectin Ligands		
P-selectin	Sialyl–Lewis X–modified proteins	Rolling
E-selectin	Sialyl–Lewis X–modified proteins	Rolling and adhesion
GlyCam-1, CD34	L-selectin*	Rolling (neutrophils, monocytes)
Integrin Ligands	Integrins	
ICAM-1 (immunoglobulin family)	CD11/CD18 integrins (LFA-1, Mac-1)	Firm adhesion, arrest, transmigration
VCAM-1 (immunoglobulin family)	VLA-4 integrin	Adhesion
Others		
CD31	CD31 (homotypic interaction)	Transmigration of leukocytes through endothelium

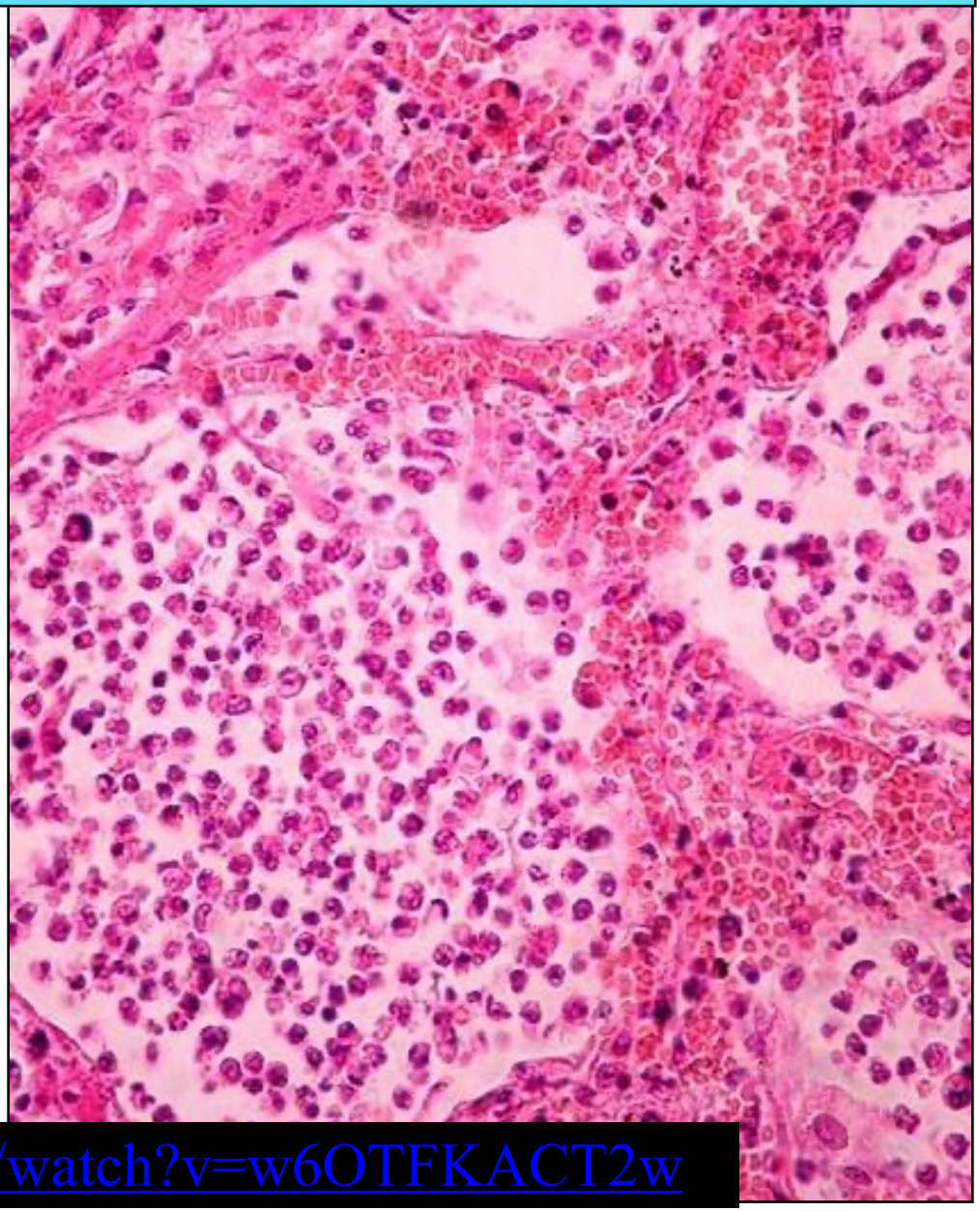
*L-selectin is also involved in the binding of circulating lymphocytes to the high endothelial venules in lymph nodes and mucosal lymphoid tissues, and subsequent homing of lymphocytes to these tissues.
ICAM-1, intercellular adhesion molecule-1; LFA-1, leukocyte function–associated antigen-1; Mac-1, macrophage-1 antigen; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.



Normal lung

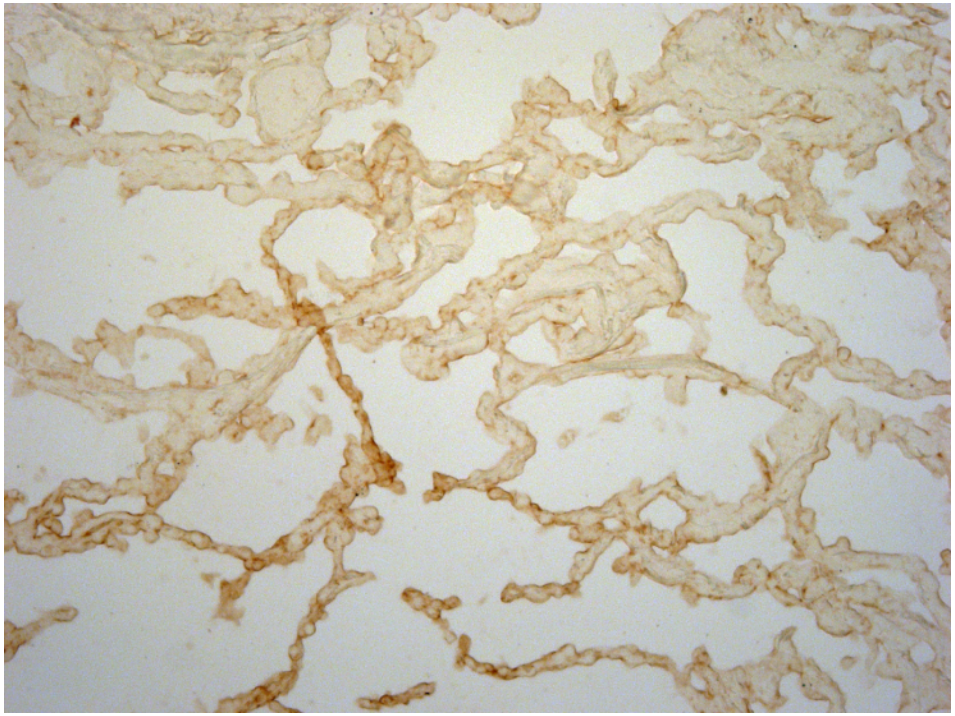


Inflammed lung in **lobar pneumonia**: **neutrophils** accumulate into alveoli

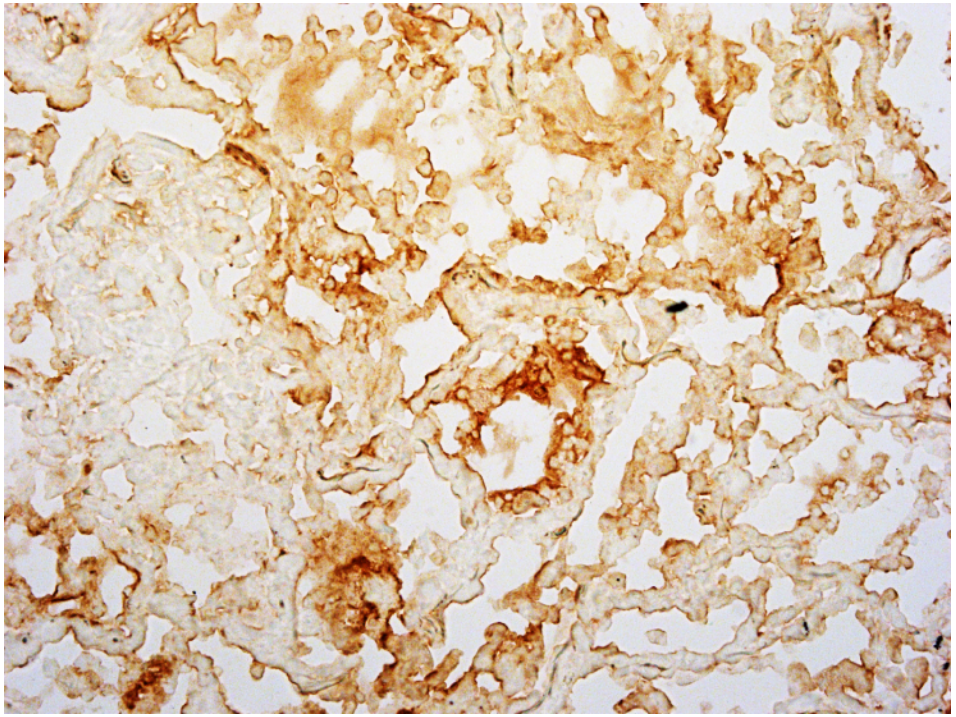


http://www.youtube.com/watch?v=w6OTFKACT2w

Lung: Low Inflammation



COVID-19 Lung: High Inflammation



Moving toward the inflammatory stimulus: **chemotaxis**

- After extravasating from the blood, leukocytes move toward sites of infection or injury **along a chemical gradient**
- Both **exogenous** and **endogenous** substances can be **chemotactic** for leukocytes, including the following:

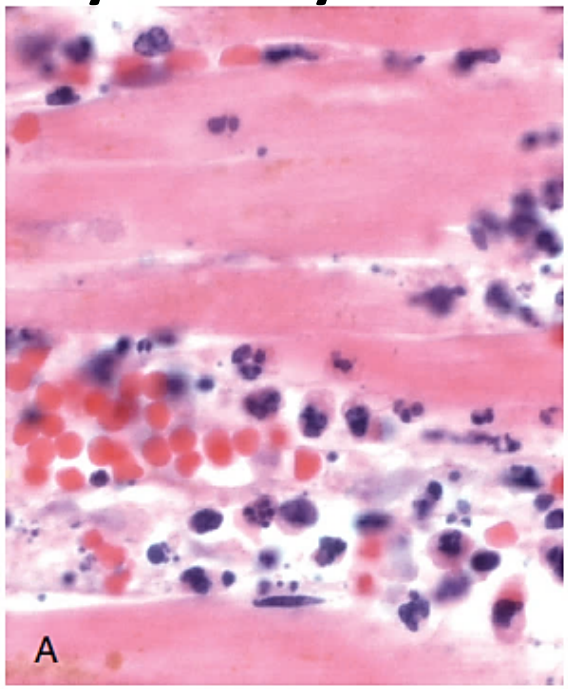
- **Bacterial products**, mainly peptides with N-formylmethionine residues
- **Cytokines**, especially those of the **chemokine** family (**IL-8**)

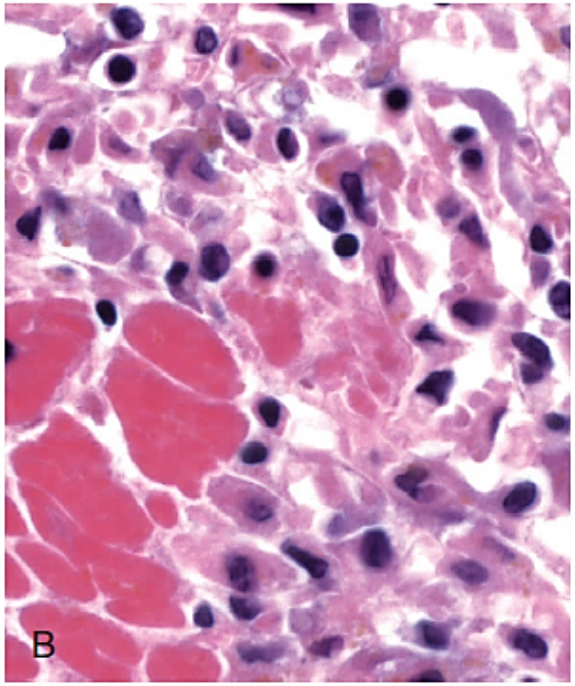
- Components of the **complement** system, particularly **C5a**

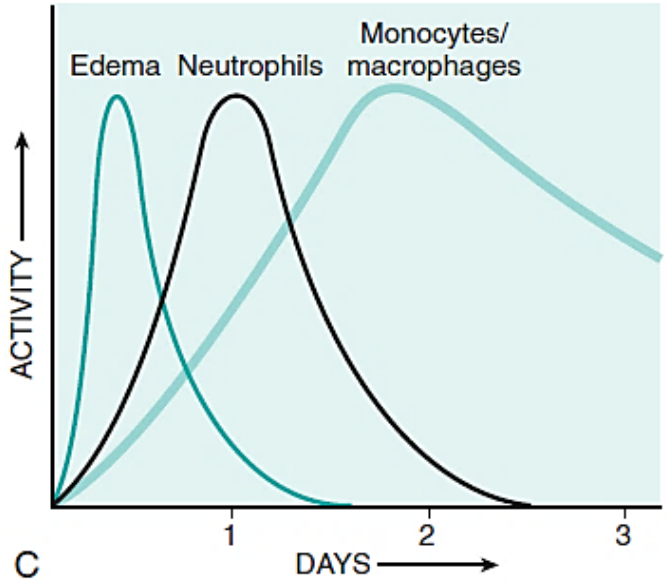
- Products of the lipoxygenase pathway of **arachidonic acid** metabolism, particularly leukotriene B4 (**LTB4**)

Leukocyte recruitment into tissue

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







Inflammatory reaction in the myocardium after **ischemic necrosis**.
A, Early neutrophilic infiltrates and congested blood vessels. **B,** Later mononuclear cellular infiltrates. **C,** Kinetics of edema and cellular infiltration.

Leukocyte recruitment into tissue

- The **relevance** of all the events leading to leukocyte recruitment into tissues is witnessed by the existence of **pathologic syndromes** characterized **by defects in cell migration** and consequent **recurrent infection**

This topic will be tackled later in more details in the chapter «IMMUNOPATHOLOGY», section «**Immunodeficiencies**»

Acute inflammation

Leukocyte activation: a prerequisite for leukocyte to complete their mission

Leukocyte **activation**

- Stimuli for leukocyte activation include **microbes**, products of **necrotic cells**, and several **other mediators**
- Leukocytes use **various receptors** to sense the presence of microbes, dead cells, and other foreign substances
- **Leukocyte activation** results in the **enhancement** of the following **functions**:
  - **Phagocytosis** (typical of professional phagocytes, such as neutrophils, monocytes and macrophages)
  - **Liberation of substances** (e.g., granular **lytic enzymes**) that **destroy** extracellular microbes and dead tissues
  - Production of **mediators**, including arachidonic acid metabolites and cytokines that **amplify the inflammatory reaction** by recruiting and activating more leukocytes

Major steps and consequences of leukocytes activation

Recognition of stimulus

Leukocyte activation

PROFESSIONAL PHAGOCYTES

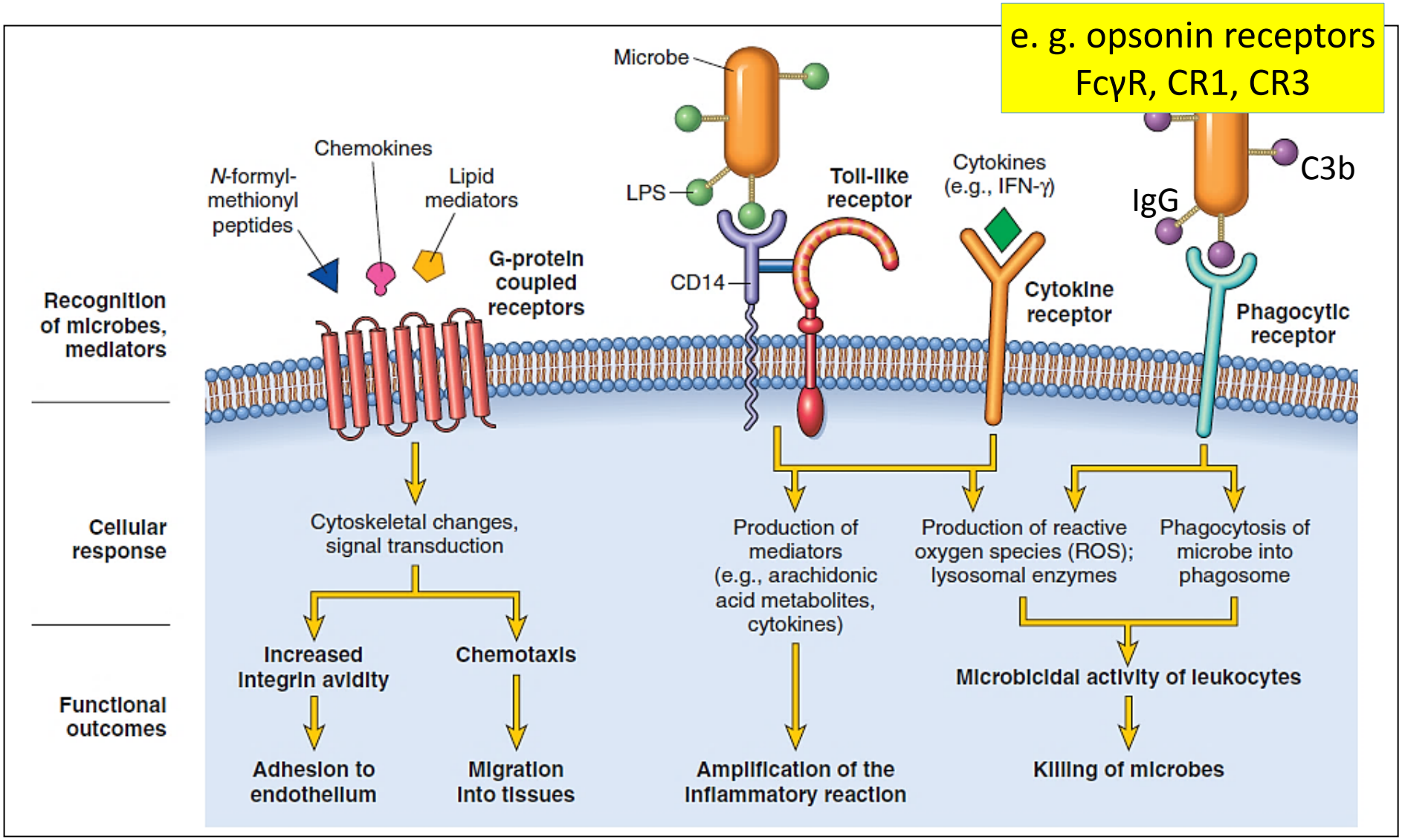
- **Engulf** particles
- **Kill** microbes
- Release **lytic enzymes**
- Release **mediators**

AMPLIFICATION OF THE INFLAMMATORY REACTION

OTHER LEUKOCYTES

- Approach the inflammatory site
- Release **mediators**

Different classes of **cell surface receptors** can mediate leukocyte activation



Main steps of **phagocytosis** and microbial **killing**

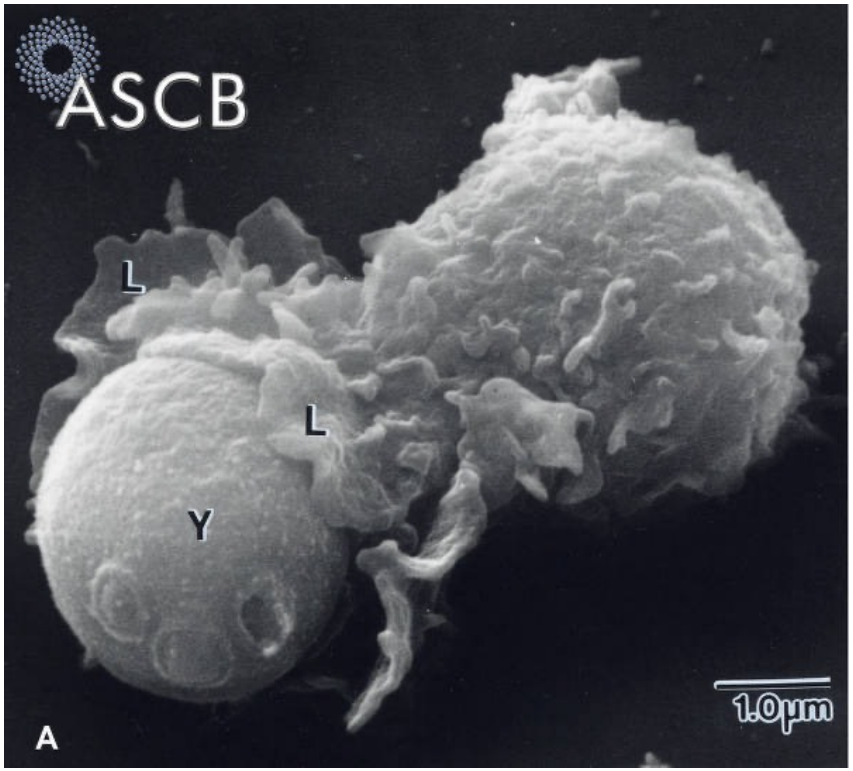
- Three main steps:

- (1) **recognition** and attachment of the microbe to the ingesting leukocyte [role of opsonins (IgG, C3b) that coat microbes and target them for phagocytosis; **opsonization**]

- (2) **engulfment**: pseudopods are extended around the microbe

Phagocytosis:

crucial to defeat infections



A phagocyte (L) engulfing a blastospore of *Candida albicans* (Y)

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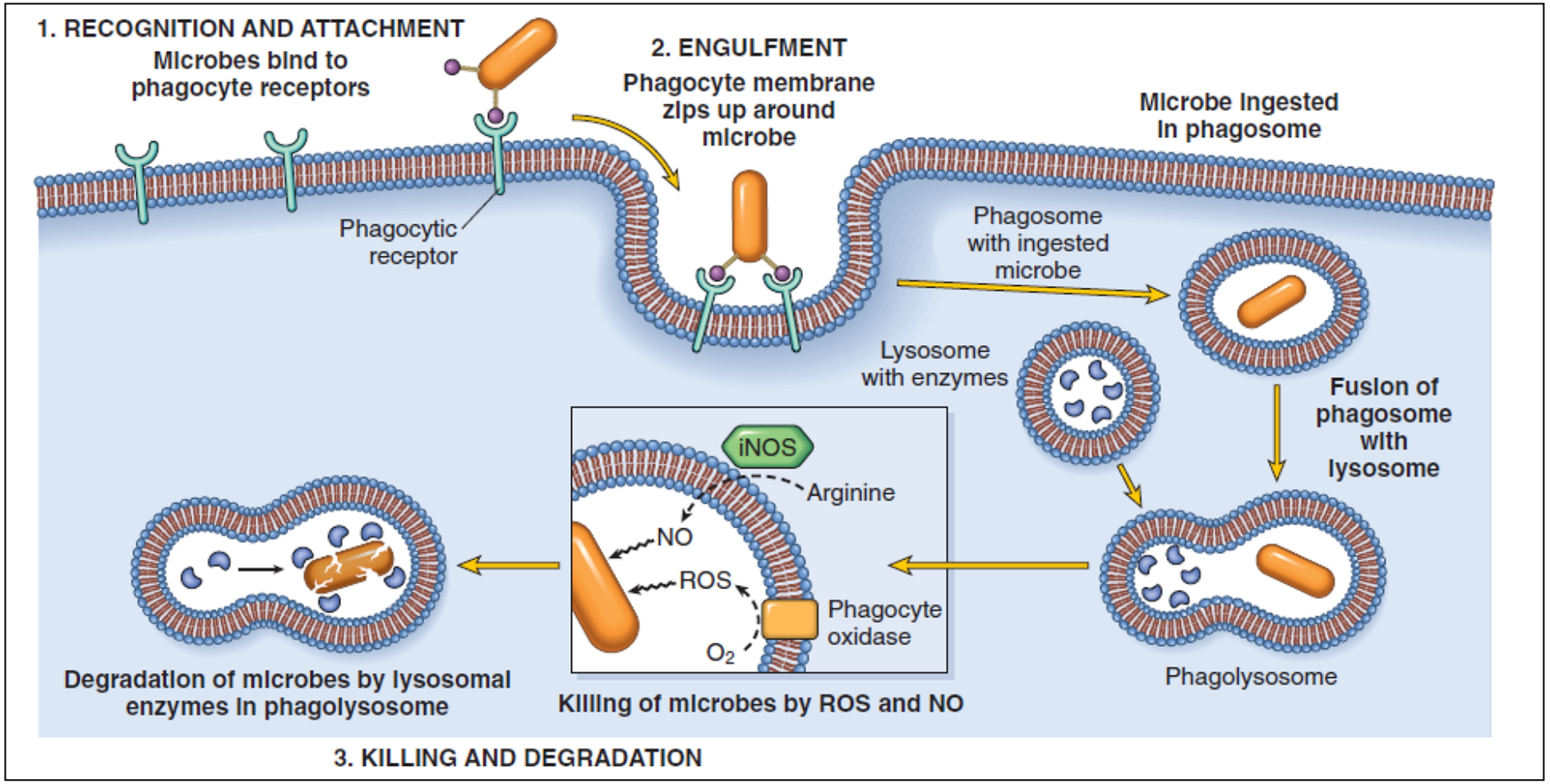
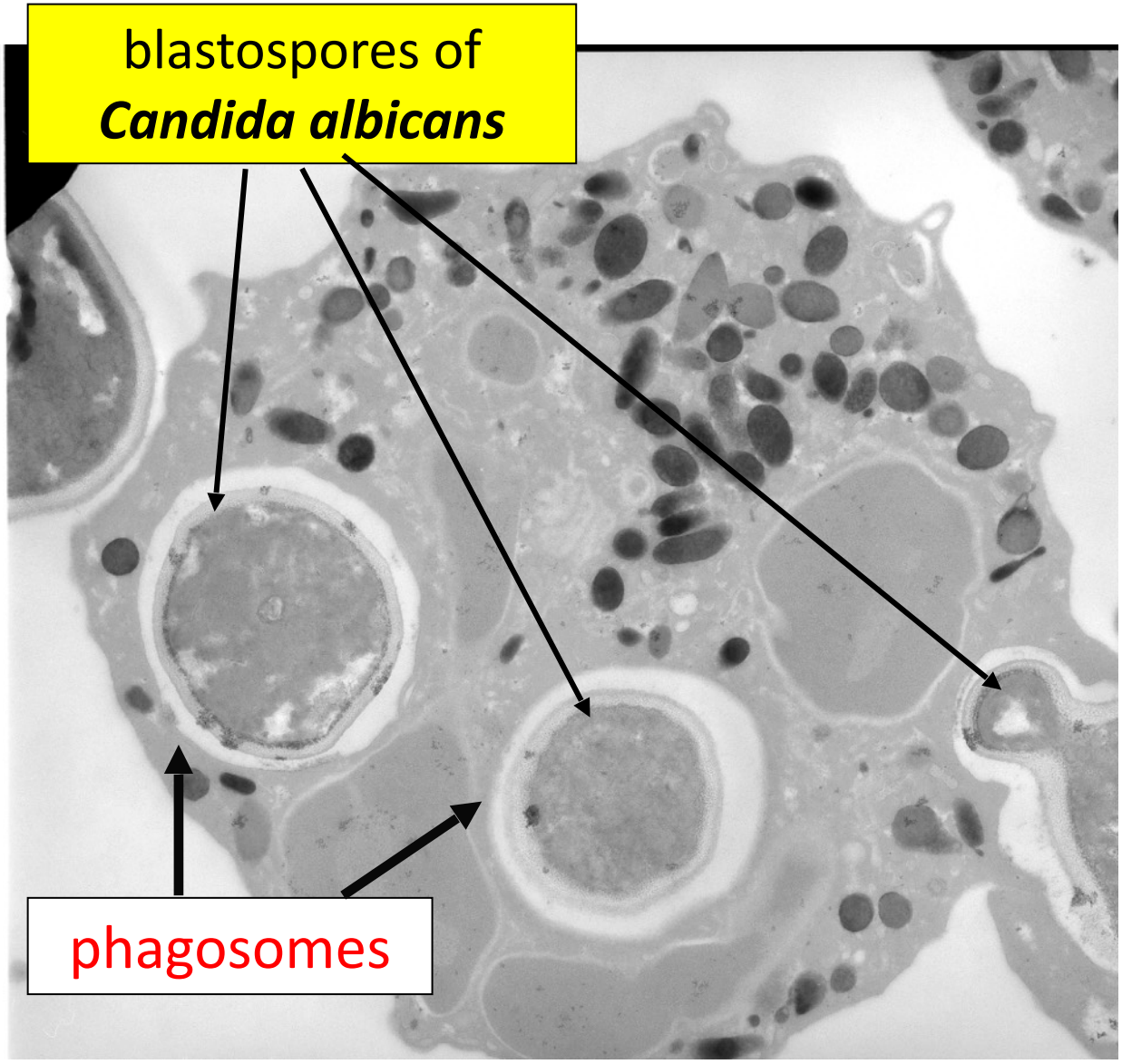
Main steps of **phagocytosis** and microbial **killing**



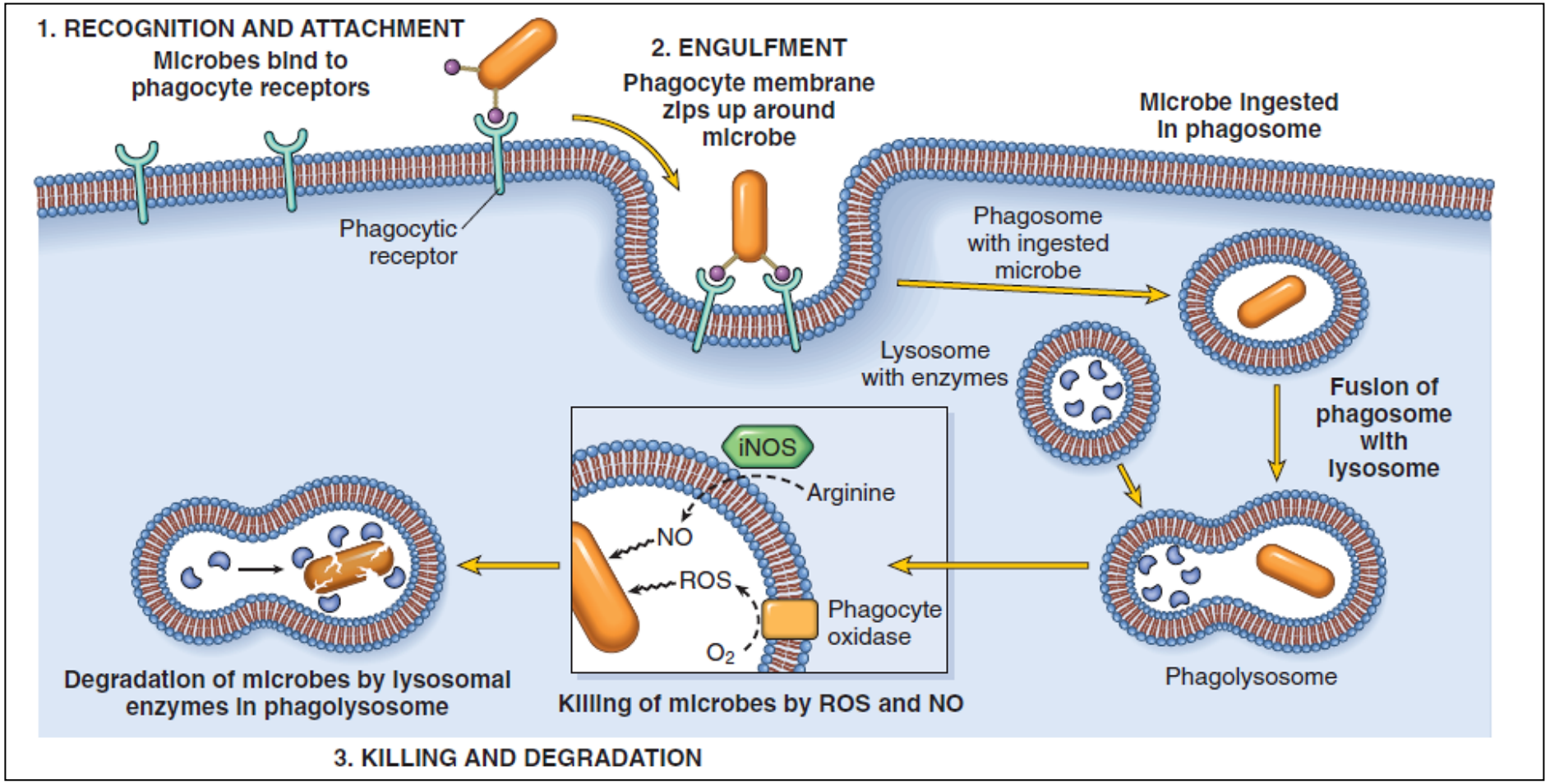
iNOS, inducible nitric oxide synthase; **NO**, nitric oxide; **ROS**, reactive oxygen species

Phagocytosis by electron microscopy



Section of a neutrophil engulfing *C. albicans* observed by transmission electron microscopy

Main steps of **phagocytosis** and microbial **killing**



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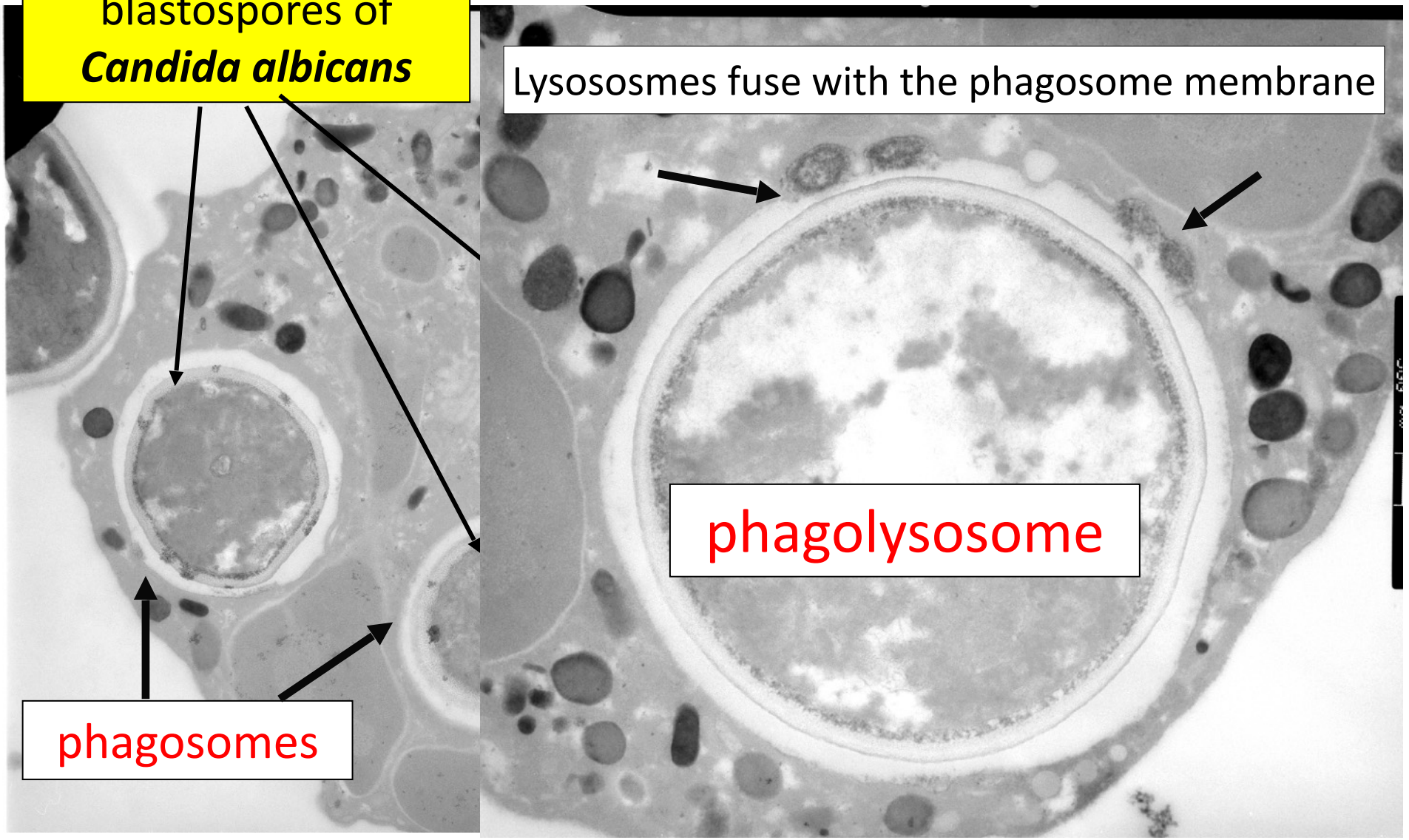

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- (3) **killing** and degradation of the ingested microbe

How can neutrophils kill microbes?

- Two main mechanisms (often acting together)

Oxygen-dependent

- Phagocytosis and engagement of various cellular receptors stimulate an **oxidative burst**, which is characterized by a **rapid increase in oxygen consumption**, glycogen catabolism, increased glucose oxidation, and **production of ROS**

Oxygen-independent

- **Microbicidal substances** stored in neutrophil **granules** are discharged into the phagolysosome

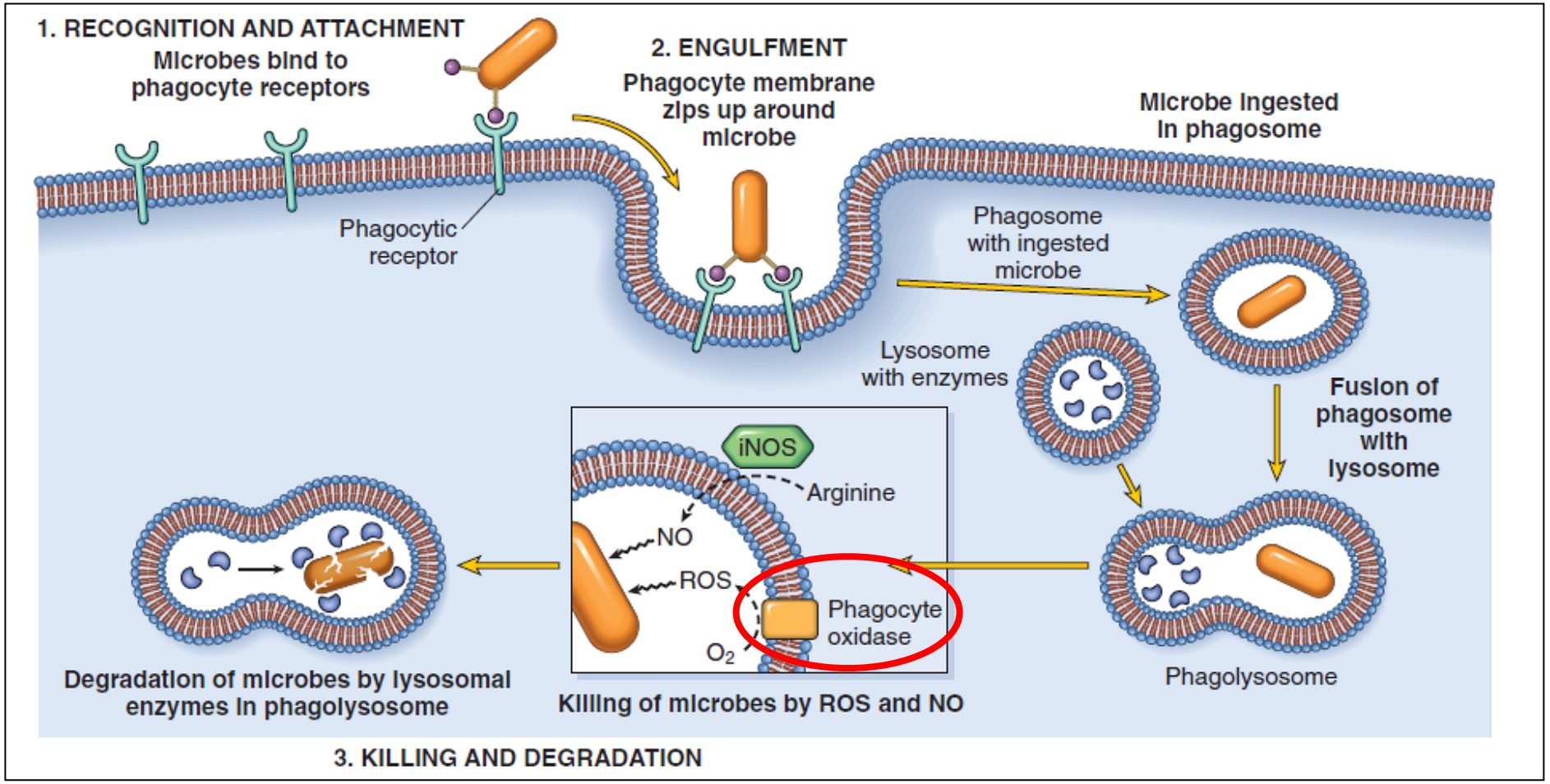
How can neutrophils kill microbes?
oxygen-dependent microbicidal mechanisms

- ROS generation is due to rapid activation of a leukocyte **NADPH oxidase** (the phagocyte oxidase, ***phox***) which oxidizes NADPH thereby **converting oxygen to superoxide anion** ($O_2^{-}\bullet$)

- **Superoxide** is then converted by **dismutation** into **hydrogen peroxide** ($O_2^{-}\bullet + 2H+ \rightarrow H_2O_2$)

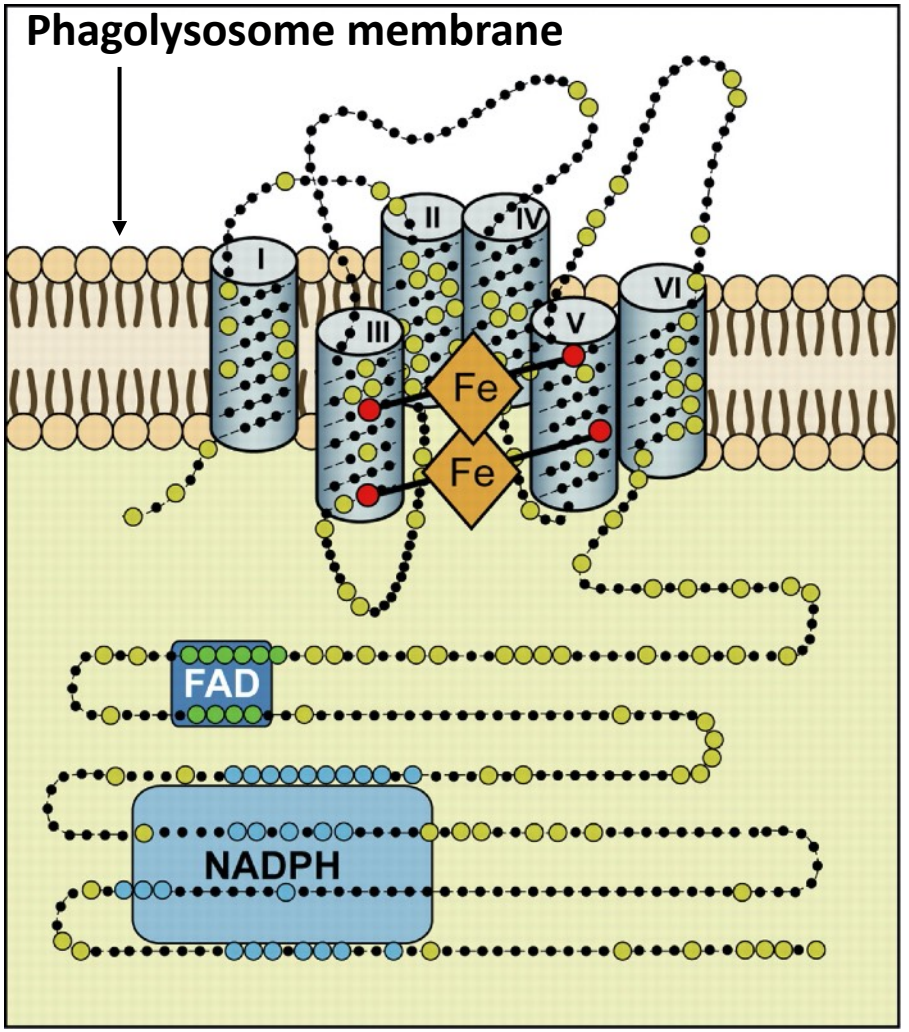
- The lysosomes of neutrophils (azurophilic granules) contain the enzyme **myeloperoxidase** (MPO) that in the presence of a halide, such as Cl^-, converts H_2O_2 to **HOCl** (hypochlorous acid), a **powerful antimicrobial agent** that kills bacteria by **protein and lipid chlorination and/or peroxidation**

Main steps of **phagocytosis** and microbial **killing**



iNOS, inducible nitric oxide synthase; **NO**, nitric oxide; **ROS**, reactive oxygen species

The cytoplasmic COOH terminus contains conserved flavin adenine dinucleotide (**FAD**) and **NADPH binding domains**. NOX enzymes are **single electron transporters**: they pass electrons from NADPH to FAD, to the first heme, to the second heme, and finally to oxygen



How can neutrophils kill microbes? **oxygen-independent** microbicidal mechanisms

- Constituents of **neutrophil granules** mostly involved in killing infectious pathogens:
  - **Bactericidal permeability-increasing protein (BPI)**: causes phospholipase activation and membrane phospholipid degradation
  - **Lysozyme**: degrades bacterial coat oligosaccharides
  - **Defensins**: peptides that kill microbes by creating holes in their membranes (osmotic lysis)

Defensins: antimicrobial peptides

- Small (29–35 amino acids), positively-charged proteins produced mainly by circulating white blood cells and epithelial cells
- Antimicrobial properties: disrupt bacteria, fungi, parasites, and some enveloped viruses **mainly by forming multimeric pores in the cell membranes of these pathogens**
- Two families, the **α-** and **β-defensins**
- Neutrophils mainly contain **α-defensins (HD, human defensins)**
- **HD 1** to **HD 4** are produced by neutrophils and are found in the airways; **HD 5** and **HD 6** are found in the small intestine and female urogenital tract
- The **β-defensins** are produced by most mucosal epithelial tissues, but appear to be **preferentially expressed in the urogenital tract**, particularly in the testis and epididymis in the male, and their production is stimulated by TLR ligands and cytokines

Leukocyte activation

- **Alterations** of leukocyte functions underlie many **human diseases**

- Syndromes featured by **defective** or **excessive** activation are known

- Given the irreplaceable role of leukocytes in defending from invading microorganisms, **defects** are inevitably associated with an **increased susceptibility to infections**

- **Excessive** leukocyte activation is peculiar of **hypersensibility reactions**

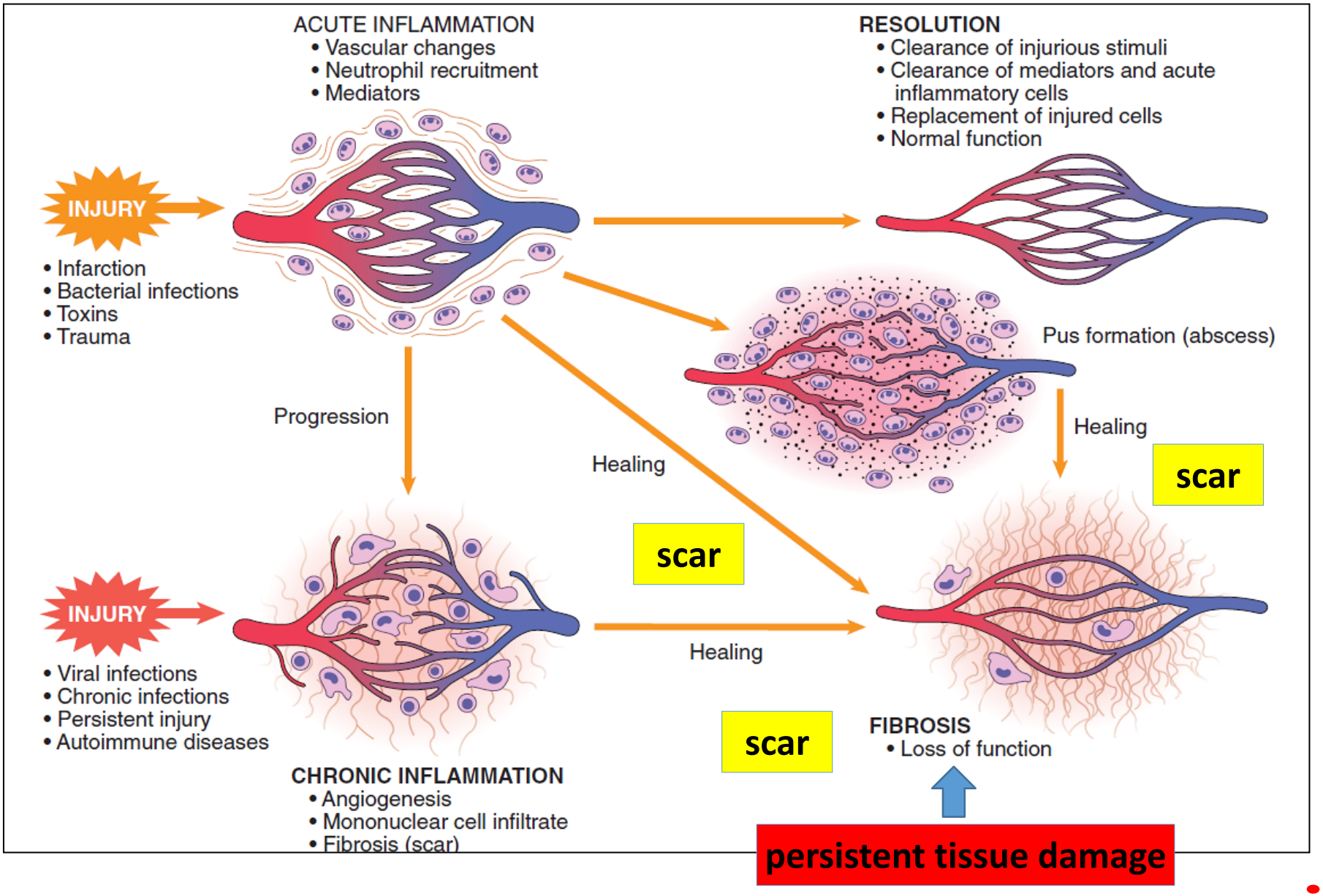
Outcomes of acute inflammation

- The consequences of acute inflammation depend on the **nature** and **intensity** of the injury, the **site** and **tissue** affected and, notably, the **ability of the host** to mount a response

- Acute inflammation generally has one of the three following outcomes:

  - **resolution** (with regeneration and repair)
  - **progression** to **chronic inflammation**
  - **scarring**

Outcomes of acute inflammation



Outcomes of acute inflammation: **resolution**

- The usual outcome is restoration to structural and functional normalcy (*restitutio ad integrum*) when:
  - the injury is **limited** or **short-lived**
  - there has been **no** or **minimal** tissue damage
  - the **injured tissue** is capable of **regenerating**

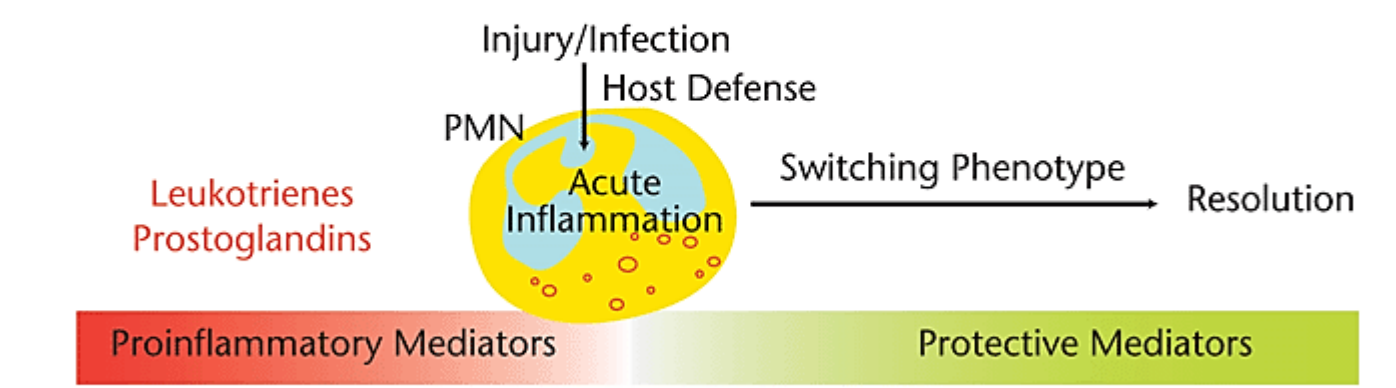
Resolution: main events involved in the termination of acute inflammation

- **Neutralization** (e.g. decay, enzymatic degradation) **of the various chemical mediators** (e.g. histaminase)
- **Normalization** of vascular permeability
- **Cessation of leukocyte migration**, with subsequent death (by apoptosis) of extravasated neutrophils
- **Decreased** synthesis of **pro-inflammatory** mediators (e.g, **TNF**)
- **Increased** synthesis of **anti-inflammatory** mediators (e.g. **TGFβ**)

Resolution: endogenous **anti-inflammatory** pro-resolving **lipid mediators**

- Specific pro-resolving lipid-based mediators belong to a family of endogenous chemical mediators that include **lipoxins**, **resolvins** and **protectins**

- Potent modulators of the **duration** and **magnitude** of inflammation; synthesized by leukocytes, epithelial cells and p



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Dimensions of Dental Hygiene, April 2009 Supplement

Pro-resolving lipid mediators

- **Lipoxins** (from **arachidonic acid**): powerful inhibitors of neutrophil infiltration; potentiate macrophage-mediated clearance of apoptotic neutrophils. **Aspirin** (acetylsalicylic acid), alone among the non-steroidal anti-inflammatory drugs (**NSAIDs***), stimulates the early formation of pro-resolving lipoxins
- **Resolvins** and **protectins** (from **omega-3 polyunsaturated fatty acids**); potent anti-inflammatory capabilities at very low concentrations (nM, pM). Like lipoxins, both halt PMN infiltration and transmigration

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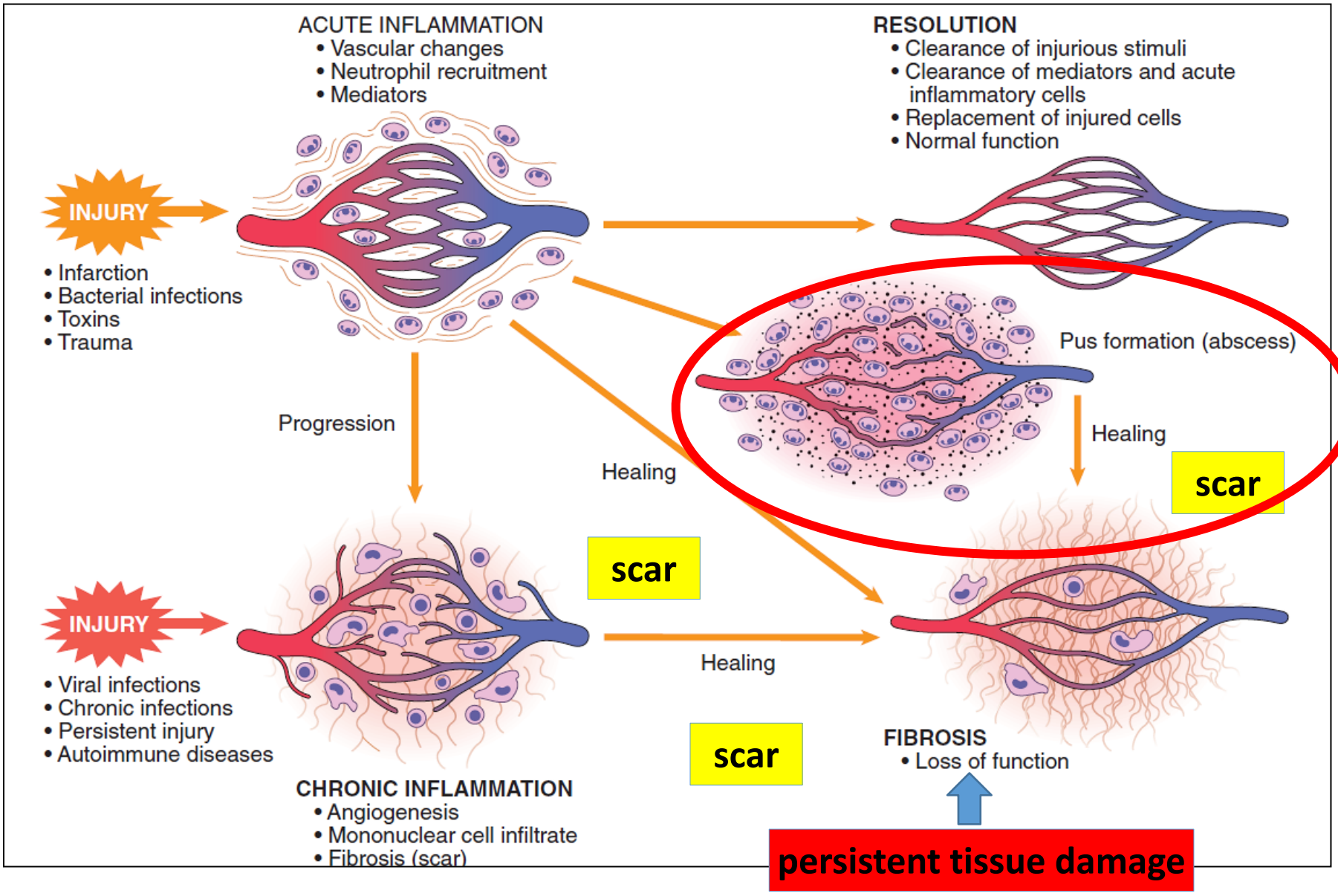
* Noti anche come farmaci anti-infiammatory non-steroidei (FANS)

Outcomes of acute inflammation: **scarring**

- Scarring is a type of **repair after** substantial **tissue destruction** or when inflammation occurs in tissues that do not regenerate, in which the injured tissue is filled in by connective tissue (e. g., myocardial infarction, deep excisional wounds)

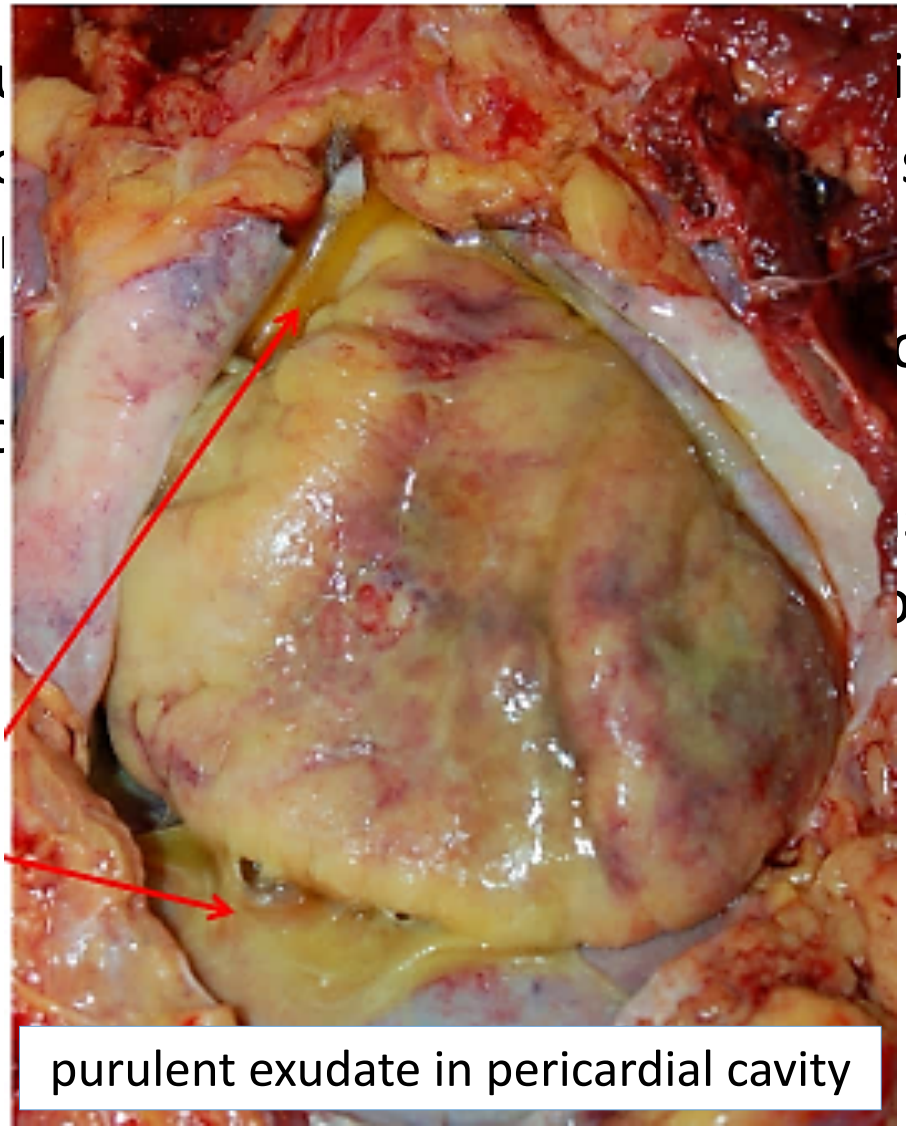
- In organs in which **extensive connective tissue deposition** occurs in attempts to heal the damage (or as a consequence of chronic inflammation, *discussed later*) the outcome is **fibrosis**, a process that **can significantly compromise function**

Outcomes of acute inflammation



Outcomes of acute inflammation: **suppuration** (pus formation)

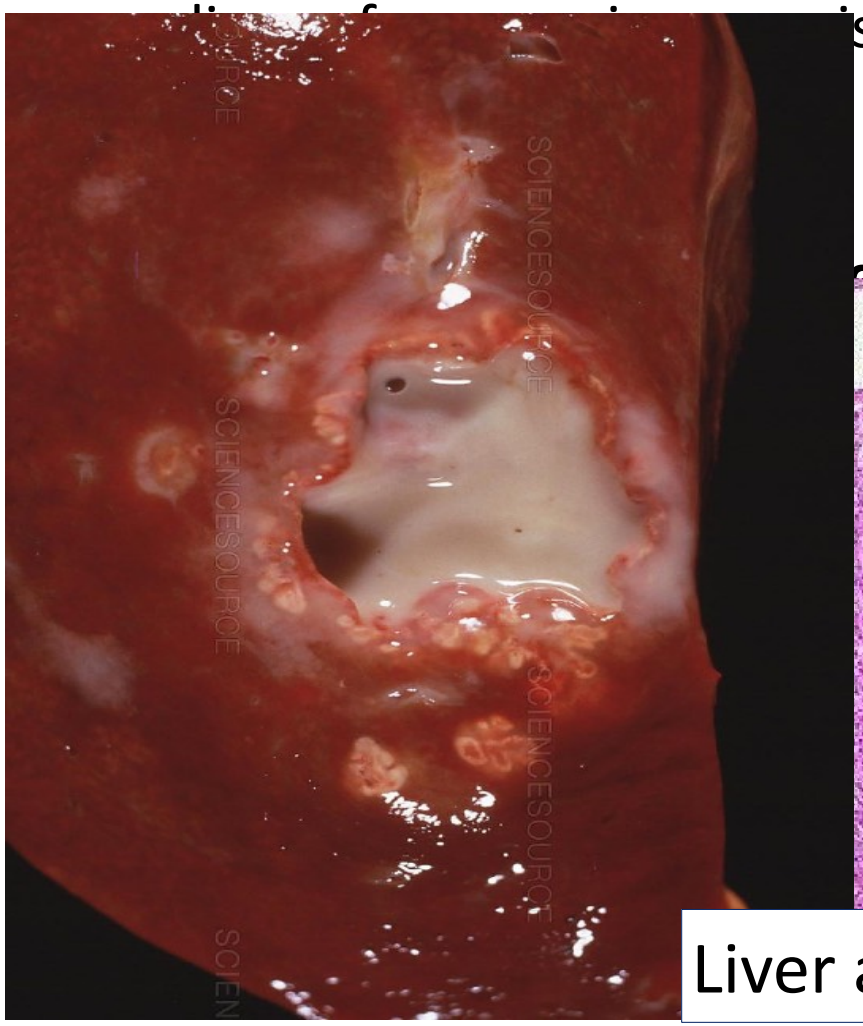
- A peculiar ou is the collection of large amo s) consisting of neutrophils,
- **Certain org** ci, gonococci, meningococc such localized suppuration s **pyogenic**, i. e. pus-forming) phils



purulent exudate in pericardial cavity

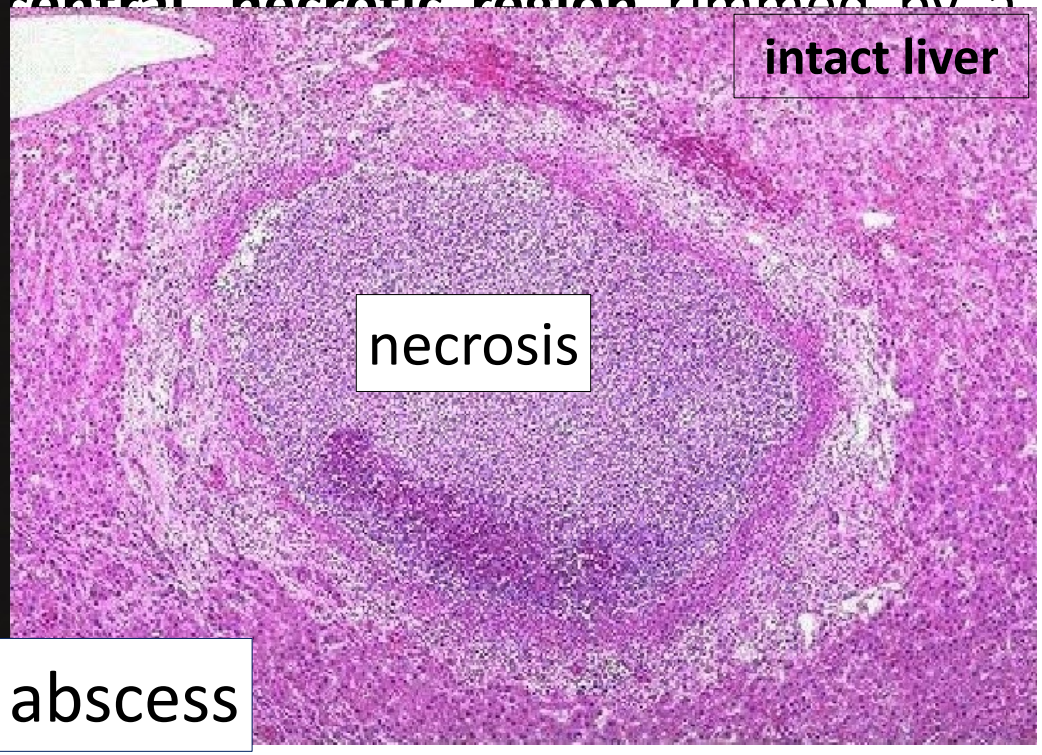
Outcomes of acute inflammation: **suppuration** with **abscess** formation

- **Abscesses** are **focal collections of pus** that may be caused by [illegible] sms into a tissue or by secondary



Liver abscess

central necrotic region rimmed by a



Outcomes of acute inflammation