

Inflammation - an overview

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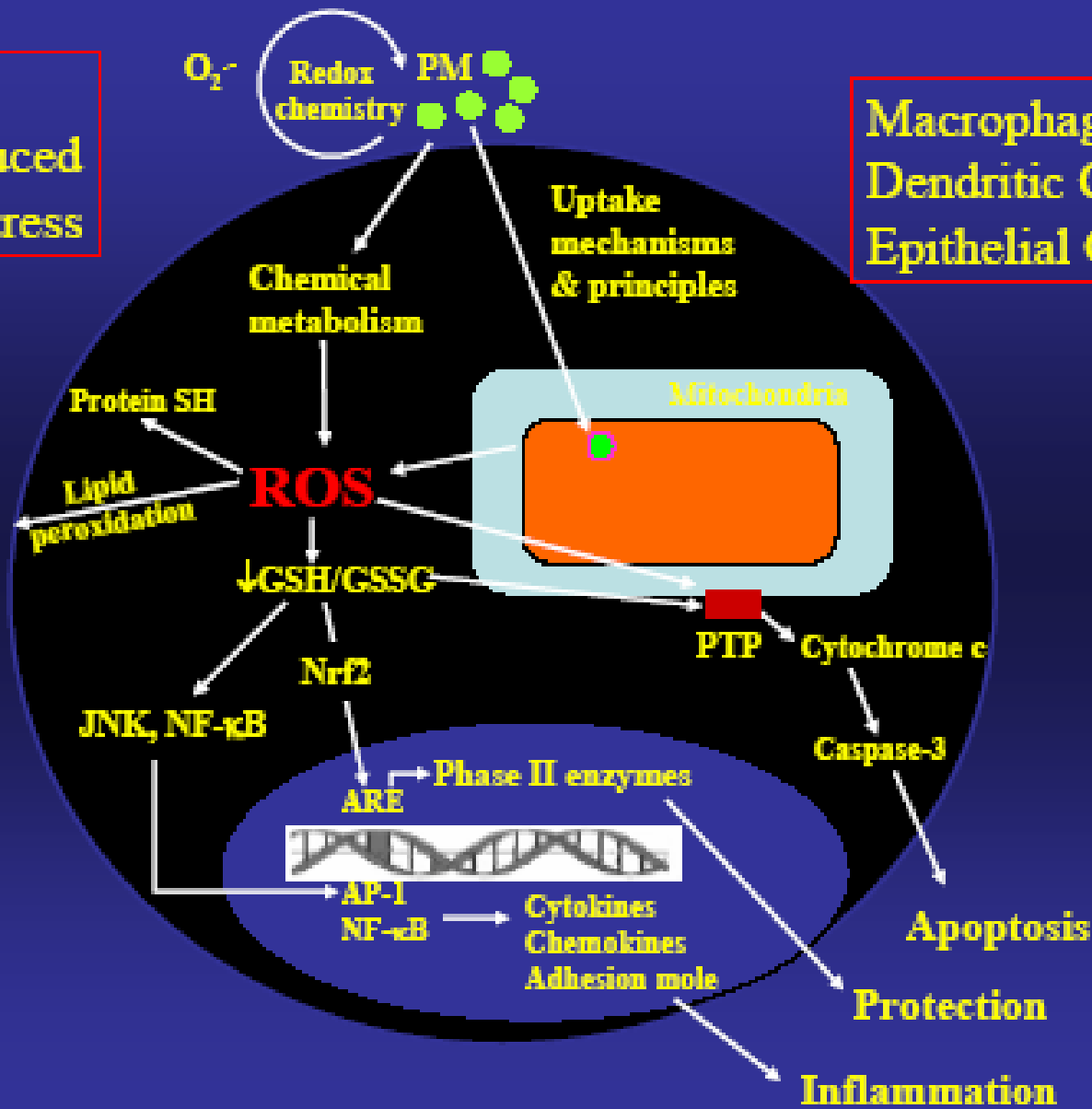
Society of Toxicology

Spring Symposium

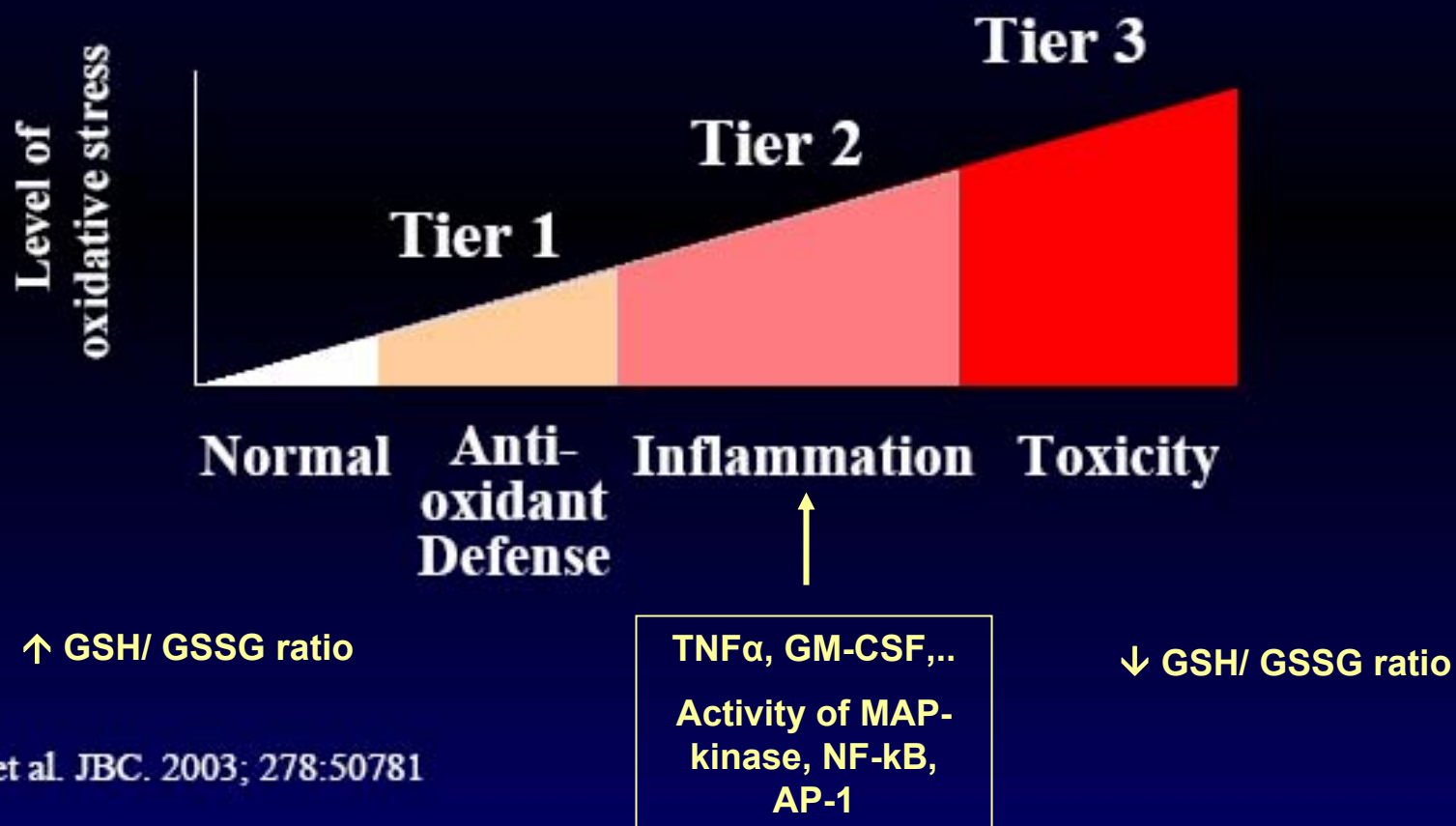
May 23, 2007

Biology of Particle-induced Oxidative Stress

**Macrophages
Dendritic Cells
Epithelial Cells**



Stratified Oxidative Stress Hypothesis



Nel et al. JBC. 2003; 278:50781

Acute Inflammation

Innate Immune Response

- **The purpose is to serve as the body's first line of defense:**
 - neutralize/ destroy injurious pathogens
 - create a barrier to limit injury
 - set in place cells and factors required for healing
 - alert the host to injury → clinical signs
(to bring protective measures to bear)
- **Two major components**
 - Vascular response
 - Cellular response

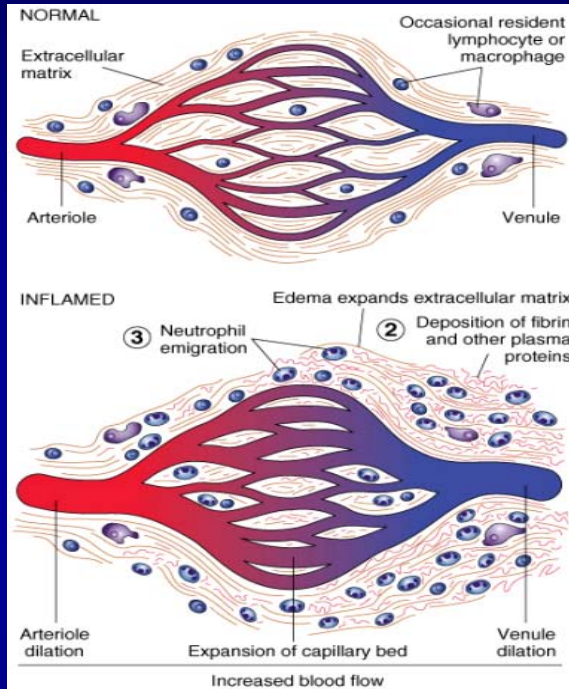
Classic Signs of Acute Inflammation



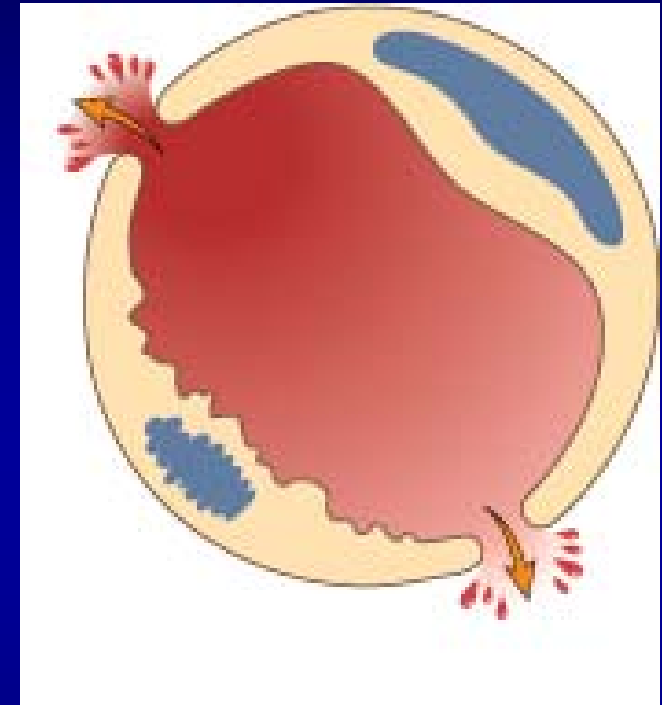
- **Redness (Rubor)** - ↑ blood flow
- **Warmth (Calor)** - ↑ blood flow
- **Swelling (Tumor)** - edema
- **Pain (Dolor)** - edema
pulse flow
- **Lost Function (Functio Laesa)**

Vascular changes in Acute Inflammation

Vascular Dilation



Vascular Permeability



Post-capillary
venules
EC contract
↓
Interendothelial gaps

- ↑ blood flow - smooth muscle relaxation dilates arterioles
- ↑ permeability - inter-endothelial gaps → edema (exudate)
 - Water, salts, immunoglobulins
 - Fibrinopeptides → polymerizes in tissue to fibrin

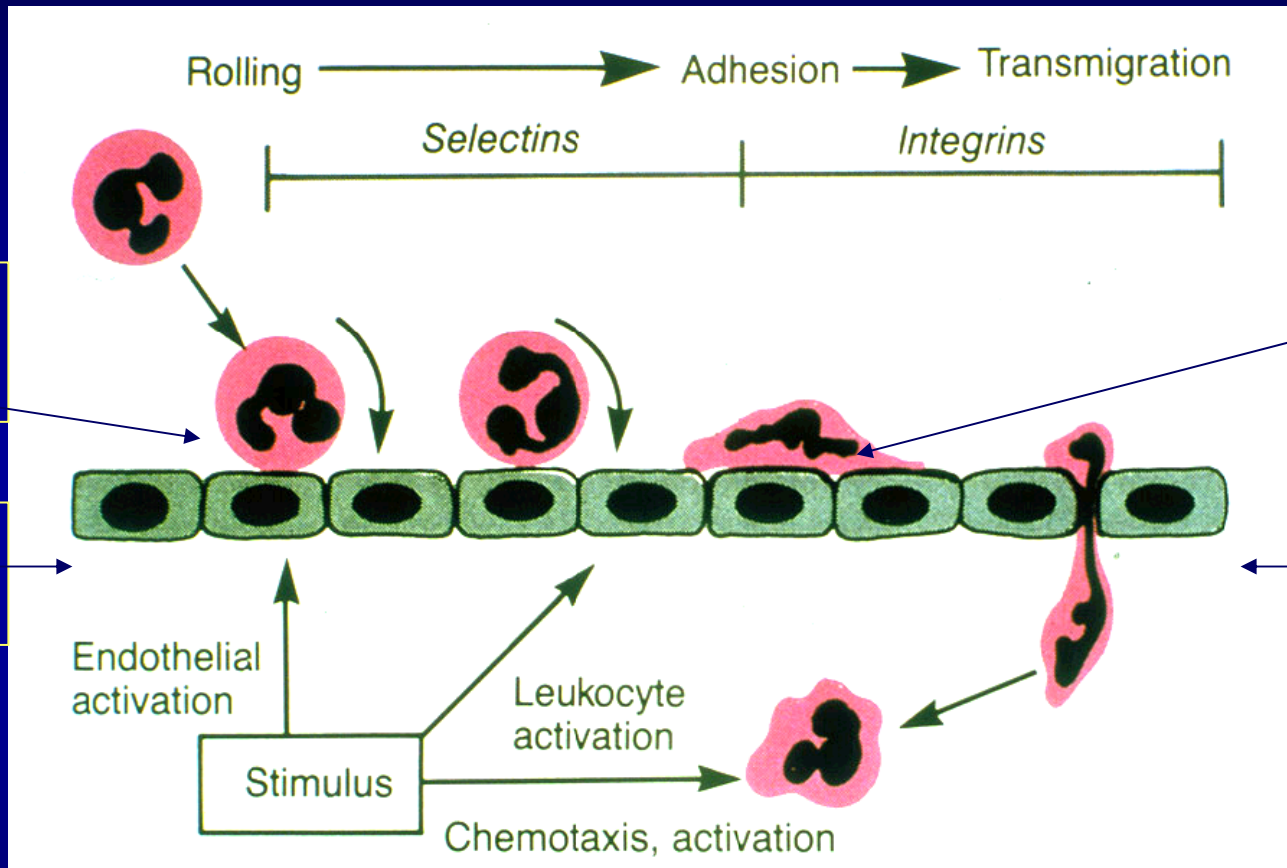
Mediators – histamine, PG, NO, bradykinin, PAF

Sequence of Events in Acute Inflammation

cellular response

- **< 24 hrs - Neutrophils (polymorphonuclear cells, PMNs) migrate out of post-capillary venule at site of injury**
 - Neutrophils survive 2-3 days
- **24-48 hrs – Monocytes in circulation and tissue macrophages**
 - Macrophages survive 60-90 days

Cellular Response Neutrophil Migration



Addressin
Sialyl-
Lewis X

P-selectin
E-selectin

Mono-
Integrins
MAC-1

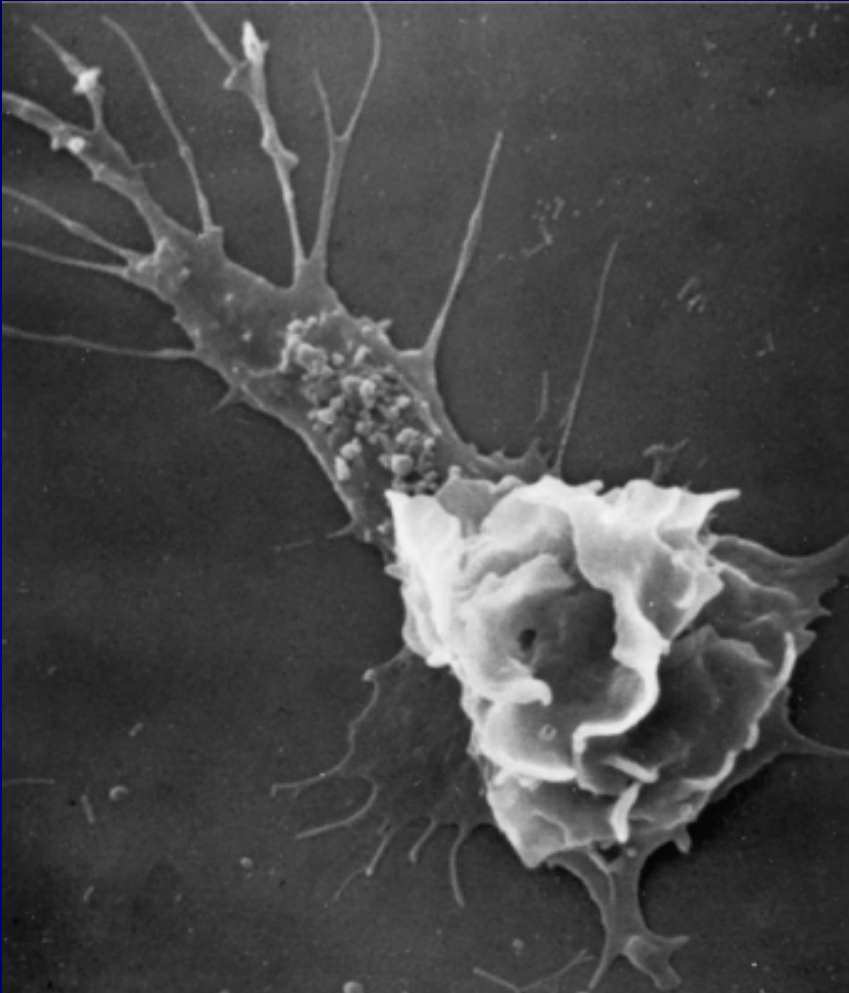
PMN
Integrins
 $\beta 1,2$ -
integrins

Endo-
Integrins
ICAM-1
VCAM-1

- 1. Marginate** with rolling
- 2. Adhere** to endothelial cells expressing up-regulated selectins and integrins
- 3. Emigrate** through endothelial cell gaps
PMNs \rightarrow elastase
- 4. Chemotaxis** migration along chemokine gradient

Mediators - PG, IL-1, TNF α , PAF

Chemotaxis



Chemokines - two subgroups based on N-terminal cysteine

- C-X-C chemokines act primarily on neutrophils (IL-8)
- C-C chemokines act primarily on macrophages, lymphocytes, basophils and eosinophils (MCPs)
- Bind to matrix to form gradient

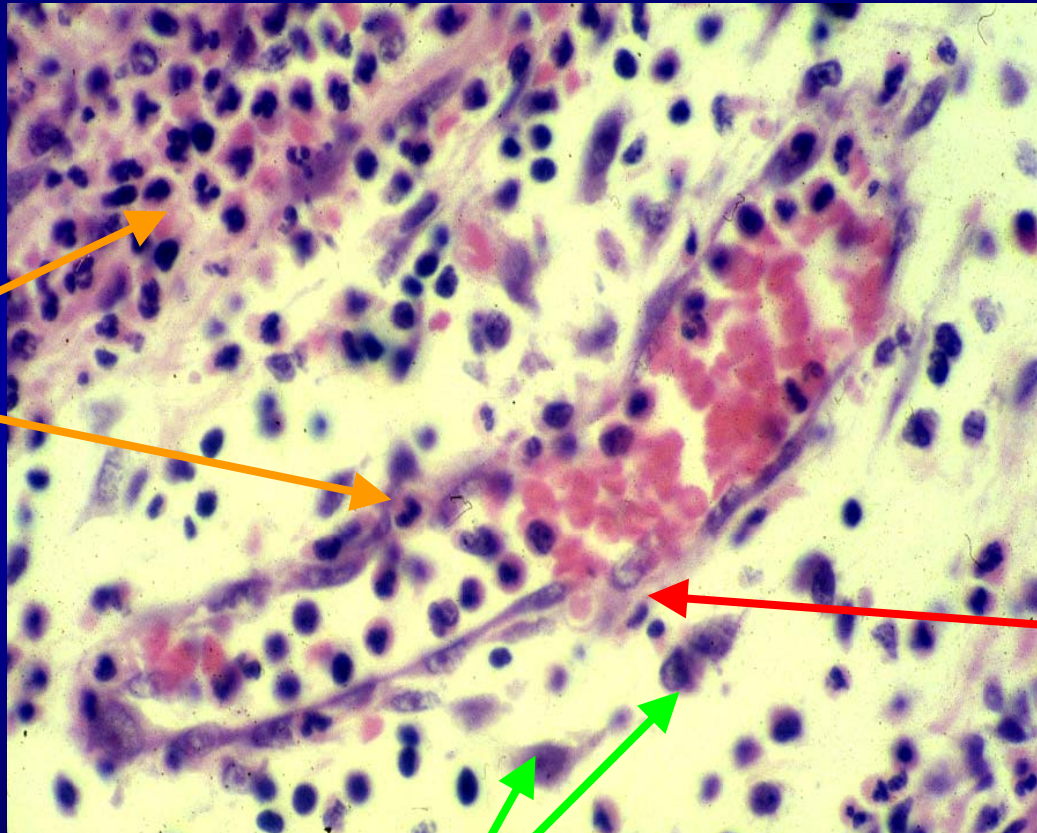
Chemokine receptors

- ligand binds to G-protein coupled receptors, activates phospholipase C → \uparrow Ca^{++} , activates GTPase → actin/myosin polymerization and directional filopodia

Chemotaxins – Chemokines, LT(B4), C5a, fibrinopeptides

Neutrophil emigration

Post-capillary venule



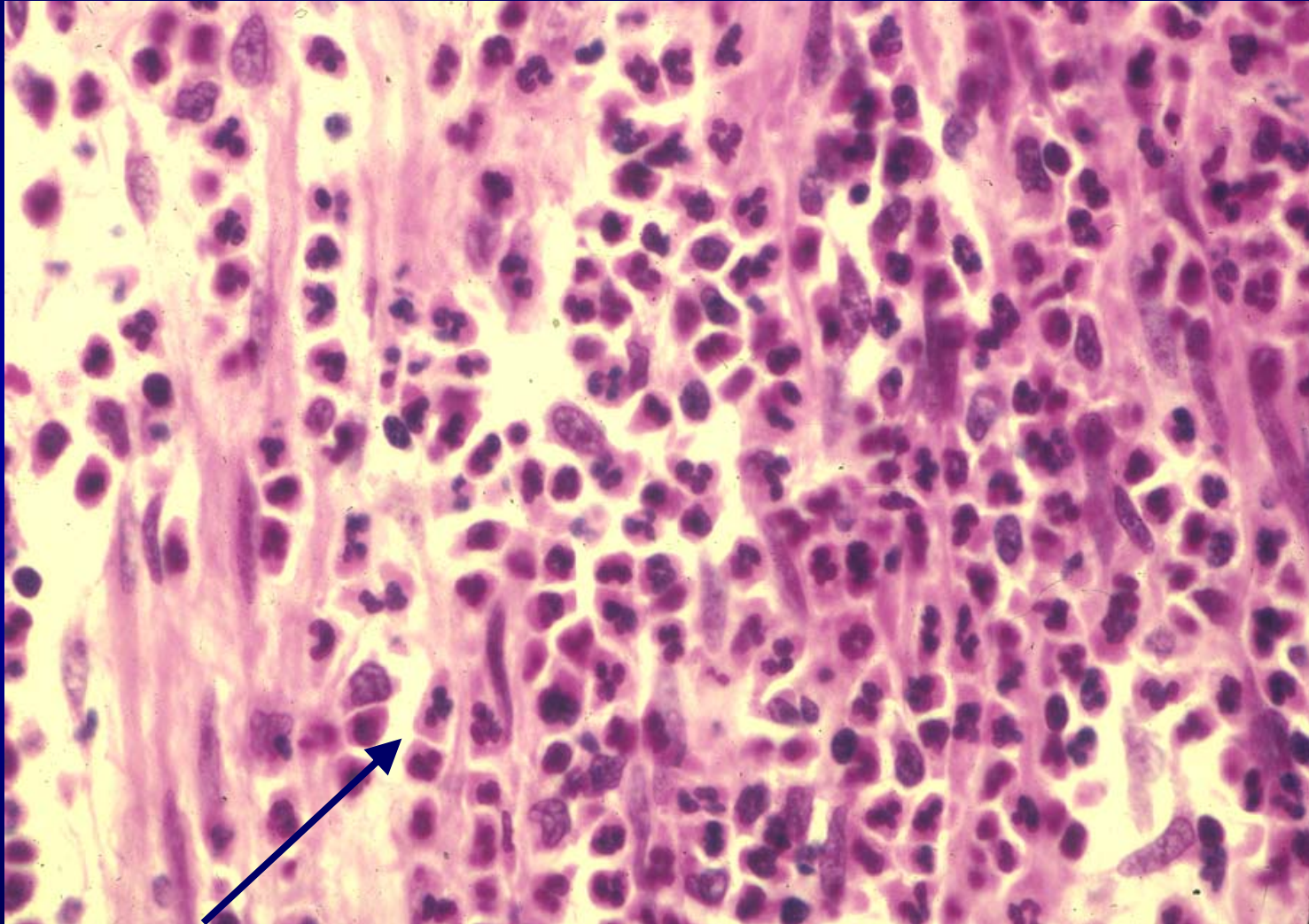
Polys
(6-24 hrs)

Inter-endothelial
gaps

RBC diapedesis

Macrophages (increase after 24-48 hrs)

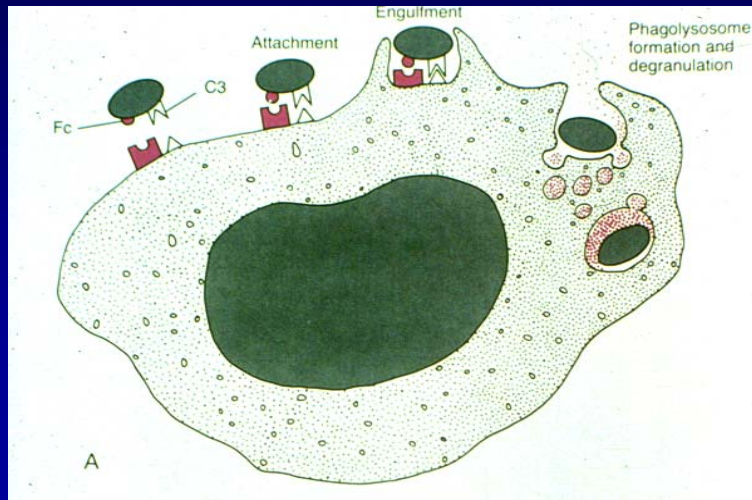
Neutrophil tissue infiltration



neutrophil

Role of Phagocytes

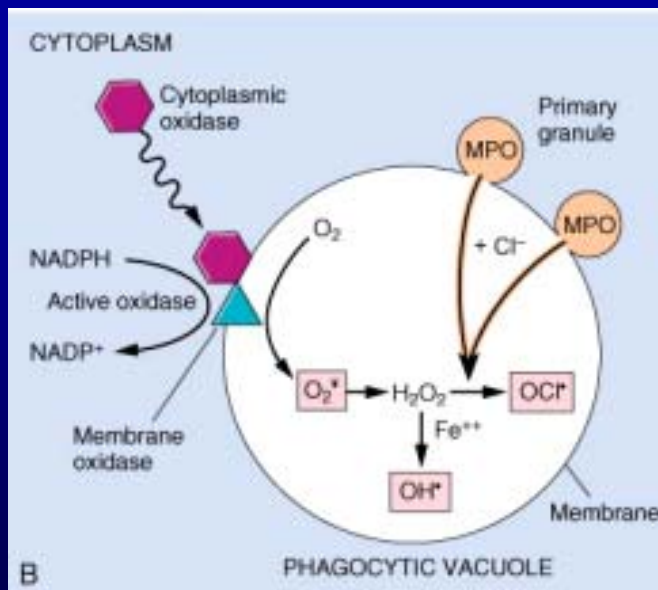
Neutrophils and Activated Macrophages



• Phagocytosis

- ingest and destroy microorganisms, cell debris, particulate matter
- **Anaerobic**
 - ligand-binding
 - non-specific (opsonin- C3b)
 - specific (Fc antibody, endotoxin)
 - engulfment into phagosome
 - phagolysosome (degranulation)
- **Aerobic metabolism**
 - respiratory burst (O₂ consumed)
 - intracellular killing by oxygen radicals

- Tissue may suffer “innocent bystander” toxicity



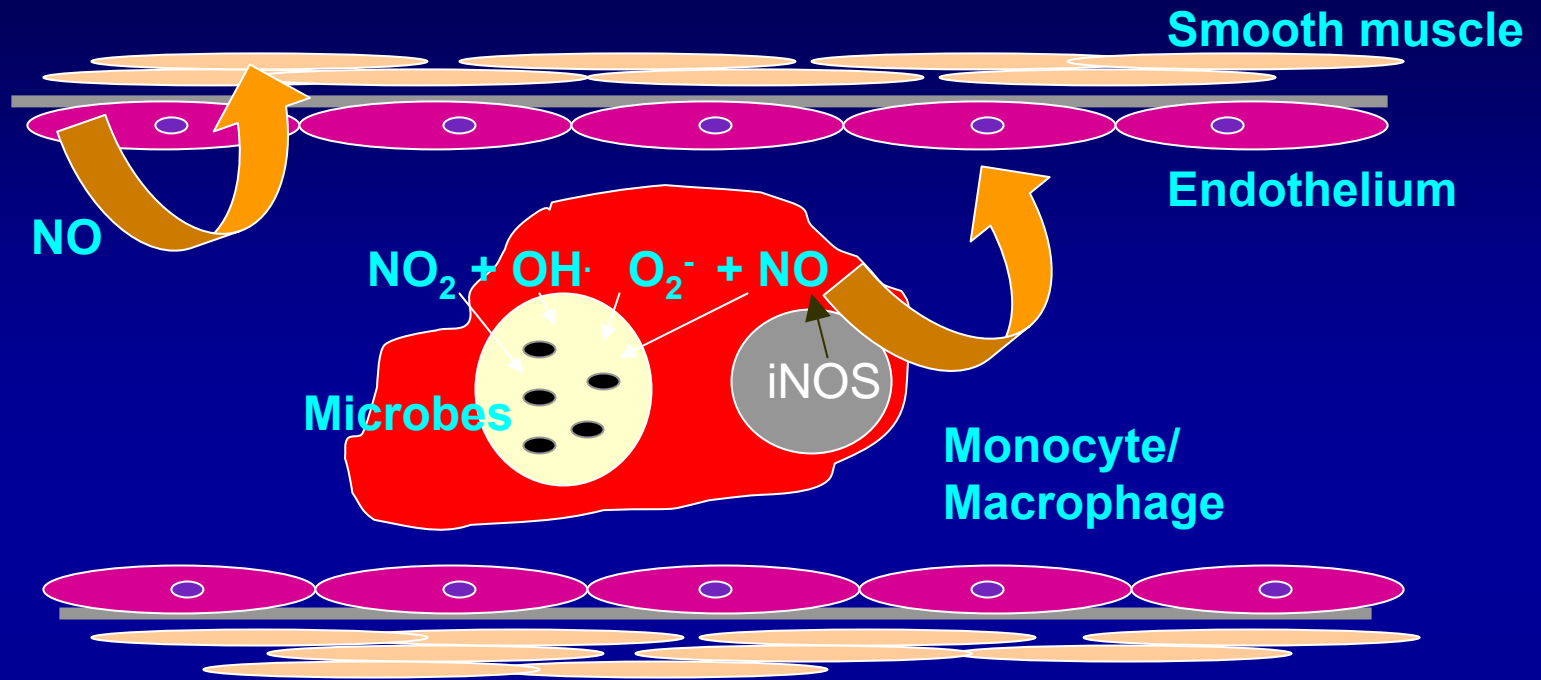
Activated Macrophages

- Activation - many factors, especially:
 - innate immunity – Endotoxin (LPS) receptor, CD14 and Toll-like receptor TLR4
 - Cytokine mediated – γ IFN (Th1 classic pathway)
- Products of Activation
 - Lysosomal enzymes and oxygen metabolites
 - Cytokines, such as TNF α , IL-1, IL-6
 - Chemokines, such as IL-8, MCP-1
 - Arachadonic acid metabolites – PG, LT
 - Plasminogen activator

Cell-derived Mediators of Acute Inflammation

- Histamine (cell-derived, preformed granules) - first response
 - → early vasodilation/ permeability
- Prostaglandin (PG)
 - arachadonic acid metabolite (cyclo-oxygenase)
 - → vasodilation > permeability, pain
- Leukotrienes (LT)
 - arachadonic acid metabolite (lipoxygenase)
 - Chemotaxin to neutrophils (LTB₄)
 - Vascular permeability
- Platelet Activating Factor (PAF)
 - Platelet activation, multiple effects
 - Vasodilation/ permeability
- Cytokines
 - TNF α , IL-1 → activate macrophages
 - IL-1 → fever
 - IL-8 → chemotaxin for polys

Nitric oxide Cell-derived



- Released preformed from stimulated endothelial cells to affect smooth muscle relaxation → **vasodilation**
- Release after synthesis from arginine in macrophages when iNOS (inducible-nitric oxide synthetase) is up-regulated (by $\text{TNF}\alpha$,...)
- **NO is antimicrobial in macrophages**

Inflammation: Plasma Mediators

Bradykinin

vasodilation
permeability
pain

Complement

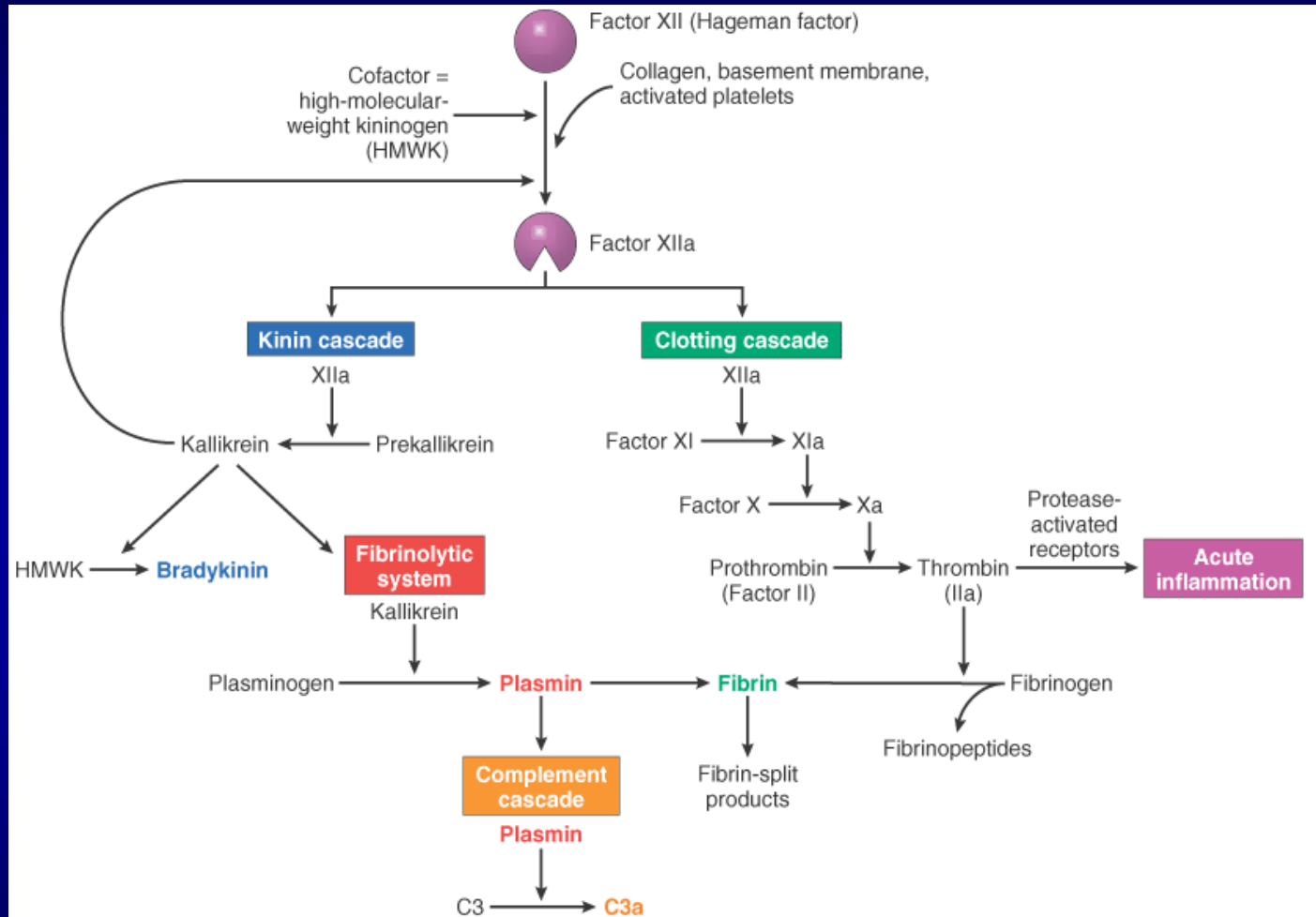
activated by -
Ag:Ab (classic)
LPS (alternative)
mannose (lectin)

C3a, C5a -

vasodilation/
permeability

C5a - chemotaxin

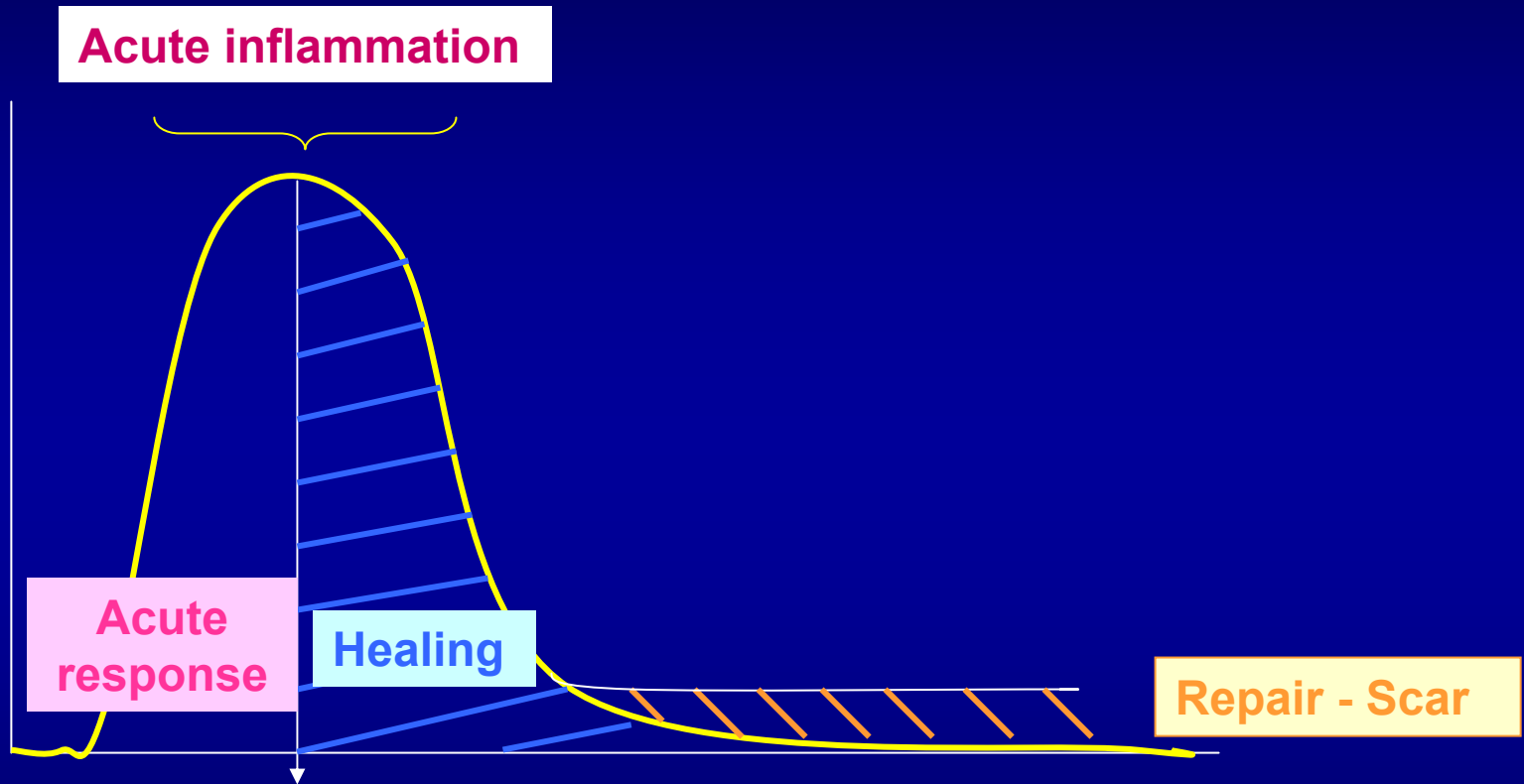
C3b - opsonin



Fibrinopeptides → vascular permeability, chemotaxin

Fibrinolytics → activates factor XII, C3a and C5a

Outcomes of Acute Inflammation



Sequence of Events in Healing and Repair

1. **Down-regulation of inflammatory mediators and up-regulation of anti-inflammatory mediators**
 - **IL-10, TGF- β**

2. **Reabsorption of edema and removal of debris**
 - **Lymphatic drainage**
 - **Macrophage phagocytosis of necrotic debris**

3. **Cell regeneration – native cells capable of mitosis**
(Growth factors - EGF, HGF, TGF α , FGF, TGF β)

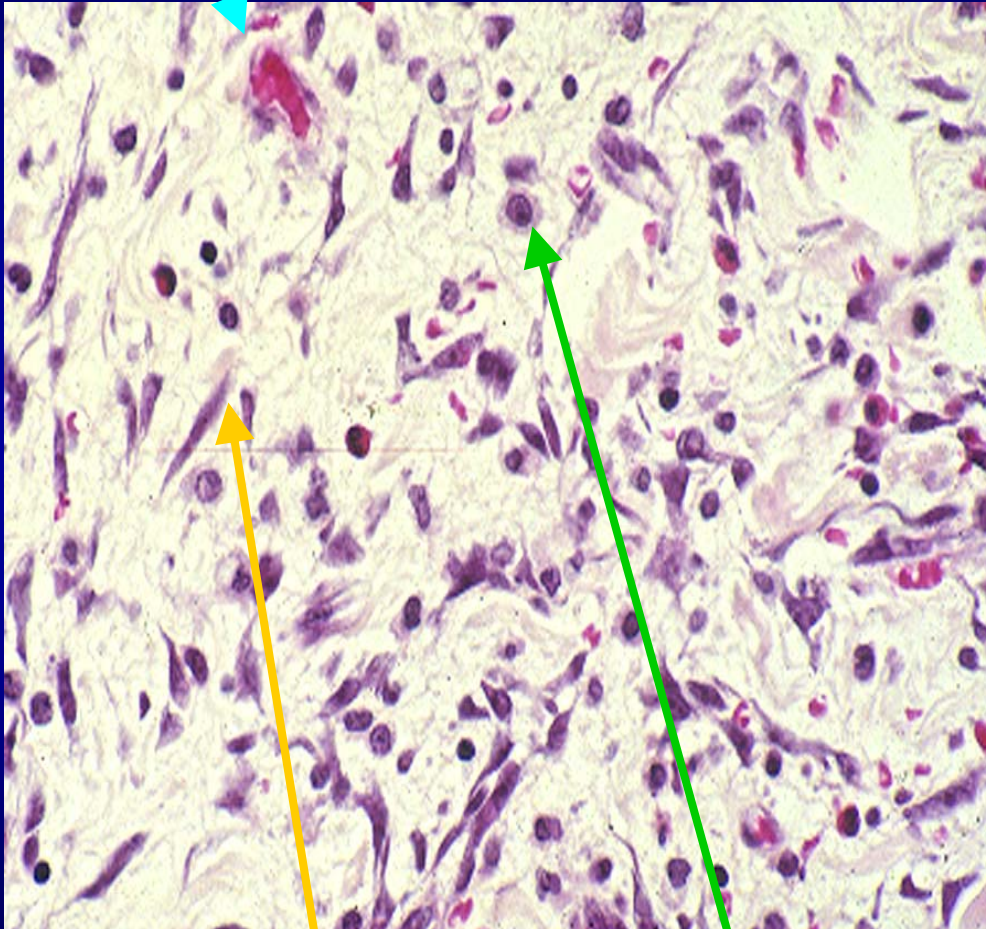
4. **Organization of new provisional tissue - granulation tissue**
 - **Accumulation of activated macrophages → cytokines**
 - **Proliferation of endothelial cells → angiogenesis**
 - **Proliferation of fibroblasts synthesizing matrix proteins**

4. **Fibrosis – scar**
 1. **Macrophages reorganize tissue and stimulate fibroblasts**
 - **Fibroblasts synthesize collagen and myofibroblasts contract to create wound closure**

Granulation Tissue

early wound organization

new arterioles

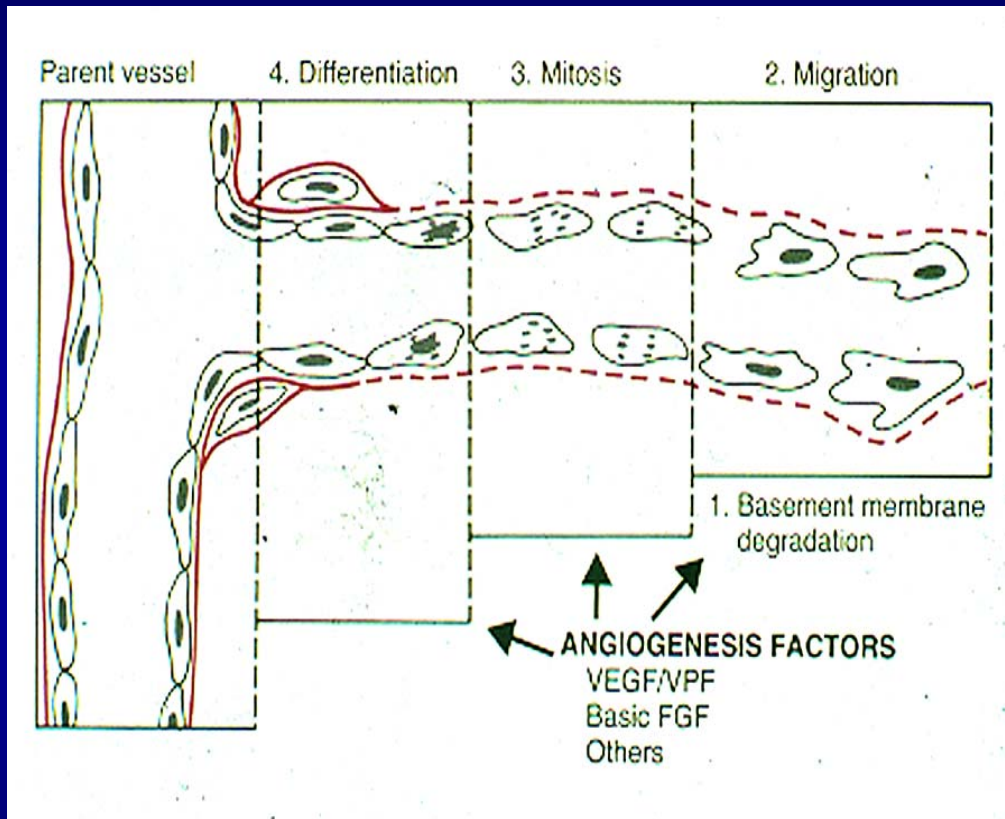


(myo)fibroblasts

macrophage

- accumulation of activated macrophages
- proliferation of fibroblasts, with a fibrin matrix
- fine collagen strands
- new capillaries - neovascularity, angiogenesis

Angiogenesis



Angiogenesis

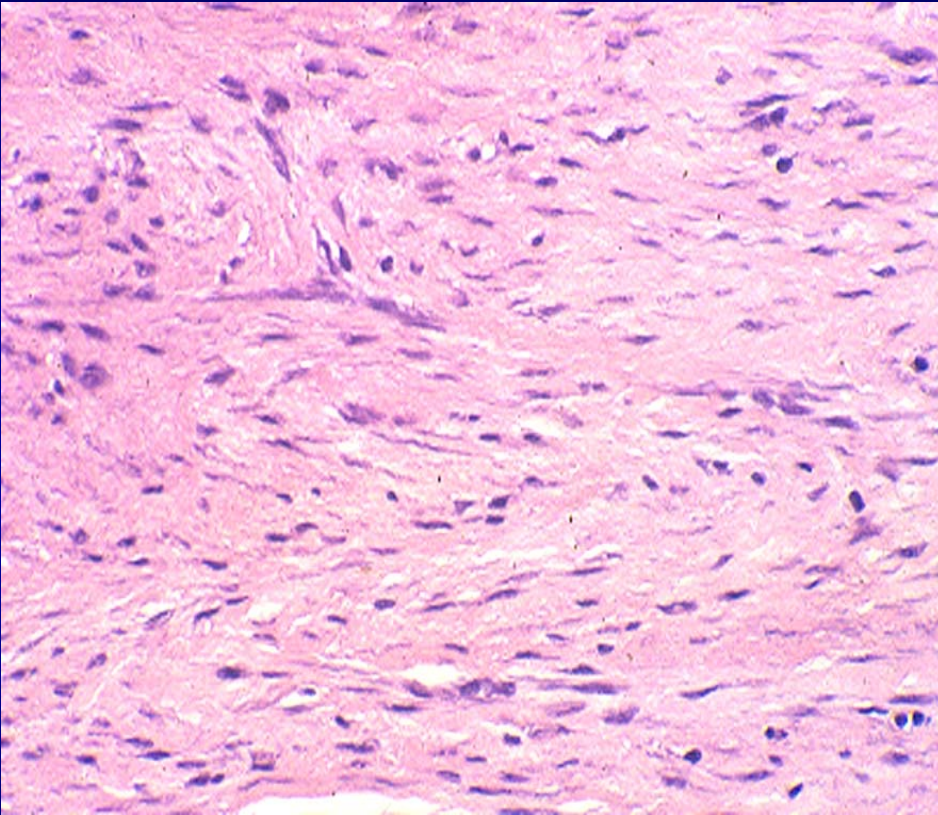
- **EPC (endothelial progenitors) from marrow enter site of injury and differentiate**
- **endothelial buds grow from pre-existing capillaries**
- **Neovascularization**

VEGF

stimulates EPC from marrow, and proliferation/ motility of endothelial cells buds

Granulation Tissue

mid-late wound organization



Fibroblasts

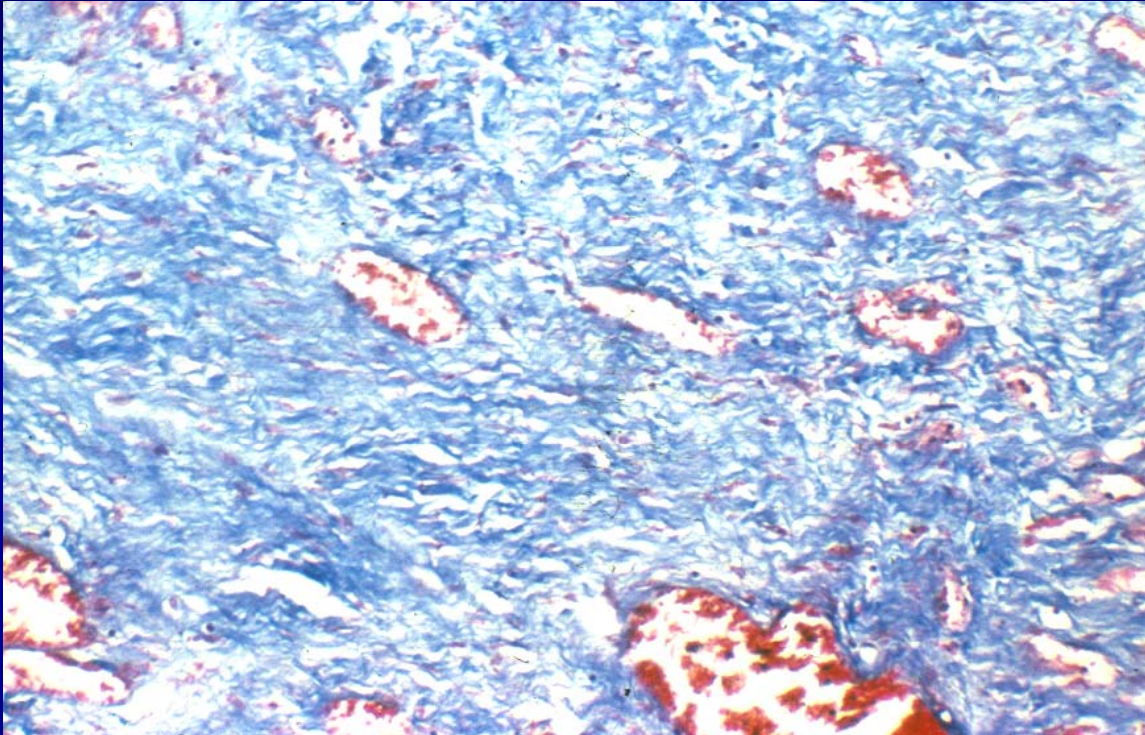
- Proliferating of fibroblasts
- Differentiation to myofibroblasts
(? epithelial cells can also differentiate to myofibroblasts)
- Circulating fibrocytes

TGF_β, FGF, PDGF

stimulate fibroblast proliferation/ motility, collagen and fibronectin synthesis

Granulation Tissue late wound organization

Image from Robbins



Fibrosis - Scar

- dense collagen sclerosis
- few cells
- Vascular regression

Masson stain -
collagen stains blue

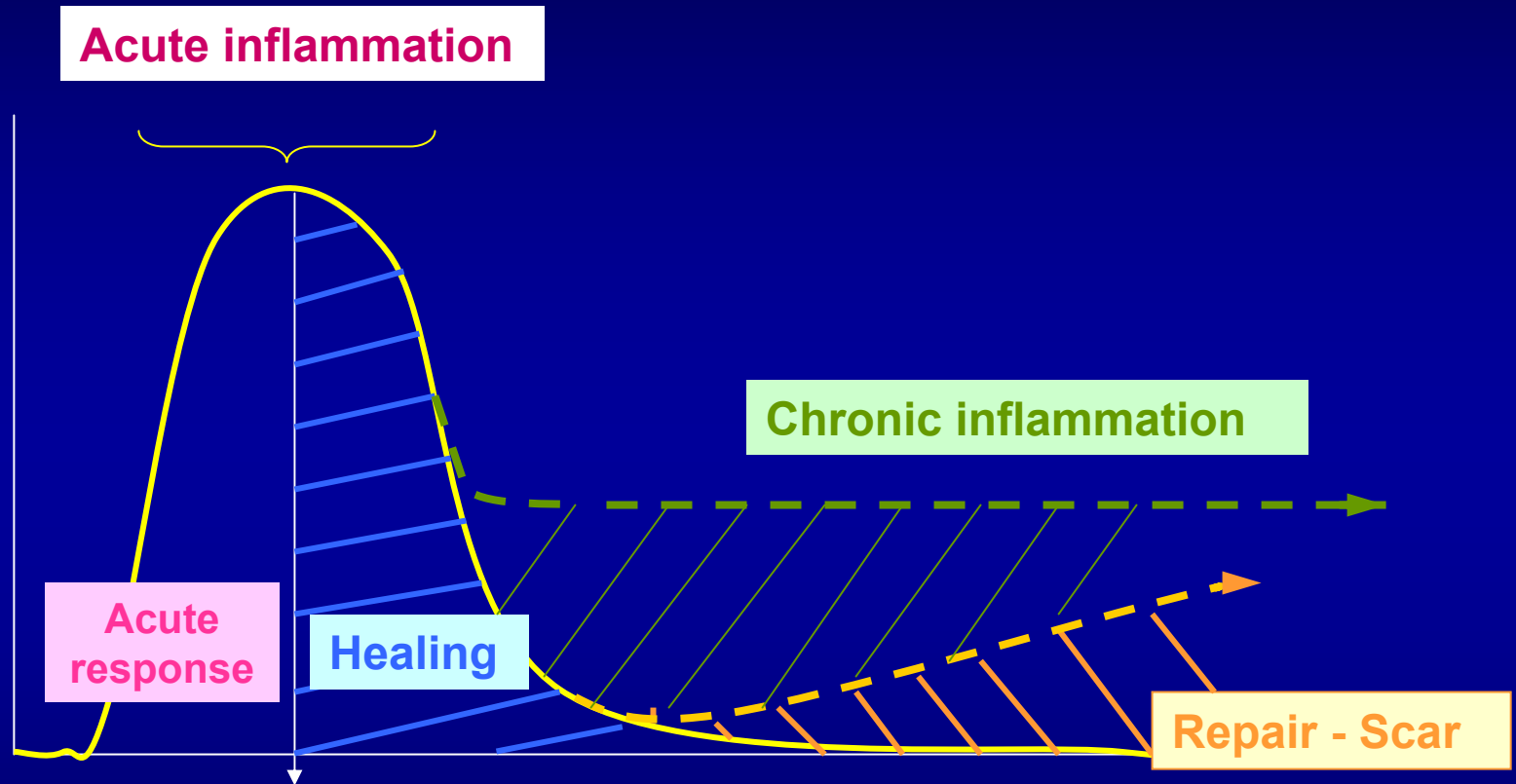
Remodeling of scar

requires metalloproteinases (Zn-dependent collagenase, stromelysin)
produced by macrophages and fibroblasts
regulated by TGF β - induced inhibitors (TIMP)

Tissue Requirements for Complete Healing

- **The architectural structure of the tissue must remain**
- **Native cells must have the capacity to regenerate**
- **No inhibitory factors to healing may be present**
 - **persistence of the causative agent**
 - **poor blood supply**
 - **defects in leukocytes**
 - **protein deficiency, malnutrition**
 - **vitamin C deficiency – inhibits collagen synthesis**
 - **suppressed immune response or steroids**

Outcomes of Acute Inflammation

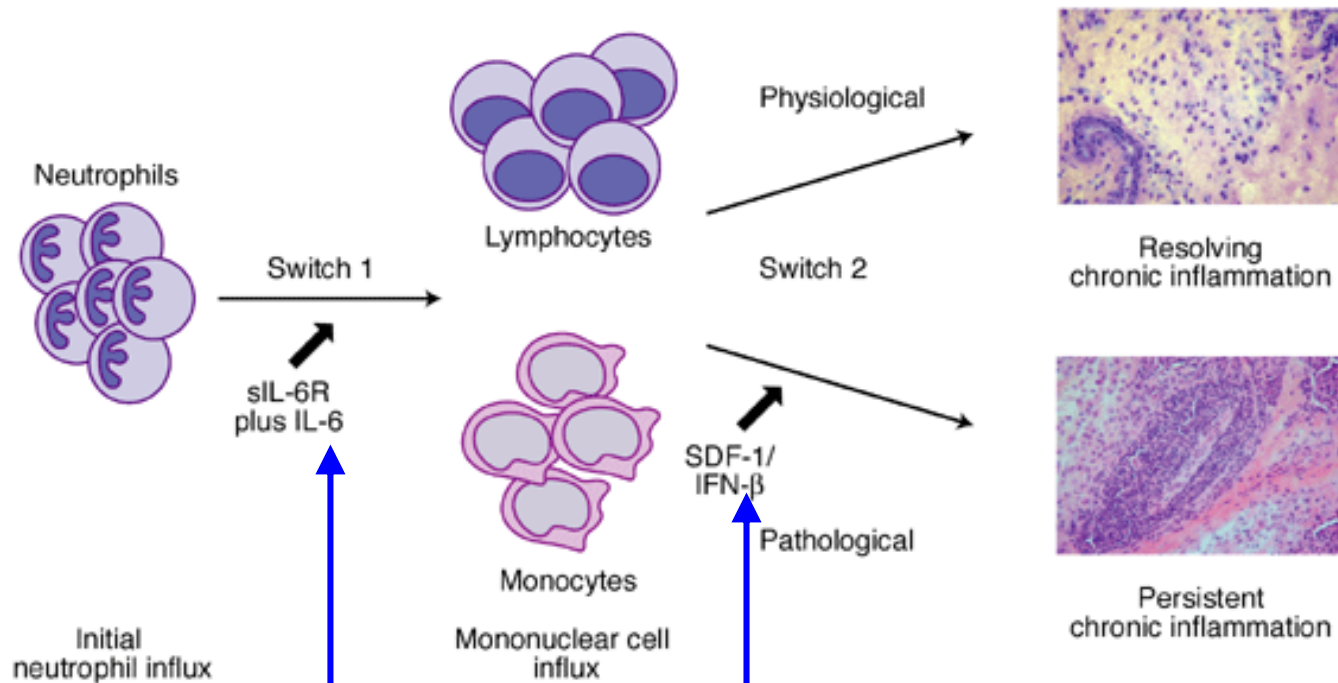


Chronic Inflammation

- **Prolonged active inflammation**
- **Concurrent healing and repair**
- **Adaptive immune-mediated component includes lymphocytes and plasma cells**
- **Macrophages are key effector cells**
- **Lymphocyte/ macrophage infiltrate**

Acute → Chronic Inflammation role of chemokines

Hurst, et al Immunity 14:705, 2001



Two switches are involved in the development of persistent chronic inflammation

Expert Reviews in Molecular Medicine © 2002 Cambridge University Press

↑ CC chemokine, MCP-1

↓ CXC chemokine, IL-8

(MCP= monocyte chemoattractant protein)

↑ IFN-β

↑ CXC chemokine, SDF-1

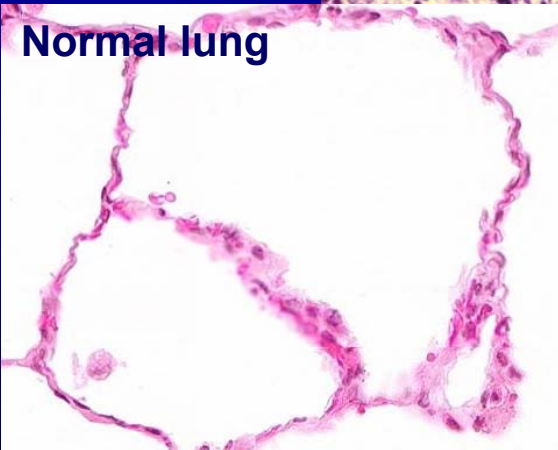
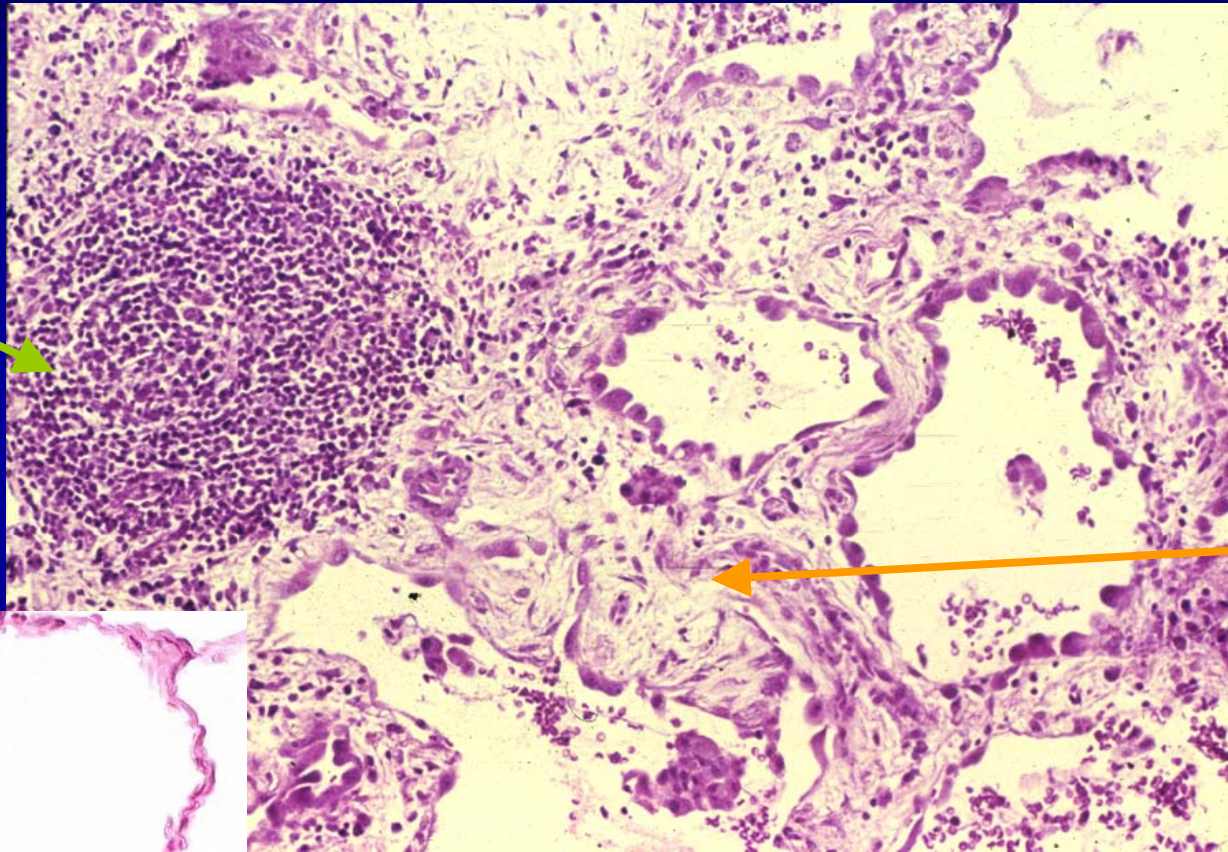
(SDF=stromal cell-derived factor)

Chronic Inflammation

Histological Patterns

- **Simple**
 - **formless “mononuclear” cell infiltrate**
lymphocytes > plasma cells, macrophages
- **Granulomatous**
 - **nodular macrophage aggregates**
macrophage clusters > rim of peripheral lymphocytes

Histopathology of Chronic inflammation Lymphocytic infiltrate



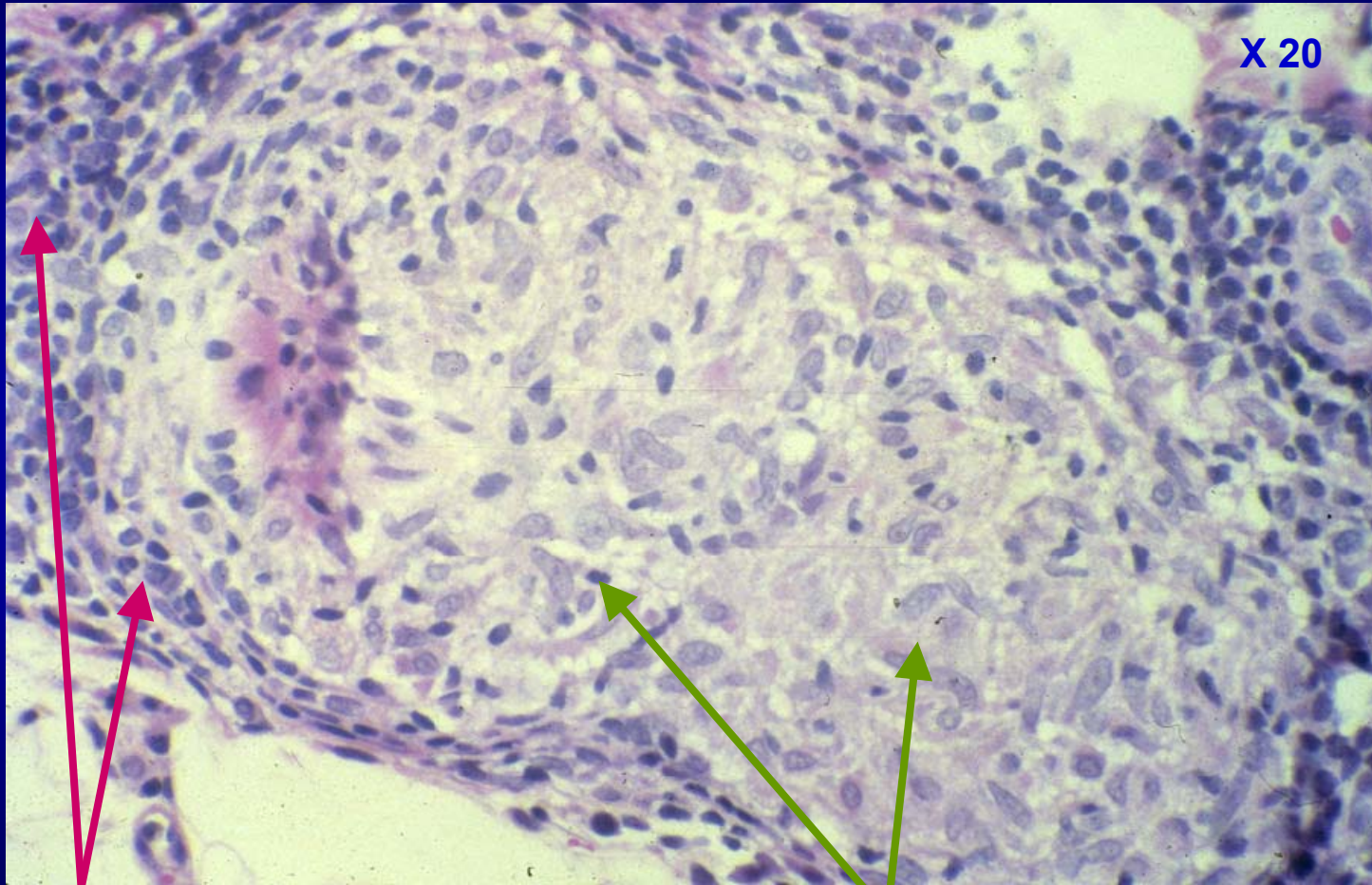
Chronic inflammation of the lung

Granuloma

strong association with (T cell) mediated immunity

- **Cluster of modified “epithelioid” macrophages**
 - Induced by agents that are insoluble with high lipid content
 - Cells develop elongated and vesicular nuclei and generous pink cytoplasm (epithelial cell-like, or epithelioid)
 - Decreased phagocytosis
 - Increased cytokine production - $\text{TNF}\alpha$ (secretory phenotype)
- **Variable peripheral rim of lymphocytes**
 - T lymphocytes \rightarrow $\text{IFN}\gamma$ (activates macrophages)
- **Etiologies:**
 - Inorganic dusts (indigestible foreign particulate matter)
 - Hypersensitivity (molds, etc...)
 - M. tuberculosis (typified by central “caseous” necrosis)
 - Fungi
 - Sarcoidosis (unknown mechanism)

Granuloma



Peripheral rim of lymphocytes

Cluster of epithelioid macrophages

Acute Inflammation pulse of injury

- Host response is nonspecific
- Onset is abrupt, well-defined
- Symptoms are prominent
- Prominent vascular effects and exudate
- Exudate is **neutrophils**
- CT proliferation occurs after inflammation subsides

Chronic Inflammation persistent injury

- Host response is variably immune-mediated
- Onset is vague
- Symptoms are often subdued
- Mild tissue effects
- Exudate is **lymphocytes and macrophages**
- CT proliferation is concurrent with on-going inflammation

Chronic Inflammation

Potential Adverse Outcome

- **Loss of organ cell function**
 - **Example: liver failure**
- **Irreversible (?) accumulation of collagen that distorts functional architecture**
 - **Example: liver cirrhosis and portal hypertension**
- **Persistent regeneration of surviving cells favors those adapted to abnormal microenvironments and those with mutations → cancer**
 - **Example: chronic hepatitis C → hepatocellular carcinoma**