INFLAMMATION III

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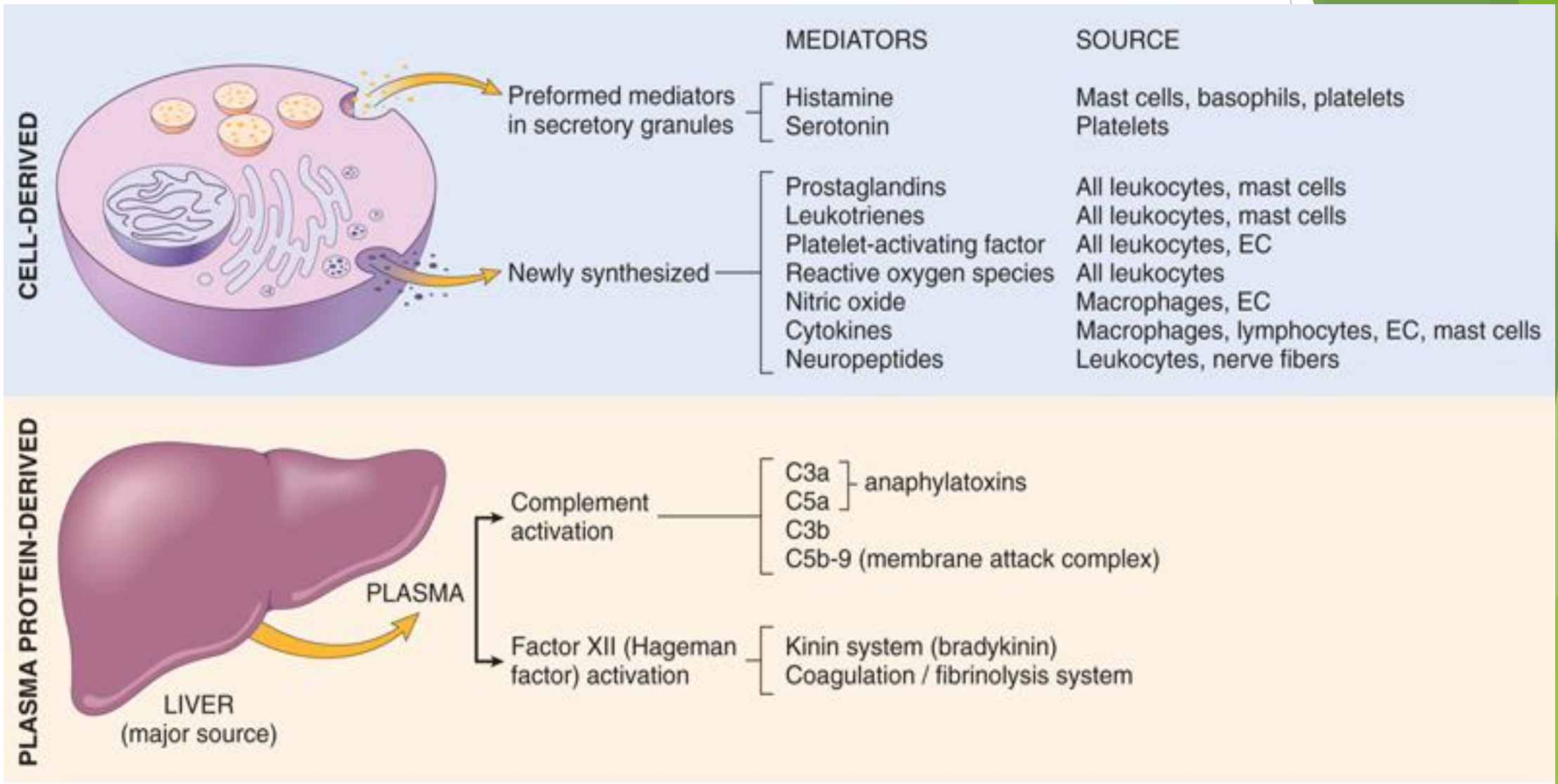
Chemical Mediators of Inflammation

- The mediators of inflammation are the substances that **initiate** and **regulate** inflammatory reactions.
- Harried student may find the list of mediators daunting (as do professors!)
- This knowledge has been used to design a large armamentarium of **anti-inflammatory** agents that are used every day by many people and which include familiar drugs such as aspirin and acetaminophen.
- Mediators may be **produced locally** by cells at the site of inflammation, or may be derived from **circulating inactive precursors** that are activated at the site of inflammation.

Chemical Mediators of Inflammation

- What are their sources?
  - **Circulating plasma proteins**
    - Coagulation / fibrinolytic factors
    - Complement System
    - Kinins
  - **Cell derived**
    - Formed elements normally sequestered in granules:
      - Vasoactive amines
    - Newly synthesized in response to stimulation
      - PGs, LT, O_2 species, NO, Cytokines, PAF

Systemic Mediators of Inflammation



Kumar et al: Robbins Basic Pathology, 9e.

Chemical Mediators of Inflammation

- General characteristics
  - Bind to specific cellular receptors, or have enzymatic activity
  - May stimulate target cells to release secondary mediators with similar or opposing functions
  - May have limited targets, or wide spread activities
  - Short lived function
    - Short half-life (AA metabolites)
    - Inactivated by enzymes (kininase on bradykinin)
    - Eliminated (antioxidants on O2 species)
    - Inhibited (complement inhibitory proteins)
  - If unchecked and uncontrolled, cause harm

Chemical Mediators of Inflammation

- The major cell types that produce mediators of acute inflammation are tissue macrophages, dendritic cells, and mast cells, but platelets, neutrophils, endothelial cells, and most epithelia also can be induced to elaborate some of the mediators.

- Cell-derived mediators are most important for reactions against offending agents in tissues.

- Plasma-derived mediators (e.g., complement proteins), produced mainly by the liver, are present in the circulation as inactive precursors that must be activated, usually by a series of proteolytic cleavages, to acquire their biologic properties and are effective against circulating microbes, but also can be recruited into tissues.

Principal Mediators of Inflammation

Mediator	Source	Action
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion, and activation
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
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Complement	Plasma (produced in liver)	Leukocyte chemotaxis and activation, direct target killing (membrane attack complex), vasodilation (mast cell stimulation)
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction,

Vasoactive Amines
Histamine and Serotonin

- So named because they have important actions on blood vessels.
- Stored in granules in mast cells (histamine), and platelets (serotonin)
- Cause arteriolar dilatation and increases permeability of venules (immediate phase reaction)
- Induce endothelial cell contraction in venules
- Binds to H1 receptors on microvascular endothelial cells
- Inactivated by histaminase
- The antihistamine drugs that are commonly used to treat some inflammatory reactions, such as allergies, are H1 receptor antagonists that bind to and block the receptor.

Vasoactive Amines
Histamine and Serotonin

Release of histamine

- Physical injury (trauma, cold, heat)
- Binding of IgE to Fc receptors
- Anaphylatoxins (C3a, C5a) binding
- Histamine releasing protein derived from PMNs
- Neuropeptides (substance P)
- Cytokines (IL-1, IL-8)

Release of serotonin

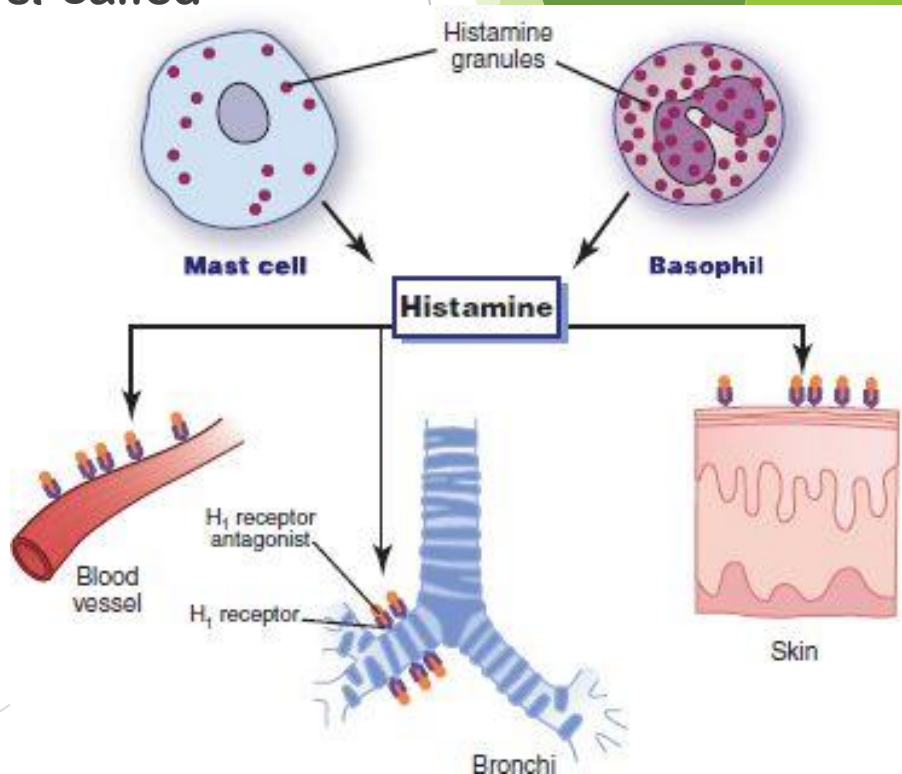
- Platelets aggregation
- PAF

ACTION OF HISTAMINE:

- Histamine causes dilation of arterioles and increases the permeability of venules. Histamine is considered the principal mediator of the immediate transient phase of increased vascular permeability, producing interendothelial gaps in postcapillary venules

- Its vasoactive effects are mediated mainly via binding to receptors. called H1 receptors, on microvascular endothelial cells.

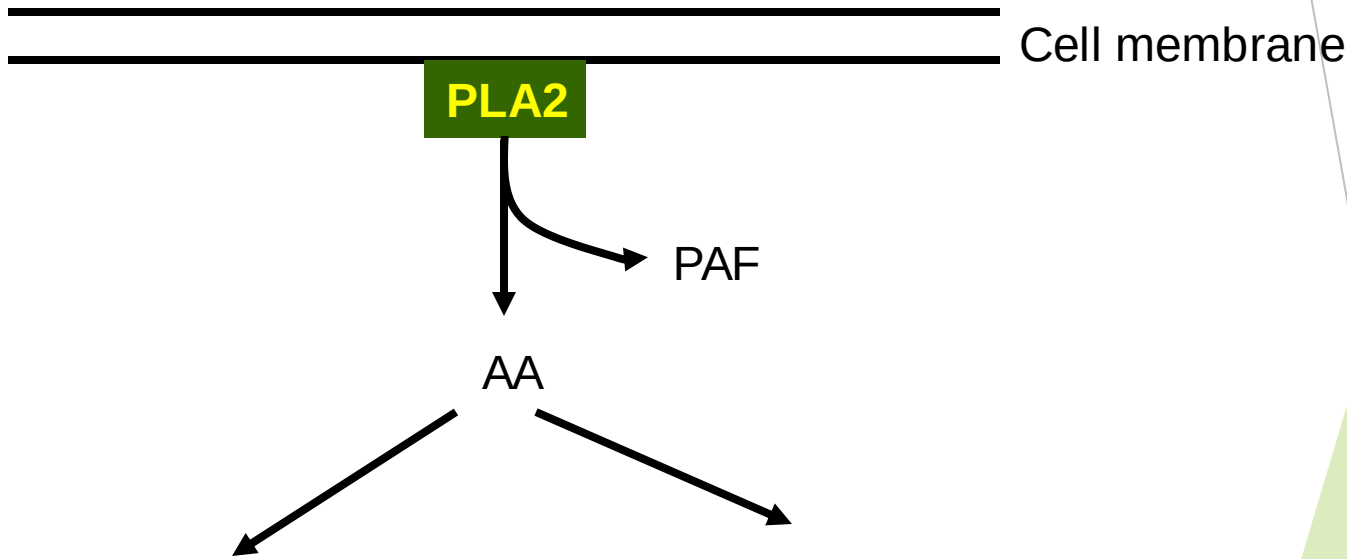
- Histamine also causes contraction of some smooth muscles



Arachidonic Acid Metabolites

- The lipid mediators prostaglandins and leukotrienes are produced from arachidonic acid present in membrane phospholipids, and they stimulate vascular and cellular reactions in acute inflammation.
- Arachidonic acid is a 20-carbon polyunsaturated fatty acid that is derived from dietary sources or by conversion from the essential fatty acid linoleic acid.
- Most cellular arachidonic acid is esterified and incorporated into membrane phospholipids.
- Mechanical, chemical, and physical stimuli or other mediators (e.g., C5a) trigger the release of arachidonic acid from membranes by activating cellular phospholipases, mainly phospholipase A2.
- Once freed from the membrane, arachidonic acid is rapidly converted to bioactive mediators. These mediators, also called eicosanoids (because they are derived from 20-carbon fatty acids; Greek eicosa = 20)

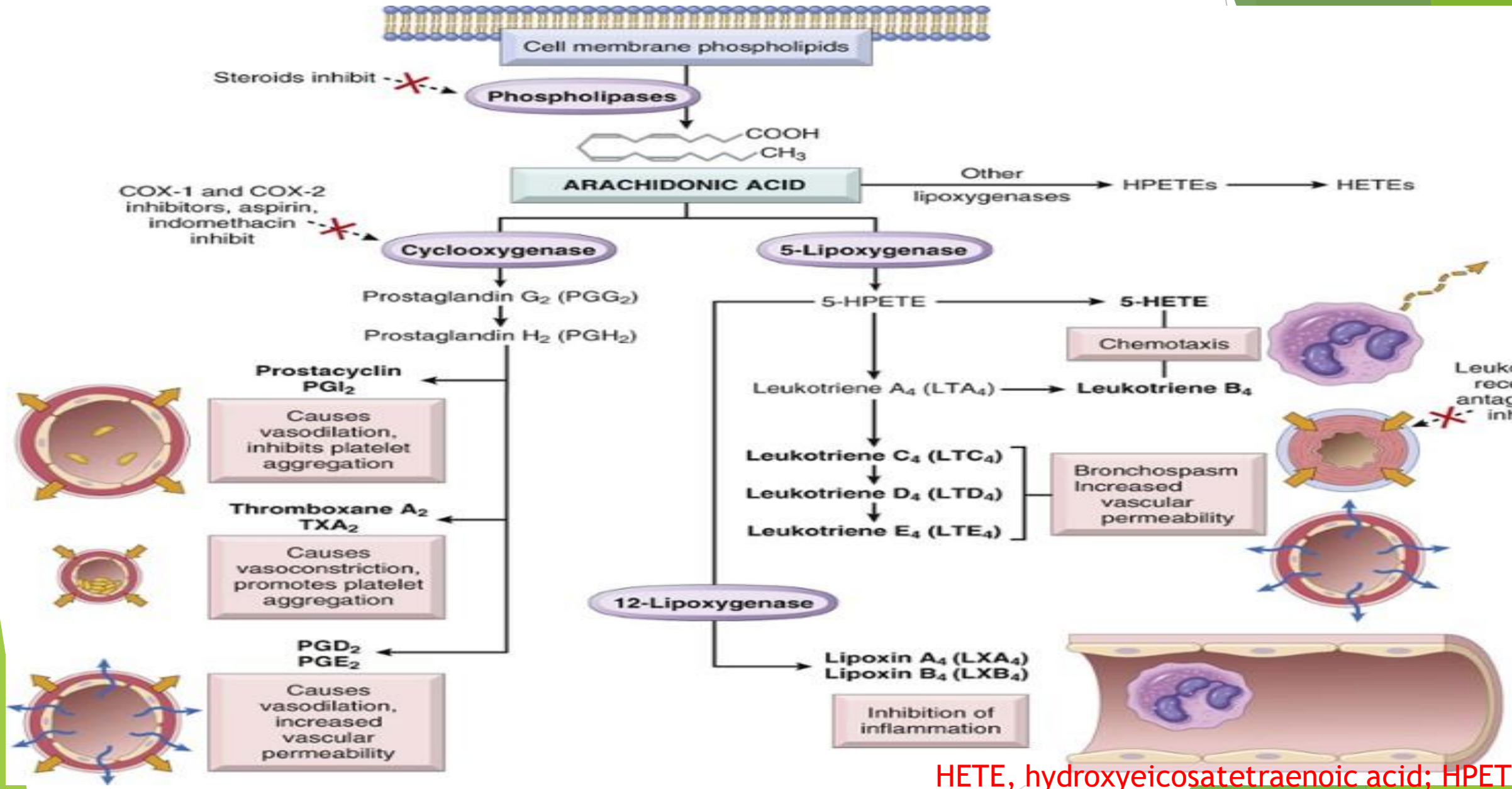
Arachidonic Acid Metabolism



Prostaglandins
PGE2 PGF2
PGD2 PGI2
TXA2

Leukotrienes & lipoxins
LTB4
LTC4, D4, E4
HETE

Generation of AA Metabolites



HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

Products of the Cycloxygenase pathway of AA metabolism

- **TXA2**
  - Vasoconstriction
  - Stimulates platelets aggregation and thrombosis.
- **PGI2 (Prostacyclin)**
  - Vasodilatation
  - Inhibits platelets aggregation
- **PGD2, PGE2, PGF2a (Prostaglandins)**
  - Vasodilatation
  - Edema formation
  - Pain (PGE2)

- **Prostaglandins are also involved in the pathogenesis of pain and fever, two common systemic manifestations of inflammation.**
- **PGE2 makes the skin hypersensitive to painful stimuli, and causes fever during infections**

Products of the Lipoxygenase pathway of AA metabolism

- **5-HETE and LTB4**
  - Chemotactic
- **LTC4, LTD4 and LTE4**
  - Vasoconstriction
  - Bronchospasm
  - Increased vascular permeability
- **Lipoxins (LXA4 & LXB4)**
  - Vasodilatation
  - Inhibit neutrophil chemotaxis and adhesion
  - Stimulate monocyte adhesion

Principal Actions of Arachidonic Acid Metabolites in Inflammation

Action	Eicosanoid
Vasodilation	Prostaglandins PGI_2 (prostacyclin), PGE_1, PGE_2, PGD_2
Vasoconstriction	Thromboxane A_2, leukotrienes C_4, D_4, E_4
Increased vascular permeability	Leukotrienes C_4, D_4, E_4
Chemotaxis, leukocyte adhesion	Leukotriene B_4
Smooth muscle contraction	Prostaglandins PGC4, PGD4, PGE4

Pharmacologic Inhibitors of Prostaglandins and Leukotrienes

1. Cyclooxygenase inhibitors (aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen).

- They inhibit both COX-1 and COX-2 and thus block all prostaglandin synthesis

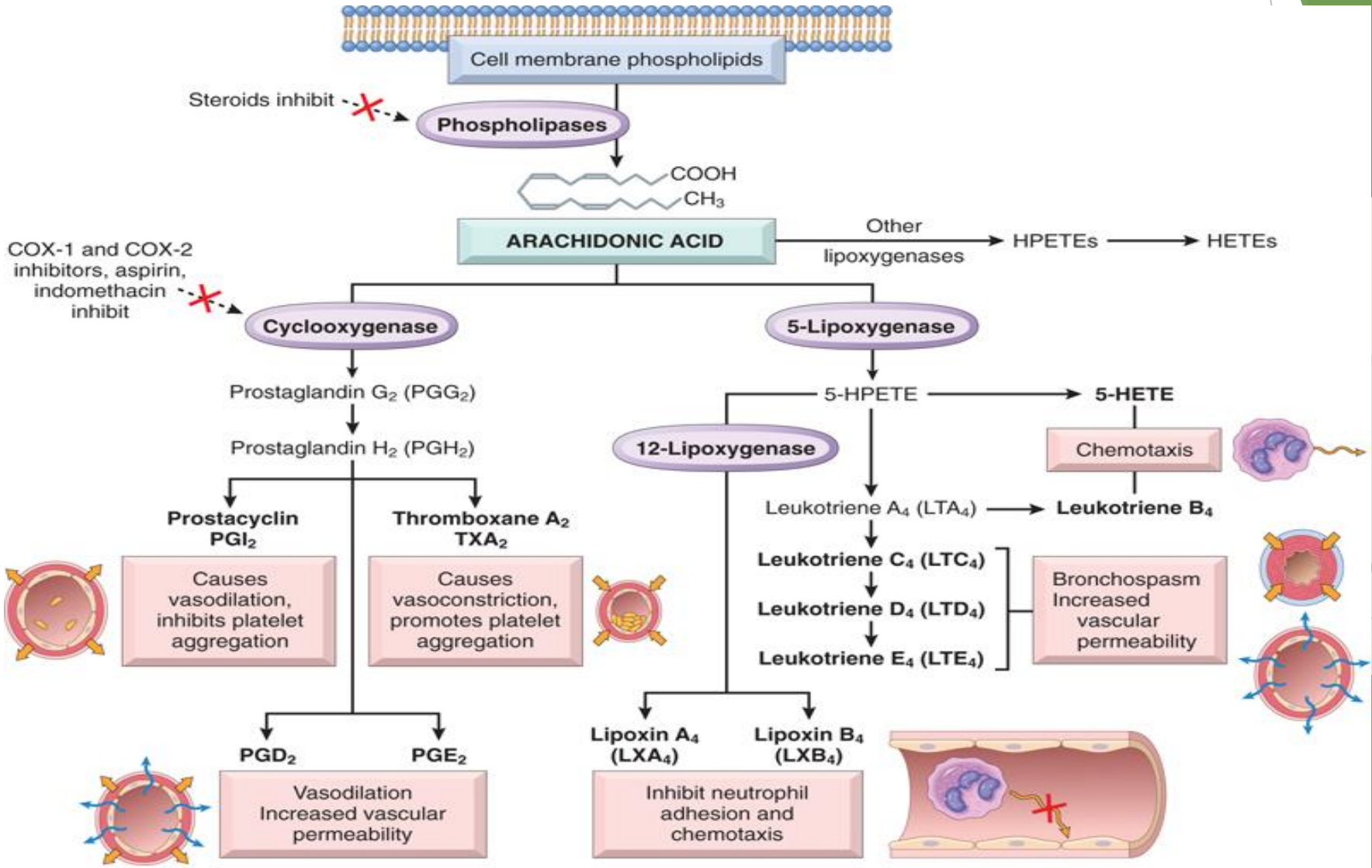
- Efficient in treating pain and fever

- Selective COX-2 inhibitors are a newer class of these drugs that are 200- to 300- fold more potent in blocking COX-2 than COX-1.

- COX-1 is responsible for the production of prostaglandins that are involved in both inflammation and physiologic functions such as protecting gastric epithelial cells from acid- induced injury (Gastric Protective).

- COX-2 generates prostaglandins that are involved only in inflammation (risk of thrombosis).

Generation of AA Metabolites



Pharmacologic Inhibitors of Prostaglandins and Leukotrienes

2. Lipoxygenase inhibitors

- 5-lipoxygenase is not affected by NSAIDs
  - Many new inhibitors of this enzyme pathway have been developed.
  - Pharmacologic agents that inhibit leukotriene production (e.g., zileuton) are useful in the treatment of asthma

3. Corticosteroids

- Broad-spectrum anti-inflammatory agents.
- Reduce the transcription of genes encoding COX-2, phospholipase A2, proinflammatory cytokines (e.g., IL-1 and TNF), and iNOS.

4. Leukotriene receptor antagonists

- block leukotriene receptors and prevent the actions of the leukotrienes.
- These drugs (e.g., Montelukast) are useful in the treatment of asthma.

Platelet-activating Factor (PAF)

- Generated from membranes phospholipids by Phospolipase A2
- Discovered as a factor that caused platelet aggregation, but it is now known to have multiple inflammatory effects
- Aggregates and degranulates platelets
- Potent vasodilator and bronchoconstrictor
- At low concentrations it induces vasodilation and increased vascular permeability

- Effects on leukocytes
  - Increase adhesion to endothelial cells
  - Chemotactic
  - Degranulation
  - Oxygen burst

Cytokines

- Cytokines are proteins secreted by many cell types (principally activated lymphocytes, macrophages, and dendritic cells, but also endothelial, epithelial, and connective tissue cells) that mediate and regulate immune and inflammatory reactions.

- By convention, growth factors that act on epithelial and mesenchymal cells are not grouped under cytokines.

- Hormone-like polypeptides produced by cells, involved in cell to cell communication
- Cytokines have diverse and pleiotropic effects including immunologic, hematopoietic and pro-inflammatory activities.

- Secretion is transient
- Effects: autocrine, paracrine, endocrine

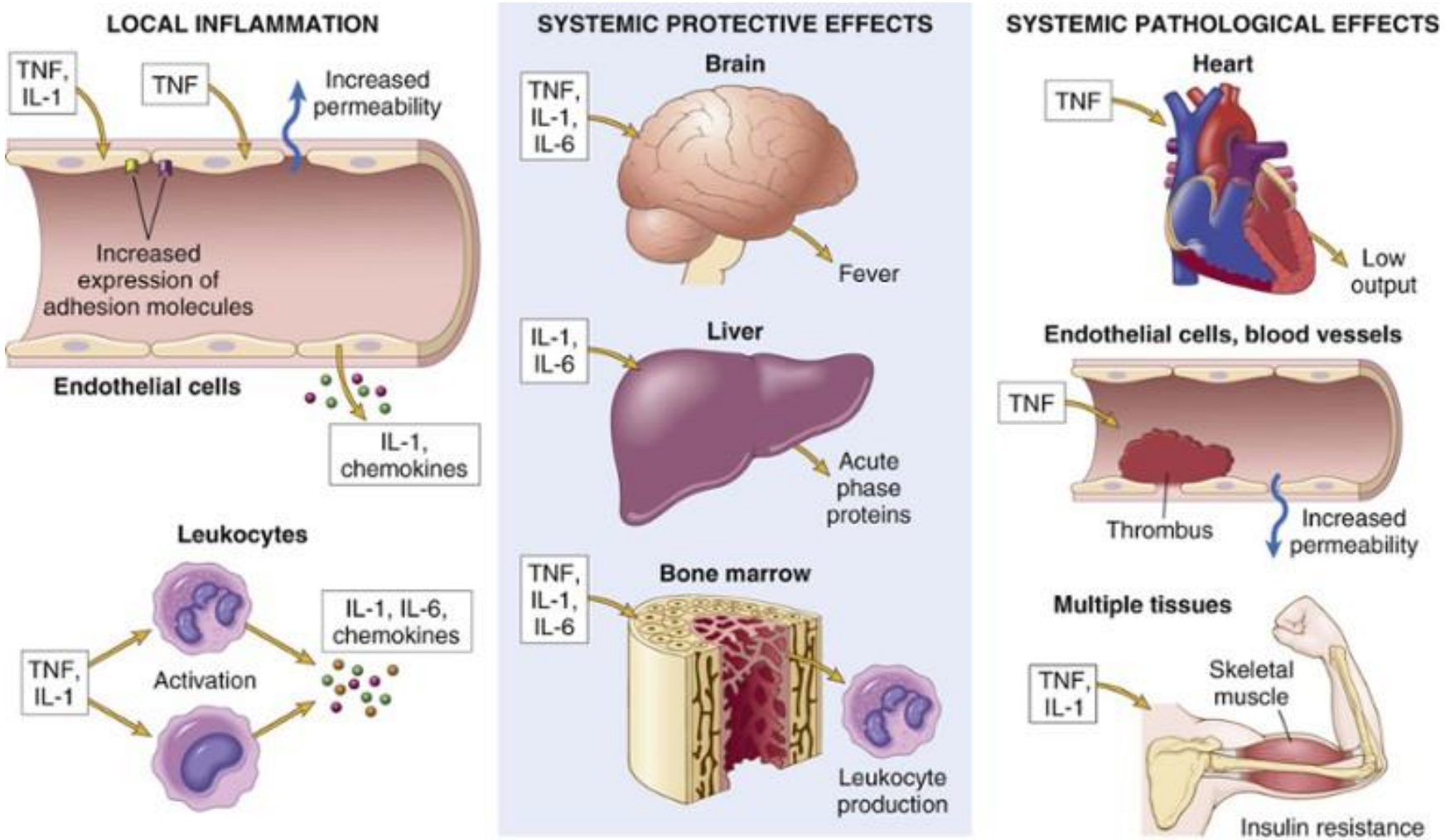
Classes of cytokines

- Regulators of lymphocyte function
  - IL-2 stimulates proliferation
  - TGFb inhibits lymphocytes growth
- Primary responders to injury (innate immunity)
  - IL-1 & TNF
- Activators of cell mediated immunity
  - INF-g & IL-12
- Chemotactics
  - IL-8
- Hematopoietic growth factors
  - IL-3 & GM-CSF

Cytokines in Inflammation

Cytokine	Principal Sources	Principal Actions in Inflammation
In Acute Inflammation		
TNF	Macrophages, mast cells, T lymphocytes	Stimulates expression of endothelial adhesion molecules and secretion of other cytokines; systemic effects
IL-1	Macrophages, endothelial cells, some epithelial cells	Similar to TNF; greater role in fever
IL-6	Macrophages, other cells	Systemic effects (acute phase response)
Chemokines	Macrophages, endothelial cells, T lymphocytes, mast cells, other cell types	Recruitment of leukocytes to sites of inflammation; migration of cells in normal tissues
IL-17	T lymphocytes	Recruitment of neutrophils and monocytes
In Chronic Inflammation		
IL-12	Dendritic cells, macrophages	Increased production of IFN-γ
IFN-γ	T lymphocytes, NK cells	Activation of macrophages (increased ability to kill microbes and tumor cells)
IL-17	T lymphocytes	Recruitment of neutrophils and monocytes

Major roles of cytokines in acute inflammation



TNF & IL-1

- Produced mainly by macrophages
- Secretion stimulated by: bacterial products, immune complexes, endotoxins, physical injury, other cytokines.
- Effects on endothelial cell, leukocytes, fibroblasts, and acute phase reactions.
- TNF antagonists have been remarkably effective in the treatment of chronic inflammatory diseases, particularly rheumatoid arthritis, psoriasis, and some types of inflammatory bowel disease.

Major Effects of IL-1 & TNF

Bacterial products, immune complexes, toxins, physical injury, other cytokines

↓

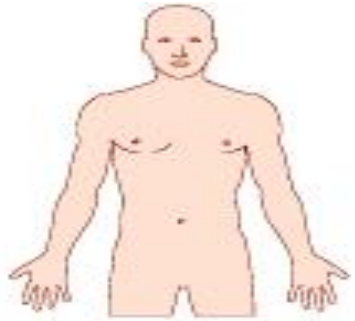
MACROPHAGE (and other cell) ACTIVATION

↓

IL-1 / TNF

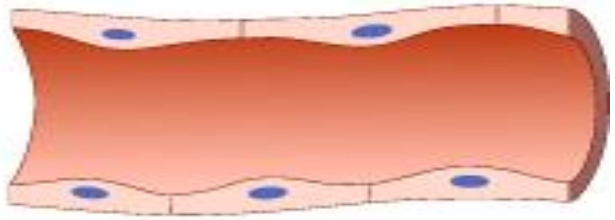
ACUTE-PHASE REACTIONS

Fever
↑ Sleep
↓ Appetite
↑ Acute-phase proteins
Hemodynamic effects (shock)
Neutrophilia



ENDOTHELIAL EFFECTS

↑ Leukocyte adherence
↑ PGI synthesis
↑ Procoagulant activity
↓ Anticoagulant activity
↑ IL-1, IL-8, IL-6, PDGF



FIBROBLAST EFFECTS

↑ Proliferation
↑ Collagen synthesis
↑ Collagenase
↑ Protease
↑ PGE synthesis

LEUKOCYTE EFFECTS

↑ Cytokine secretion (IL-1, IL-6)

Chemokines

- A group of related chemotactic polypeptides, all of which have 4 cysteine residues.
- Chemokines are a family of small (8–10 kD) proteins that act primarily as chemoattractants for specific types of leukocytes.
- About 40 different chemokines and 20 different receptors for chemokines have been identified.
- Regulate adhesion, chemotaxis and activation of leukocytes.
- Important for proper targeting of leukocytes to infection sites.

Chemokines

- Chemokines mediate their activities by binding to seven-transmembrane G protein–coupled receptors.
- They have **two main** functions:

1- ***Acute inflammation (inflammatory chemokines)***

Most chemokines stimulate leukocyte attachment to endothelium by acting on leukocytes to increase the affinity of integrins, and also serve as chemoattractants, thereby guiding leukocytes to sites of infection or tissue damage. Because they mediate aspects of the inflammatory reaction, they are sometimes called inflammatory chemokines. Their production is induced by microbes and other stimuli

Chemokines

- ***2 - Maintenance of tissue architecture (homeostatic chemokines)***

- Some chemokines are produced constitutively by stromal cells in tissues and are sometimes called homeostatic chemokines.

- These organize various cell types in different anatomic regions of the tissues, such as T and B lymphocytes in discrete areas of the spleen and lymph nodes -

Chemokines

- The largest family consists of CC chemokines, so named because the first 2 of the 4 cysteine residues are adjacent to each other.

- Examples of CC chemokines:
  - CCL2: Monocyte chemoattractant protein 1 (MCP-1)
  - CCL3 & CCL4: Macrophage inflammatory protein 1 (MIP-1a & 1b)
  - CCL5: RANTES **(regulated and normal T-cell expressed and secreted)**
  - CCL11: Eotaxin

- Examples of CXC chemokines:
  - CXCL8: IL-8, neutrophil chemotactic

- Difficult to develop chemokine antagonists that suppress inflammation (functional redundancy of these proteins).

The End