Charting New Horizons in Education

Mediators of inflammation

07

Pathology

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Definition and properties

- Inflammatory mediators are the substances that initiate and regulate inflammatory reactions.

- The most important mediators of acute inflammation are:

1. Vasoactive amines
2. Lipid products (prostaglandins and leukotrienes) (Most important)
3. Cytokines (including chemokines)
4. Products of complement activation

Definition and properties

- Mediators may be produced locally by cells at the site of inflammation **(resident cells)**, or may be derived from circulating inactive precursors that are activated at the site of inflammation.
- Cell-derived mediators are rapidly released from intracellular granules or synthesized de novo in response to a stimulus → They are most important for reactions against **offending agents in tissues.**

- Plasma-derived mediators (e.g., complement proteins) are present in the circulation → They are produced mainly in the liver, are effective against **circulating microbes**

- Active mediators are produced only in response to various molecules that stimulate inflammation, including microbial products and substances released from necrotic cells.

Definition and properties

- Most of the mediators are short-lived.

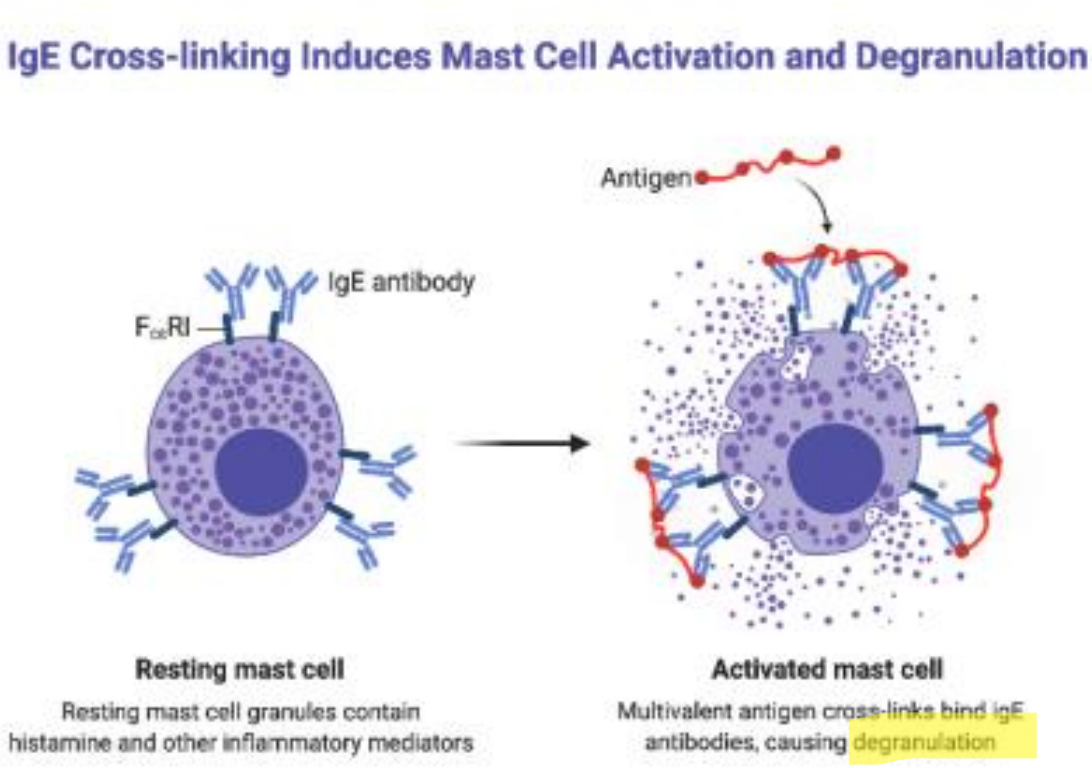
- One mediator can stimulate the release of other mediators.

- **Major groups:**
- Vasoactive Amines: Histamine and Serotonin.
- Arachidonic Acid Metabolites.
- Cytokines and Chemokines.
- Complement System.

Vasoactive Amines:

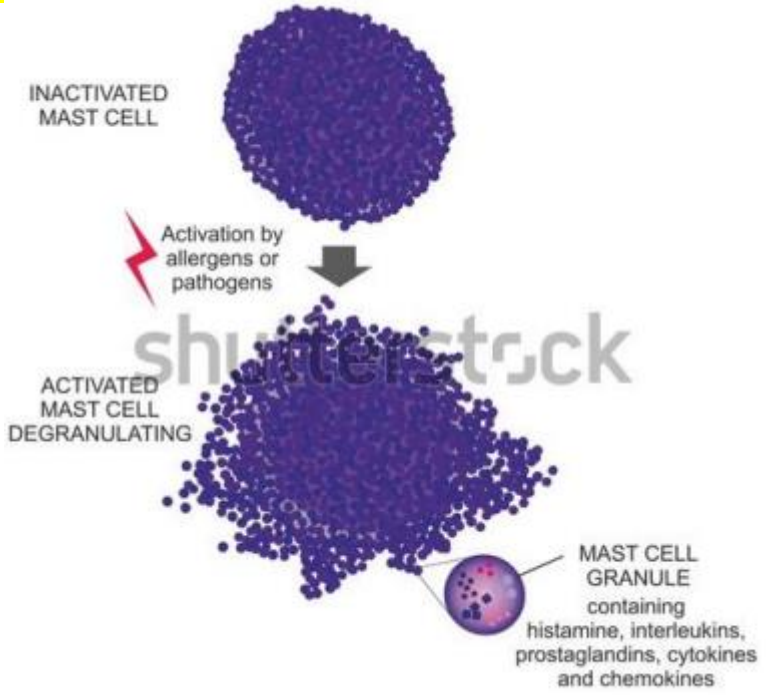
1. Hi*stam*ine:

- Vasoactive means: act on blood vessels.
- They are stored as preformed molecules in cells and are therefore among **the first mediators** to be released during inflammation.
- Released from ***mast* cells**, which are normally present in the connective tissue adjacent to blood vessels.
- Histamine also is found in blood basophils and platelets



Vasoactive Amines:

- It is stored in mast cell granules and is released by degranulation in response to a variety of stimuli, including:



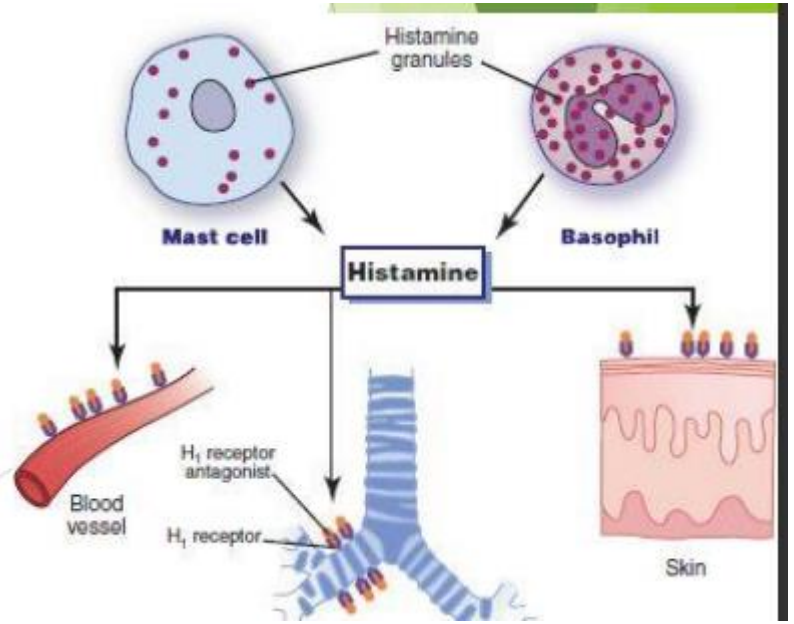
1. Physical injury, such as trauma, cold, or heat, by unknown mechanisms.

2. Binding of antibodies to mast cells, which underlies immediate hypersensitivity (allergic) reactions.

3. Products of complement called anaphylatoxins (C3a and C5a)

Vasoactive Amines:

- Its vasoactive effects are mediated mainly via binding to **H1 receptors.**



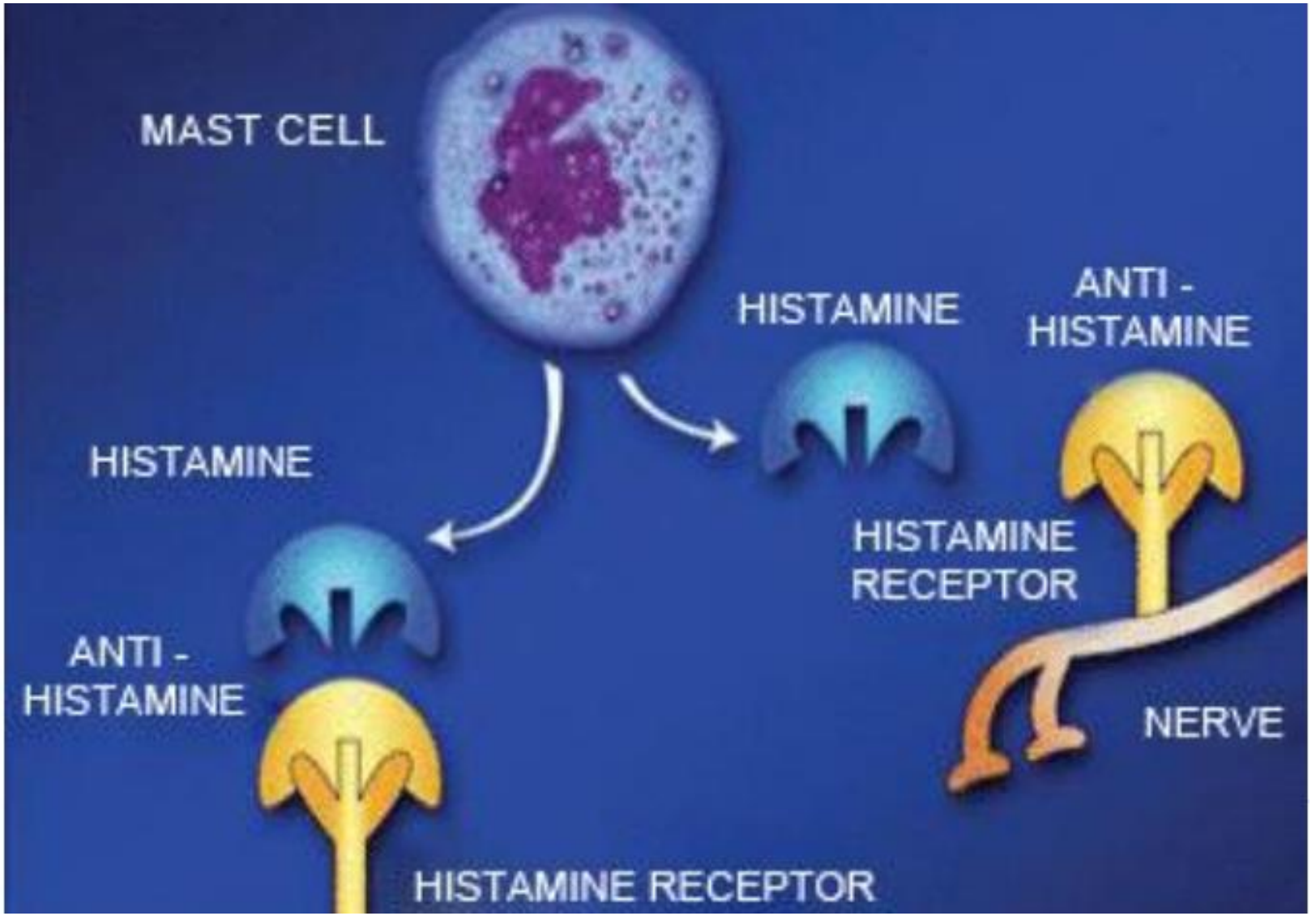
- Histamine actions:

1. Vasodilation.
2. Increases vascular permeability of venules, producing interendothelial gaps in postcapillary venules.
3. Contraction of some smooth muscles

- In an autopsy of an asthma patient, you would find evidence of bronchoconstriction and hypersecretion of mucus, leading to obstructed airways and inflammation due to histamine release.

- **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:



Vasoactive Amines:

2. Serotonin:

- Serotonin (5-hydroxytryptamine) is a preformed vasoactive mediator present in platelets and certain neuroendocrine cells, such as in the gastrointestinal tract, and in mast cells in rodents but not humans.

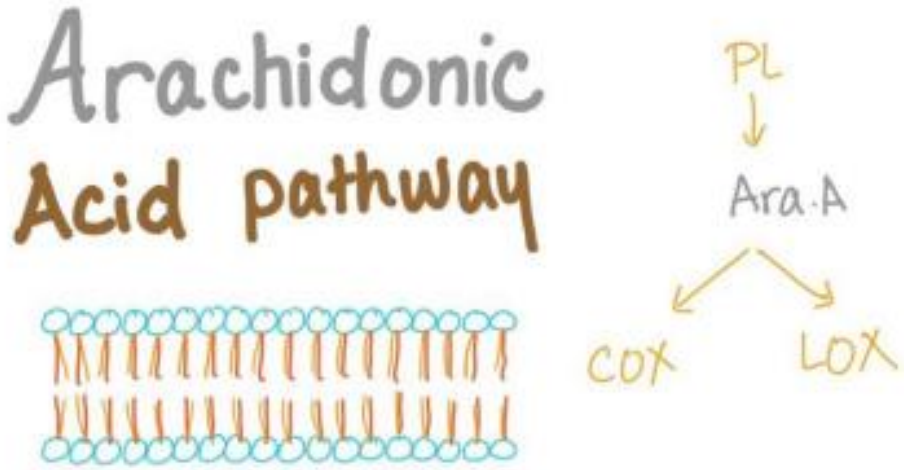
- Its primary function is as a neurotransmitter in the gastrointestinal tract.

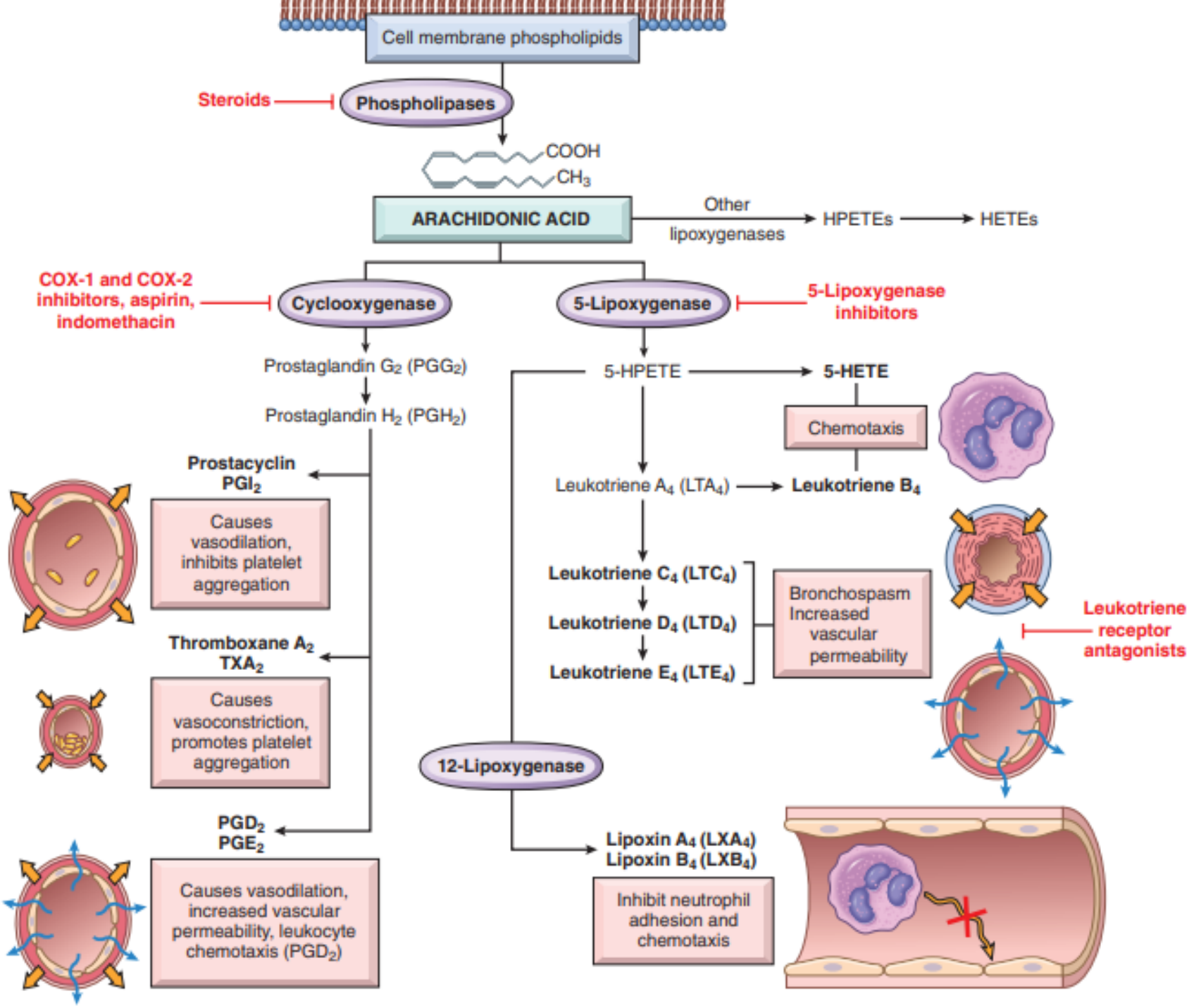
- It also is a vasoconstrictor, but the importance of this action in inflammation is unclear

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.




Cell membrane phospholipids
Steroids
Phospholipases
COOH
CH3
ARACHIDONIC ACID
Other lipoxygenases
HPETEs
HETEs
COX-1 and COX-2 inhibitors, aspirin, indomethacin
Cyclooxygenase
5-Lipoxygenase
5-Lipoxygenase inhibitors
Prostaglandin G2 (PGG2)
Prostaglandin H2 (PGH2)
5-HPETE
5-HETE
Chemotaxis
Leukotriene A4 (LTA4)
Leukotriene B4
Prostacyclin PGI2
Causes vasodilation, inhibits platelet aggregation
Leukotriene C4 (LTC4)
Leukotriene D4 (LTD4)
Leukotriene E4 (LTE4)
Bronchospasm Increased vascular permeability
Leukotriene receptor antagonists
Thromboxane A2 TXA2
Causes vasoconstriction, promotes platelet aggregation
12-Lipoxygenase
PGD2 PGE2
Causes vasodilation, increased vascular permeability, leukocyte chemotaxis (PGD2)
Lipoxin A4 (LXA4) Lipoxin B4 (LXB4)
Inhibit neutrophil adhesion and chemotaxis

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	Leukotrienes B_4, HETE

HETE, Hydroxyeicosatetraenoic acid.

Arachidonic Acid Metabolites

A. Prostaglandins:

- Prostaglandins (PGs) are produced by mast cells, macrophages, endothelial cells, and many other cell types.
- They are involved in the vascular and systemic reactions of inflammation.
- They are generated by the actions of two cyclooxygenases called COX-1 and COX-2.
- Prostaglandins are named based on structural features coded by a letter (e.g., PGD, PGE, PGF, PGG, and PGH) and a subscript numeral (e.g., 1, 2), which indicates the number of double bonds in the compound.
- The most important prostaglandins in inflammation are PGE2, PGD2, PGF2a, PGI2 (prostacyclin), and TXA2 (thromboxane A2).
- In addition to their local effects, prostaglandins are 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

Arachidonic Acid Metabolites

Characteristics comparison between COX-1 and COX-2

	COX-1	COX-2
Synthesis	intrinsic	induced
Functions	physiological: gastrointestinal protection platelet aggregation regulation vascular resistance regulation renal blood flow regulation	physiological: production of PG elevated during pregnancy pathological: producing proteinase, PG, and other inflammatory mediators

Arachidonic Acid Metabolites

B. Leukotrienes:

- Leukotrienes are produced in leukocytes and mast cells by the action of lipoxygenase and are involved in vascular and smooth muscle reactions and leukocyte recruitment.

i. LTB4 → Produced by neutrophils and some macrophages.

a) Potent chemotactic agent and activator of neutrophils, causing aggregation and adhesion of the cells to venular endothelium.

b) Generation of ROS.

c) Release of lysosomal enzymes.

ii. LTC4 ,LTD4 and LTE4 → Produced mainly in mast cells.

a) Cause intense vasoconstriction, bronchospasm (important in asthma), and increased permeability of venules.

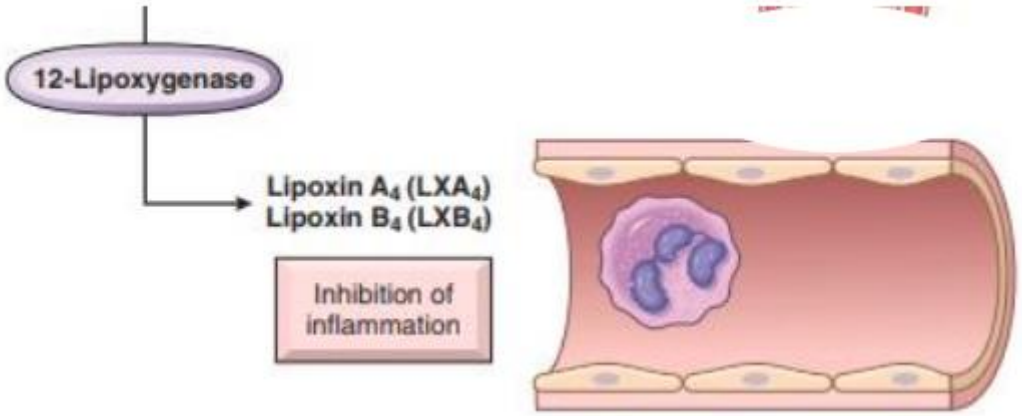
Arachidonic Acid Metabolites

C. Lipoxins

- Are generated from arachidonic acid by the lipoxygenase pathway.
- Unlike prostaglandins and leukotrienes, the lipoxins suppress inflammation by inhibiting the recruitment of leukocytes.
- They inhibit neutrophil chemotaxis and adhesion to endothelium.

- So we have 4 inhibitors:

1. IL-10
2. TGF-B
3. LXA4
4. LXB4



Arachidonic Acid Metabolites

- Pharmacologic Inhibitors of Prostaglandins and Leukotrienes:

A. The importance of eicosanoids in inflammation has driven attempts to develop drugs that inhibit their production or actions and thus suppress inflammation.

B. These anti-inflammatory drugs include the following:

1. Cyclooxygenase inhibitors
2. Lipoxygenase inhibitors:
3. Corticosteroids
4. Leukotriene receptor antagonists

Arachidonic Acid Metabolites

1. Cyclooxygenase inhibitors:

- ✓ It include aspirin and other (NSAIDs), such as ibuprofen.
- ✓ They inhibit both COX-1 and COX-2 and thus block all prostaglandin synthesis (hence their efficacy in treating pain and fever).
- ✓ Selective COX-2 inhibitors → 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, whereas COX-2 generates prostaglandins that are involved only in inflammation.
- ✓ So in patient with gastric ulceration we prefer Selective COX-2 inhibitors.

- ✓ Selective COX-2 inhibitors may increase the risk of cardiovascular and cerebrovascular events, possibly because they impair endothelial cell production of prostacyclin (PGI2), which prevents thrombosis, while leaving intact the COX-1-mediated production by platelets of TXA2, which induces platelet aggregation. Thus, selective COX-2 inhibition may tilt the balance toward vascular thrombosis

Arachidonic Acid Metabolites

2. Lipoxygenase inhibitors:

- Pharmacologic agents that inhibit leukotriene production (e.g., zileuton) are useful in the treatment of asthma.

3. Corticosteroids:

4. Are broad-spectrum anti-inflammatory agents that reduce the transcription of genes encoding COX-2, phospholipase A2, proinflammatory cytokines (e.g., IL-1 and TNF), and iNOS.

4. Leukotriene receptor antagonists:

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

Cytokines and Chemokines

1. 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.

Cytokines and Chemokines

A. Tumor Necrosis Factor and Interleukin-1:

- **Serve critical roles in leukocyte recruitment by promoting adhesion of leukocytes to endothelium and their migration through vessels.**

- Produced by activated macrophages and dendritic cells under the effect of Microbial products, foreign bodies, necrotic cells.

- The most important roles of these cytokines in inflammation are the following:

1. Endothelial activation.
2. Activation of leukocytes and other cells.
3. Systemic acute-phase response.
4. TNF regulates energy balance by promoting lipid and protein catabolism and by suppressing appetite.

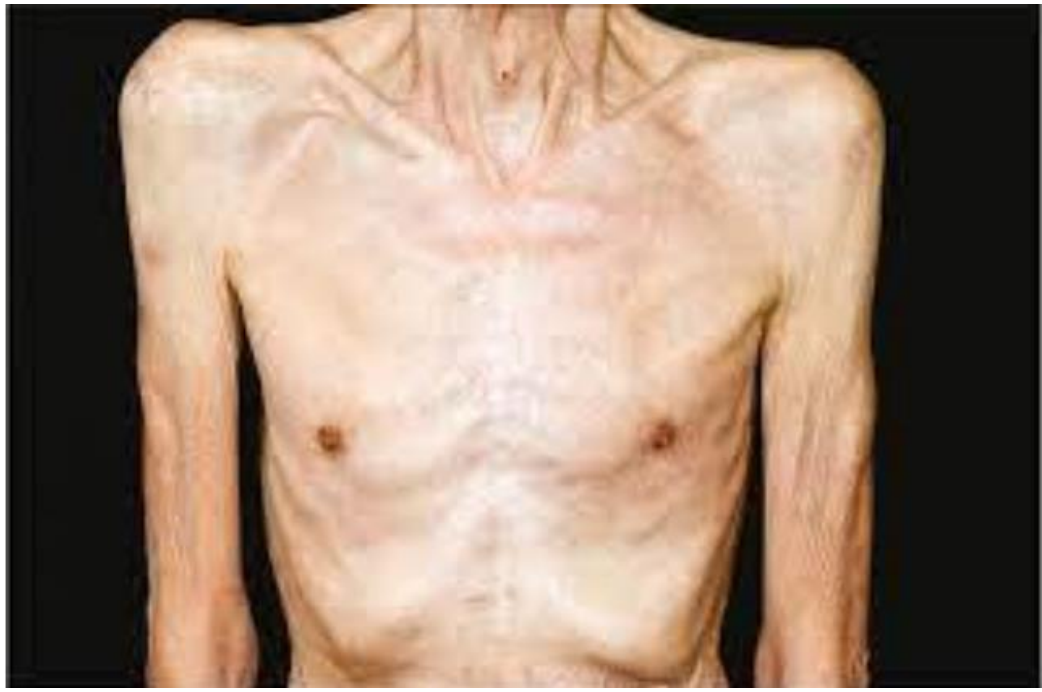
Cytokines and Chemokines

- TNF antagonists have been remarkably effective in the treatment of chronic inflammatory diseases:

A. Rheumatoid arthritis.

B. Psoriasis.

C. Some types of inflammatory bowel disease.



✓ Sustained production of TNF contributes to **cachexia,** a pathologic state characterized by weight loss, muscle atrophy, and anorexia that accompanies some chronic infections and cancers

Cytokines and Chemokines

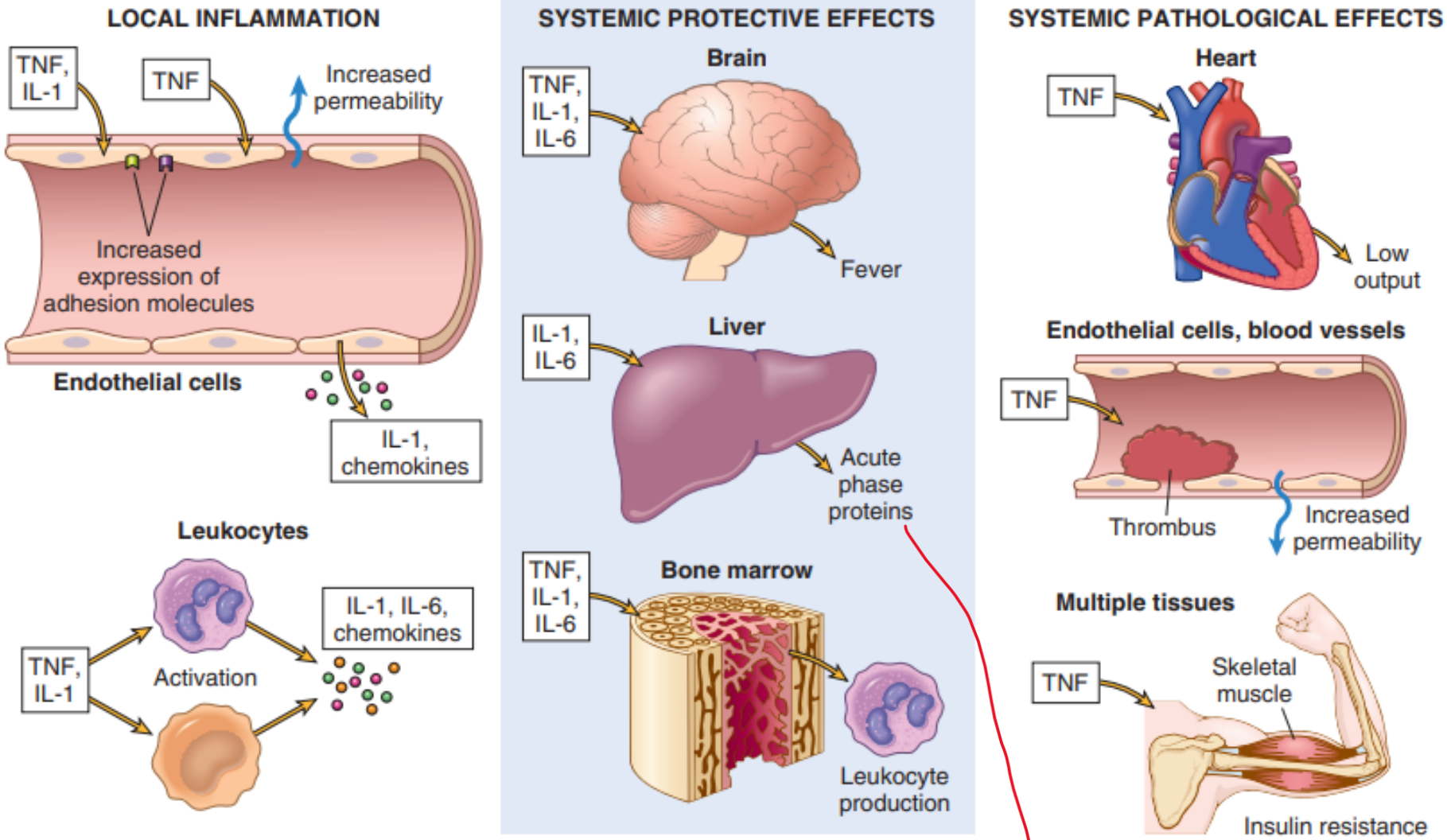


Figure 3.11 Major roles of cytokines in acute inflammation. *IL*, Interleukin; *TNF*, tumor necrosis factor.

ESR + CRP

Cytokines and Chemokines

B. Chemokines:

- Chemokines are a family of small proteins that act primarily as **chemoattractants** for specific types of leukocytes.
- Chemokines mediate their activities by binding to seven-transmembrane G protein–coupled receptors.
- They are classified into four major groups, according to the arrangement of cysteine (C) residues in the proteins:

1. C-X-C chemokines:

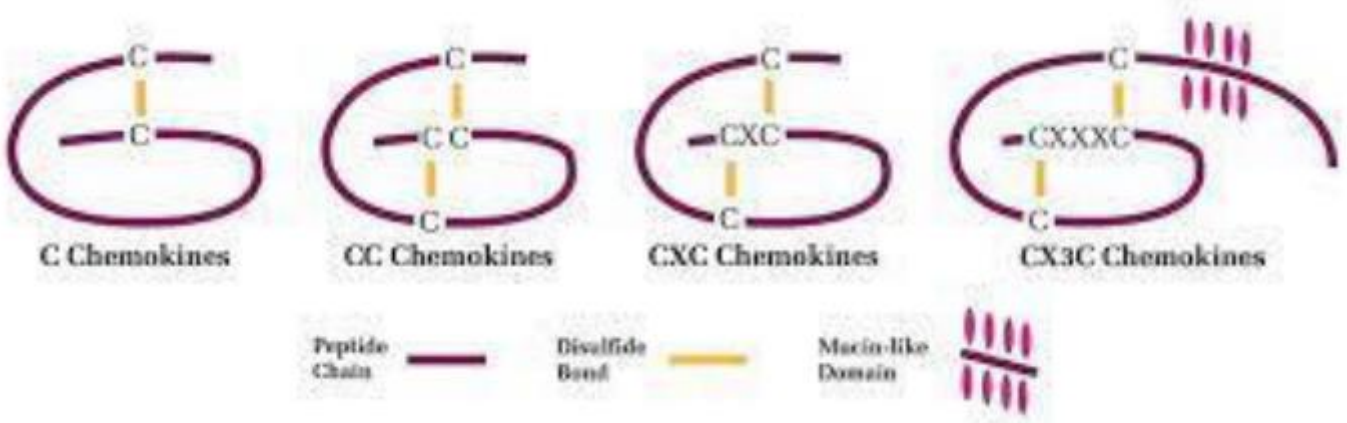
✓ Have one amino acid residue separating the first two of the four conserved cysteines.

✓ These chemokines act primarily as neutrophils chemoattractant. IL-8 (now called CXCL8) is typical of this group.

Cytokines and Chemokines

2. C-C chemokines:

- ✓ Have the first two conserved cysteine residues adjacent.
- ✓ Include monocyte chemoattractant protein (MCP-1, CCL2), eotaxin (CCL11), and macrophage inflammatory protein-1α (MIP-1α, CCL3).
- ✓ Mainly serve as chemoattractants for monocytes, eosinophils, basophils, and lymphocytes.



Cytokines and Chemokines

3. C chemokines :

- ✓ Lack the first and third of the four conserved cysteines.
- ✓ The C chemokines (e.g., lymphotactin, XCL1) are relatively specific for lymphocytes.

4. CX3C chemokines :

- ✓ contain three amino acids between the first two cysteines
- ✓ The only known member of this class is called fractalkine (CX3CL1).

Cytokines and Chemokines

- Chemokines bind to proteoglycans and are displayed at high concentrations on the surface of endothelial cells and in the extracellular matrix.

- They have two main functions:

✓ Acute inflammation:

- 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.

✓ Maintenance of tissue architecture:

- 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

Complement System

- The complement system is a collection of soluble proteins and their membrane receptors that function mainly in host defense against microbes and in pathologic inflammatory reactions.

- There are more than 20 complement proteins, some of which are numbered C1 through C9.

- In the process of complement activation, several cleavage products of complement proteins are elaborated that cause increased vascular permeability, chemotaxis, and opsonization

Complement System

- The critical step in complement activation is the proteolysis of the third (and most abundant) component, C3. Cleavage of C3 can occur by one of three pathways:

1. The classical pathway:

- Which is triggered by fixation of C1 to antibody (IgM or IgG) that has combined with antigen.

2. The alternative pathway:

- Which can be triggered by microbial surface molecules (e.g., endotoxin), complex polysaccharides, and other substances, in the absence of antibody.

3. The lectin pathway:

- In which plasma mannose-binding lectin binds to carbohydrates on microbes and directly activates C1.

Complement System

- All three pathways of complement activation lead to the formation of an enzyme called the C3 convertase, which splits C3 into two functionally distinct fragments, C3a and C3b.
- C3a is released, and C3b becomes covalently attached to the cell or molecule where the complement is being activated.
- More C3b then binds to the previously generated fragments to form C5 convertase, which cleaves C5 to release C5a and leave C5b attached to the cell surface.
- C5b binds the late components (C6–C9), culminating in the formation of the membrane attack complex (MAC, composed of multiple C9 molecules).
- Complement proteins are present in inactive forms in the plasma, and many of them are activated to become proteolytic enzymes that degrade other complement proteins.

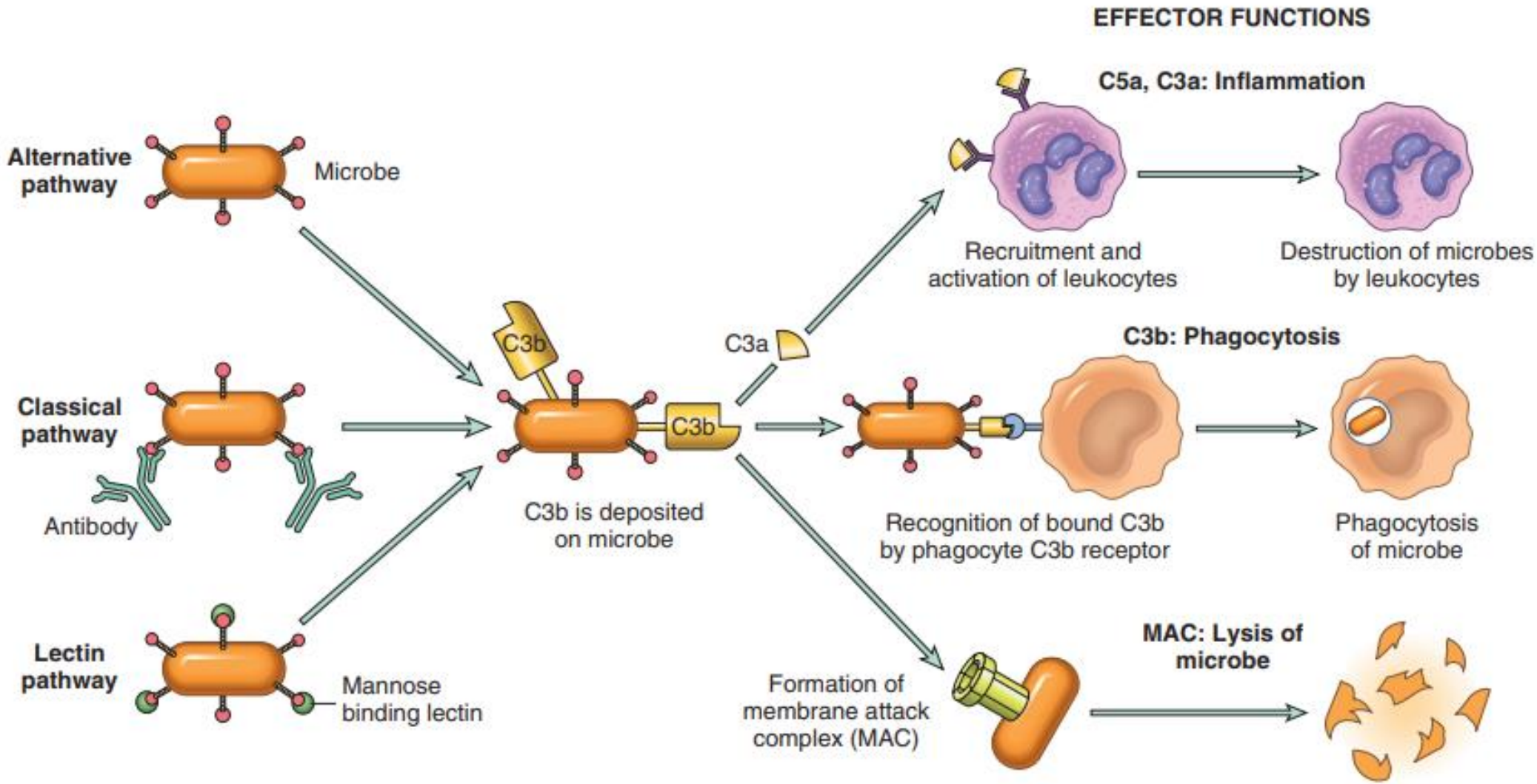
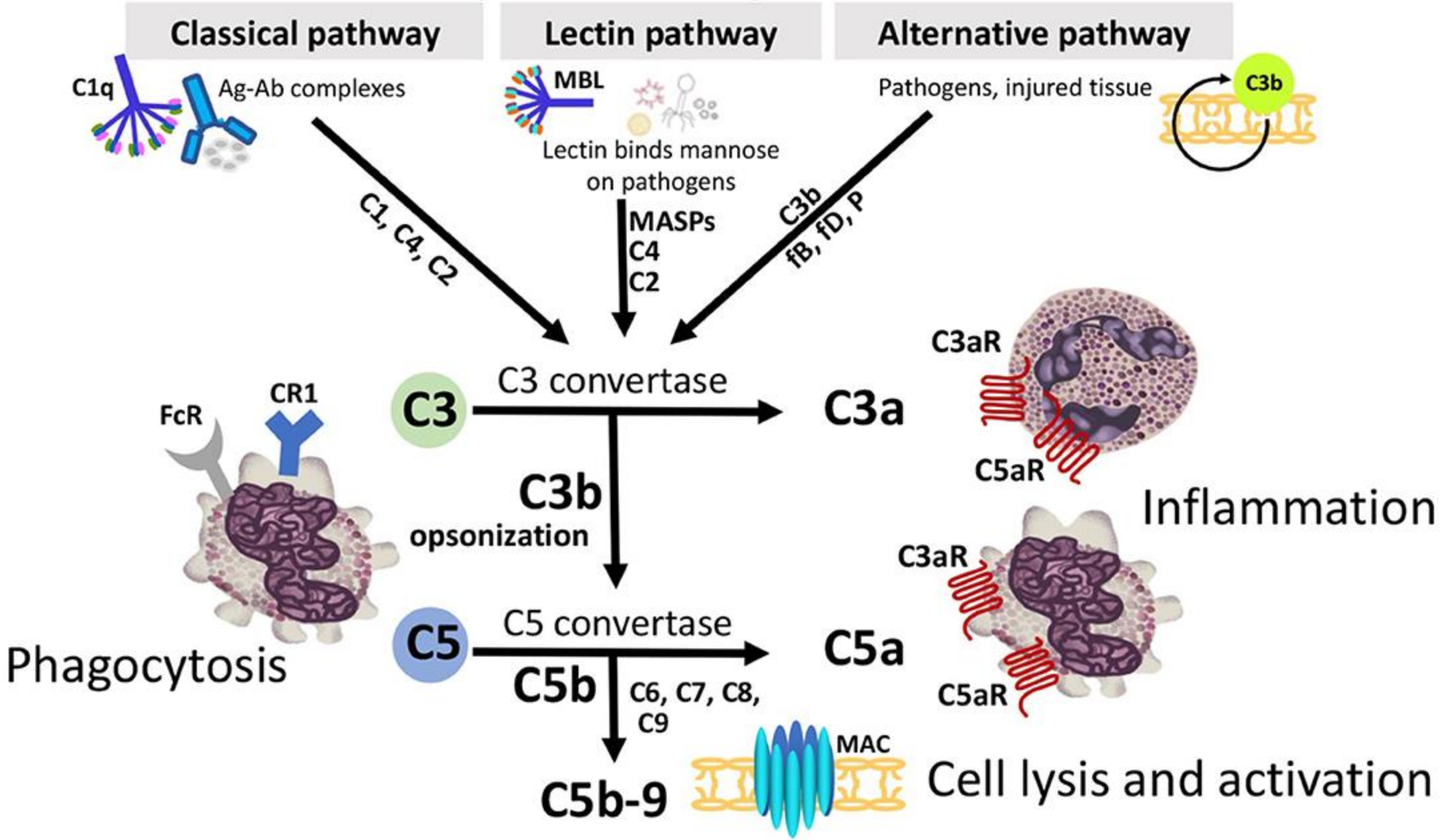


Figure 3.12 Activation and functions of the complement system. Activation of complement by different pathways leads to cleavage of C3. The functions of the complement system are mediated by breakdown products of C3 and other complement proteins and by the membrane attack complex *(MAC)*.

Complement system



Complement System

- The complement system has three main functions:

A. Inflammation:

- C5a, C4a and C3a are called **anaphylatoxins.**
- They stimulate histamine release from mast cells and thereby increase vascular permeability and cause vasodilation.

- C5a also is:

A. A chemotactic agent for neutrophils, monocytes, eosinophils, and basophils.

B. Activates the lipoxygenase pathway of arachidonic acid metabolism in neutrophils and monocytes, causing release of more inflammatory mediators.

Complement System

B. Opsonization and phagocytosis:

- C3b and its cleavage product iC3b (inactive C3b) act as opsonins.
- Promote phagocytosis by neutrophils and macrophages, which bear cell surface receptors for these complement fragments.

B. Cell lysis:

- The deposition of the MAC on cells drills holes in the cell membrane, making the cells permeable to water and ions and resulting in their osmotic death (lysis).
- This function of complement is important mainly for the killing of microbes with thin cell walls, such as **Neisseria bacteria.**

Complement System

- Regulatory proteins for complement system

A. C1 inhibitor → blocks the activation of C1.

- Inherited deficiency of this inhibitor is the cause of hereditary angioedema.

B. Decay accelerating factor (DAF) and CD59 :

- DAF prevents formation of C3 convertases.
- CD59 inhibits formation of the MAC.
- An acquired deficiency of these regulators and excessive complement activation and lysis of red cells This gives rise to a disease called paroxysmal nocturnal hemoglobinuria (PNH)

Complement System

C. Factor H:

- Is a plasma protein that serves as a cofactor for the proteolysis of the C3 convertase.
- its deficiency results in excessive complement activation.
- Mutations in Factor H are associated with hemolytic uremic syndrome, as well as in wet macular degeneration of the eye

- The complement system contributes to disease in several ways:

1. The activation of complement by antibodies or antigen–antibody complexes deposited on host cells and tissues.
2. Inherited deficiencies of complement proteins cause increased susceptibility to infections.
3. Deficiencies of regulatory proteins cause a variety of disorders.

«Wherever the art of medicine is loved,
there is also a love of humanity.»

- Hippocrates-

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