

2. Arachidonic Acid MetabolitesAA:

- Called *eicosanoids* (because they are derived from 20-carbon fatty acids-Greek *eicosa*, twenty"),
- Leukocytes, mast cells, endothelial cells, and platelets are the major sources of AA metabolites in inflammation
- AA-derived mediators act locally at the site of generation and then decay spontaneously or are enzymatically destroyed .
- AA is a 20-carbon polyunsaturated fatty acid produced primarily from dietary linoleic acid and present as a component of cell membrane phospholipids

- It is released from phospholipids through the action of cellular phospholipases.
- AA metabolism proceeds along one of two major pathways

A. Cyclooxygenase pathway

B. Lipoxygenase Pathway

A. Prostaglandins and thromboxanes

- Products of the cyclooxygenase pathway include :.
 1. Prostaglandins E_2 (PGE_2), PGD_2 , $PGF_{2\alpha}$, PGI_2
 2. And thromboxane A_2 (TXA_2),

- a. Endothelial cells, contain prostacyclin synthase, responsible for the formation of PGI₂, which is :
1. A vasodilator and
 2. A potent inhibitor of platelet aggregation
- b. Platelets contain the enzyme *thromboxane synthase*, and hence TXA₂ (thromboxane A₂) which is a potent platelet-aggregating agent and vasoconstrictor
- c. Mast cells: PGD₂ is the major metabolite of the cyclooxygenase pathway in mast cells; and along with PGE₂ and PGF₂ it causes vasodilation

Note PGE2 contributes to the pain and fever in acute inflammation

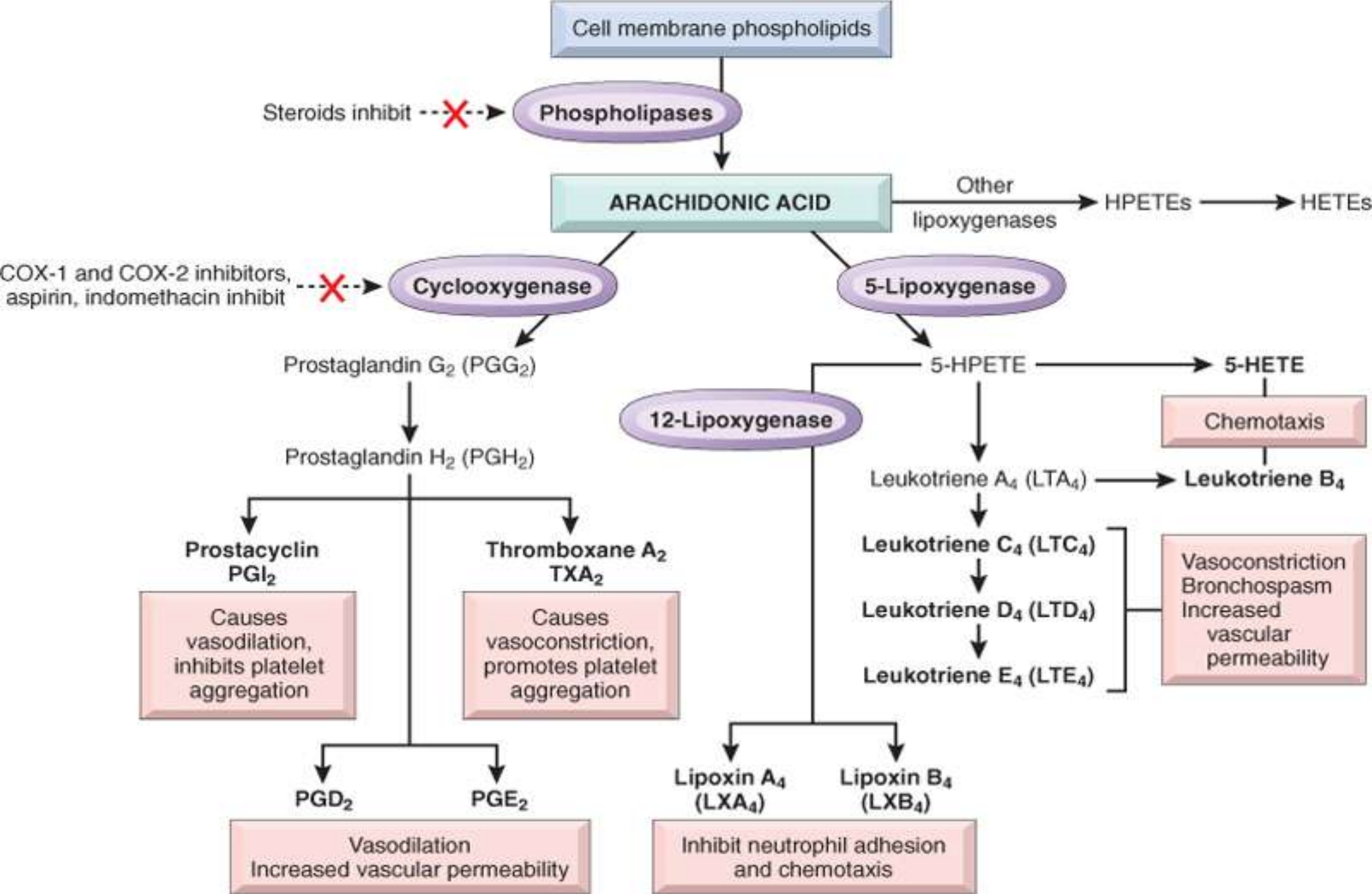
B. Leukotrienes: Are produced by the action of 5-lipoxygenase, the major AA-metabolizing enzyme in neutrophils and their synthesis involves multiple steps

- The first step generates leukotriene A₄ (LTA₄), which in turn gives rise to LTB₄ or LTC₄
 1. LTB₄ is produced by neutrophils and is a potent chemotactic agent for neutrophils
 2. LTC₄ and its subsequent metabolites, LTD₄ and LTE₄, are produced mainly in mast cells and cause

- a. Bronchoconstriction
- b. And increased vascular permeability

C. Lipoxins.

- Once leukocytes enter tissues, they gradually change their major lipoxygenase-derived AA products from leukotrienes to anti-inflammatory mediators called lipoxins which inhibit neutrophil chemotaxis and adhesion to endothelium and thus serve as endogenous antagonists of leukotrienes
- Platelets that are activated and adherent to leukocytes also are important sources of lipoxins



Anti-inflammatory Drugs That Block Prostaglandin Production

- Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, inhibit cyclooxygenase activity, thereby blocking all prostaglandin synthesis (are efficacy in treating pain and fever)
- There are two forms of the cyclooxygenase enzyme, COX-1 and COX-2.
 - a. COX-1 is produced in response to inflammatory stimuli and also is constitutively expressed in most tissues, where it stimulates the production of prostaglandins that serve a

homeostatic function (e.g., fluid and electrolyte balance in the kidneys, cytoprotection in the gastrointestinal tract).

b. By contrast, COX-2 is induced by inflammatory stimuli but it is absent from most normal tissues.

Note:

- Therefore, COX-2 inhibitors have been developed with the expectation that they will inhibit harmful inflammation but will not block the protective effects of constitutively produced prostaglandins.⁸

COX-2 inhibitors may increase the risk for cardiovascular and cerebrovascular events, possibly because they impair endothelial cell production of prostacyclin (PGI_2), an inhibitor of platelet aggregation, but leave intact the COX-1-mediated production by platelets of TXA_2 , a mediator of platelet aggregation.

c. Glucocorticoids, which are powerful anti-inflammatory agents, act by inhibiting the activity of phospholipase A_2 and thus the release of AA from membrane lipids.

3. Cytokines :

- Are polypeptide products of many cell types acting as mediators of inflammation and immune responses
- Some cytokines stimulate bone marrow precursors to produce more leukocytes, thus replacing the ones that are consumed during inflammation and immune responses
- *The major cytokines in acute inflammation are TNF, IL-1, IL-6, and chemokines*
- Cytokines important in chronic inflammation include interferon- γ (IFN- γ) and IL-12

A. Tumor necrosis factor and IL-1

- Their secretion is stimulated by bacterial endotoxin, and immune complexes and IL-1 is also the cytokine induced by activation of the inflammasome.

NOTE- The principal role of these cytokines in inflammation is in endothelial activation

Both TNF and IL-1:

- a. Stimulate the expression of adhesion molecules on endothelial cells
- b. Enhance the production of chemokines) and eicosanoids

- c. They may enter the circulation and act at distant sites to induce the systemic acute-phase reaction
- d. IL-1 activates tissue fibroblasts, resulting in increased proliferation and production of ECM.

B. Chemokines : The main functions of chemokines are:

- a. Chemotactic factors for leukocytes
- b. Activate leukocytes; resulting in increased affinity of leukocyte integrins for their ligands on endothelial cells
- c. Two of the chemokine receptors (called CXCR4 and CCR5) are important coreceptors for the binding and entry of the human immunodeficiency virus into lymphocytes

- Chemokines are classified into four groups
- The two major groups are the CXC and CC
 - a. *CXC chemokines*: Have one amino acid separating the conserved cysteines and act primarily on neutrophils and IL-8 is typical of this group
 - b. *CC chemokines* :Have adjacent cysteine residues and :
 - A. Monocyte chemoattractant protein-1 (MCP-1
 - B. Macrophage inflammatory protein-1 α (MIP-1 α)
- Both (a&b) chemotactic predominantly for monocytes),
- C. Eotaxin (chemotactic for eosinophils

4. Nitric Oxide (NO): Is a short-lived, soluble, free radical gas produced by many cell types and capable of mediating a variety of functions that include:

- a. In the central nervous system it regulates neurotransmitter release as well as blood flow
 - . b. The macrophages use it as a cytotoxic agent for killing microbes and tumor cells
 - c. When produced by endothelial cells it relaxes vascular smooth muscle and causes vasodilation.
- NO is synthesized de novo from L-arginine and NADPH by the enzyme nitric oxide synthase (NOS).

--, here are three isoforms of NOS,

- a. Type I, neuronal NOS (nNOS), is constitutively expressed in neurons, and has no significant role in inflammation
- b. Type II, inducible NOS (iNOS), is induced in macrophages and endothelial cells by a number of inflammatory cytokines and mediators, most notably by IL-1, TNF, and IFN- γ , and by bacterial endotoxin,
 - Is responsible for production of NO in inflammatory reactions and this inducible form is also present in hepatocytes, cardiac myocytes, and respiratory epithelial cells

- c. Type III, endothelial NOS, (eNOS), is constitutively synthesized primarily (but not exclusively) in endothelium.
 - An important function of NO is as a microbicidal (cytotoxic) agent in activated macrophages
 - NO plays other roles in inflammation, including:
 - a. Vasodilation
 - b. Antagonism of all stages of platelet activation (adhesion, aggregation, and degranulation),
 - c. And reduction of leukocyte recruitment at inflammatory sites

5. Neuropeptides

- these are small proteins, such as substance P, that transmit pain signals, and modulate vascular permeability.
- Nerve fibers that secrete neuropeptides are especially prominent in the lung and gastrointestinal

6. Lysosomal Enzymes of Leukocytes

- Acid proteases generally are active only in the low-pH - environment of phagolysosomes
- Neutral proteases, including elastase, collagenase, and cathepsin, are active in extracellular locations and cause

tissue injury by degrading elastin, collagen, basement membrane, and other matrix proteins

- The damaging effects of lysosomal enzymes are limited by antiproteases present in the plasma and tissue fluids
- These include
 1. α_1 -antitrypsin, the major inhibitor of neutrophil elastase,
 2. α_2 -macroglobulin
- Deficiencies of these inhibitors may result in sustained activation of leukocyte proteases, resulting in tissue destruction at sites of leukocyte accumulation

II. Plasma Protein-Derived Mediators

- Circulating proteins of three interrelated systems- the complement, kinin, and coagulation systems-are involved in several aspects of the inflammatory reaction.

I. The complement system: Consists of plasma proteins that Upon activation, different complement proteins produced and these complement-derived factors contribute to a variety of phenomena in acute inflammation

A. Vascular effects.: Mediated by C3a and C5a:

1. Increase vascular permeability

2. Cause vasodilation by inducing mast cells to release histamine

Note: These complement products are also called anaphylatoxins because their actions mimic those of mast cells, which main cellular effectors of the severe allergic reaction called anaphylaxis

B. Leukocyte activation, adhesion, and chemotaxis.

- C5a, and to lesser extent, C3a and C4a, :

1. Activate leukocytes increasing their adhesion to endothelium,

:

c. Complement activation ultimately generates a porelike membrane attack complex (MAC) that punches holes in the membranes of invading microbes.

2. and is a potent. chemotactic agent for neutrophils, monocytes, eosinophils, and basophils

C. Phagocytosis.

1. Product iC3b act as opsonins, augmenting phagocytosis by binding to opsonin receptor
2. The MAC (membrane attack complex), which is made up of multiple copies of the final component C9, kills some bacteria (especially thin-walled *Neisseria*) by creating pores that disrupt osmotic balance.

NOTE: The activation of complement is tightly controlled by cell-associated and circulating regulatory proteins

- The presence of these inhibitors in host cell membranes protects normal cells from inappropriate damage during protective reactions against microbes
- Inherited deficiencies of these regulatory proteins lead to spontaneous complement activation
- 1. A protein called *C1 inhibitor* blocks activation of C1, and its inherited deficiency causes a disease called *hereditary angioedema*, in which

excessive to complement activation results in edema in multiple tissues, including the larynx.

2. *decay-accelerating factor* (DAF)

- In a disease called *paroxysmal nocturnal hemoglobinuria*, there is an acquired deficiency of DAF that results in complement-mediated lysis of red cells (which are more sensitive to lysis than most nucleated cells)

3. *Factor H deficiency* : called the *hemolytic uremic syndrome* as well as spontaneous vascular permeability in *macular degeneration* of the eye.

2. Kinin system: Its activation leads to the formation of bradykinin which causes

a. Increased vascular permeability

b. Arteriolar dilation

c. Bronchial smooth muscle contraction

d. It causes pain when injected into the skin

Note : Actions of bradykinin are short –lived because are it is rapidly degraded by kininases present in the plasma

Role of mediators in different reactions of inflammation

vasodilation	Prostaglandins Nitric oxide histamine
Increased vascular permeability	Histamine and serotonin C3a and C5a Bradykinin Leukotriens C, D4,E4
Leukoyte recruitment and activation	TNF,IL-1 Chemokines(IL-8) C3a C5a LTB4 Bacterial products
fever	IL-1, TNF Prostaglandin E2
pain	Prostaglandins E2 Bradykinin neurpptides
Tissue damage	Lysosomal enzymes of leukocytes Reactive oxygen species Nitric oxide

1. Degradative *enzymes such as histaminase and kininase.*
2. Mechanisms that counteract inflammatory mediators
 - a. Lipoxins,
 - b. and complement regulatory proteins
 - c. IL-10: down-regulate the responses of activated macrophages
 - an inherited disease in which IL-10 receptors are mutated, affected patients develop severe colitis in infancy.
 - d. TGF- β and tyrosine

- a. Is inflammation of prolonged duration (weeks to years)
- b. In which continuing inflammation, tissue injury, and healing by fibrosis, proceed simultaneously
- c. Chronic inflammation is characterized by:
 - 1. Infiltration with mononuclear cells, including macrophages, lymphocytes, and plasma cells
 - 2. Repair involving new vessel formation and fibrosis

Acute inflammation may progress to chronic inflammation if the acute response cannot be resolved, either:

- a. Because of the persistence of the injurious agent
- b. Because of interference with the normal process of healing, - For example, a peptic ulcer of the duodenum initially shows acute inflammation followed by the beginning stages of resolution. However, recurrent bouts of duodenal epithelial injury interrupt this process, resulting in a lesion characterized by both acute and chronic inflammation.

c. Alternatively, some forms of injury (e.g., immunologic reactions, some viral infections) engender a chronic inflammatory response from the outset

- Chronic inflammation may arise in the following settings:

1. *Persistent infections* by microbes that are difficult to eradicate.: These include *Mycobacterium tuberculosis*, *Treponema pallidum* (cause syphilis), and certain viruses and fungi, all of which tend to elicit a T lymphocyte-mediated immune response called *delayed-type hypersensitivity reaction*

2. *Immune-mediated inflammatory diseases* :

- a. *Autoimmune diseases* that results in tissue damage and persistent inflammation and autoimmunity plays an important role in common chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis
- b. Immune responses against environmental substances are the cause of *allergic diseases*, such as bronchial asthma

Note: Immune-mediated diseases may show morphologic patterns of mixed acute and chronic inflammation because they are characterized by repeated bouts of inflammation

- In most cases, the eliciting antigens cannot be eliminated, these disorders tend to be chronic

3. Prolonged exposure to potentially toxic agents.

- a. Exogenous materials such as inhaled silica
- b. cholesterol which may contribute to atherosclerosis

4. Mild forms of chronic inflammation may be important in the pathogenesis of many diseases that are not conventionally thought of as inflammatory disorders. Such diseases include as Alzheimer disease, atherosclerosis, type 2 diabetes.

Chronic Inflammatory Cells and Mediators

1. Macrophages: The dominant cells of chronic inflammation

- Are tissue cells derived from circulating blood monocytes .
- Are normally diffusely scattered in most connective tissues and are also found in organs such as
 - a. The liver (called Kupffer cells),
 - b. Spleen and lymph nodes (where they are called sinus histiocytes)
 - c. Central nervous system (microglial cells),
 - d. and lungs (alveolar macrophages)

- All these cells constitute the so-called *mononuclear phagocyte system*, or reticuloendothelial system
- Monocytes arise from bone marrow precursors and circulate in the blood for about a day and under the influence of chemokines, they migrate to a site of injury within 24 to 48 hours after the onset of acute inflammation
- When monocytes reach the extravascular tissue, they undergo transformation into macrophages, which are somewhat larger and have a longer lifespan and a greater capacity for phagocytosis than do blood monocytes

- Two major pathways of macrophage activation
 1. *Classical macrophage activation*:- Is induced by :
 - a. microbial products such as endotoxin,
 - b. by T cell-derived signals mainly the cytokine IFN- γ ,
 - c. and by foreign substances including crystals
- Classically activated macrophages produce lysosomal enzymes, NO, and ROS, all of which enhance their ability to kill ingested organisms

2. *Alternative macrophage activation*: Is induced by cytokines IL-4 and IL-13, produced by T lymphocytes .
- Alternatively activated macrophages are not actively microbicidal; instead, their role is in tissue repair.
 - So they secrete growth factors that promote angiogenesis, activate fibroblasts and stimulate collagen synthesis

NOTE:

- In response to most injurious stimuli, macrophages are initially activated by the classical pathway, designed to destroy the offending agents, and this is followed by alternative activation, which initiates tissue repair

- Roles of macrophages include:
 1. *ingest and eliminate microbes and dead tissues*
 2. Initiate the process of tissue repair and are involved in scar formation and fibrosis
 3. *Secrete mediators of inflammation, such as cytokines (TNF, IL-1, chemokines, and eicosanoids.*
 4. Display antigens *to T lymphocytes and respond to signals from T cells*, thus setting up a feedback loop that is essential for defense against many microbes by cell-mediated immune responses

B. Lymphocytes

- Are mobilized in the setting of infections as well as non-immune-mediated inflammation (due to ischemic necrosis or trauma), and are the major drivers of inflammation in many autoimmune and other chronic inflammatory diseases
- In the tissues
 - a. B lymphocytes may develop into *plasma cells*, which secrete antibodies,
 - b. and CD4+ T lymphocytes are activated to secrete cytokines

- There are three subsets of CD4+ helper T cells that secrete different sets of cytokines and elicit different types of inflammation
 1. T_H1 cells produce the cytokine IFN- γ , which activates. . macrophages in the classical pathway
 2. T_H2 cells secrete IL-4, IL-5, and IL-13, which recruit and activate eosinophils and are responsible for the alternative- pathway of macrophage activation
 3. T_H17 cells secrete IL-17 and other cytokines that induce the secretion of chemokines responsible for recruiting neutrophils and monocytes into the reaction.

- T_H2 cells are important in defense against helminthic parasites and in allergic inflammation.
- Lymphocytes and macrophages interact in a bidirectional way, and so, they play an important role in propagating chronic inflammation
- Macrophages display antigens to T cells, and produce cytokines (IL-12 and others) that stimulate T cell responses and activated T lymphocytes, in turn, produce cytokines, which recruit and activate macrophages and thus promote more antigen presentation and cytokine secretion,.

Eosinophils:

- Are present in parasitic infections and as part of immune reactions mediated by IgE, such as with allergies.
- Their recruitment is driven mainly by (eotaxin)
- Eosinophil granules contain major basic protein, a protein toxic to parasites and causes epithelial cell necrosis.

D. Mast cells: Are widely distributed in connective tissues throughout the body and they can participate in both acute and chronic inflammatory responses and important in allergic reactions), to environmental antigens.

NOTE:

- *Although the presence of neutrophils is the hallmark of acute inflammation, many forms of chronic inflammation may continue to show extensive neutrophilic infiltrates, as a result of either persistent microbes or necrotic cells, or mediators elaborated by macrophages.*
- Such inflammatory lesions are sometimes called "acute on chronic"-for example, in inflammation of bones (osteomyelitis)

Granulomatous inflammation

- Is a distinctive pattern of chronic inflammation characterized by aggregates of activated macrophages with scattered lymphocytes.
- Granulomas are characteristic of certain specific pathologic states; Consequently, recognition of the granulomatous pattern is important because of the limited number of conditions (some life-threatening) that cause it

Causes of granulomas are:

A. Infections: With persistent T-cell responses to certain microbes (such as *Mycobacterium tuberculosis*)

, *T. pallidum*, or fungi),

- *Tuberculosis is the prototype of a granulomatous disease caused by infection and should always be excluded as the cause when granulomas are identified*

B. Crohn disease: Gastrointestinal disease

C. Sarcoidosis: a disease of unknown etiology

D. Foreign Bodies: Relatively inert foreign bodies (suture,) forming so-called *foreign body granulomas*

NOTE:

- The formation of a granuloma effectively "walls off" the offending agent

and is therefore a useful defense mechanism

- Granuloma formation does not always lead to eradication of the causal agent, which is frequently resistant to killing
- Granulomatous inflammation with subsequent fibrosis may even be the major cause of organ dysfunction in some diseases, such as tuberculosis

MORPHOLOGY :In the usual H&E preparations:

- a. The activated macrophages in granulomas have pink, granular cytoplasm with indistinct cell boundaries; called **epithelioid macrophages**

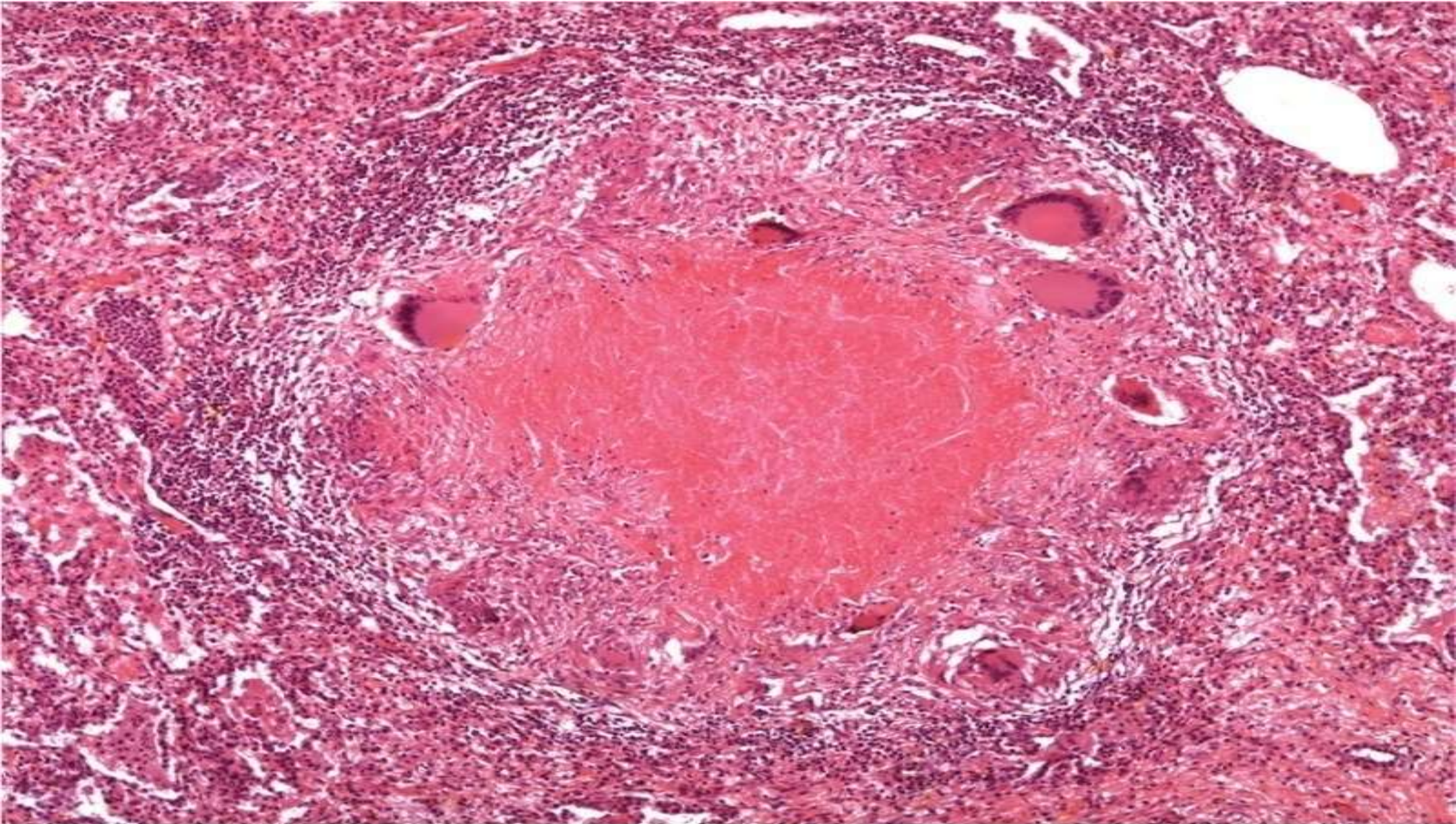
- b. The aggregates of epithelioid macrophages are surrounded by a collar of lymphocytes
 - c. Older granulomas may have a rim of fibroblasts and connective tissue
 - d. Frequently, multinucleate **giant cells** 40 to 50 μ m in diameter are found in granulomas. and such cells consist of a large mass of cytoplasm and many nuclei, and they derive from the fusion of multiple activated macrophages
- If the nuclei are arranged at the periphery, it is called Langhans multinucleated cells , but if nuclei are present haphazardly , it is called foreign body type
 -

In granulomas associated with certain infectious organisms (tubercle bacillus), a combination of hypoxia and free radical injury leads to a central zone of necrosis and

On gross examination: This has cheesy appearance called **caseous necrosis**

- The granulomas associated with Crohn disease, sarcoidosis, tend to be "noncaseating."

Granuloma



Called the *acute-phase reaction*,

- The cytokines TNF, IL-1, and IL-6 are the most important mediators of the acute-phase reaction
- These cytokines are released systemically.

- IL-6 stimulates the hepatic synthesis of a number of plasma proteins.

The acute-phase response consists of :

a. Fever: Characterized by an elevation of body temperature,

- Is produced in response to substances called pyrogens that act by stimulating prostaglandin synthesis in the vascular and perivascular cells of the hypothalamus

1. *Exogenous pyrogens such as lipopolysaccharide (LPS)* stimulate leukocytes to release cytokines
- 2.- *Endogenous pyrogens*) such as IL-1 and TNF (

increase the levels of cyclooxygenases that convert Arachidonic acids into prostaglandins.

3. In the hypothalamus the prostaglandins, especially PGE₂, stimulate the production of neurotransmitters, which function to reset the temperature set point at a higher level

- NSAIDs, including aspirin, reduce fever by inhibiting cyclooxygenase and thus blocking prostaglandin synthesis

b. ***Elevated plasma levels of acute-phase proteins.***

- These plasma proteins are synthesized in the liver, and in acute inflammation, their concentrations may increase

several hundred-fold and the best known are:

1. C-reactive protein (CRP)
2. Fibrinogen,
3. Serum amyloid A (SAA) protein

Synthesis of these molecules by hepatocytes is by IL-6

1. CRP and SAA, bind to microbial walls, and act as opsonins and fix complement, so promoting the elimination of the microbes
2. Fibrinogen binds to erythrocytes and causes them to form stacks (rouleaux) that sediment more rapidly at unit gravity than individual erythrocytes and this is the basis for measuring the erythrocyte sedimentation rate (ESR)

as a simple test for the systemic inflammatory response, caused by any number of stimuli, including LPS

- Serial measurements of ESR and CRP are used to assess therapeutic responses in patients with inflammatory disorders such as rheumatoid arthritis.
- It is believed that inflammation is involved in the development of atherosclerosis ;therefore, elevated serum levels of CRP are now used as a marker for increased risk of myocardial infarction or stroke in patients with atherosclerotic vascular disease

c. Leukocytosis

- The leukocyte count usually climbs to 15,000 to 20,000 cells/mL, but in some extraordinary cases it may reach 40,000 to 100,000 cells/mL.
- These extreme elevations are called *leukemoid reactions*
 1. The leukocytosis occurs initially because of accelerated release of cells under the influence of cytokines (TNF and IL-1) from the bone marrow postmitotic reserve pool.
 2. Prolonged infection also stimulates production of colony-stimulating factors (CSFs), which increase the bone marrow output of leukocytes, thus compensating for the

consumption of these cells in the inflammatory reaction

- Most bacterial infections induce an increase in the blood neutrophil count, called neutrophilia
- Viral infections, such as infectious mononucleosis, mumps increase numbers of lymphocytes (lymphocytosis).
- Bronchial asthma and parasite infestations cause an increase in the number of eosinophils, called eosinophilia
- Typhoid fever, rickettsiae, are associated with a decreased number of circulating white cells (leukopenia)

d. Rigors(shivering) **chills** (perception of being cold