CHAPTER THREE
INFLAMMATION

General Features
Inflammation is defined as "the response of living vascularized tissues to harmful agents." It consists principally of vascular changes associated with leukocytes infiltration and systemic reactions." Inflammation is a fundamental and common pathologic process seen in many disease states. It is essentially a protective response, the aim of which is to get rid of the injurious agents (e.g., microbes, toxins) as well as its consequences (e.g., necrotic cells and tissues). Inflammation is concurrently tangled with another process (repair) that tries to mend the damaged tissues resulting from the battle between the offending agent and the host. Without inflammation, infections would go uninhibited, wounds would never heal, and injured organs might remain permanently damaged. Some times, however, inflammation and its associated repair may be potentially harmful. For this reason, pharmacies flourish with anti-inflammatory drugs, which ideally control the harmful sequelae of inflammation yet do not interfere with its beneficial effects.

Many tissues and cells are involved in the inflammatory reaction, including plasma fluid proteins, circulating leukocytes, blood vessels, and cellular and extracellular constituents of connective tissues. The circulating leukocytes include neutrophils, monocytes, eosinophils, lymphocytes, basophils, in addition to platelets. The connective tissue cells are mast cells, fibroblasts, macrophages, and lymphocytes. The extracellular matrix consists of structural proteins (collagen, elastin), adhesive glycoproteins (fibronectin, laminin), and proteoglycans.

Inflammation is divided into acute or chronic. The latter includes also a specific form called granulomatous inflammation.

Acute inflammation is rapid in onset (seconds or minutes), of relatively short duration (minutes, hours, or at most a few days), characterized by the exudation of fluid and plasma proteins, & the emigration of leukocytes, predominantly neutrophils.

Chronic inflammation, in contradistinction, is of insidious onset, of longer duration, and is associated histologically with the presence of lymphocytes, macrophages, plasma cells, proliferation of blood vessels and fibroblasts.

In both forms tissue necrosis of varying extent occurs. The vascular and cellular reactions of both acute and chronic inflammation are mediated by chemical substances (chemical mediators) that are derived from plasma proteins or cells. Such substances, acting singly, in combinations, or in sequence, amplify the inflammatory response and influence its evolution.

The five cardinal signs of inflammation are rubor (redness), tumor (swelling), calor (heat), dolor (pain), and loss of function (functio laesa). The first four signs are typically more prominent in acute inflammations than in chronic ones.

ACUTE INFLAMMATION
Stimuli of acute inflammation
Acute inflammatory reactions are triggered by a variety of stimuli that include
1. Infections: bacterial, viral, parasitic and microbial toxins
2. Physical and chemical agents (trauma, thermal injuries, irradiation, toxins, strong acids, etc.)
3. Tissue necrosis (of any from or cause)
4. Foreign bodies (splinters, dirt, sutures)
5. Immune reactions (hypersensitivity and autoimmune reactions)

Exudation is the escape of fluid, proteins, and blood cells from the vascular system into the interstitial tissue. An exudate is an extravascular fluid that has a high protein concentration and a specific gravity above 1.020. It involves significant alteration in the normal permeability of small blood vessels in the area of injury. In contrast, a transudate is a fluid with low protein content (most of which is albumin) and a specific gravity of less than 1.012.
It is essentially an ultrafiltrate of blood plasma that results from osmotic or hydrostatic imbalance across the vessel wall without an increase in vascular permeability. Edema refers to an excess of fluid in the interstitial tissues or body cavities; the accumulated fluid can be either an exudate or a transudate. Pus (purulent exudate) is an inflammatory exudate rich in leukocytes (mostly neutrophils), the debris of dead cells and, in many cases, microbes (pyogenic bacteria).

Acute inflammation has three major components: (Fig. 3-1)
A. Vasodilation associated with increased blood flow
B. Increased vascular permeability associated with decreased blood flow
C. Emigration and activation of leukocytes and phagocytosis

A. Vasodilation and increased blood flow
This is, sometimes, preceded by a transient constriction of arterioles, lasting a few seconds. Vasodilation first involves the arterioles, which leads to an increase in blood flow; this in turn leads to opening of new capillary beds in the area with subsequent dilation of capillaries & venules. This process allows more blood to flow into the area, a process known as “active hyperemia” (hyper- = increased; -emia = blood). These changes explain the clinically noted heat and redness. Vasodilation is induced by the action of several mediators (such as histamine) on vascular smooth muscles. It is possible that autonomic nerve impulses may also play a role in relaxation of arteriolar smooth muscle leading to their dilation.

B. Increased Vascular Permeability and decreased blood flow
Increased vascular permeability leads to the escape of exudates into the extravascular tissue. This is driven by the increased hydrostatic pressure owing to increased blood flow through the dilated vessels and is perpetuated through the loss of proteins from the plasma that reduces the intravascular osmotic pressure and increases the osmotic pressure of the interstitial fluid.

Several mechanisms have been proposed for the increased vascular permeability, that include
1. Formation of endothelial gaps in venules due to endothelial cells contraction. This is the most common mechanism & is elicited by several mediators e.g. histamine, bradykinin, and leukotrienes. Binding of these mediators to receptors on endothelial cells leads to stimulation of contractile proteins (such as myosin). The result is contraction of the endothelial cells and separation of intercellular junctions that eventuate in intercellular gaps formation.
2. Junctional retraction caused by chemical mediators such as TNF and IL-1; these induce structural reorganization of the cytoskeleton of the cells.
3. Direct endothelial cell injury as by burns or infections. Because of endothelial damage and exposure of the subendothelial thrombogenic collagen, this type is frequently associated with platelets adhesion with subsequent thrombosis.
4. Leukocyte-dependant injury due to accumulation of leukocytes and their activation products (such as toxic oxygen radicals and proteolytic enzymes) during the inflammatory response. These lead to endothelial cell damage.

According to the above mechanisms, there are three basic patterns of increased permeability
1. Immediate transient response lasting for 30 minutes or less, mediated mainly by the actions of histamine and leukotrienes on endothelium
2. Delayed response starting at about 2 hours and lasting for about 8 hours, mediated principally by kinins, complement products.
3. Prolonged response that is most noticeable after direct endothelial injury, e.g. after burns.

The inflammatory exudate, in addition to leukocytes, is composed of plasma proteins; of these, two play a particularly important role
1. Immunoglobulins; a group of antibodies that have the ability to react with certain antigens, making them vulnerable to the actions of neutrophils and macrophages (opsonization)
1. Binding of leukocytes to the endothelial cells. Normally, the vascular endothelium does not bind circulating cells or impede their passage. In inflammation, however, the endothelium becomes activated to permit binding of leukocytes to its surface. This is followed by

2. Transmigration of leukocytes across the endothelium (diapedesis)

3. Migration of leukocytes within the interstitial tissues toward the focus of tissue injury.

Because blood flow slows down in inflammation, more white cells assume a peripheral position along the endothelial surface. This process is called margination. Subsequently, leukocytes tumble and roll over slowly along the endothelium and eventually come to rest through firm adhesions with the endothelial cells. In time, the endothelium becomes virtually lined by white cells, an appearance called pavementing. After firm adhesion, leukocytes insert pseudopods into the junctions between the endothelial cells, squeeze through interendothelial junctions, and eventually, traverse the basement membrane and escape into the extravascular space.

Neutrophils, monocytes, lymphocytes, eosinophils, and basophils, all use the same pathway to migrate from the blood into tissues. Leukocyte adhesion and transmigration are achieved by the binding of complementary adhesion molecules on the leukocyte and endothelial surfaces, a process regulated by chemical mediators. The adhesion receptors involved belong to several molecular families including selectins and integrins. The next step in the process is migration of the leukocytes through the endothelium, called transmigration or diapedesis. Chemokines (chemoattractants) act on the adherent leukocytes and stimulate the cells to migrate toward the site of injury or infection. Certain adhesion molecules, present in the intercellular junction of endothelium, are involved in the migration of leukocytes. Leukocyte diapedesis, similar to increased vascular permeability, occurs predominantly in the venules. After traversing the endothelium, leukocytes eventually pierce the basement membrane, probably by secreting degrading enzymes such as collagenases & elastases. Once leukocytes enter the extravascular connective tissue, they are able to adhere to the extracellular matrix by virtue of integrins and CD44. Thus, the leukocytes are retained at the site where they are needed. (Fig. 3-3) The type of emigrating leukocyte varies with the age of the inflammatory response and with the type of stimulus. In most forms of acute inflammation, neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 hours, and then are replaced by monocytes in 24 to 48 hours. After entering tissues, neutrophils are short-lived; they undergo apoptosis (self destruction) and disappear after 24 to 48 hours, whereas monocytes (by now called macrophages: macro- = large and phage = eater) survive longer and thus outline neutrophils and become more apparent. There are, however, exceptions to this pattern of cellular exudation. In certain infections—for example, those produced by Pseudomonas organisms—neutrophils predominate over 2 to 4 days; in viral infections, lymphocytes may be the first cells to arrive; in some hypersensitivity reactions and parasitic infestations, eosinophils may be the main cell type.

Chemotaxis

After extravasation, leukocytes emigrate in tissues toward the site of injury; this is achieved by a process called chemotaxis. Chemotaxis is defined as locomotion oriented along a chemical gradient of chemoattractants. All granulocytes, monocytes and, to a lesser extent, lymphocytes respond to chemoattractants (chemotactic stimuli) with varying rates of speed. Both exogenous and endogenous substances can act as chemoattractants. The former is exemplified by bacterial products. Endogenous chemoattractants, however, include several chemical mediators:

1. Components of the complement system, particularly C5a
2. Products of the lipoxygenase pathway, mainly leukotriene B4 (LTB4)
3. Cytokines (secreted from cells) e.g., IL-8

All the chemoattractants mentioned above bind to specific receptors on the surface of leukocytes. Signals initiated from these receptors result in recruitment & activation of specific leukocytic proteins including tyrosine kinases. These changes eventuate in polymerization of actin that results in increased amounts of this contractile protein at the leading edge of the cell. The leukocyte moves by extending filopodia that pull the back of the cell in the direction of extension, much as a car with front-wheel drive is pulled by the wheels in front.

Leukocyte Activation

This refers to induction of a number of responses within leukocytes, which are mediated by microbes, products of necrotic cells, antigen-antibody complexes, and cytokines. These mediators trigger several signaling
pathways in leukocytes that result in an increase in cytoplasmic Ca\(^{++}\) and activation of enzymes. The activation of leukocytes is reflected functionally as follows:

1. Production of arachidonic acid (AA) metabolites
2. Secretion of lysosomal enzymes and other microbicidal substances
3. Modulation of leukocyte adhesion molecules allowing firm adhesion to endothelium
4. Activation of macrophages: through the release of IFN-\(\gamma\) (major macrophage-activating cytokine), which is secreted by natural killer (NK) cells.
5. Activation of phagocytosis through stimulation of opsonins-receptors. The process of coating a particle, such as a microbe, to make it vulnerable for phagocytosis is called opsonization; substances that do this are opsonins. Phagocytosis (Fig. 3-4)

Phagocytosis is one of the major functions of the accumulated neutrophils and macrophages at the inflammatory focus, being responsible for eliminating the injurious agents.

Phagocytosis involves three distinct but interrelated steps:

1. Recognition and attachment of the particle to be ingested by the leukocyte
2. Its engulfment, with subsequent formation of a phagocytic vacuole
3. Killing and degradation of the ingested material.

Recognition and Attachment

Although neutrophils and macrophages can engulf bacteria without attachment to specific receptors, typically the phagocytosis of microbes and dead cells is initiated by recognition of these particles by receptors expressed on the leukocyte surface. The efficiency of phagocytosis is greatly enhanced when microbes are opsonized by specific proteins (opsonins) for which the phagocytes express high-affinity receptors. The major opsonins are IgG antibodies, the C3b breakdown product of complement, and certain plasma lectins.

Engulfment

Binding of a particle to phagocytic leukocyte receptors initiates the process of active phagocytosis. During engulfment, extensions of the cytoplasm (pseudopods) flow around the particle to be engulfed, eventually resulting in complete enclosure of the particle within a phagosome created by the plasma membrane of the cell. The limiting membrane of this phagocytic vacuole then fuses with the limiting membrane of a lysosomal granule forming phagolysosome. This fusion results in discharge of lysosomal contents into the phagolysosome.

Killing and Degradation

The ultimate step in the elimination of infectious agents and necrotic cells is their killing and degradation within neutrophils and macrophages, which occur most efficiently after activation of these phagocytes. Microbial killing is accomplished largely by oxygen-dependent mechanisms, which depends on the production of reactive oxygen species, particularly H2O2. The latter is generally not able to efficiently kill microbes by itself. However, the azurophilic granules of neutrophils contain the enzyme myeloperoxidase (MPO), which, in the presence of Cl\(^{-}\), converts H2O2 to hypochlorite (HOCl). The latter is a potent antimicrobial agent that destroys microbes by halogenation or by oxidation of proteins and lipids (lipid peroxidation). The H2O2-MPO-halide system is the most efficient bactericidal system in neutrophils. Oxygen-independent degradation depends on the release of granules, containing proteolytic enzymes such as defensins (antibacterial peptide attacking bacterial cell membrane), proteolytic enzymes such as elastases, lysozymes, and cationic proteins. The major basic protein of eosinophils has limited bactericidal activity but is cytotoxic to many parasites. After killing, acid hydrolases, which are normally stored in lysosomes, degrade the microbes within phagolysosomes.

Macrophages are excellent phagocytes and are particularly good at engulfing and processing antigenic substances and presenting altered antigens to other cells (lymphocytes) for ultimate destruction.

Release of leukocyte products and leukocyte-induced Tissue Injury

During activation and phagocytosis, leukocytes release microbicidal and other products not only within the phagolysosome but also into the extracellular space. The most important of these substances are lysosomal enzymes, reactive oxygen radicals, and products of AA metabolism (including prostaglandins and leukotrienes). These products are capable of causing injuries of the host endothelium and tissues, and may thus amplify the effects of the initial injurious agent. Products of monocytes/macrophages and other leukocyte types have
additional potentially harmful products (see chronic inflammation). Thus, if persistent and unchecked, the leukocyte infiltrate itself becomes harmful. Leukocyte-dependent tissue injury underlies many acute and chronic human diseases as listed in the following table.

Clinical Examples of Leukocyte-Induced Injury

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>Arthritis</td>
</tr>
<tr>
<td>Acute transplant rejection</td>
<td>Asthma</td>
</tr>
<tr>
<td>Asthma</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>Chronic lung disease</td>
</tr>
<tr>
<td>Reperfusion injury</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>Septic shock</td>
<td></td>
</tr>
<tr>
<td>Vasculitis</td>
<td></td>
</tr>
</tbody>
</table>

Defects in Leukocyte Function

Leukocytes play a central role in host defense. Not surprisingly, therefore, defects in leukocyte function, genetic or acquired, lead to increased vulnerability to infections. Impairments of virtually every phase of leukocyte function—from adherence to vascular endothelium to microbicidal activity—have been identified, and the existence of clinical genetic deficiencies in each step in the process has been described. These defects are manifested clinically by recurrent bacterial infections and impaired wound healing. In practice, the most frequent cause of leukocyte defects is bone marrow suppression, leading to reduced production of leukocytes. This is seen following therapies for cancer (radiation and chemotherapy) and when the marrow space is replaced and destroyed by metastatic cancers to bone.

Contribution of tissue cells to the inflammatory process

There are in addition to leukocytes other cells that are resident in tissues. These also serve important functions in initiating acute inflammation. The two most important of these cell types are mast cells and tissue macrophages. Mast cells react to physical trauma, breakdown products of complement, microbial products, etc. The cells release histamine, leukotrienes, enzymes, and many cytokines (including TNF, IL-1, and chemokines), all of which contribute to inflammation. Macrophages recognize microbial products and secrete most of the cytokines important in acute inflammation. These cells are stationed in tissues to rapidly recognize potentially injurious stimuli and initiate the host defense reaction.

CHEMICAL MEDIATORS OF INFLAMMATION

Chemical mediators are substances that are responsible for many of the inflammatory events. According to their origin, they are either

1. **Plasma-derived** (e.g. complements & kinins): these are present in plasma in precursor forms and need to be activated to function.
2. **Cell-derived:** either
   a. ready-made within intracellular granules (e.g., histamine in mast cell granules) or
   b. synthesized when needed (e.g., prostaglandins, cytokines) in response to a stimulus.

The major cellular sources are platelets, neutrophils, monocytes/macrophages, and mast cells. Most mediators perform their job by binding to specific receptors on target cells. Most mediators have the potential to cause harmful effects that is why their biological actions are short-lived or they are inactivated or degraded rapidly by other substances. One mediator can stimulate the release of other mediators. These secondary mediators may be have identical or similar action to the initial mediators but may also have opposing activities. The more important mediators of acute inflammation are

1. **Vasoactive amines**
Histamine and serotonin are stored in cells and are therefore among the first mediators to be released during inflammation.

a. Histamine
The richest source of this amine is the mast cells that are normally present in the connective tissue adjacent to blood vessels. It is also found basophils and platelets. Histamine causes dilation of the arterioles and increases the permeability of venules by binding to receptors on endothelial cells.

b. Serotonin (5-hydroxytryptamine) is present in platelets (and enterochromaffin cells). It has actions similar to those of histamine.
Release of serotonin and histamine from platelets (platelet release reaction) occurs when platelets aggregate after contact for e.g. with collagen, thrombin, and antigen-antibody complexes and platelet activating factors (PAF). They are released by mast cells during IgE-mediated immune reactions.

2. Plasma proteins
These belong to three interrelated systems, the complement, kinin, and clotting systems.

a. The complement System is composed of specific proteins found in greatest concentration in plasma. In the process of complement activation, a number of complement components are elaborated to mediate a variety of phenomena in acute inflammation:
   i. Vascular phenomena: C3a, C5a stimulate histamine release from mast cells and thereby increase vascular permeability and cause vasodilation.
   ii. Chemoattractants: for e.g. C5a is a powerful chemotactic agent for neutrophils, monocytes, eosinophils, and basophils.
   iii. Opsonins: when fixed to the bacterial cell wall, C3b acts as an opsonin and favor phagocytosis by neutrophils and macrophages.

b. The kinin System
Initial activation of the kinin system is through the action of XIIa on prekallikrein that lead to the formation of kallikrein. This occurs following the exposure of blood plasma to vascular basement membrane collagen after injury to endothelial cells. Kallikrein has a chemotactic activity, and also directly converts C5 to the chemoattractant C5a. One of the important kinins is the vasoactive bradykinin, which has actions similar to those of histamine.

c. Clotting System
Activation of the clotting system results in the formation of thrombin. Thrombin generates insoluble fibrin clot. It also binds to specific receptors expressed on platelets, endothelial and smooth muscle cells, triggering recruitment of leukocytes. Factor XIIa has two opposing actions; induces clotting and activating the fibrinolytic system through generation of plasmin, which is important in lysing fibrin clots. Such degradation, leads to the formation fibrin degradation (split) products (FDP), which may increase vascular permeability. It is evident from the preceding that coagulation and inflammation are tightly linked. Acute inflammation, by activating or damaging the endothelium, can trigger coagulation and induce thrombus formation. Conversely, the coagulation cascade induces inflammation, primarily via the actions of thrombin.

3. PHOSPHOLIPIDS-DERIVED MEDIATORS
A. Arachidonic acid metabolites: prostaglandins, leukotriens, & lipoxins
On cell activation, arachidonic acid (AA), which is a fatty acid, is released from membrane phospholipids through the action of cellular phospholipase A2 (activated by C5a). AA metabolites are synthesized by two major classes of enzymes:
   1. Cyclooxynegenases (COX) leading to the generation of prostaglandins (PGs) including thromboxane (TxA2)
   2. Lipoxynegenases that generate leukotrienes and lipoxins
AA metabolites bind to specific receptors on many cell types and can mediate virtually every step of inflammation. Suppressors of cyclooxygenase activity (aspirin, nonsteroidal anti-inflammatory drugs, and COX-2 inhibitors [coxibs]) reduce inflammation in vivo.

Several PGs are important in inflammation including PGI2 (prostacyclin), and thromboxane (TxA2). Platelets contain the enzyme thromboxane synthetase, and hence TxA2 is the major product in these cells. TxA2 is a potent platelet-aggregating agent and a vasoconstrictor. Vascular endothelium (unlike platelets) lacks thromboxane synthetase but possesses prostacyclin synthetase, which leads to the formation of prostacyclin. Prostacyclin, has actions opposing that of TxA2 in that it is a vasodilator, a potent inhibitor of platelet aggregation. The prostaglandins are also involved in the pathogenesis of pain and fever in inflammation. PGD2 is the major metabolite of the cyclooxygenase (COX) pathway in mast cells; along with PGE2, it causes vasodilation and increases the permeability of postcapillary venules, thus potentiating edema formation.

In the lipoxygenase pathway, the main products are a family of compounds collectively called leukotrienes. LTB4 is a potent chemotactic agent and activator of neutrophils. Lipoxins are a recent addition to the family of bioactive products generated from AA. Leukocytes, particularly neutrophils, produce lipoxins through their interaction with platelets. The principal actions of lipoxins are to inhibit neutrophil chemotaxis and adhesion to endothelium.

B. Platelet-activating factor (PAF) is another bioactive phospholipid-derived mediator. A variety of cell types, including platelets, basophils (and mast cells), neutrophils, monocytes/macrophages, and endothelial cells, can elaborate PAF. In addition to platelet stimulation, PAF causes vasoconstriction (and bronchospasm), and at extremely low concentrations it induces vasodilation and increased venular permeability with a potency 100 to 10,000 times greater than that of histamine. PAF also causes increased leukocyte adhesion to endothelium (by enhancing integrin-mediated leukocyte binding), chemotaxis, and leukocytes activation. Thus, PAF can elicit most of the cardinal features of inflammation.

4. CYTOKINES AND CHEMOKINES

Cytokines are proteins produced principally activated lymphocytes and macrophages. In addition to being involved in cellular immune responses, they also play important roles in both acute and chronic inflammation. Those relevant to the inflammatory response include Tumor Necrosis Factor (TNF) and Interleukin-1 (IL-1), which are the major cytokines that mediate inflammation. The secretion of TNF and IL-1 can be stimulated by endotoxin and other microbial products, immune complexes, and physical injury. Their most important actions in inflammation are

a. Induce the synthesis of endothelial adhesion molecules and chemical mediators
b. Increase the surface thrombogenicity of the endothelium.
c. Induce the systemic acute-phase responses associated with infection or injury (e.g. fever, loss of appetite, release of neutrophils into the circulation, the release of corticosteroids).

Chemokines are a family of small proteins that act primarily as chemoattractants for specific types of leukocytes, for e.g. IL-8 acts primarily on neutrophils. It is secreted by activated macrophages, endothelial cells, and other cell types and causes activation and chemotaxis of neutrophils, with limited activity on monocytes and eosinophils. Its most important inducers are microbial products and other cytokines, mainly IL-1 and TNF.

5. NITRIC OXIDE (NO)

NO is a soluble gas that is produced by endothelial cells & macrophages (and some neurons in the brain). Since the in vivo half-life of NO is only seconds, the gas acts only on cells in close proximity to where it is produced. NO is a potent vasodilator by virtue of its actions on vascular smooth muscle. In addition, NO reduces platelet aggregation and adhesion & other inflammatory responses. Thus, production of NO reduces many inflammatory responses. Abnormalities in endothelial production of NO occur in atherosclerosis, diabetes, and hypertension. NO and its derivatives are microbicidal, and thus NO is also a mediator of host defense against infection.

6. LYSOSOMAL CONSTITUENTS OF LEUKOCYTES
Neutrophils and monocytes/macrophages contain lysosomal granules, which when released may contribute to the inflammatory response. Neutrophils have two main types of granules

1. The smaller specific (or secondary) granules that contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, etc.
2. The large azurophil (or primary) granules that contain myeloperoxidase, bactericidal factors (lysozyme, defensins), acid hydrolases, and neutral proteases (e.g. collagenases, proteinase 3).

Both types of granules can empty into phagocytic vacuoles that form around engulfed material, or the granule contents can be released into the extracellular space. Different granule enzymes serve different functions. Acid proteases degrade bacteria and debris within the phagolysosomes, in which a low (acid) pH is readily reached. Neutral proteases are capable of degrading various extracellular components. These enzymes can attack collagen, basement membrane, fibrin, elastin, and cartilage, resulting in the tissue destruction that accompanies inflammatory processes. Neutrophil elastase has been shown to degrade virulence factors of bacteria and thus combat bacterial infections. Monocytes and macrophages also contain acid hydrolases, collagenase, elastase, phospholipase, and plasminogen activator. These may be particularly active in chronic inflammatory reactions.

Because of the destructive effects of lysosomal enzymes, the initial leukocytic infiltration, if unchecked, can potentiate further increases in vascular permeability and tissue damage. These harmful proteases, however, are held in check by a system of antiproteases in the serum and tissue fluids. Foremost among these is α1-antitrypsin, which is the major inhibitor of neutrophil elastase. A deficiency of these inhibitors may lead to sustained action of leukocyte proteases (progressive tissue damage), as is the case in patients with α1-antitrypsin deficiency.

7. OXYGEN-DERIVED FREE RADICALS
Oxygen-derived free radicals may be released extracellularly from leukocytes after exposure to microbes, chemokines, and immune complexes. Superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH) are the major species produced within the cell. Extracellular release of low levels of these potent mediators can amplify the inflammatory response. The physiologic function of these reactive oxygen intermediates is to destroy phagocytosed microbes. At higher levels, release of these potent mediators can damage the tissues. Serum, tissue fluids, and host cells possess antioxidant mechanisms that protect against these potentially harmful oxygen-derived radicals. The influence of oxygen-derived free radicals in any given inflammatory reaction depends on the balance between the production and the inactivation of these metabolites by cells and tissues.

8. NEUROPEPTIDES
Neuropeptides, similar to the vasoactive amines and the AA metabolites, play a role in the initiation and propagation of an inflammatory response. They include substance P, which has many biologic functions, including the transmission of pain signals, regulation of blood pressure, and increasing vascular permeability.

9. OTHER MEDIATORS
The mediators described above account for inflammatory reactions to microbes, toxins, and many types of injury, but may not explain why inflammation develops in some specific situations. Recent studies are providing clues about the mechanisms of inflammation in two frequently encountered pathologic conditions.

a. Response to hypoxia
It is known that hypoxia causes cell injury and necrosis. However, it is also an inducer of the inflammatory response. The latter is mediated by a protein called hypoxia-induced factor 1α, which is produced by cells deprived of oxygen and activates many genes involved in inflammation; one of these leads to the production of vascular endothelium growth factor (VEGF), which increases vascular permeability.

b. Response to necrotic cells
It is well known that necrotic cells elicit inflammatory reactions that serve to eliminate these cells. One participant may be uric acid, which is a product of necrotic cell’s DNA breakdown. Uric acid crystallizes when present at sufficiently high concentrations in extracellular tissues. Uric acid crystals stimulate inflammation and
subsequent immune response. This inflammatory action of uric acid is the basis of the disease gout, in which excessive amounts of uric acid are produced and crystals deposit in joints and other tissues.

MORPHOLOGIC PATTERNS OF ACUTE INFLAMMATION
Many variables may modify the basic inflammatory response; these include
1. The nature and intensity of the injury
2. The site and tissues affected
3. The responsiveness of the host
Several types of inflammation are recognized, which vary in their morphology and clinical correlates.

Serous inflammation is characterized by the outpouring of a thin fluid that is derived from either the plasma or the secretions of mesothelial cells lining the peritoneal, pleural, and pericardial cavities. In these serous cavities the accumulated fluid is called effusion. (Fig. 3-5) The skin blister resulting from a burn or viral infection represents a large accumulation of serous fluid, either within or immediately beneath the epidermis of the skin.

Fibrinous inflammation
With more severe injuries and the resulting greater vascular permeability, larger molecules such as fibrinogen pass the vascular barrier, and fibrin is formed and deposited in the extracellular space. A fibrinous exudate develops in such cases. The latter also occurs when there is a stimulus for coagulation in the interstitium (e.g., cancer cells). A fibrinous exudate is characteristic of inflammation in the lining of body cavities, such as the meninges, pericardium, and pleura. (Fig. 3-6) Microscopically, fibrin appears as an eosinophilic meshwork of threads or amorphous coagulated mass. Fibrinous exudates may be removed by fibrinolysis and clearing of other debris by macrophages. However, when the fibrin is not removed, it may stimulate the ingrowth of fibroblasts and blood vessels and thus lead to scarring. Conversion of the fibrinous exudate to scar tissue is called organization. When this occurs within the pericardial sac it leads either to opaque fibrous thickening of the pericardium or, more often, to the development of fibrous strands that reduce and may even obliterate the pericardial space.

Suppurative (purulent) inflammation
This is characterized by the production of large amounts of pus or purulent exudate consisting of neutrophils, necrotic cells, and edema fluid. Certain bacteria (e.g., staph. aureus, St. pyogenes, Pneumococci, gonococci, meningococci and E. coli) produce this localized suppuration and are therefore called pyogenic (pus-producing) bacteria. A common example of an acute suppurative inflammation is acute (suppurative) appendicitis. (Fig. 3-7)

An abscess is a localized collection of purulent inflammatory fluid (pus) caused by suppuration buried in a tissue, an organ, or a confined space. Pus is a thick creamy yellow or blood-stained fluid. Abscesses are produced by deep seeding of pyogenic bacteria into a tissue. They have a central region that appears as a mass of necrotic leukocytes and tissue cells. There is usually a zone of preserved neutrophils around this necrotic focus, and outside this region vascular dilation and fibroblastic proliferation occur, indicating the beginning of repair. In time, the abscess may become walled off and ultimately replaced by connective tissue. A common example of an abscess is the skin furuncle. (Fig. 3-8)

Ulcers
An ulcer is a local defect, or excavation of the surface of an organ or tissue that is produced by the sloughing (shedding) of inflammatory necrotic tissue. Ulceration occurs only when tissue necrosis and resultant inflammation exist on or near a surface. It is most commonly encountered in:
1. Inflammatory necrosis of mucosa-lined cavities e.g. mouth, larynx, stomach, intestines, or genitourinary tract. (Fig. 3-9)
2. Subcutaneous inflammation of the lower extremities in older persons who have circulatory disturbances that predispose to extensive necrosis.
Ulcerations are best exemplified by peptic ulcer of the stomach or duodenum, in which acute and chronic inflammation coexist.
Pseudomembranous inflammation of mucous membranes
Severe injury may be associated with extensive epithelial necrosis with sloughing. This creates large shallow ulcers. Fibrin, dead epithelium, neutrophils, red cells and bacteria mix together to produce a white or cream-colored false (pseudo-) membrane covering the affected mucosa. Diphtheria and pseudomembranous colitis are typical examples. (Fig. 3-10)

EFFECTS OF ACUTE INFLAMMATION

Beneficial Effects
1. Dilution of Toxins by the edema fluid
2. Production of protective Antibodies & promotion of immunity
3. Fibrin meshwork formation that forms a scaffold for inflammatory cell migration & also limits the spread of infections
4. Cell Nutrition

Harmful Effects
1. Swelling & edema that can be detrimental for e.g. acute epiglottitis that may be life threatening (Fig. 3-11)
2. Rise in tissue pressure that contributes to tissue necrosis
3. Digestion of adjacent viable tissue
4. Sever damaging allergic reaction
5. Generalized increase in vascular permeability can cause shock as seen in anaphylactic reactions.

OUTCOMES OF ACUTE INFLAMMATION

In general, acute inflammation may have one of three outcomes
1. Complete resolution
   The battle between the injurious agent and the host may end with restoration of the site of acute inflammation to normal. This is called resolution and is the usual outcome when
   a. the injury is limited or short-lived
   b. there has been little tissue destruction
   c. the damaged parenchymal cells can regenerate
2. Healing by fibrosis
   This occurs
   a. after extensive tissue destruction
   b. when the inflammatory injury involves tissues that are incapable of regeneration
   c. when there is abundant fibrin exudation.
   When the fibrinous exudate in tissue or serous cavities (pleural, peritoneal, synovial) cannot be adequately cleared, connective tissue grows into the area of exudate, converting it into a mass of fibrous tissue—a process also called organization.
3. Progression to chronic inflammation
   Acute to chronic transition occurs when the acute inflammatory response persists, owing either to the perseverance of the injurious agent or to some interference with the normal process of healing. For example, failure of acute bacterial pneumonia to resolve may lead to extensive tissue destruction and formation of a cavity in which the inflammation continues to smolder, leading eventually to a chronic lung abscess.

CHRONIC INFLAMMATION

Although it may follow acute inflammation, it frequently begins from the outset as a chronic (chronic inflammation ab initio), insidious, and low-grade, smoldering response. Chronic inflammation is the cause of tissue damage in some of the most common and disabling human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases.
Chronic Inflammation may complicate acute inflammation. The latter is almost always a suppurative type of inflammation that presents as a purulent discharge (pus) as seen in abscess. The cause is either a delay in the evacuation of an abscess, or presence of foreign-body within inflamed area (dirt, wood, metal or a sequestrated bone).

Causes of chronic inflammation ab initio include
1. Persistent infections by certain microorganisms such as tubercle bacilli, Treponema pallidum, certain viruses, fungi, and parasites. These organisms are of low toxicity and evoke delayed type hypersensitivity reaction.
2. Prolonged exposure to toxic agents either exogenous as inhaled silica particles, or endogenous such as toxic plasma lipids that are thought to be responsible for atherosclerosis. The latter is thought to be a chronic inflammatory process of the arterial wall.
3. Autoimmunity

Under certain conditions, immune reactions develop against the individual's own tissues, leading to autoimmune diseases. In these diseases, autoantigens activate a self-perpetuating immune reaction that results in chronic inflammation with associated tissue damage. Examples of this type include several common chronic inflammatory diseases, such as rheumatoid arthritis and lupus erythematosus.

Morphologic features of chronic inflammation
In contrast to acute inflammation, which is manifested by vascular changes, edema, and predominantly neutrophilic infiltration, chronic inflammation is characterized by:
1. Infiltration with mononuclear cells including macrophages, lymphocytes, and plasma cells.
2. Tissue destruction, induced by the persistent offending agent or by the inflammatory cells.
3. Attempts at healing by fibrosis of the damaged tissue, achieved by proliferation of small blood vessels (angiogenesis) & fibroblasts. (Fig. 3-12)

Mononuclear cell infiltration
The macrophage is the dominant cell in chronic inflammation. The mononuclear phagocyte system (reticuloendothelial system) consists of closely related cells of bone marrow origin, including blood monocytes and tissue macrophages. The latter are diffusely scattered in connective tissues or located in organs such as the liver (Kupffer cells), spleen and lymph nodes (sinus histiocytes), and lungs (alveolar macrophages). From the blood, monocytes migrate into various tissues and differentiate into macrophages. The half-life of blood monocytes is about 1 day, whereas the life span of tissue macrophages is several months or years. When the monocyte reaches the extravascular tissue, it undergoes transformation into a larger phagocytic cell, the macrophage. Macrophages may be activated by a variety of stimuli, including cytokines (e.g., IFN-γ) secreted by sensitized T lymphocytes, NK cells, bacterial endotoxins, and other chemical mediators. Activation results in increased cell size, and greater ability to phagocytose and kill ingested microbes. Activated macrophages secrete a wide variety of biologically active products that result in tissue injury and fibrosis. In short-lived acute inflammation, if the irritant is eliminated, macrophages eventually disappear (dying off or travel through lymphatics to lymph nodes). In chronic inflammation, macrophage accumulation persists, and this is mediated by the following:
1. Recruitment from circulating monocytes; a process fundamentally similar to that of neutrophils.
2. Local proliferation of macrophages after their emigration from the bloodstream. This is now known to occur prominently in some chronic inflammatory lesions, such as atheromatous plaques.
3. Immobilization of macrophages within the site of inflammation. Certain cytokines and oxidized lipids can cause such immobilization (migration inhibiting factors).
The products of activated macrophages serve to eliminate injurious agents such as microbes and to initiate the process of repair, but are also responsible for much of the tissue injury in chronic inflammation; these products include
1. Toxic substances to microbes and host cells (e.g., toxic O2 species, NO, and proteases)
2. Chemoattractants to other inflammatory cells
3. Growth factors the cause of fibroblast proliferation, collagen deposition, and angiogenesis.

Other cells in chronic inflammation
Other cell types present in chronic inflammation include lymphocytes, plasma cells, eosinophils, and mast cells: **Lymphocytes** are mobilized in immune and nonimmune inflammation. Antigen-stimulated T and B-cells use various adhesion molecules (predominantly the integrins) and chemokines to migrate into inflammatory sites. Lymphocytes and macrophages interact in a bidirectional way and these reactions play an important role in chronic inflammation. Macrophages display antigens to T cells that stimulate them. Activated T lymphocytes produce cytokines, and one of these, IFN-γ, which is a major activator of macrophages. **Plasma cells** develop from activated B lymphocytes and produce antibody directed against persistent antigen in the inflammatory site. **Eosinophils** are abundant in immune reactions mediated by IgE and in parasitic infections. The recruitment of eosinophils involves extravasation from the blood and their migration into tissue by processes similar to those for other leukocytes. One of the chemokines that is especially important for eosinophil recruitment is eotaxin. Eosinophils have granules that contain major basic protein that is toxic to parasites. **Mast cells** are widely distributed in connective tissues and participate in both acute and persistent inflammatory reactions. Mast cells express on their surface the receptor that binds the Fc portion of IgE antibody. In acute reactions, IgE antibodies bound to the cells' Fc receptors specifically recognize antigen, and the cells degranulate and release mediators, such as histamine and products of AA oxidation. Mast cells are also present in chronic inflammatory reactions, and may produce cytokines that contribute to fibrosis. **Neutrophils** although characteristic of acute inflammation, many forms of chronic inflammation continue to show large numbers of neutrophils, induced either by persistent microbes or by mediators produced by macrophages and T lymphocytes. In chronic bacterial infection of bone (osteomyelitis), a neutrophilic exudate can persist for many months. Neutrophils are also important in the chronic damage induced in lungs by smoking and other irritant stimuli.

Mediators of chronic inflammation (Fig. 3-13)

Examples of chronic inflammation

**Chronic Cholecystitis** may be the sequel to repeated bouts of acute cholecystitis, but in most instances it develops de novo. **Like acute cholecystitis it is almost always associated with gallstones** but these do not seem to have a direct role in the initiation of inflammation. Rather, supersaturation of bile predisposes to both chronic inflammation and, in most instances, stone formation. Microorganisms, usually E. coli and enterococci, can be cultured from the bile in only about one-third of cases. The gallbladder may be contracted, of normal size, or enlarged. The submucosa and subserosa are often thickened from fibrosis. In the absence of superimposed acute cholecystitis, mural lymphocytes are the only feature of inflammation. (Fig. 3-14)

**GRANULOMATOUS INFLAMMATION**

This is a distinctive pattern of chronic inflammatory reaction characterized by focal accumulations of activated macrophages, which often develop an epithelioid (epithelial-like) appearance.

**Causes**

Granulomatous inflammation is encountered in a number of immunologically mediated infectious and some noninfectious conditions, these include

1. Tuberculosis
2. Sarcoidosis
3. Cat-scratch disease
4. Lymphogranuloma inguinale
5. Leprosy
6. Brucellosis
7. Syphilis.
8. Some fungal infections
9. Berylliosis
10. Reactions of irritant lipids

Recognition of granulomas in a biopsy specimen is important because it shortens the list of the differential diagnosis. A granula is a focus of chronic inflammation consisting of a microscopic aggregation of macrophages that are transformed into epithelioid cells surrounded by a collar of mononuclear leukocytes, principally lymphocytes and occasionally plasma cells. The epithelioid cells have a pale pink granular cytoplasm with indistinct cell borders and a vesicular nucleus that is oval or elongate. Older granulomas
develop an enclosing rim of fibroblasts and connective tissue. Frequently, epithelioid cells fuse to form giant cells in the periphery or sometimes in the center of granulomas. These giant cells may attain diameters of 40 to 50 µm. (Fig. 3-15) They have a large mass of cytoplasm containing 20 or more small nuclei arranged either peripherally (Langhans-type giant cell) or haphazardly (foreign body-type giant cell). There are two types of granulomas, which differ in their pathogenesis.

1. **Foreign body granulomas**, which are provoked by foreign bodies. Typically, foreign body granulomas form when material such as talc (associated with intravenous drug abuse), sutures, or other fibers are large enough to preclude phagocytosis by a single macrophage and do not incite any specific inflammatory or immune response. Epithelioid cells and giant cells form and are apposed to the surface of the foreign body and/or actually include it. The foreign material can usually be identified in the center of the granuloma, particularly if viewed with polarized light, in which it appears refractile. (Fig. 3-16)

2. **Immune granulomas**; these are caused by insoluble, poorly degradable or particulate particles, typically microbes that are capable of inducing a cell-mediated immune response. In these responses, macrophages engulf the inciting agent, process it, and present some of it to appropriate T lymphocytes, causing them to become activated. The responding T cells produce cytokines, such as IL-2, which activates other T cells, perpetuating the response, and IFN-γ, which is important in activating macrophages and transforming them into epithelioid cells and multinucleate giant cells. The typical example of an immune granuloma is that caused M. tuberculosis. In tuberculosis, the granulomatous reaction is referred to as a tubercle and is classically characterized by the presence of central caseous necrosis, whereas caseation is rare in other granulomatous diseases. (Fig. 3-17) It is always necessary to identify the specific etiologic agent by special stains for organisms (e.g., acid-fast stains for tubercle bacilli), by culture methods (e.g., in tuberculosis and fungal diseases), by molecular techniques (e.g., the polymerase chain reaction in tuberculosis), and by serologic studies (e.g., in syphilis). In sarcoidosis, the etiologic agent is unknown and the diagnosis is that of exclusion. (Fig. 3-18)

**LYMPHATICS IN INFLAMMATION**
Lymph nodes filter the extravascular fluids brought to them by lymphatic vessels. They represent a secondary line of defense that operates whenever a local inflammatory reaction fails to contain and neutralize an external agent, such as a microbe.
Lymphatics are delicate channels that are difficult to visualize in ordinary tissue sections because they readily collapse. In inflammation lymph flow is increased and helps drain the edema fluid from the extravascular space. Not only fluid, but also leukocytes and cell debris may find their way into lymph. The drainage may transport the offending agent (chemical or microbial). The lymphatics may become secondarily inflamed (lymphangitis), as may the draining lymph nodes (lymphadenitis). Therefore, it is not uncommon in infections of the hand, for example, to observe red streaks along the entire arm up to the axilla following the course of the lymphatics (lymphangitis), accompanied by painful enlargement of the axillary lymph nodes (lymphadenitis). The nodal enlargement is usually caused by hyperplasia of the lymphoid follicles as well as by hyperplasia of the phagocytic cells lining the sinuses of the lymph nodes (reactive or inflammatory lymphadenitis). In severe infections, the lymph nodes may be overwhelmed and fail to halt the spread of infection. The organisms gain access to the vascular circulation, thus inducing a bacteremia. The phagocytic cells of the liver, spleen, and bone marrow constitute the next line of defense, but in massive infections, bacteria seed distant tissues of the body. The heart valves, meninges, kidneys, and joints are favored sites of implantation for blood-borne organisms, and when this happens; endocarditis, meningitis, renal abscesses, and septic arthritis may develop.

**SYSTEMIC EFFECTS OF INFLAMMATION**
The systemic changes associated with inflammation, especially infections, are collectively called the acute phase response (Systemic inflammatory response syndrome [SIRS]). These changes are reactions to cytokines produced in response to bacterial infections and other inflammatory stimuli. The acute phase response consists of several clinical and pathologic changes:
1. Fever is a prominent manifestation; it is produced in response to pyrogens that act by stimulating PG synthesis in the vascular and perivascular cells of the hypothalamus.
2. Acute-phase proteins are plasma proteins, mostly synthesized in the liver, and whose plasma concentrations may increase several hundred times in inflammation. The best-known of these are
   a. C-reactive protein (CRP)
   b. Fibrinogen
   c. Serum amyloid A protein (SAA).
CRP and SAA, bind to microbial cell walls acting as opsonins and fixing complement. The rise in fibrinogen causes erythrocytes to form stacks (rouleaux) that sediment more rapidly than individual erythrocytes. This is the basis for the elevation of the ESR. Prolonged production of SAA causes secondary amyloidosis in destructive chronic inflammations (e.g. rheumatoid arthritis). Elevated serum levels of CRP are now used as a marker for increased risk of myocardial infarction in patients with atherosclerotic coronary artery disease. The inflammation involving atherosclerotic plaques in the coronary arteries may predispose to thrombosis and subsequent infarction, and CRP is produced during inflammation. On this basis, anti-inflammatory agents are being tested in patients to reduce the risk of myocardial infarction.
3. Leukocytosis is a common feature of the acute phase response, especially those induced by bacterial infection. The leukocyte count usually rises to 15,000 or 20,000 cells/µl, but sometimes it may reach very high levels of 40,000 to 100,000 cells/µl. These extreme elevations are referred to as leukemoid reactions because they are similar to the white cell counts obtained in leukemia. The leukocytosis occurs initially because of accelerated release of cells from the bone marrow reserve pool (induced by cytokines, including IL-1 and TNF) and is therefore associated with a rise in the number of more immature neutrophils in the blood (shift to the left). Prolonged infection also induces proliferation of precursors in the bone marrow, caused by increased production of colony stimulating factors (CSFs). Neutrophilia refers to an increase in the blood neutrophil count. Most bacterial infections induce neutrophilia. Viral infections such as infectious mononucleosis, mumps, and German measles produce a leukocytosis due to absolute lymphocytosis. In bronchial asthma, hay fever, and parasitic infestations, there is an absolute increase in the number of eosinophils, creating an eosinophilia. Certain infections (typhoid fever and infections caused by viruses, rickettsiae, and certain protozoa) are associated with a decreased number of circulating white cells (leukopenia). Leukopenia is also encountered in infections that overwhelm patients debilitated by disseminated cancer or uncontrolled tuberculosis.
4. Other manifestations of the acute phase response include increased pulse and blood pressure; decreased sweating; rigors, and anorexia.
5. Disseminated intravascular coagulation (DIC) & septic shock: in severe bacterial infections (sepsis), the large amounts of organisms and lipopolysaccharides (LPS) in the blood stimulate the production of enormous quantities of TNF and IL-1. High levels of TNF cause DIC. LPS and TNF induce tissue factor (TF) expression on endothelial cells, which initiates coagulation; the same agents inhibit natural anticoagulation mechanisms. Cytokines cause liver injury and impaired liver function, resulting in a failure to maintain normal blood glucose levels due to a lack of gluconeogenesis from stored glycogen. Overproduction of NO by cytokine-activated cardiac myocytes and vascular smooth muscle cells leads to heart failure and loss of perfusion pressure, respectively, resulting in cardiogenic shock. The clinical triad of DIC, hypoglycemia, and cardiovascular failure is described as septic shock. Multiple organs show inflammation and intravascular thrombosis, which can produce organ failure. Lung damage (adult respiratory distress syndrome [ARDS]) results when neutrophil-mediated endothelial injury allows fluid to escape from the blood into the airspaces. The kidney and the bowel are also injured, largely due to reduced perfusion. Septic shock is often fatal.

CONSEQUENCES OF DEFECTIVE OR EXCESSIVE INFLAMMATION
Defective inflammation typically results in
1. Increased susceptibility to infections
2. Delayed healing or repair of wounds
3. Tissue damage
Delayed repair is due to the fact that the inflammatory response provides the necessary stimulus to get the repair process started.

**Excessive inflammation** is the basis of many categories of human disease that include allergies and autoimmune diseases.

Recent studies, however, are pointing to an important role of inflammation in a wide variety of human diseases that are not primarily disorders of the immune system. These include

1. Cancer
2. Atherosclerosis
3. Ischemic heart disease
4. Some neurodegenerative diseases such as Alzheimer disease.

In addition, prolonged inflammation and the fibrosis that accompanies it are responsible for much of the pathology in many chronic infectious, metabolic and other diseases. Since these disorders are some of the major curses of mankind, it is not surprising that the normally protective inflammatory response is being called the "silent killer".