General Pathology
Chapter 2
Acute and Chronic Inflammation

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In order to survive, man and other organisms requires to eliminate foreign invaders, such as infectious pathogens, & damaged tissue.

These functions are mediated by a protective, complex host response called inflammation.

In the following lectures, we will discuss

(1) Acute inflammation (stimuli; vascular changes; leukocyte recruitment & activation; & the leukocyte-induced tissue injury, morphologic patterns & outcomes of acute Inflammation).

(2) Cell-derived & plasma protein-derived chemical mediators of inflammation.

(3) Chronic inflammation (cells, mediators, & granulomatous inflammation).

(4) Systemic effects of inflammation.
Overview of Inflammation

**Inflammation** is reaction of living tissues to injury.

It is also a **protective** response intended to:

1. **Eliminate** the initial cause of cell injury, &
2. **Remove** the necrotic cells & tissues resulting from the original insult.

This is accomplished by diluting, neutralizing, or destroying the harmful agents, microbes or toxins.

Inflammation leads eventually to healing of the injured sites by **repair processes**, whereby damaged tissue is replaced by the regeneration of parenchymal cells, and/or by filling of any residual defect by fibrous scar.
Although protective & beneficial, both inflammation & repair, are capable of causing tissue damage.

Three examples:

(1) Inflammatory responses are the basis of life-threatening anaphylactic reactions to insect bites or drugs.

(2) Peritonitis heals with fibrous bands may cause intestinal obstruction,

(3) Pericarditis may result in dense, encase fibrous scarring of pericardium which prevent normal diastolic ventricular dilatation & filling by blood, leading to heart failure.
Five groups of players of inflammatory response interact to resolve the local injury & restore normal function:

1. **Circulating bone marrow-derived cells** include the leukocytes, neutrophils, eosinophils & basophils; lymphocytes, monocytes, & platelets.

2. **Circulating proteins** include clotting factors, kininogens, & complement components, synthesized by the liver.
3. **Vascular wall cells**: include
(a) Endothelial cells (EC) are in direct contact with the blood,
(b) The underlying smooth muscle cells (SMC) that impart tone to the vessels.

4. **Connective tissue cells** include:
(a) Guard to invasion such as *mast cells, macrophages, & lymphocytes*;
(b) The *fibroblasts* that synthesize the extracellular matrix (ECM) & can proliferate to fill in a wound.

5. **The extracellular matrix (ECM)** consist of fibrous structural proteins (e.g., *collagen & elastin*), gel forming proteoglycans, & the glycoproteins (e.g., *fibronectin*) that are the Cell-ECM & ECM-ECM connectors
The components of acute & chronic inflammatory responses & their principal functions.
The components of inflammation are:
- Vascular reaction.
- Cellular response.

Both are activated by mediators that are derived from plasma proteins & various cells.

**The 5 steps of the inflammatory response can be remembered as the 5 R:**

1. **Recognition** of the injurious agent,
2. **Recruitment** of leukocytes (WBC),
3. **Removal** of the agent,
4. **Regulation** (control) of the response,
5. **Resolution** (repair).
The **two outcomes** of acute inflammation are either:

1- **Elimination** of the noxious stimulus, followed by decline of the reaction & repair of damaged tissue, or

2- **Persistent** injury resulting in chronic inflammation.
**Nomenclature**: Inflammation in tissue or organ, is designated by attaching the suffix **itis** to the affected tissue/organ lateen name, e.g.

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Inflammation is divided into two basic patterns:

**Acute inflammation:**
is of relatively short duration, lasting from a few minutes up to a few days, characterized by fluid & plasma protein exudation, & neutrophilic WBC Infiltration.

**Chronic inflammation:**
is of longer duration (days to years) & is characterized by influx of lymphocytes & macrophages, and vascular proliferation & scarring.
Acute Inflammation

Is a rapid host response that serves to deliver leukocytes and plasma proteins, such as antibodies, to sites of infection or tissue injury and has three major components:

(1) alterations in vascular caliber increase in blood flow.
(2) structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation.
(3) emigration of the leukocytes from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent.
The above vascular & cellular changes account for three of the five classic local signs of acute inflammation: **heat** (calor), **redness** (rubor), and **swelling** (tumor).

The two additional cardinal features of acute inflammation, **pain** (dolor) and **loss of function** (functio laesa), occur as consequences of local release of chemical mediators, and by leukocyte- mediated damage.
Branchial cysts.  
Non-inflamed cyst, typically located near the jaw angle, anterior to the sternomastoid muscle; & Inflamed cyst, with evident redness & swelling (due to suppuration = abscess formation).
Stimuli for Acute Inflammation

1- **Infections** (bacterial, viral, fungal, parasitic) and microbial toxins. Among the most important receptors for microbial products are the family of Toll-like receptors (TLRs), and several cytoplasmic receptors which can detect bacteria, viruses, and fungi.

2- **Tissue necrosis** from any cause, including ischemia, trauma, and physical and chemical injury.

3- **Foreign bodies**.

4- **Immune reactions** (also called hypersensitivity reactions).
Reactions of Blood Vessels in Acute Inflammation

A. NORMAL
- Hydrostatic pressure
- Plasma proteins

B. TRANSUDATE
- Increased hydrostatic pressure (venous outflow obstruction, e.g., congestive heart failure)
- Fluid leakage
- Decreased colloid osmotic pressure (decreased protein synthesis, e.g., liver disease; increased protein loss, e.g., kidney disease)

C. EXUDATE
- Fluid and protein leakage
- Vasodilation and stasis
- Increased interendothelial spaces
**Exudate** is an extravascular fluid that has a high protein concentration, contains cellular debris, and has a high specific gravity. Its presence implies an increase in the normal permeability of small blood vessels in an area of injury and, therefore, an inflammatory reaction.

**Transudate** is a fluid with low protein content (most of which is albumin), little or no cellular material, and low specific gravity.

**Edema** denotes an excess of fluid in the interstitial tissue or serous cavities; it can be either an exudate or a transudate. **Pus, a purulent exudate**, is an inflammatory exudate rich in leukocytes (mostly neutrophils), the debris of dead cells and, in many cases, microbes.
Changes in Vascular Flow and Caliber

- **Vasodilation** is first involves the arterioles and then leads to opening of new capillary beds in the area. It is induced by the action of several mediators, notably histamine and nitric oxide (NO), on vascular smooth muscle.

- **Increased permeability** of the microvasculature, with the outpouring of protein-rich fluid into the extravascular tissues.

- **Blood Stasis** slow blood flow, concentration of red cells in small vessels, and increased viscosity of the blood (vascular congestion producing localized redness).

- **Blood leukocytes**, principally neutrophils, **accumulate** along the vascular endothelium, then adhere to the endothelium, and soon afterward they migrate through the vascular wall into the interstitial tissue.
Acute inflammation X335. A capillary in the inflamed appendix is enormously dilated (X15 times its normal resting).
The polymorphs accumulate at the periphery of vessels, forming almost a continuous layer, this is called **margination** or of the EC (arrow). This is followed later by → **rolling** → **adhesion** to EC → **transmigration** between EC & → **migration** in interstitial tissues to chemotactic stimulus.
Several mechanisms are responsible for the increased vascular permeability.

1- Contraction of endothelial cells resulting in increased interendothelial spaces is the most common mechanism of vascular leakage and is elicited by histamine, bradykinin, leukotrienes, the neuropeptide substance P, and many other chemical mediators, short-lived (15-30 minutes).
2- Endothelial injury, resulting in endothelial cell necrosis and detachment. Direct damage to the endothelium is encountered in severe injuries, for example, in burns, or by the actions of microbes that target endothelial cells.

C. ENDOTHELIAL INJURY
- Occurs in arterioles, capillaries, venules
- Caused by burns, some microbial toxins
- Rapid; may be long-lived (hours to days)

D. LEUKOCYTE-MEDIATED VASCULAR INJURY
- Occurs in venules, pulmonary capillaries
- Associated with late stages of inflammation
- Long-lived (hours)
3- Increased transport of fluids and proteins, called transcytosis, through the endothelial cell. (involve channels called the vesiculovacuolar organelle), many of which are located close to intercellular junctions. Certain factors, such as VEGF, seem to promote vascular leakage in part by increasing the number and perhaps the size of these channels.
Responses of Lymphatic Vessels

In inflammation, lymph flow is increased and helps drain edema fluid that accumulates due to increased vascular permeability.

In addition to fluid, leukocytes and cell debris, as well as microbes, may find their way into lymph. Lymphatic vessels, like blood vessels, proliferate during inflammatory reactions to handle the increased load.
The lymphatics may become secondarily inflamed (*lymphangitis*), as may the draining lymph nodes (*lymphadenitis*).

Inflamed lymph nodes are often enlarged because of hyperplasia of the lymphoid follicles and increased numbers of lymphocytes and macrophages.

This constellation of pathologic changes is termed reactive, or inflammatory, *lymphadenitis*. 
Reactions of Leukocytes in Inflammation

The most important leukocytes in typical inflammatory reactions are the ones capable of phagocytosis, (neutrophils and macrophages).

Leukocytes also produce growth factors that aid in repair.

The leukocyte products that destroy microbes and necrotic tissues can also injure normal host tissues.

The processes involving leukocytes in inflammation consist of:
- Their recruitment from the blood into extravascular tissues.
- Recognition of microbes and necrotic tissues, and
- Removal of the offending agent.
Recruitment of Leukocytes to Sites of Infection and Injury

The journey of leukocytes from the vessel lumen to the interstitial tissue, called **Extravasation**, can be divided into the following steps:

1. **In the lumen:** *margination, rolling, and adhesion* to endothelium (EC). In inflammation the endothelium is activated and can bind leukocytes, as a prelude to their exit from the blood vessels.
2. **Migration** across the endothelium and vessel wall.
3. **Migration** in the tissues toward a chemotactictic stimulus.
Because of blood **stasis**, hemodynamic conditions change, and more white cells assume a peripheral position along the endothelial surface (**margination**). Subsequently, individual and then rows of leukocytes adhere transiently to the endothelium, detach and bind again, thus rolling on the vessel wall. **The cells finally come to rest at some point where they adhere firmly.**

ICAM-1, intercellular adhesion molecule 1; TNF, tumor necrosis factor.
Leukocyte Adhesion to Endothelium.

The adhesion of leukocytes to endothelial cells is mediated by complementary adhesion molecules on the two cell types whose expression is enhanced by secreted proteins called cytokines. Cytokines are secreted by cells in tissues in response to microbes and other injurious agents, thus ensuring that leukocytes are recruited to the tissues where these stimuli are present.
The initial rolling interactions are mediated by a family of proteins called selectins. **There are three types of selectins:**

one expressed on **leukocytes** (L-selectin),

one on **endothelium** (E-selectin), and

one in **platelets** and on **endothelium** (P-selectin).
The ligands for selectins are sialylated oligosaccharides bound to mucin-like glycoprotein backbones.

The expression of selectins and their ligands is regulated by cytokines produced in response to infection and injury.

Tissue macrophages, mast cells, and endothelial cells that encounter microbes and dead tissues respond by secreting several cytokines, including tumor necrosis factor (TNF), interleukin-1 (IL-1), and chemokines (chemoattractant cytokines).
TNF and IL-1 act on the endothelial cells of post-capillary venules adjacent to the infection and induce the coordinate expression of numerous adhesion molecules.

Redistribution of P-selectin from intracellular stores to the cell surface. Within 1-2 hours.

Increased surface expression of selectins and ligands for integrins upon cytokine activation of endothelium.

Increased binding avidity of integrins induced by chemokines. Clustering of integrins contributes to their increased binding avidity (not shown).
Firm adhesion is mediated by a family of heterodimeric leukocyte surface proteins called **integrins**. TNF and IL-1 induce endothelial expression of ligands for integrins, mainly vascular cell adhesion molecule 1 (VCAM-1, the ligand for the VLA-4 integrin) and intercellular adhesion molecule-1 (ICAM-1, the ligand for the LFA-1 and Mac-1 integrins).

As a result, the bound leukocytes bind, detach, and bind again, and thus begin to roll along the endothelial surface. These weak rolling interactions slow down the leukocytes and give them the opportunity to bind more firmly to the endothelium.
Leukocyte Migration through Endothelium.

The next step in the process of leukocyte recruitment is migration of the leukocytes through the endothelium, called **transmigration or diapedesis**, which occurs mainly in post-capillary venules.

Chemokines act on the adherent leukocytes and stimulate the cells to migrate through interendothelial spaces toward the chemical concentration gradient, that is, toward the site of injury or infection where the chemokines are being produced.
Several adhesion molecules present in the intercellular junctions between endothelial cells are involved in the migration of leukocytes. These molecules include a member of the immunoglobulin superfamily called PECAM-1 (platelet endothelial cell adhesion molecule) or CD31 and several junctional adhesion molecules.
After traversing the endothelium, leukocytes pierce the basement membrane, probably by secreting collagenases, and enter the extravascular tissue. The cells then migrate toward the chemotactic gradient created by chemokines and accumulate in the extravascular site.

In the connective tissue, the leukocytes are able to adhere to the extracellular matrix by virtue of integrins and CD44 (is a cell-surface glycoprotein) binding to matrix proteins.

Thus, leukocytes are retained at the site where they are needed.

(1) the submucosa contains dilated & congested capillaries (thick arrow). (2) The interstitial connective tissue is pale & edematous due to the presence of inflammatory exudate (center), (3) Polymorphs (double arrow) are visible within capillaries (margination), as well as in the submucosa & within the surface stratified squamous epithelium (migration).
Chemotaxis of Leukocytes.

After exiting the circulation, leukocytes emigrate in tissues toward the site of injury by a process called chemotaxis, which is defined as locomotion oriented along a chemical gradient.

Both exogenous and endogenous substances can act as chemoattractants.

The most common exogenous agents are bacterial products, including peptides that possess an $N$-formylmethionine terminal amino acid, and some lipids.
Endogenous chemoattractants include several chemical mediators:

(1) **cytokines**, particularly those of the chemokine family (e.g., IL-8);

(2) components of the **complement system**, particularly C5a; and

(3) **arachidonic acid (AA) metabolites**, mainly leukotriene B$_4$ (LTB$_4$).

All these chemotactic agents bind to specific seven-transmembrane **G protein–coupled** receptors on the surface of leukocytes.
The nature of the leukocyte infiltrate varies with the age of the inflammatory response and the type of stimulus. In most forms of acute inflammation neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 hours and are replaced by monocytes in 24 to 48 hours.

The photomicrographs are representative of the early (neutrophilic) (A) and later (mononuclear) cellular infiltrates (B) seen in an inflammatory reaction in the myocardium following ischemic necrosis (infarction). The kinetics of edema and cellular infiltration (C) are approximations.
Recognition of Microbes and Dead Tissues

Once leukocytes (neutrophils and monocytes) have been recruited to a site of infection or cell death, they must be activated to perform their functions.

The responses of leukocytes consist of two sequential sets of events:

(1) recognition of the offending agents, which deliver signals that (2) activate the leukocytes to ingest and destroy the offending agents and amplify the inflammatory reaction.
Leukocytes express several receptors that recognize external stimuli and deliver activating signals.
1- **Receptors for microbial products: Toll-like receptors (TLRs)** recognize components of different types of microbes. Thus far 10 mammalian TLRs have been identified, and each seems to be required for responses to different classes of infectious pathogens.

2- **G protein–coupled** receptors found on neutrophils, macrophages, and most other types of leukocytes recognize short bacterial peptides containing N-formylmethionyl residues.

3- **Receptors for opsonins:** Leukocytes express receptors for proteins that coat microbes. The process of coating a particle, such as a microbe, to target it for ingestion (phagocytosis) is called opsonization, and substances that do this are opsonins.
4- Receptors for cytokines: Leukocytes express receptors for cytokines that are produced in response to microbes. One of the most important of these cytokines is interferon-γ (IFN-γ), which is secreted by natural killer cells reacting to microbes and by antigen-activated T lymphocytes during adaptive immune responses.

IFN-γ is the major macrophage-activating cytokine.
Activation results from signaling pathways that are triggered in leukocytes, resulting in increases in cytosolic $\text{Ca}^{2+}$ and activation of enzymes such as protein kinase C and phospholipase A$_2$. The functional responses that are most important for destruction of microbes and other offenders are phagocytosis and intracellular killing. Several other responses aid in the defensive functions of inflammation and may contribute to its injurious consequences.
Phagocytosis involves three sequential steps:

(1) Recognition and attachment of the particle to be ingested by the leukocyte;
(2) Its engulfment, with subsequent formation of a phagocytic vacuole; and
(3) Killing or degradation of the ingested material.
Phagocytosis of a particle involves binding to receptors on the WBC membrane, engulfment, and fusion of lysosomes with phagocytic vacuoles. This is followed by destruction of ingested particles lysosomal enzymes and by reactive oxygen and nitrogen species. The microbicidal products generated from superoxide are hypochlorite (HOCl\(^{\cdot}\)) and hydroxyl radical (\(\cdot\)OH), and from nitric oxide (NO) it is peroxynitrite (OONO\(^{\cdot}\)).

MPO, myeloperoxidase; iNOS, inducible NO synthase.
Mannose receptors, scavenger receptors, and receptors for various opsonins all function to bind and ingest microbes.

The macrophage mannose receptor is a lectin that binds terminal mannose and fucose residues of glycoproteins and glycolipids.

The efficiency of phagocytosis is greatly enhanced when microbes are opsonized by specific proteins (opsonins) for which the phagocytes express high-affinity receptors.
Engulfment:

After the formation of pseudopods and phagosoms, the phagosome then fuses with a lysosomal granule, resulting in discharge of the granule's contents into the phagolysosome. **During this process the phagocyte may also release granule contents into the extracellular space.**

The process of phagocytosis is involves the integration of many receptor-initiated signals to lead to membrane remodeling and cytoskeletal changes. Phagocytosis is dependent on polymerization of actin filaments.

The signals that trigger phagocytosis are many of the same that are involved in chemotaxis.
Phagocytosis of cells: LN X860. Dilated lymphatic sinus in an axillary LN with a deposit of metastatic cancer. Within the sinus are numerous very large phagocytic cells (thin A), the nuclei of which are very large, pale & vesicular (thick A) & in their abundant cytoplasm are many ingested pyknotic, necrotic or fragmented neoplastic cells & lymphocytes (Double A).
Killing and Degradation:

Microbial killing is accomplished largely by reactive oxygen species (ROS, also called reactive oxygen intermediates) and reactive nitrogen species, mainly derived from NO.
The generation of ROS is due to the rapid assembly and activation of a multi-component oxidase (NADPH oxidase, also called phagocyte oxidase), which oxidizes NADPH (reduced nicotinamide-adenine dinucleotide phosphate) and, in the process, reduces oxygen to superoxide anion.

Phagocyte oxidase is an enzyme complex consisting of at least seven proteins.

In response to activating stimuli, the cytosolic protein components translocate to the phagosomal membrane, where they assemble and form the functional enzyme complex.

Thus, the **ROS are produced within the lysosome** where the ingested substances are segregated, and the cell's own organelles are protected from the harmful effects of the ROS.
Superoxide anion is then converted into hydrogen peroxide (H₂O₂), mostly by spontaneous dismutation. H₂O₂ is not able to efficiently kill microbes by itself.

However, the azurophilic granules of neutrophils contain the enzyme *myeloperoxidase* (MPO), which, in the presence of a halide such as Cl⁻, converts H₂O₂ to hypochlorite (OCl⁻, the active ingredient in household bleach). The latter is a potent antimicrobial agent that destroys microbes by *halogenation* (in which the halide is bound covalently to cellular constituents) or by oxidation of proteins and lipids (lipid peroxidation).

**The H₂O₂-MPO-halide system is the most efficient bactericidal system of neutrophils.**
Nitric oxide (NO), produced from arginine by the action of nitric oxide synthase (NOS), also participates in microbial killing. NO reacts with superoxide to generate the highly reactive free radical peroxynitrite (ONOO•).

These oxygen- and nitrogen-derived free radicals attack and damage the lipids, proteins, and nucleic acids of microbes as they do with host macromolecules. Microbial killing can also occur through the action of other substances in leukocyte granules. Neutrophil granules contain many enzymes, such as elastase, that contribute to microbial killing.
Importantly, leukocytes, especially macrophages, produce a number of growth factors that stimulate the proliferation of endothelial cells and fibroblasts and the synthesis of collagen, and enzymes that remodel connective tissues. These products drive the process of repair after tissue injury and are mainly involved in tissue repair and fibrosis.
Different stimuli activate leukocytes to secrete mediators of inflammation as well as inhibitors of the inflammatory response, and thus serve to both amplify and control the reaction.

This may be another distinction between classically and alternatively activated macrophages—the former trigger inflammation and the latter function to limit inflammatory reactions.
Classically activated macrophages are induced by microbial products and cytokines, particularly IFN-γ, and are microbicidal and involved in potentially harmful inflammation. Alternatively activated macrophages are induced by other cytokines and in response to helminths (not shown), and are important in tissue repair and the resolution of inflammation (and may play a role in defense against helminthic parasites, also not shown).
Leukocytes are important causes of injury to normal cells and tissues under several circumstances:

1- As part of a normal defense reaction against infectious microbes, when adjacent tissues suffer “collateral damage.”

2- When the inflammatory response is inappropriately directed against host tissues, as in certain autoimmune diseases.

3- When the host reacts excessively against usually harmless environmental substances, as in allergic diseases, including asthma.
During activation and phagocytosis, neutrophils and macrophages 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, present in the granules, and reactive oxygen and nitrogen species.

These released substances are capable of damaging normal cells and vascular endothelium, and may thus amplify the effects of the initial injurious agent.
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<td>Pulmonary fibrosis</td>
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Defects in Leukocyte Function

Because leukocytes play a central role in host defense, defects in leukocyte function, both inherited and acquired, lead to increased vulnerability to infections. Impairments of virtually every phase of leukocyte function have been identified—from adherence to vascular endothelium to microbicidal activity. These include the following:
1- Inherited defects in leukocyte adhesion: the genetic defects of integrins and selectin-ligands that cause leukocyte adhesion deficiencies types 1 and 2. The major clinical problem in both is recurrent bacterial infections.

2- Inherited defects in phagolysosome function. The main leukocyte abnormalities are neutropenia (decreased numbers of neutrophils), defective degranulation, and delayed microbial killing. Leukocytes contain giant granules, which can be readily seen in peripheral blood smears and are thought to result from aberrant phagolysosome fusion.
3- Inherited defects in microbicidal activity. The importance of oxygen-dependent bactericidal mechanisms is shown by the existence of a group of congenital disorders called chronic granulomatous disease, which are characterized by defects in bacterial killing and render patients susceptible to recurrent bacterial infection.

4- Acquired deficiencies. Clinically, the most frequent cause of leukocyte defects is bone marrow suppression, leading to decreased production of leukocytes. This is seen following therapies for cancer (radiation and chemotherapy) and when the marrow space is compromised by tumors, which may arise in the marrow (e.g., leukemias) or be metastatic from other sites.
Cells resident in tissues also serve important functions in initiating acute inflammation. The two most important of these cell types are **mast cells** and **tissue macrophages**.

These “sentinel” cells are stationed in tissues to rapidly recognize potentially injurious stimuli and initiate the host defense reaction.
Mast cells react to physical trauma, breakdown products of complement, microbial products, and neuropeptides.

These 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.
Termination of The Acute Inflammatory Response

In part, inflammation declines simply because the mediators of inflammation are produced in rapid bursts, only as long as the stimulus persists, have short half-lives, and are degraded after their release. Neutrophils also have short half-lives in tissues and die by apoptosis within a few hours after leaving the blood.

In addition, as inflammation develops the process also triggers a variety of stop signals that serve to actively terminate the reaction.
These active termination mechanisms include a switch in the type of arachidonic acid metabolite produced, from pro-inflammatory leukotrienes to anti-inflammatory lipoxins; the liberation of anti-inflammatory cytokines, including transforming growth factor-β (TGF-β) and IL-10, from macrophages and other cells; the production of anti-inflammatory lipid mediators, called resolvins and protectins, derived from polyunsaturated fatty acids; and neural impulses (cholinergic discharge) that inhibit the production of TNF in macrophages.
Mediators of Inflammation

How mediators function in a coordinated manner is still not fully understood.

The mediators of inflammation have some shared properties and general principles of their production and actions.
1- Mediators are generated either from cells or from plasma proteins.
2- Active mediators are produced in response to various stimuli.
3- One mediator can stimulate the release of other mediators.
4- Mediators vary in their range of cellular targets.
5- Once activated and released from the cell, most of these mediators are short-lived.
Cell-Derived Mediators

Vasoactive Amines: Histamine and Serotonin.

The major vasoactive amines, which have an important actions on blood vessels.

They are stored as preformed molecules in cells and are therefore among the first mediators to be released during inflammation. The richest sources of histamine are the mast cells (in mast cell granules) that are normally present in the connective tissue adjacent to blood vessels.

It is also found in blood basophils and platelets.
Histamine is released by mast cell degranulation in response to a variety of stimuli, including:

(1) Physical injury such as trauma, cold, or heat.
(2) Binding of antibodies to mast cells, which underlies allergic reactions.
(3) Fragments of complement called anaphylatoxins (C3a and C5a).
(4) Histamine-releasing proteins derived from leukocytes.
(5) Neuropeptides (e.g., substance P); and
(6) Cytokines (IL-1, IL-8).
Histamine causes dilation of arterioles and increases the permeability of venules.
Its vasoactive effects are mediated mainly via binding to $\text{H}_1$ receptors on microvascular endothelial cells.
Serotonin (5-hydroxytryptamine) is a preformed vasoactive mediator with actions similar to those of histamine. It is present in platelets and certain neuroendocrine cells, e.g. in the GIT.

Release of serotonin (and histamine) from platelets is stimulated when platelets aggregate after contact with collagen, thrombin, adenosine diphosphate, and antigen-antibody complexes.

Thus, the platelet release reaction, which is a key component of coagulation, also results in increased vascular permeability. This is one of several links between clotting and inflammation.
Arachidonic Acid (AA) Metabolites: Prostaglandins, Leukotrienes, and Lipoxins

When cells are activated by diverse stimuli, such as microbial products and various mediators of inflammation, membrane AA is rapidly converted by the actions of enzymes to produce *prostaglandins* and *leukotrienes*.

These biologically active lipid mediators serve as intracellular or extracellular signals to affect a variety of biologic processes, including inflammation and hemostasis.
AA is a 20-carbon polyunsaturated fatty acid (5,8,11,14-eicosatetraenoic acid) that is derived from dietary sources or by conversion from the essential fatty acid linoleic acid.

It does not occur free in the cell but is normally esterified in membrane phospholipids.

Mechanical, chemical, and physical stimuli or other mediators (e.g., C5a) release AA from membrane phospholipids through the action of cellular phospholipases, mainly phospholipase A₂. (biochemical signals include an increase in cytoplasmic Ca²⁺ and activation of various kinases in response to external stimuli)
AA-derived mediators, also called eicosanoids, are synthesized by two major classes of enzymes: cyclooxygenases (which generate prostaglandins) and lipoxygenases (which produce leukotrienes and lipoxins). Eicosanoids bind to G protein–coupled receptors on many cell types and can mediate virtually every step of inflammation.
Prostaglandins (PGs) are produced by mast cells, macrophages, endothelial cells, and many other cell types, and are involved in the vascular and systemic reactions of inflammation.

They are produced by the actions of two cyclooxgenases, the constitutively expressed COX-1 and the inducible enzyme COX-2.
The most important ones in inflammation are PGE$_2$, PGD$_2$, PGF$_{2\alpha}$, PGI$_2$ (prostacyclin), and TxA$_2$ (thromboxane), each of which is derived by the action of a specific enzyme on an intermediate in the pathway.

Some of these enzymes have restricted tissue distribution. For example, platelets contain the enzyme thromboxane synthetase, and hence TxA$_2$ is the major product in these cells.
TxA₂, a potent platelet-aggregating agent and vasoconstrictor, is itself unstable and rapidly converted to its inactive form TxB₂.

Vascular endothelium lacks thromboxane synthetase but possesses prostacyclin synthetase, which leads to the formation of prostacyclin (PGI₂) and its stable end product PGF₁α.

**Prostacyclin** is a vasodilator, a potent inhibitor of platelet aggregation, and also markedly potentiates the permeability-increasing and chemotactic effects of other mediators.
**PGD$_2$ is the major prostaglandin made by mast cells;** along with PGE$_2$ (which is more widely distributed), it causes vasodilation and increases the permeability of post-capillary venules, thus potentiating edema formation.

PGF$_{2\alpha}$ stimulates the contraction of uterine and bronchial smooth muscle and small arterioles, and PGD$_2$ is a chemoattractant for neutrophils.
The prostaglandins are also involved in the pathogenesis of pain and fever in inflammation. \( \text{PGE}_2 \) is hyperalgesic and makes the skin hypersensitive to painful stimuli, such as intradermal injection of suboptimal concentrations of histamine and bradykinin. It is involved in cytokine-induced fever during infections.
The **lipoxygenase** enzymes are responsible for the production of **leukotrienes**, which are secreted mainly by leukocytes, are chemoattractants for leukocytes, and also have vascular effects.

There are three different lipoxygenases, **5-lipoxygenase** being the predominant one in **neutrophils**.

**LTB\textsubscript{4}** is a potent chemotactic agent and activator of neutrophils, causing aggregation and adhesion of the cells to venular endothelium, generation of ROS, and release of lysosomal enzymes.
The cysteinyl containing leukotrienes C\textsubscript{4}, D\textsubscript{4}, and E\textsubscript{4} (LTC\textsubscript{4}, LTD\textsubscript{4}, LTE\textsubscript{4}) cause intense vasoconstriction, bronchospasm (important in asthma), and increased vascular permeability.

The vascular leakage, as with histamine, is restricted to venules.

Leukotrienes are much more potent than is histamine in increasing vascular permeability and causing bronchospasm.
Lipoxins are also generated from AA by the lipoxygenase pathway, but unlike prostaglandins and leukotrienes, the lipoxins are inhibitors of inflammation. The principal actions of lipoxins are to inhibit leukocyte recruitment and the cellular components of inflammation. They inhibit neutrophil chemotaxis and adhesion to endothelium. The lipoxins may be endogenous negative regulators of leukotrienes and may thus play a role in the resolution of inflammation.
The molecular targets of action of some anti-inflammatory drugs are indicated by a red X. Not shown are agents that inhibit leukotriene production by inhibition of 5-lipoxygenase (e.g., Zileuton) or block leukotriene receptors (e.g., Monteleukast).

COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.
Many anti-inflammatory drugs work by inhibiting the synthesis of eicosanoids:

1- **Cyclooxygenase** inhibitors include aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin. They inhibit both COX-1 and COX-2 and thus inhibit prostaglandin synthesis; **aspirin** does this by irreversibly acetylatlng and inactivating cyclooxygenases.

**Selective COX-2 inhibitors** are a newer class of these drugs.
**COX-2** is induced by a variety of inflammatory stimuli and is absent from most tissues under normal “resting” conditions and generates prostaglandins that are involved only in inflammatory reactions.

**COX-1** is produced in response to inflammatory stimuli and is also constitutively expressed in most tissues and is responsible for the production of prostaglandins that are involved in both inflammation and homeostatic functions (e.g., fluid and electrolyte balance in the kidneys, cytoprotection in the GIT).
2- Lipoxygenase inhibitors. 5-lipoxygenase is not affected by NSAIDs, and many new inhibitors of this enzyme pathway have been developed.

Pharmacologic agents that inhibit leukotriene production (e.g. Zileuton) or block leukotriene receptors (e.g. Montelukast) are useful in the treatment of asthma.
**3- Broad-spectrum inhibitors** include corticosteroids. These powerful anti-inflammatory agents may act by reducing the transcription of genes encoding COX-2, phospholipase A$_2$, pro-inflammatory cytokines (such as IL-1 and TNF), and iNOS.
Another approach to manipulating inflammatory responses has been to modify the intake and content of dietary lipids by increasing the consumption of fish oil.

The polyunsaturated fatty acids in fish oil serve as poor substrates for conversion to active metabolites by both the cyclooxygenase and lipoxygenase pathways but are excellent substrates for the production of anti-inflammatory lipid products called resolvins and protectins.
Platelet-Activating Factor (PAF)

PAF is another phospholipid-derived mediator, causes platelet aggregation, and have multiple inflammatory effects.

A variety of cell types, including platelets themselves, basophils, mast cells, neutrophils, macrophages, and endothelial cells, can elaborate PAF, in both secreted and cell-bound forms.
**PAF** causes vasoconstriction and bronchoconstriction, 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, degranulation, and the oxidative burst.

**PAF can elicit** most of the vascular and cellular reactions of inflammation, and also boosts the synthesis of other mediators, particularly eicosanoids, by leukocytes and other cells.
Reactive Oxygen Species

Oxygen-derived free radicals may be released extracellularly from leukocytes after exposure to microbes, chemokines, and immune complexes, or following a phagocytic challenge.

Their production is dependent, on the activation of the NADPH (nicotinamide adenine dinucleotide phosphate) oxidase system. Superoxide anion, hydrogen peroxide ($\text{H}_2\text{O}_2$), and hydroxyl radical (‘OH) are the major species produced within cells, and superoxide anion can combine with NO to form reactive nitrogen species.
Extracellular release of low levels of these potent mediators can increase the expression of chemokines (e.g., IL-8), cytokines, and endothelial leukocyte adhesion molecules, amplifying the inflammatory response.

They are implicated in the following responses in inflammation:
1- **Endothelial cell damage**, with resultant increased vascular permeability. Adherent neutrophils, produce their own toxic species and also stimulate production of ROS in the endothelial cells.

2- **Injury to other cell types** (parenchymal cells, RBCs).

3- **Inactivation of antiproteases**, such as α₁-antitrypsin. This leads to unopposed protease activity, with increased destruction of extracellular matrix.
Serum, tissue fluids, and host cells possess antioxidant mechanisms that protect against these potentially harmful oxygen-derived radicals. They include:

1- The enzyme superoxide dismutase, which is found in or can be activated in a variety of cell types.
2- The enzyme catalase, which detoxifies hydrogen peroxide.
3- Glutathione peroxidase, another powerful H₂O₂ detoxifier.
4- The copper-containing serum protein ceruloplasmin; and
5- The iron-free fraction of serum transferrin.
Nitric Oxide (NO)

NO is a soluble gas that is produced not only by endothelial cells but also by macrophages and some neurons in the brain. It acts in a paracrine manner on target cells through induction of cyclic guanosine monophosphate, which, in turn, initiates a series of intracellular events leading to a response, such as the relaxation of vascular smooth muscle cells.
NO is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). There are three different types of NOS: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS).

eNOS and nNOS are constitutively expressed at low levels and can be activated rapidly by an increase in cytoplasmic Ca\(^{2+}\). iNOS, in contrast, is induced when macrophages and other cells are activated by cytokines (e.g., TNF, IFN-γ) or microbial products.
NO has dual actions in inflammation:
it relaxes vascular smooth muscle and promotes vasodilation, thus contributing to the vascular reaction, but it is also an inhibitor of the cellular component of inflammatory responses.

NO reduces platelet aggregation and adhesion, inhibits several features of mast cell–induced inflammation, and inhibits leukocyte recruitment (endogenous mechanism for controlling inflammatory responses.).

NO and its derivatives are microbicidal, and thus NO is a mediator of host defense against infection.
Cytokines and Chemokines

**Cytokines**: are proteins produced by many cell types (principally activated lymphocytes and macrophages, but also endothelial, epithelial, and connective tissue cells) that modulate the functions of other cell types. Cytokines are involved in cellular immune responses, and have additional effects that play important roles in both acute and chronic inflammation.
# Cytokines in Inflammation

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Principal Sources</th>
<th>Principal Actions in Inflammation</th>
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<tbody>
<tr>
<td><strong>IN ACUTE INFLAMMATION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>Macrophages, mast cells, T lymphocytes</td>
<td>Stimulates expression of endothelial adhesion molecules and secretion of other cytokines; systemic effects</td>
</tr>
<tr>
<td>IL-1</td>
<td>Macrophages, endothelial cells, some epithelial cells</td>
<td>Similar to TNF; greater role in fever</td>
</tr>
<tr>
<td>IL-6</td>
<td>Macrophages, other cells</td>
<td>Systemic effects (acute-phase response)</td>
</tr>
<tr>
<td>Chemokines</td>
<td>Macrophages, endothelial cells, T lymphocytes, mast cells, other cell types</td>
<td>Recruitment of leukocytes to sites of inflammation; migration of cells to normal tissues</td>
</tr>
<tr>
<td><strong>IN CHRONIC INFLAMMATION</strong></td>
<td></td>
<td></td>
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<tr>
<td>IL-12</td>
<td>Dendritic cells, macrophages</td>
<td>Increased production of IFN-γ</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>T lymphocytes, NK cells</td>
<td>Activation of macrophages (increased ability to kill microbes and tumor cells)</td>
</tr>
<tr>
<td>IL-17</td>
<td>T lymphocytes</td>
<td>Recruitment of neutrophils and monocytes</td>
</tr>
</tbody>
</table>
Tumor Necrosis Factor and Interleukin-1

Are two of the major cytokines that mediate inflammation.

They are produced mainly by activated macrophages. The secretion of TNF and IL-1 can be stimulated by endotoxin and other microbial products, immune complexes, physical injury, and a variety of inflammatory stimuli.
Microbial products, other cytokines, toxins → ACTIVATION OF MACROPHAGES (and other cells) → TNF / IL-1

**LOCAL EFFECTS**
- **Vascular endothelium**
  - ↑ Expression of leukocyte adhesion molecules
  - Production of IL-1, chemokines
  - ↑ Procoagulant and ↓ anticoagulant activity

- **Leukocytes**
  - Activation
  - Production of cytokines

- **Fibroblasts**
  - Proliferation
  - ↑ Collagen synthesis

**INFLAMMATION**

**REPAIR**

**SYSTEMIC EFFECTS**
- Fever
- Leukocytosis
- ↑ Acute-phase proteins
- ↓ Appetite
- ↑ Sleep

**SYSTEMIC MANIFESTATIONS OF INFLAMMATION**
Their most important actions in inflammation are their effects on endothelium, leukocytes, and fibroblasts, and induction of systemic acute-phase reactions.

**TNF** also regulates energy balance by promoting lipid and protein mobilization and by suppressing appetite. **Therefore**, sustained production of TNF contributes to **cachexia**, a pathologic state characterized by weight loss and anorexia that accompanies some chronic infections and neoplastic diseases.
Chemokines are a family of small (8 to 10 kilodatons) proteins that act primarily as chemoattractants for specific types of leukocytes. About 40 different chemokines and 20 different receptors for chemokines have been identified. They are classified into four major groups, according to the arrangement of the conserved cysteine (C) residues in the mature proteins.
C-X-C chemokines (also called α chemokines).

Have one amino acid residue separating the first two conserved cysteine residues.

Act primarily on neutrophils. (e.g. IL-8). 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.
**C-C chemokines** (also called β chemokines) have the first two conserved cysteine residues adjacent. These C-C chemokines, which include monocyte chemoattractant protein (MCP-1), eotaxin, macrophage inflammatory protein-1α (MIP-1α), and RANTES (regulated and normal T-cell expressed and secreted), generally attract monocytes, eosinophils, basophils, and lymphocytes but not neutrophils. Although most of the chemokines in this class have overlapping actions, eotaxin selectively recruits eosinophils.
**C chemokines** *(also called γ chemokines).* lack two (the first and third) of the four conserved cysteines (e.g., lymphotactin) are relatively specific for lymphocytes.

**CX3C chemokines** contain three amino acids between the two cysteines. The only known member of this class is called *fractalkine*.

This chemokine exists in two forms: the **cell surface-bound protein** can be induced on endothelial cells by inflammatory cytokines and promotes strong adhesion of monocytes and T cells, and a **soluble form**, derived by proteolysis of the membrane-bound protein, has potent chemoattractant activity for the same cells.
Chemokines mediate their activities by binding to seven-transmembrane G protein–coupled receptors and have two main functions: they stimulate leukocyte recruitment in inflammation and control the normal migration of cells through various tissues.

Some chemokines are produced transiently in response to inflammatory stimuli and promote the recruitment of leukocytes to the sites of inflammation.

Other chemokines are produced constitutively in tissues and function to organize different cell types in different anatomic regions of the tissues.
The Cytokines:
- IL-6, made by macrophages and other cells, which is involved in local and systemic reactions; and
- IL-17, produced mainly by T lymphocytes, which promotes neutrophil recruitment.
Lysosomal Constituents of Leukocytes

The lysosomal granules of neutrophils & monocytes contain molecules that can mediate acute inflammation. These:

1. May be released after cell death,
2. May leaked during the formation of phagocytic vacuole,
3. May leaked by frustrated phagocytosis against large, indigestible surfaces.
While **acid proteases** have **acidic** optima, active only within phagolysosomes; **Neutral proteases**, including **elastase, collagenase, & cathepsin**, are

(a) active in the ECM causing **destructive, deforming** injury by degrading elastin, collagen, BM, and others.

(b) can also **cleave C3 & C5** to generate the vasoactive mediators C3a & C5a.

**Thus**, if the initial WBC infiltration is **left unchecked**, substantial vascular permeability and tissue damage may result.
Fortunately, these effects are checked, by the following antiproteases present in the serum and tissue fluids:

1. $\alpha_2$-macroglobulin, and
2. $\alpha_1$-antitrypsin, a major inhibitor of neutrophils elastase.

Deficiencies of these inhibitors result in tissue destruction at sites of WBC accumulation, e.g., in the lung, $\alpha_1$-antitrypsin deficiency can gives rise to severe panacinar emphysema.
Neuropeptides.

Neuropeptides are secreted by sensory nerves and various leukocytes, and play a role in the initiation and propagation of an inflammatory response. Neuropeptides are small proteins, such as *substance P*, that transmit pain signals, regulate vessel tone, and modulate vascular permeability. Nerve fibers that secrete neuropeptides are especially prominent in the lung and GIT.
Plasma Protein–Derived Mediators that belong to three interrelated systems: the complement, kinin, and clotting systems.

The complement system consists of more than 20 proteins, some of which are numbered C1 through C9. This system functions in both innate and adaptive immunity for defense against microbial pathogens. Upon activation, different complement proteins:

1. coat (opsonize) particles, such as microbes for phagocytosis & destruction,
2. increased vascular permeability,
3. induce WBC chemotaxis.
Complement activation ultimately generates a pore like **membrane attack complex (MAC)** that punches holes in the membranes of microbes.

Complement components (numbered C1 to C9), are present in plasma as inactive forms.
**Briefly,** the most critical step in the elaboration of the biologic functions of complement is the **activation** of the third component, C3:

**(1) via the **classic pathway**, triggered by **fixation of C1** to antibody (IgM or IgG); or

**(2) alternative pathway**, triggered by microbial surface molecule (e.g., endotoxin), complex polysaccharides, cobra venom, and other substances, in the absence of antibody.

**(3) lectin pathway**, in which plasma mannose-binding lectin binds to carbohydrates on microbes and directly activates C1. (classic pathway but in the absence of antibodies).
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).
The biologic functions of the complement system fall into three general categories:

**Inflammation**: (Vascular effects) \textbf{C3a & C5a} (anaphylatoxins) increase vascular permeability & cause vasodilation (through what?)

**Phagocytosis**: C3b and its cleavage product iC3b (inactive C3b), when fixed to a microbial cell wall, act as opsonins and promote phagocytosis by neutrophils and macrophages, which bear cell surface receptors for the complement fragments.

**Cell lysis**: The deposition of the MAC on cells makes these cells permeable to water and ions and results in death (lysis) of the cells.
The activation of complement is tightly controlled by cell-associated & circulating regulatory proteins. The presence of these inhibitors in cell membranes protects normal cells from inappropriate damage during protective reactions against microbes. However, inappropriate or excessive complement activation (e.g., in antibody-mediated diseases, such as Glomerulonephritis) can overwhelm the regulatory systems, and this is why complement activation is responsible for serious tissue injury in some immunologic disorders (e.g., GN).
Coagulation & Kinin Systems

Inflammation and blood clotting are often intertwined, with each promoting the other. The clotting system is divided into two pathways that converge, culminating in the activation of thrombin and the formation of fibrin.
Activation of Hageman factor (XII) to activated Hageman factor (XIIa) initiates four systems involved in inflammation:

1. **Kinin system** producing vasoactive kinins (bradykinin);
2. **Clotting system** including the activation of thrombin, fibrinopeptides, & factor X, all with inflammatory properties;
3. **Fibrinolytic system** producing plasmin & inactivating thrombin; and
4. **Complement system** producing anaphylatoxins C3a & C5a.
Hageman factor = factor XII of the intrinsic coagulation cascade, is a protein synthesized by the liver, circulate in an inactive form, until it encounters (I) collagen, BM, or activated platelets (as at a site of EC injury), or (II) plasmin. Each can activate Hageman factor, thereby amplifying the entire set of responses.
Interrelationship among the 4 plasma mediator systems triggered by activation of factor XII.
With the assistance of a high-molecular-weight kininogens (HMWK) cofactor, factor XII then undergo a conformational change (becoming active, factor XIIa), exposing an active serine center, that can cleave a number of protein substrates of the kinin & coagulation systems.

# In the clotting system, factor XIIa activate factor XI to XIa which in turn convert factor X to Xa which convert Prothrombin into thrombin which convert circulating soluble fibrinogen to an insoluble fibrin clot.

(1) Factor Xa increase vascular permeability & WBC emigration.
(2) Thrombin enhances WBC adhesion to EC.
(3) Fibrinogen cleavage results in the generation of fibrinopeptides that increase vascular permeability & are chemotactic for WBC.
Fibrinolytic system
while activated Hageman factor is inducing clotting, it is concurrently (at the same time) activating the: Fibrinolytic system.

This mechanism exists to counter-regulate clotting by cleaving fibrin, thereby solubilizing the fibrin clot.
Without fibrinolysis, & other regulatory mechanisms, initiation of the coagulation cascade, even by trivial (very mild) injury, would culminate in continuous & irreversible clotting of the entire vasculature!

(I) Plasminogen activator {PA} (released from EC, WBC, & other tissues), & (II) kallikrein, Both cleave plasminogen, a plasma protein bound up in the evolving fibrin clot, result in Plasmin, a multifunctional protease that cleave fibrin & is therefore important in lysing clots.
However, fibrinolysis also participates in the vascular phenomena of inflammation.

Plasmin, also, cleaves the complement C3 component to C3a, resulting in vasodilation & increase vascular permeability.

Plasmin, also, activate Hageman factor, hereby amplifying the entire set of responses. Fibrin-split products increase vascular permeability,
Kinin system activation

in which factor **XIIa** converts plasma **prekallikrein** into **kallkrein**, which act on the circulating HMWK leads finally to the formation of **bradykinin**.

**Bradykinin**, *like Histamine* causes arteriolar *dilatation*, *increases* vascular permeability, & *bronchial smooth muscle contraction*, causes *pain* when injected in skin.

Bradykinin actions are short-lived, because it is rapidly inactivated by degradative **kininases** present in the plasma & tissues.

So, **kallikrein is a:**

1. A potent activator of Hageman factor,
2. Activate plasminogen→ into plasmin.
3. Convert HMWK → to bradykinin.
Role of Mediators in Different Reactions of Inflammation

- **Vasodilation**: Histamine + NO + PGs
- **Increased Vascular Permeability**: Histamine, serotonin + C3a & C5a {by liberating histamine & serotonin from their cells} + Bradykinin + LTC4, LTD4, LTE4 + PAF + Substance P.
- **Leukocyte recruitment & Activation**: TNF & IL-1 + Chemokines (IL-8) + C3a & C5a + LTB4, + Bacterial products (e.g., N-formyl methyl peptides).
- **Fever**: IL-1, TNF + PG
- **Pain**: PG + Bradykinin + Neuropeptides.
- **Tissue Damage**: lysosomal enzymes of WBC + NO + ROS.
We still do **not fully understand** why some stimuli elicit inflammatory reactions, e.g., necrotic cells are a powerful stimulus for inflammation, but how dead cells trigger this reaction? is not yet established!

Hypoxia, itself induces an inflammatory response, partly by stimulating the production of mediators, e.g., VEGF that increases vascular permeability.
Outcomes of Acute Inflammation

All acute inflammatory reactions may have one of three outcomes:

**ACUTE INFLAMMATION**
- Vascular changes
- Neutrophil recruitment
- Limited tissue injury

**RESOLUTION**
- Clearance of injurious stimuli
- Clearance of mediators and acute inflammatory cells
- Replacement of injured cells
- Normal function

**INJURY**
- Infarction
- Bacterial infections
- Toxins
- Trauma

**CHRONIC INFLAMMATION**
- Angiogenesis
- Mononuclear cell infiltrate
- Fibrosis (scar)
- Progressive tissue injury

**FIBROSIS**
- Collagen deposition
- Loss of function
Morphologic Patterns of Acute Inflammation

The morphologic hallmarks of all acute inflammatory reactions are dilation of small blood vessels, slowing of blood flow, and accumulation of leukocytes and fluid in the extravascular tissue.
However, special morphologic patterns are often superimposed on these general features, depending on the severity of the reaction, its specific cause, and the particular tissue and site involved.
Serous inflammation is marked by the outpouring of a thin fluid that may be derived from the plasma or from the secretions of mesothelial cells lining the peritoneal, pleural, and pericardial cavities. Accumulation of fluid in these cavities is called an effusion.

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 greater increase in vascular permeability, large molecules such as fibrinogen pass the vascular barrier, and fibrin is formed and deposited in the extracellular space. A fibrinous exudate develops when the vascular leaks are large or there is a local procoagulant stimulus (e.g., cancer cells).

A fibrinous exudate is characteristic of inflammation in the lining of body cavities, such as the meninges, pericardium and pleura.
Histologically, fibrin appears as an eosinophilic meshwork of threads or sometimes as an amorphous coagulum. Fibrinous exudates may be removed by fibrinolysis and clearing of other debris by macrophages. If the fibrin is not removed, over time it may stimulate the ingrowth of fibroblasts and blood vessels and thus lead to scarring (organization).

A pink meshwork of fibrin exudate (F) overlies the pericardial surface (P).
This type of inflammation is characterized by the production of large amounts of pus or purulent exudate consisting of neutrophils, liquefactive necrosis, and edema fluid. **Certain bacteria** (e.g., staphylococci) produce this localized suppuration and are therefore referred to as **Pyogenic** (pus-producing) bacteria.

A common example of an acute suppurative inflammation is acute appendicitis.
Abscesses are localized collections of purulent inflammatory tissue caused by suppurative burials in a tissue, an organ, or a confined space. They are produced by deep seeding of pyogenic bacteria into a tissue. Abscesses have a central region that appears as a mass of necrotic leukocytes and tissue cells.

A, Multiple bacterial abscesses in the lung, in a case of bronchopneumonia. B, The abscess contains neutrophils and cellular debris, and is surrounded by congested blood vessels.
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 inflamed necrotic tissue and can occur only when tissue necrosis and resultant inflammation exist on or near a surface.

A - A chronic duodenal ulcer. B - Low-power cross-section of a duodenal ulcer crater with an acute inflammatory exudate in the base.
Chronic Inflammation

is inflammation of prolonged duration (weeks or months) in which inflammation, tissue injury, and attempts at repair coexist, in varying combinations. It may follow acute inflammation, as described earlier, or chronic inflammation may begin insidiously, as a low-grade, smoldering response without any manifestations of an acute reaction.
Causes of Chronic Inflammation

1- Persistent infections by microorganisms that are difficult to eradicate, such as mycobacteria, and certain viruses, fungi, and parasites (delayed-type hypersensitivity) and (specific pattern called a granulomatous reaction).

2- Prolonged exposure to potentially toxic agents, either exogenous or endogenous. An example of an exogenous agent is particulate silica, a non-degradable inanimate material that, when inhaled for prolonged periods, results in an inflammatory lung disease called silicosis.
Immune-mediated inflammatory diseases, caused by excessive and inappropriate activation of the immune system (examples of such diseases are rheumatoid arthritis and multiple sclerosis). In other cases, chronic inflammation is the result of unregulated immune responses against microbes, as in inflammatory bowel disease. Immune responses against common environmental substances are the cause of allergic diseases, such as bronchial asthma (mixed acute and chronic inflammation because they are characterized by repeated bouts of inflammation. Fibrosis may dominate the late stages.).
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, which include macrophages, lymphocytes, and plasma cells.**

2- **Tissue destruction, induced by the persistent offending agent or by the inflammatory cells.**

3- **Attempts at healing by connective tissue replacement of damaged tissue, accomplished by proliferation of small blood vessels (angiogenesis) and, in particular, fibrosis.**
Chronic inflammation in the lung, showing all three characteristic histologic features:

A- (1) collection of chronic inflammatory cells (*),
(2) destruction of parenchyma (normal alveoli are replaced by spaces lined by cuboidal epithelium, *arrowheads*), and
(3) replacement by connective tissue (fibrosis, *arrows*).

B- By contrast, in acute inflammation of the lung (*acute bronchopneumonia*), neutrophils fill the alveolar spaces and blood vessels are congested.
Role of Macrophages in Chronic Inflammation

The *macrophage* is the dominant cellular player in chronic inflammation. *Macrophages* are one component of the *mononuclear phagocyte system*.
The macrophages are diffusely scattered in the connective tissue or located in organs such as the:

- Liver (Kupffer cells).
- Spleen and Lymph nodes (sinus histiocytes).
- Lungs (alveolar macrophages), and
- Central nervous system (microglia).

The half-life of blood monocytes is about 1 day, whereas the life span of tissue macrophages is several months or years.
Extravasation of monocytes is governed by the adhesion molecules and chemical mediators with chemotactic and activating properties and in the extravascular tissue, it undergoes transformation into a larger phagocytic cell, the *macrophage*. (may be *activated* by a variety of stimuli, including microbial products that engage TLRs and other cellular receptors, cytokines (e.g., IFN-γ) secreted by sensitized T lymphocytes and by natural killer cells, and other chemical mediators.)

AA, arachidonic acid; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TGFβ, transforming growth factor β.
Activated macrophages serve to eliminate injurious agents such as microbes and to initiate the process of repair, and are responsible for much of the tissue injury in chronic inflammation.

Activation of macrophages results in increased levels of lysosomal enzymes and reactive oxygen and nitrogen species, and production of cytokines, growth factors, and other mediators of inflammation.
Some of these products are toxic to microbes and host cells (e.g., reactive oxygen and nitrogen species) or to extracellular matrix (proteases); some cause influx of other cell types (e.g., cytokines, chemotactic factors); and still others cause fibroblast proliferation, collagen deposition, and angiogenesis (e.g., growth factors).

different macrophage populations may serve distinct functions: some may be important for microbial killing and inflammation, and others for repair.
Other Cells in Chronic Inflammation include lymphocytes, plasma cells, eosinophils, and mast cells:

1- **Lymphocytes** are mobilized in both antibody-mediated and cell-mediated immune reactions. Antigen-stimulated lymphocytes of different types (T and B cells) use various adhesion molecule pairs (selectins, integrins and their ligands) and chemokines to migrate into inflammatory sites.
Different subsets of T cells (called $T_{H1}$ and $T_{H17}$) may produce different sets of cytokines.

Activated macrophages in turn stimulate T cells by presenting antigens and via cytokines (such as IL-12).

Activated T cells produce cytokines that recruit macrophages (TNF, IL-17, chemokines) and others that activate macrophages (IFN$\gamma$).

Macrophage-lymphocyte interactions in chronic inflammation.
2- **Plasma cells** develop from activated B-cells and produce antibodies directed either against persistent foreign or self antigens in the inflammatory site or against altered tissue components. In some strong chronic inflammatory reactions, the accumulation of lymphocytes, antigen-presenting cells, and plasma cells may assume the morphologic features of lymphoid organs, particularly lymph nodes, even containing well-formed germinal centers (tertiary lymphoid organs).
3- Eosinophils are abundant in immune reactions mediated by IgE and in parasitic infections. A chemokine that is especially important for eosinophil recruitment is eotaxin.

Eosinophils have granules that contain major basic protein, a highly cationic protein that is toxic to parasites but also causes lysis of mammalian epithelial cells. This is why eosinophils are of benefit in controlling parasitic infections, but they contribute to tissue damage in immune reactions such as allergies.
4- **Mast cells** are widely distributed in connective tissues and participate in both acute and chronic inflammatory reactions. Mast cells express on their surface the receptor (FcεRI) that binds the Fc portion of IgE antibody. This type of response occurs during allergic reactions. Mast cells are also present in chronic inflammatory reactions, and because they secrete a plethora of cytokines, they have the ability to both promote and limit inflammatory reactions in different situations.
Although neutrophils are characteristic of acute inflammation, many forms of chronic inflammation, lasting for months, continue to show large numbers of neutrophils, induced either by persistent microbes or by mediators produced by activated 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.
In addition to cellular infiltrates, growth of blood vessels and lymphatic vessels is often prominent in chronic inflammatory reactions. This growth of vessels is stimulated by growth factors, such as VEGF, produced by macrophages and endothelial cells.
Granulomatous Inflammation

Is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and some noninfectious conditions. Immune reactions are usually involved in the development of granulomas.

A granuloma is a cellular attempt to contain an offending agent that is difficult to eradicate.

In this attempt there is often strong activation of T lymphocytes leading to macrophage activation, which can cause injury to normal tissues.
# Examples of Diseases with Granulomatous Inflammation

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cause</th>
<th>Tissue Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Caseating granuloma (tubercle): focus of activated macrophages (epithelioid cells), rimmed by fibroblasts, lymphocytes, histiocytes, occasional Langhans giant cells; central necrosis with amorphous granular debris; acid-fast bacilli</td>
</tr>
<tr>
<td>Leprosy</td>
<td><em>Mycobacterium leprae</em></td>
<td>Acid-fast bacilli in macrophages; noncaseating granulomas</td>
</tr>
<tr>
<td>Syphilis</td>
<td><em>Treponema pallidum</em></td>
<td>Gumma: microscopic to grossly visible lesion, enclosing wall of histiocytes; plasma cell infiltrate; central cells necrotic without loss of cellular outline</td>
</tr>
</tbody>
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### Examples of Diseases with Granulomatous Inflammation

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<tr>
<th>Disease</th>
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<tbody>
<tr>
<td>Cat-scratch disease</td>
<td>Gram-negative bacillus</td>
<td>Rounded or stellate granuloma containing central granular debris and recognizable neutrophils; giant cells uncommon</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Unknown etiology</td>
<td>Noncaseating granulomas with abundant activated macrophages</td>
</tr>
<tr>
<td>Crohn disease (inflammatory bowel disease)</td>
<td>Immune reaction against intestinal bacteria, self-antigens</td>
<td>Occasional noncaseating granulomas in the wall of the intestine, with dense chronic inflammatory infiltrate</td>
</tr>
</tbody>
</table>
A granuloma is a focus of chronic inflammation consisting of a microscopic aggregation of macrophages that are transformed into epithelium-like cells, surrounded by a collar of mononuclear leukocytes, principally lymphocytes and occasionally plasma cells.

In the usual H & E – stained tissue sections, the epithelioid cells have a pale pink granular cytoplasm with indistinct cell boundaries. The nucleus is less dense than that of a lymphocyte, is oval or elongate, and may show folding of the nuclear membrane.
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. 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).

Older granulomas develop an enclosing rim of fibroblasts and connective tissue.

Typical tuberculous granuloma showing an area of central necrosis surrounded by multiple Langhans-type giant cells, epithelioid cells, and lymphocytes.
There are two types of granulomas, which differ in their pathogenesis.

**Foreign body granulomas** are incited by relatively inert foreign bodies. Typically, foreign body granulomas form around material such as talc (associated with intravenous drug abuse), sutures, or other fibers that are large enough to preclude phagocytosis by a single macrophage and do not incite any specific inflammatory or immune response.

**Immune granulomas** are caused by a variety of agents that are capable of inducing a cell-mediated immune response. This type of immune response produces granulomas usually when the inciting agent is poorly degradable or particulate. In such responses macrophages engulf foreign protein antigen, process it, and present peptides to antigen-specific T lymphocytes, causing their activation.
**systemic effects of inflammation**

These effects are collectively called: **acute-phase reaction**. They include **fever, malaise** (feeling of being sick), **anorexia** (loss of apatite), **insomnia, hypotension**, accelerated degradation of skeletal muscle proteins, **hepatic synthesis** of a variety of proteins (e.g., complement & coagulation proteins), & alteration in the circulating WBC.
The most important mediators of the acute-phase reaction are the cytokines **TNF, IL-1, & IL-6**, produced mainly by WBC in response to infection, or to immune & toxic injury, and are released systemically, frequently in a cascade. Thus, TNF induces the production of IL-1, which stimulates the production of IL-6. **TNF & IL-1** cause similar effects, both act on the thermoregulatory center of the hypothalamus-via local PGE production- to **induce fever** (hence the efficacy of aspirin & NSAIDs in reducing fever).
**IL-6 stimulates** the hepatic synthesis of several plasma proteins,

(1) **Fibrinogen**; elevated fibrinogen levels cause RBC to agglutinate more readily, explaining why inflammation is associated with a higher ESR

(2) **C-reactive protein (CRP) & serum amyloid A (SAA) proteins**, both bind to microbial cell walls, and they may act as **opsonins** and fix complement, thus promoting the elimination of the microbes.

Elevated serum levels of **CRP are now used as marker for increased risk of MI or stroke in patients with atherosclerosis**, which is believed to be inflammatory in nature & increased CRP is a measure of inflammation.
Leukocytosis (increased, mature, white blood cell count in blood) is a common feature of inflammatory reactions, especially those induced by bacterial infection. WBC count typically increases from a normal 4,000 to 10,000 to 15,000 - 20,000 cells per micro liter, but may climb as high as 40,000 to 100,000, a so-called Leukemoid (leukemia-like) reaction.

This must be differentiated from leukemia, a malignant neoplastic proliferation of WBC in the bone marrow.
Most **bacterial infections** induce selective increase in polymorphonuclear cells (**neutrophilia**), while **parasitic** infections and **allergic** responses characteristically induce **eosinophilia**.

Certain **viruses**, like infectious mononucleosis, mumps, & rubella cause selective increase in lymphocytes (**lymphocytosis**).

However, most viral infections, rickettsial, protozoal, and certain types of bacterial infections (e.g., typhoid fever), are associated with a deceased number of circulating WBC (**leucopenia**).
Severe bacterial infections (sepsis), especially by gram-negative bacteria stimulate the production of huge quantities of several cytokines, notably TNF, IL-1, IL-6, & IL-8, resulting in septic shock, which is usually fatal.

REPAIR begins almost as soon as the inflammatory changes have started and involves cell proliferation, differentiation and ECM deposition.
END of acute & chronic inflammation.

Lectures prepared by:
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Next lecture

Chapter 3

Tissue Renewal, Regeneration, and Repair