INFLAMMATION

II. Chronic inflammation (The Bad Side of Inflammation)

When the inflammatory response persists for longer durations (weeks or months up to years), inflammation is said to be chronic, during which the inflammation remains active with tissue destruction and healing processes, occurs at the same time. It can also occur as a low-grade, asymptomatic, prolonged response to an inciting agent.

Causes of chronic inflammation

1. Failure to eliminate the causative agent of **acute inflammation**
2. **Infections**: low-grade, low-toxicity but persistent caused by slowly progressing organisms: like Mycobacterium tuberculosis, and some viruses, fungi, & parasites.
3. Continued exposures to **low-level irritant or foreign material** & some **toxic agents**: a) exogenous (e.g. silica, talc, suture material or b) endogenous. These agents that cannot be eliminated by enzymatic breakdown or phagocytosis.
4. Inflammatory & biochemical inducers causing **oxidative stress** and mitochondrial dysfunction.
5. **Immune-mediated inflammatory** diseases: defect in inflammatory cells response:

Hypersensitivity diseases: excessive and improper reaction of the immune system.
Autoimmune diseases: immune reactions against "self" i.e. normal body's tissues (such as type 1 diabetes; rheumatoid arthritis (RA); systemic lupus erythematosus (SLE)).

Morphological features

Certain chronic inflammatory conditions may occur from the onset without the prior acute phase. Most of the features of acute inflammation continue as the inflammation becomes chronic, including vasodilation, increased blood flow & capillary permeability, and infiltration migration of neutrophils into the tissues through *diapedesis*.

The main features of chronic inflammation are:

- Infiltration of chief inflammatory cells in the tissue site, including: macrophages, lymphocytes, and plasma cells. Such cells produce inflammatory *cytokines*, *growth factors*, and *enzymes*, contributing to the progression of tissue damage and secondary repair, including fibrosis and, in some infections, granuloma formation.
- Tissue destruction, mostly produced by the products of inflammatory cells.
- Repair, including new vessel proliferation (angiogenesis) and fibrosis.

Chronic inflammatory cells

1. MACROPHAGES

The main cells of chronic inflammation derived from circulating blood monocytes. Circulatory monocytes live for only 1 day, after migration into different tissues, they form *tissue macrophages* that scatter diffusely in connective tissues. Macrophages are larger, live longer and have greater capacity for phagocytosis than monocytes. Collectively are called the mononuclear phagocyte system (reticulo-endothelial cells (RECs)).

Tissue macrophage are named according to the site or organ where they migrate to: Kupffer cells (liver), sinus histiocytes (spleen and lymph nodes), alveolar macrophages (lungs) and Microglial cells (central nervous system).

Macrophages are **activated** through two pathways:

1. Classical macrophage activation, which is induced by
   - microbial products such as endotoxin
   - T cell–derived signals, importantly the cytokine IFN-γ
   - foreign substances
2. Alternative macrophage activation, which is induced by *cytokines* such as IL-4 and IL-13produced by T lymphocytes and other cells (mast cells and eosinophils).

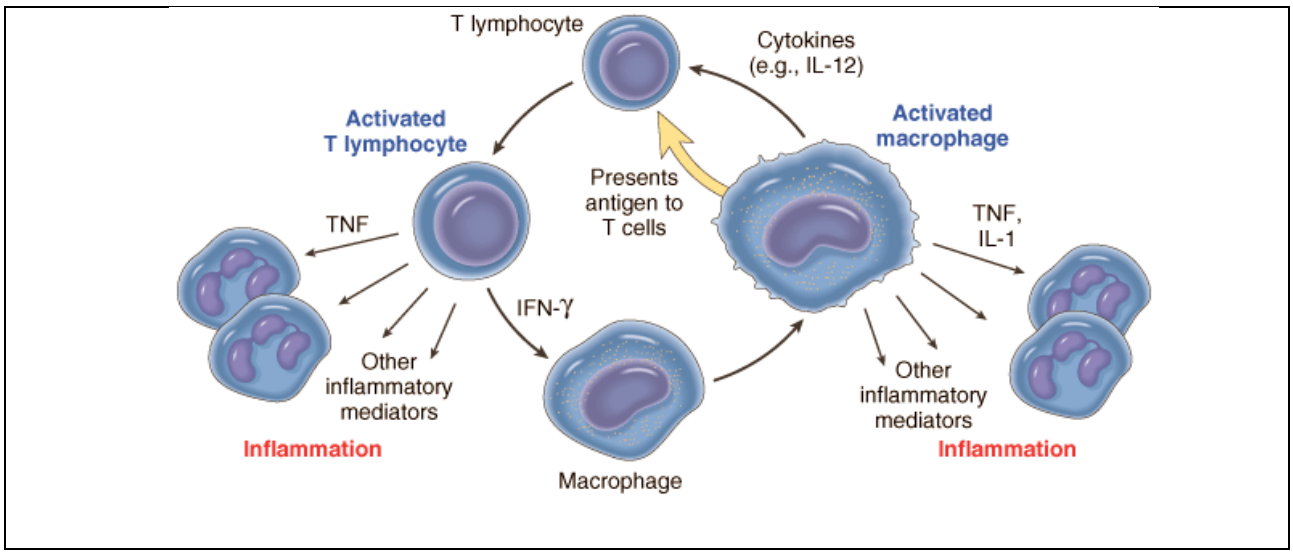
Products of activated macrophages, facilitate different functions:

- Engulf & eradicate microbes & dead tissue through: ROS, NO, lysosomal enzyme
- Initiate tissue repair processes and involved in scar formation (growth factor)
- Secrete mediators of inflammation such as: Tumor necrotic factor (TNF), Interleukin-1 (IL-1) and other cytokines that promote leukocyte recruitment.
- Macrophages display antigens to T lymphocyte and respond to signal from T cell

2. LYMPHOCYTES

During the stage of leukocytes recruitment, both T and B lymphocytes migrate into inflammatory sites using the same adhesion molecule pairs and chemokines. These lymphocytes interact with macrophages in a *bidirectional way*, and these interactions play an important role in chronic inflammation. *Macrophages produce cytokines (**IL-12**) that stimulate T-cell responses*.

The activated T lymphocytes produce cytokines, among which, **IFN-γ**, that is a powerful activator of macrophages, promoting more antigen presentation and cytokine secretion. *The result is a cycle of cellular reactions that fuel and sustain chronic inflammation.*



Macrophage-lymphocyte interactions in chronic inflammation

3. PLASMA CELLS

Plasma cells are fully differentiated B-lymphocyte that is rich in the rough endoplasmic reticulum (rER), where they can produce immunoglobulins or antibodies against:

1) Persistent antigens in the inflammatory site.
2) Altered tissue components.

The presence of lymphocytes, macrophages and plasma cells together in certain intense chronic inflammatory responses may show similar physical characteristics of lymphoid organs (resembling lymph nodes and possibly contain fully developed germinal centers).

4. EOSINOPHILS

Eosinophils are classically involved in inflammations cause by parasitic infections and in *IgE-mediated* immune responses associated with allergies.

The granules of eosinophils have *major basic protein*, which is highly charged cationic protein that is involved in inactivating and killing of parasites (parasitic infestations). However, this protein may also cause necrosis of epithelial cells.

5. MAST CELLS

Mast cells are residents mainly in connective tissues, contain granules rich in histamine (an inflammatory mediator) and heparin (an anticoagulant) and are involved in both acute and chronic inflammatory reactions.

Mast cells have single round nucleus, with granulated cytoplasm express on their surface the receptor that binds the *Fc portion of IgE antibody*. They may produce cytokines that contribute to fibrosis in chronic inflammatory reactions.

Types of Chronic Inflammation

1) Non-specific Proliferative

Non-specific granulation tissue is formed by infiltration of mononuclear cells (lymphocytes, macrophages, and plasma cells) and multiplying fibroblasts, connective tissue, vessels, and epithelial cells. Examples are inflammatory polyp-like nasal polyps, lung abscesses, and pyogenic granuloma (Angio-granuloma) of the gingivae.

2) Granulomatous Inflammation

Granulomatous lesions that are usually nodular created from collection of activated macrophages or epithelioid (epithelial-like) cells, which have a pale-pink granular cytoplasm with indistinct cell boundaries and usually surrounded by lymphocytes.
The macrophages inside the granulomas usually fuse to form giant multinucleated cells (40-50 µm in size) consist of a large cytoplasm and many nuclei (20 or more) arranged either at the cell periphery as in "Langhans giant cells in tuberculosis" or randomly distributed nuclei as in foreign body giant cells.

Types of granulomas

A) *Foreign body granuloma* (e.g. silicosis, suture, or splinter) … or
B) *Granuloma due to* persistent *T-cell-mediated immune response*, where T cell–derived cytokines cause chronic macrophage activation in response to certain slowly progressing infections such as tuberculosis, T. pallidum, fungi and leprosy.
C) Granulomas may also develop in certain immune-mediated inflammatory diseases such as Crohn disease (a type of inflammatory bowel diseases).
D) Finally, granulomas may develop in a disease of unknown cause such as sarcoidosis.

Microscopically

Granulomas are classically seen as aggregates of epithelioid macrophages that are encircled by a collar of lymphocytes. Multinucleate giant cells may be seen. A rim of fibroblasts and connective tissue may older granulomas, which may be extensive during healing stages.

In granulomas of certain slowly progressing infectious such as tuberculosis a central necrotic zone is seen that result from cellular injury caused by hypoxia and free radical. Grossly, this type of necrosis looks as a granular, cheesy appearance "*caseous necrosis*". Microscopically, the necrotic area appears as eosinophilic amorphous, that lack a proper structure containing granular debris, with complete loss of cellular details.

Other granulomas seen in Crohn disease, Sarcoidosis or foreign body granuloma, they lack necrotic centers and are therefore called "non-caseating granulomas".

Morphologic Patterns of Inflammation

1. *Granulomatous* Inflammation, as described above.
2. *Fibrinous* Inflammation
3. *Serous* Inflammation
4. *Suppurative* (Purulent) Inflammation
5. *Ulcerative* Inflammation
6. *Catarrhal* Inflammation
7. *Pseudomembranous* Inflammation

Fibrinous Inflammation is seen in acute or chronic, where fluid containing large plasma proteins (mainly fibrinogen) extensively leak from blood vessels into tissue. Commonly seen in throat streptococcal infection, bacterial pericarditis, and bacterial pneumonia. Finally, the fibrinogen organization may occur with rigid fibrous tissue accumulation.

Serous Inflammation, like fibrinous type, is also a general morphological pattern of acute or chronic inflammation. The exudate of serum is generated from mild vascular leak or synthesized by mesothelial cells. Examples seen in pneumonia, skin blisters of herpes virus or burns, serous pericarditis, pleuritis and joint fluid in rheumatoid arthritis.

Suppurative Inflammation may be seen in acute or chronic inflammation. Large amount of pus, consists of neutrophils, dead cells, and fluid produced by pyogenic bacterial infections such as Staphylococci.

Ulcerative inflammation result in the necrotic loss of tissue, exposing deeper layers occurring of *epithelial surfaces (skin or mucosa)*. Examples: gastric or duodenal ulcers. An ulcer is a general morphological pattern of both acute and chronic inflammation.

Catarrhal inflammation is a form affecting mainly *mucosal surfaces*, marked by a non-suppurative, copious discharge of mucus, and epithelial debris. Examples include acute rhinitis and catarrhal bronchitis.

Pseudomembranous is a form of ulcerative/exudative inflammation affecting mucous and serous membranes. Exudate made of fibrin, pus, cellular debris, and mucus forms a pseudo-membrane on the surface of the ulcers. Examples pseudomembranous colitis and throat pseudo-membrane in diphtheria.

CHEMICAL MEDIATOR OF ACUTE INFLAMMATION

Inflammation chemical mediators are materials that regulate *vascular* and *cellular* events in acute inflammation and the resulting morphologic changes. They also are the MAIN cause of signs and symptoms of inflammation, beside the cause of injury.

Chemical mediators of inflammation can be:

Cell Derived Mediators

Produced locally by cells at inflammation site: Histamine, prostaglandins & cytokines.

Plasma Protein-Derived Mediators

Circulating plasma proteins (*typically synthesized by the liver*) as inactive precursors that will be activated at the site of inflammation. Plasma contains four enzymatic cascade systems: *complement system*, kinin system, coagulation system, and fibrinolytic system.

MODE OF ACTION

- Most mediators act by *binding to specific receptors* on the target cells.
- Mediators may act on single or few targets or cause widespread actions.
- Certain mediators exert direct enzymatic and/or toxic activities (ex. lysosomal proteases and Reactive Oxygen Species).

REGULATION OF ACTION:

- The actions of most mediators are strictly regulated.
- Once activated and released from the cell, mediators perform their action and then quickly *cleared out*: decayed, inactivated by enzymes, eliminated or inhibited

CELL-DERIVED MEDIATORS

ALL locally present cells at the site of inflammation including: endothelial cells, mast cells, tissue macrophages and as well as leukocytes recruited from blood are all capable of producing different inflammatory mediators.

1) Vasoactive Amines

Vasoactive amines are stored as pre-form molecules in mast cells and other cells. These amines (**histamine** and **serotonin**) are among the first mediators to be released in acute inflammatory reactions.

Histamine causes vascular dilatation and the immediate transient phase of increased vascular permeability. Mast cells are the main source of histamine but may also be produced by basophils and platelets.

2) Arachidonic Acid Metabolites (AA)

These include *Prostaglandins*, *Leukotrienes*, *Lipoxins*, which are involved in **ALL** step of inflammatory response. The AA mediators are produced by many cell types: such as leukocytes, mast cells, endothelial cells, as well as platelets.

Prostaglandins (PG) potentiate vascular permeability by causing vasodilation and potentiates edema formation. They are involved in the pathogenesis of pain and fever.

Leukotrienes are potent chemotactic agent for neutrophils that have vasoactive properties and causes increased vascular permeability.

Lipoxins are **anti-inflammatory mediators**, which serve as endogenous antagonists of leukotrienes. They inhibit neutrophil chemotaxis and adhesion to endothelium.

3) Platelet-Activating Factor (PAF)

PAF prime role is to aggregate platelets and cause their degranulation.

Their **functions** include: **a)** platelets stimulation; **b)** bronchospasm; **c)** increased vascular permeability; and **d)** vasodilation (100 up to 1000 times more potent than is histamine).

PAF induce *most of inflammatory reactions*, including enhanced leukocyte adhesion, chemotaxis, leukocyte degranulation and intracellular killing.

4) Cytokines

Cytokines, are polypeptide products of various cell types, which serve as mediators of inflammation and immune responses.

Interleukins (IL), are a subgroup of cytokines, that facilitate leukocytes interaction. They are labeled by serial numbering (IL-1, IL-2), each type of has specific functions.

The primary cytokines involved in acute inflammation are Tumor Necrosis Factor (**TNF**) and **IL-1**, along with a *group of chemo-attractant cytokines* known as **chemokines**.

In chronic inflammation, other cytokines are involved, including interferon-γ (IFN-γ) and IL-12. Tumor Necrosis Factor and Interleukin-1. Activated macrophages, mast cells, endothelial cells, as well as some other cell types, produce TNF and IL-1.

TNF and IL-1 play a principal role in activating endothelial cells through stimulating the expression of adhesion molecules on endothelial cells, resulting in increased leukocyte binding and recruitment.

TNF also increases the neutrophils aggregation and activation. While IL-1 activates tissue fibroblasts, resulting in increased proliferation.

Chemokines

Chemokines are a family small structurally related proteins. They are chemo-attractants for different subsets of leukocytes and their prime role is to selectively recruit and attract leukocytes to the site of inflammation.

Chemokines also activate leukocytes, by increasing the affinity of leukocytes adhesion site for their ligands on endothelial cells.

5) Reactive Oxygen Species (ROS)

ROS are oxygen-derived free radicals, which are synthesized inside the lysosomes and released from neutrophils and activated macrophages. The main functions of ROS are to destroy phagocytosed microbes and necrotic cells.

In low concentrations, ROS can increase chemokine, cytokine, and adhesion molecule expression. In higher concentrations, they cause tissue injury through several mechanisms, including: a) endothelial damage; b) direct injury to other cell types (e.g. tumor cells, erythrocytes, parenchymal cells).

6) Nitric Oxide (NO)

A short-lived, soluble, free-radical gas produced by endothelial cells & macrophages. NO has many roles in inflammation:

- It is used by macrophages as a cytotoxic metabolite for killing microbes and tumor cells.
- In the endothelial cells (vasodilation).
- At the end of acute inflammation, NO suppresses the inflammatory response.

7) Lysosomal Enzymes of Leukocytes:

The lysosomal granules of neutrophils and monocytes contain many molecules that can mediate acute inflammation. They may be leak-out after cell death during the formation of phagocytic vacuoles, or during unsuccessful attempts to phagocytose big molecules that are hard to digest. The most important lysosomal enzymes

- Acid proteases, which are active only within phagolysosomes.
- Neutral proteases (including elastase, collagenase) cause destructive tissue injury by degrading elastin, collagen, basement membrane & other matrix proteins.

8) Neuropeptides

Small proteins, such as substance P, that transmit *pain signals*, *regulate vessel tone*, and *modulate vascular permeability*.

PLASMA PROTEIN-DERIVED MEDIATORS

These are circulatory blood proteins involved in numerous phases of the inflammatory reaction. Plasma contains four enzymatic cascade systems: *complement system*, *coagulation* system, *fibrinolytic* system, and *kinin* system.

1) Complement

The complement system, also called the *complement cascade*, is a part of the immune system. It enhances the ability of antibodies and phagocytic cells to attack the pathogen's cell membrane, clear microbes and damaged cells, and promote inflammation.

The complement system contains several plasma proteins that work together to "complement" the action of antibodies in destroying bacteria.

Complement proteins circulate in the blood in an inactive form (enzymes or enzyme precursors), which are activated during inflammatory reactions. Once activated, it increases pathogens' removal via opsonisation and phagocytosis.

The main functions of the complement system include (1) opsonisation, (2) chemotaxis, (3) cell lysis, and (4) agglutination.

Opsonisation is the process by which opsonins (e.g. antibody molecules) bind to the surface of the antigen so that the antigen will be readily identified and engulfed by phagocytes for destruction.

Chemotaxis attracts macrophages and neutrophils through inflammatory mediators.

Cell lysis ruptures membranes due to the formation of a membrane attack complex (MAC), and **agglutination** causes the clustering and binding of pathogens.

Activated complement proteins can also increase vascular permeability, make mast cells release histamine, and work for neutrophils as chemotactic elements.

2) Coagulation factors (clotting cascade)

A group of ACTIVE proteins in the blood that convert soluble fibrinogen into fibrin, forming a protective protein mesh over injury sites. Coagulation factor XII (Hageman factor: a protein synthesized by the liver). It is activated by activated platelets at the site of inflammation. It induces clotting and generation of group of circulating inflammatory mediators of through activating coagulation, kinin, and fibrinolytic systems.

3) The Fibrinolytic system

This system opposes the coagulation system, counterbalances clotting and generates several other inflammatory mediators. Lysis of fibrin by plasmin may have a local effect on vascular permeability.

4) Kinin

A group of blood proteins that play a role in inflammation, blood pressure control, coagulation, and pain. Bradykinin and kallidin, produced after Kinin activation, are essential mediators in inflammation and are vasodilators.

Bradykinin is the most important mediator of pain, increased vascular permeability, vasodilation, bronchial smooth muscle contraction in acute inflammation. It's are short-lived because it is rapidly degraded by kininases present in plasma and tissues.

Kallikrein, an intermediate in the kinin cascade with chemotactic activity, and potent activator of factor XII. It represents a link between the kinin and clotting systems.

Systemic Manifestations of Inflammation

The inflammatory response is usually localized. Sometimes, the response may cause significant systemic manifestations induced by inflammatory mediators. The most important systemic manifestations of inflammation are:

1. **Acute phase response:**

During which the main manifestations include: *Fever*, *Lethargy*, Changes in the *plasma proteins concentrations*, raised *erythrocyte sedimentation rate* (ESR).

Fever is produced by pyrogens that reset the hypothalamic thermoregulatory center. Pyrogens can be ***endogenous*** (cytokine: like IL-1), which is found naturally in the body, or ***exogenous*** such as endotoxins from gram-negative bacteria or pyrogenic prions.

2. **Alterations in white blood cell count:**

Leukocytosis: raised leukocyte counts or *leucopenia*: reduced leukocyte count

3. **Lymphadenitis:**

Acute and chronic inflammation may cause localized or systemic enlargement of lymph nodes that drain the affected area, which may be tender & palpable.

4. **Sepsis and septic shock:**

Also known as the *systemic inflammatory response*. It represents a severe systemic inflammatory reaction to the presence of **toxins** (Toxemia) in the blood. Toxemia cause induction of histamine and thereby generalized vasodilation that leads to septic shock.

Chronic inflammation may be caused by certain infections such as hepatitis B and C viruses, Helicobacter pylori (HP) infection, schistosomiasis, etc.).

Certain chronic inflammations may be a major cause of cancer because it releases powerful oxidants which cause excessive cell division and/or mutagens.