Engulfment

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

Killing and Degradation

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

Release of leukocyte products and leukocyte-induced Tissue Injury

During activation and phagocytosis, leukocytes release microbicidal and other products not only within the phagolysosome but also into the extracellular space. The most important of these substances are

lysosomal enzymes, reactive oxygen radicals, and products of AA metabolism (including prostaglandins and leukotrienes). These products are capable of causing injuries of the host endothelium and tissues, and may thus amplify the effects of the initial injurious agent. Products of monocytes/macrophages and other leukocyte types have additional potentially harmful products (see chronic inflammation). Thus, if persistent and unchecked, the leukocyte infiltrate itself becomes harmful. Leukocyte-dependent tissue injury underlies many acute and chronic human diseases as listed in the following table

Clinical Examples of Leukocyte-Induced Injury

Acute	Chronic
Acute respiratory distress syndrome	Arthritis
Acute transplant rejection	Asthma
Asthma	Atherosclerosis
Glomerulonephritis	Chronic lung disease
Reperfusion injury	Chronic rejection
Septic shock	
Vasculitis	

Defects in Leukocyte Function

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

Contribution of tissue cells to the inflammatory process:-

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

secrete most of the cytokines important in acute inflammation. These cells are stationed in tissues to rapidly recognize potentially injurious stimuli and initiate the host defense reaction.

CHEMICAL MEDIATORS OF INFLAMMATION

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

1. Plasma-derived (e.g. complements & kinins): these are present in plasma in precursor forms and need to be activated to function.

2. Cell-derived: either

a. ready-made within intracellular granules (e.g., histamine in mast cell granules) or

b. synthesized when needed (e.g., prostaglandins, cytokines) in response to a stimulus.

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

The more important mediators of acute inflammation are:-

1. Vasoactive amines.

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

a. Histamine

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

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

2. Plasma proteins

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

a. The complement System is composed of specific proteins found in greatest concentration in plasma. In the process of complement activation, a number of complement components are elaborated to mediate a variety of phenomena in acute inflammation:

i. Vascular phenomena: C3a, C5a stimulate histamine release from mast cells and thereby increase vascular permeability and cause vasodilation.
ii. Chemoattractants: for e.g. C5a is a powerful chemotactic agent for neutrophils, monocytes, eosinophils, and basophils.
iii. Opsonins: when fixed to the bacterial cell wall, C3b acts as an opsonin and favor phagocytosis by neutrophils and macrophages.

b. The kinin System
Initial activation of the kinin system is through the action of XIIa on prekallikrein that lead to the formation of kallikrein. This occurs following the exposure of blood plasma to vascular basement membrane collagen after injury to endothelial cells. Kallikrein has a chemotactic activity, and also directly converts C5 to the chemoattractant C5a. One of the important kinins is the vasoactive bradykinin, which has actions similar to those of histamine.
c. Clotting System
Activation of the clotting system results in the formation of thrombin. Thrombin generates insoluble fibrin clot. It also binds to specific receptors expressed on platelets, endothelial and smooth muscle cells, triggering recruitment of leukocytes. Factor XIIa has two opposing actions; induces clotting and activating the fibrinolytic system through generation of plasmin, which is important in lysing fibrin clots. Such degradation, leads to the formation fibrin degradation (split) products (FDP), which may increase vascular permeability. It is evident from the preceding that coagulation and inflammation are tightly linked. Acute inflammation, by activating or damaging the endothelium, can trigger coagulation and induce thrombus formation. Conversely, the coagulation cascade induces inflammation, primarily via the actions of thrombin.

3. PHOSPHOLIPIDS-DERIVED MEDIATORS

A. Arachidonic acid metabolites: prostaglandins, leukotriens, & lipoxins.
On cell activation, arachidonic acid (AA), which is a fatty acid, is released from membrane phospholipids through the action of cellular phospholipase A_2 (activated by C5a). AA metabolites are synthesized by two major classes of enzymes:
1. Cyclooxygenases (COX) leading to the generation of prostaglandins (PGs) including thromboxane (TxA2)**.**
2. Lipoxygenases that generate leukotrienes and lipoxins.
AA metabolites bind to specific receptors on many cell types and can mediate virtually every step of inflammation. Suppressors of cyclooxygenase activity (aspirin, nonsteroidal anti-inflammatory drugs, and COX-2 inhibitors [coxib]) reduce inflammation in vivo.

Several PGs are important in inflammation including PGI2 (prostacyclin), and thromboxane (TxA2). Platelets contain the enzyme thromboxane synthetase, and hence TxA2 is the major product in these cells. TxA2 is a potent platelet-aggregating agent and a vasoconstrictor. Vascular endothelium (unlike platelets) lacks thromboxane synthetase but possesses prostacyclin synthetase, which leads to the formation of prostacyclin. Prostacyclin, has actions opposing that of TxA2 in that it is a vasodilator, a potent inhibitor of platelet aggregation. The prostaglandins are also involved in the pathogenesis of pain and fever in inflammation. PGD2 is the major metabolite of the cyclooxygenase (COX) pathway in mast cells; along with PGE2, it causes vasodilation and increases the permeability of postcapillary venules, thus potentiating edema formation.
In the lipoxygenase pathway, the main products are a family of compounds collectively called leukotrienes. LTB4 is a potent chemotactic agent and activator of neutrophils.
Lipoxins are a recent addition to the family of bioactive products generated from AA. Leukocytes, particularly neutrophils, produce lipoxins through their interaction with platelets. The principal actions of lipoxins are to inhibit neutrophil chemotaxis and adhesion to endothelium.

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

4. CYTOKINES AND CHEMOKINES

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

b. Increase the surface thrombogenicity of the endothelium.
c. Induce the systemic acute-phase responses associated with infection or injury (e.g. fever, loss of appetite, release of neutrophils into the circulation, the release of corticosteroids).
Chemokines are a family of small proteins that act primarily as chemoattractants for specific types of leukocytes, for e.g. IL-8 acts primarily on neutrophils. It is secreted by activated macrophages, endothelial cells, and other cell types and causes activation and chemotaxis of neutrophils, with limited activity on monocytes and eosinophils. Its most important inducers are microbial products and other cytokines, mainly IL-1 and TNF.

5. NITRIC OXIDE (NO)

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

6. LYSOSOMAL CONSTITUENTS OF LEUKOCYTES

Neutrophils and monocytes/mactophages contain lysosomal granules, which when released may contribute to the inflammatory response. Neutrophils have two main types of granules:-
1. The smaller specific (or secondary) granules that contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, etc.
2. The large azurophil (or primary) granules that contain myeloperoxidase, bactericidal factors (lysozyme, defensins), acid hydrolases, and neutral proteases (e.g. collagenases, proteinase 3).
Both types of granules can empty into phagocytic vacuoles that form around engulfed material, or the granule contents can be released into the extracellular space. Different granule enzymes serve different functions. **Acid proteases** degrade bacteria and debris within the phagolysosomes, in which a low (acid) pH is readily reached. **Neutral proteases** are capable of degrading various extracellular components. These enzymes can attack collagen, basement membrane, fibrin, elastin, and cartilage, resulting in the tissue destruction that accompanies inflammatory processes. **Neutrophil elastase** has been shown to degrade virulence factors of bacteria and thus combat bacterial infections.
Monocytes and macrophages also contain acid hydrolases,

collagenase, elastase, phospholipase, and plasminogen activator. These may be particularly active in chronic inflammatory reactions. Because of the destructive effects of lysosomal enzymes, the initial leukocytic infiltration, if unchecked, can potentiate further increases in vascular permeability and tissue damage. These harmful proteases, however, are held in check by a system of antiproteases in the serum and tissue fluids. Foremost among these is **α_1-antitrypsin**, which is the major inhibitor of neutrophil elastase. A deficiency of these inhibitors may lead to sustained action of leukocyte proteases (progressive tissue damage), as is the case in patients with α_1-antitrypsin deficiency.

7. OXYGEN-DERIVED FREE RADICALS

Oxygen-derived free radicals may be released extracellularly from leukocytes after exposure to microbes, chemokines, and immune complexes. Superoxide anion (O^-_2) hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) are the major species produced within the cell. Extracellular release of low levels of these potent mediators can amplify the inflammatory response. The physiologic function of these reactive oxygen intermediates is to destroy phagocytosed microbes. At higher levels, release of these potent mediators can damage the tissues. Serum, tissue fluids, and host cells possess antioxidant mechanisms that protect against these potentially harmful oxygen-derived radicals. The influence of oxygen-derived free radicals in any given inflammatory reaction depends on the balance between the production and the inactivation of these metabolites by cells and tissues.

8. NEUROPEPTIDES

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

9. OTHER MEDIATORS

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

a. Response to hypoxia

It is known that hypoxia causes cell injury and necrosis. However, it is also an inducer of the inflammatory response. The latter is mediated by a protein called **hypoxia-induced factor$_1\alpha$**, which is produced by cells deprived of oxygen and activates many genes involved in inflammation; one of these leads to the production of

vascular endothelium growth factor (VEGF), which increases vascular permeability.

b. Response to necrotic cells.

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