# Inflammation, Inflammatory Disorders, and Wound Healing

1

## INTRODUCTION

### I. INFLAMMATION

A. Allows inflammatory cells, plasma proteins (e.g., complement), and fluid to exit blood vessels and enter the interstitial space
B. Divided into acute and chronic inflammation

## ACUTE INFLAMMATION

### I. BASIC PRINCIPLES

A. Characterized by the presence of edema and neutrophils in tissue (Fig. 2.1A)
B. Arises in response to infection (to eliminate pathogen) or tissue necrosis (to clear necrotic debris)
C. Immediate response with limited specificity (innate immunity)

### II. MEDIATORS OF ACUTE INFLAMMATION

A. Toll-like receptors (TLRs)
   1. Present on cells of the innate immune system (e.g., macrophages and dendritic cells)
   2. Activated by pathogen-associated molecular patterns (PAMPs) that are commonly shared by microbes
      i. CD14 (a co-receptor for TLR4) on macrophages recognizes lipopolysaccharide (a PAMP) on the outer membrane of gram-negative bacteria.
   3. TLR activation results in upregulation of NF-κB, a nuclear transcription factor that activates immune response genes leading to production of multiple immune mediators.
   4. TLRs are also present on cells of adaptive immunity (e.g., lymphocytes) and, hence, play an important role in mediating chronic inflammation.
B. Arachidonic acid (AA) metabolites
   1. AA is released from the phospholipid cell membrane by phospholipase $A_2$ and then acted upon by cyclooxygenase or 5-lipoxygenase.
      i. Cyclooxygenase produces prostaglandins (PG).
         a. $PGI_2$, $PGD_2$, and $PGE_2$ mediate vasodilation and increased vascular permeability.
         b. $PGE_2$ also mediates pain and fever.
      ii. 5-lipoxygenase produces leukotrienes (LT).
         a. $LTB_4$ attracts and activates neutrophils.
         b. $LTC_4$, $LTD_4$, and $LTE_4$ (slow reacting substances of anaphylaxis) mediate vasoconstriction, bronchospasm, and increased vascular permeability.
C. Mast cells
   1. Widely distributed throughout connective tissue
   2. Activated by (1) tissue trauma, (2) complement proteins C3a and C5a, or (3) cross-linking of cell-surface IgE by antigen

i. Immediate response involves release of preformed histamine granules, which mediate vasodilation of arterioles and increased vascular permeability.
ii. Delayed response involves production of arachidonic acid metabolites, particularly leukotrienes.

D. Complement
1. Proinflammatory serum proteins that "complement" inflammation
2. Circulate as inactive precursors; activation occurs via
i. Classical pathway - C1 binds IgG or IgM that is bound to antigen.
ii. Alternative pathway - Microbial products directly activate complement.
iii. Mannose-binding lectin (MBL) pathway - MBL binds to mannose on microorganisms and activates complement.
3. All pathways result in production of C3 convertase (mediates C3 →C3a and C3b), which, in turn, produces C5 convertase (mediates C5 → C5a and C5b). C5b complexes with C6-C9 to form the membrane attack complex (MAC).
i. C3a and C5a (anaphylatoxins) - trigger mast cell degranulation, resulting in histamine-mediated vasodilation and increased vascular permeability
ii. C5a - chemotactic for neutrophils
iii. C3b - opsonin for phagocytosis
iv. MAC - lyses microbes by creating a hole in the cell membrane

E. Hageman factor (Factor XII)
1. Inactive proinflammatory protein produced in liver
2. Activated upon exposure to subendothelial or tissue collagen; in turn, activates
i. Coagulation and fibrinolytic systems
ii. Complement
iii. Kinin system - Kinin cleaves high-molecular-weight kininogen (HMWK) to bradykinin, which mediates vasodilation and increased vascular permeability (similar to histamine), as well as pain.

## III. CARDINAL SIGNS OF INFLAMMATION

A. Redness (rubor) and warmth (calor)
1. Due to vasodilation, which results in increased blood flow
2. Occurs via relaxation of arteriolar smooth muscle; key mediators are histamine, prostaglandins, and bradykinin.

B. Swelling (tumor)
1. Due to leakage of fluid from postcapillary venules into the interstitial space (exudate)
2. Key mediators are (1) histamine, which causes endothelial cell contraction and (2) tissue damage, resulting in endothelial cell disruption.

C. Pain (dolor)
1. Bradykinin and $PGE_2$ sensitize sensory nerve endings.

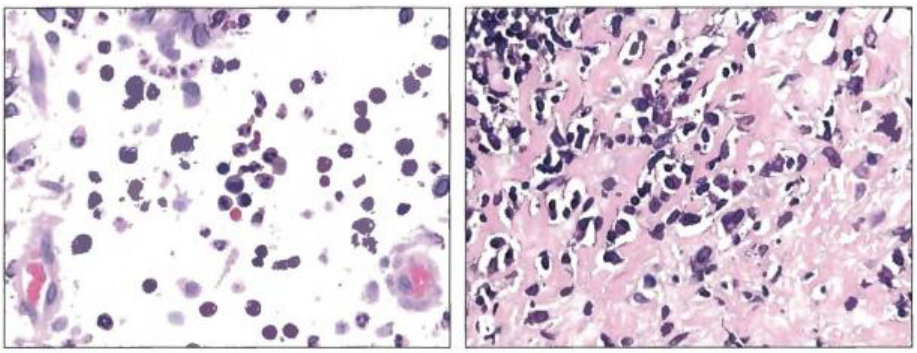


**Fig. 2.1** Inflammation. **A,** Acute inflammation with neutrophils. **B,** Chronic inflammation with lymphocytes and plasma cells.

D. Fever
1. Pyrogens (e.g., LPS from bacteria) cause macrophages to release IL-1 and TNF, which increase cyclooxygenase activity in perivascular cells of the hypothalamus.
2. Increased $PGE_2$ raises temperature set point.

## IV. NEUTROPHIL ARRIVAL AND FUNCTION

A. Step 1 - Margination
1. Vasodilation slows blood flow in postcapillary venules.
2. Cells marginate from center of flow to the periphery.

B. Step 2 - Rolling
1. Selectin "speed bumps" are upregulated on endothelial cells.
   i. P-selectin release from Weibel-Palade bodies is mediated by histamine.
   ii. E-selectin is induced by TNF and IL-1.
2. Selectins bind sialyl Lewis X on leukocytes.
3. Interaction results in rolling of leukocytes along vessel wall.

C. Step 3 - Adhesion
1. Cellular adhesion molecules (ICAM and VCAM) are upregulated on endothelium by TNF and IL-1.
2. Integrins are upregulated on leukocytes by C5a and $LTB_4$.
3. Interaction between CAMs and integrins results in firm adhesion of leukocytes to the vessel wall.
4. Leukocyte adhesion deficiency is most commonly due to an autosomal recessive defect of integrins (CD18 subunit).
   i. Clinical features include delayed separation of the umbilical cord, increased circulating neutrophils (due to impaired adhesion of marginated pool of leukocytes), and recurrent bacterial infections that lack pus formation.

D. Step 4 - Transmigration and Chemotaxis
1. Leukocytes transmigrate across the endothelium of postcapillary venules and move toward chemical attractants (chemotaxis).
2. Neutrophils are attracted by bacterial products, IL-8, C5a, and $LTB_4$.

E. Step 5 - Phagocytosis
1. Consumption of pathogens or necrotic tissue; phagocytosis is enhanced by opsonins (IgG and C3b).
2. Pseudopods extend from leukocytes to form phagosomes, which are internalized and merge with lysosomes to produce phagolysosomes.
3. Chediak-Higashi syndrome is a protein trafficking defect (autosomal recessive) characterized by impaired phagolysosome formation. Clinical features include
   i. Increased risk of pyogenic infections
   ii. Neutropenia (due to intramedullary death of neutrophils)
   iii. Giant granules in leukocytes (due to fusion of granules arising from the Golgi apparatus)
   iv. Defective primary hemostasis (due to abnormal dense granules in platelets)
   v. Albinism
   vi. Peripheral neuropathy

F. Step 6 - Destruction of phagocytosed material
1. $O_2$-dependent killing is the most effective mechanism.
2. HOCl generated by oxidative burst in phagolysosomes destroys phagocytosed microbes.
   i. $O_2$ is converted to $O_2^{\cdot-}$ by NADPH oxidase (oxidative burst).
   ii. $O_2^{\cdot-}$ is converted to $H_2O_2$ by superoxide dismutase (SOD).
   iii. $H_2O_2$ is converted to HOCl (bleach) by myeloperoxidase (MPO).

3. Chronic granulomatous disease (CGD) is characterized by poor $O_2$-dependent killing.
   i. Due to NADPH oxidase defect (X-linked or autosomal recessive)
   ii. Leads to recurrent infection and granuloma formation with catalase-positive organisms, particularly ***Staphylococcus aureus, Pseudomonas cepacia, Serratia marcescens, Nocardia,*** and ***Aspergillus***
   iii. Nitroblue tetrazolium test is used to screen for CGD. Leukocytes are incubated with NBT dye, which turns blue if NADPH oxidase can convert $O_2$ to $O_2^-$, but remains colorless if NADPH oxidase is defective.
4. MPO deficiency results in defective conversion of $H_2O_2$ to HOCl.
   i. Increased risk for Candida infections; however, most patients are asymptomatic.
   ii. NBT is normal; respiratory burst ($O_2$ to $H_2O_2$) is intact.
5. $O_2$-independent killing is less effective than $O_2$-dependent killing and occurs via enzymes present in leukocyte secondary granules (e.g., lysozyme in macrophages and major basic protein in eosinophils).

G. Step 7 - Resolution
   1. Neutrophils undergo apoptosis and disappear within 24 hours after resolution of the inflammatory stimulus.

## V. MACROPHAGES

A. Macrophages predominate after neutrophils and peak 2-3 days after inflammation begins.
   1. Derived from monocytes in blood
B. Arrive in tissue via the margination, rolling, adhesion, and transmigration sequence
C. Ingest organisms via phagocytosis (augmented by opsonins) and destroy phagocytosed material using enzymes (e.g., lysozyme) in secondary granules ($O_2$-independent killing)
D. Manage the next step of the inflammatory process. Outcomes include
   1. Resolution and healing - Anti-inflammatory cytokines (e.g., IL-10 and TGF-β) are produced by macrophages.
   2. Continued acute inflammation - marked by persistent pus formation; IL-8 from macrophages recruits additional neutrophils.
   3. Abscess - acute inflammation surrounded by fibrosis; macrophages mediate fibrosis via fibrogenic growth factors and cytokines.
   4. Chronic inflammation - Macrophages present antigen to activate $CD4^+$ helper T cells, which secrete cytokines that promote chronic inflammation.

# CHRONIC INFLAMMATION

## I. BASIC PRINCIPLES

A. Characterized by the presence of lymphocytes and plasma cells in tissue (Fig. 2.1B)
B. Delayed response, but more specific (adaptive immunity) than acute inflammation
C. Stimuli include (1) persistent infection (most common cause); (2) infection with viruses, mycobacteria, parasites, and fungi; (3) autoimmune disease; (4) foreign material; and (5) some cancers.

## II. T LYMPHOCYTES

A. Produced in bone marrow as progenitor T cells
B. Further develop in the thymus where the T-cell receptor (TCR) undergoes rearrangement and progenitor cells become $CD4^+$ helper T cells or $CD8^+$ cytotoxic T cells
   1. T cells use TCR complex (TCR and CD3) for antigen surveillance.

2. TCR complex recognizes antigen presented on MHC molecules.
   i. $CD4^+$ T cells - MHC class II
   ii. $CD8^+$ T cells - MHC class I
3. Activation of T cells requires (1) binding of antigen/MHC complex and (2) an additional 2nd signal.

C. $CD4^+$ helper T-cell activation
1. Extracellular antigen (e.g., foreign protein) is phagocytosed, processed, and presented on MHC class II, which is expressed by antigen presenting cells (APCs).
2. B7 on APC binds CD28 on $CD4^+$ helper T cells providing 2nd activation signal.
3. Activated $CD4^+$ helper T cells secrete cytokines that "help" inflammation and are divided into two subsets.
   i. $T_H1$ subset secretes IFN-γ (activates macrophage, promotes B-cell class switching from IgM to IgG, promotes $T_H1$ phenotype and inhibits $T_H2$ phenotype).
   ii. $T_H2$ subset secretes IL-4 (facilitates B-cell class switching to IgE), IL-5 (eosinophil chemotaxis and activation, and class switching to IgA), and IL-13 (function similar to IL-4).

D. $CD8^+$ cytotoxic T-cell activation
1. Intracellular antigen (derived from proteins in the cytoplasm) is processed and presented on MHC class I, which is expressed by all nucleated cells and platelets.
2. IL-2 from $CD4^+$ $T_H1$ cell provides 2nd activation signal.
3. Cytotoxic T cells are activated for killing.
4. Killing occurs via
   i. Secretion of perforin and granzyme; perforin creates pores that allow granzyme to enter the target cell, activating apoptosis.
   ii. Expression of FasL, which binds Fas on target cells, activating apoptosis

## III. B LYMPHOCYTES

A. Immature B cells are produced in the bone marrow and undergo immunoglobulin rearrangements to become naïve B cells that express surface IgM and IgD.

B. B-cell activation occurs via
1. Antigen binding by surface IgM or IgD; results in maturation to IgM- or IgD-secreting plasma cells
2. B-cell antigen presentation to $CD4^+$ helper T cells via MHC class IL
   i. CD40 receptor on B cell binds CD40L on helper T cell, providing 2nd activation signal.
   ii. Helper T cell then secretes IL-4 and IL-5 (mediate B-cell isotype switching, hypermutation, and maturation to plasma cells).

## IV. GRANULOMATOUS INFLAMMATION

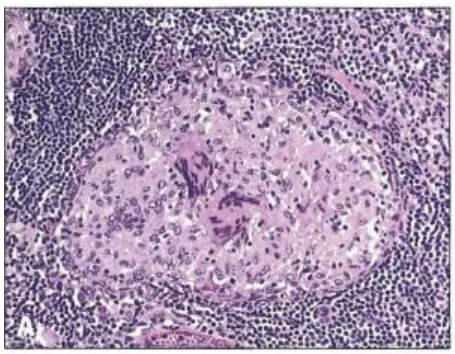
A. Subtype of chronic inflammation

B. Characterized by granuloma, which is a collection of epithelioid histiocytes (macrophages with abundant pink cytoplasm), usually surrounded by giant cells and a rim of lymphocytes

C. Divided into noncaseating and caseating subtypes
1. Noncaseating granulomas lack central necrosis (Fig. 2.2A). Common etiologies include reaction to foreign material, sarcoidosis, beryllium exposure, Crohn disease, and cat scratch disease.
2. Caseating granulomas exhibit central necrosis and are characteristic of tuberculosis and fungal infections (Fig. 2.2B).

D. Steps involved in granuloma formation

1. Macrophages process and present antigen via MHC class II to $CD4^+$ helper T cells.
2. Interaction leads macrophages to secrete IL-12, inducing $CD4^+$ helper T cells to differentiate into $T_H1$ subtype.
3. $T_H1$ cells secrete IFN-γ, which converts macrophages to epithelioid histiocytes and giant cells.



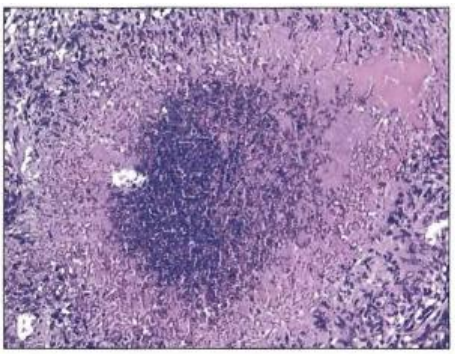




**Fig.** 2.2 Granuloma. **A,** Noncaseating. **B,** Caseating.

**Fig.** 2.3 Angioedema. (Courtesy of James Heilman, MD, Wikipedia)

## WOUND HEALING

### I. BASIC PRINCIPLES

A. Healing is initiated when inflammation begins.
B. Occurs via a combination of regeneration and repair

### II. REGENERATION

A. Replacement of damaged tissue with native tissue; dependent on regenerative capacity of tissue
B. Tissues are divided into three types based on regenerative capacity: labile, stable, and permanent.
C. Labile tissues possess stem cells that continuously cycle to regenerate the tissue.
   1. Small and large bowel (stem cells in mucosal crypts, Fig. 2.5)
   2. Skin (stem cells in basal layer, Fig. 2.6)
   3. Bone marrow (hematopoietic stem cells)
D. Stable tissues are comprised of cells that are quiescent ($G_0$), but can reenter the cell cycle to regenerate tissue when necessary.
   1. Classic example is regeneration of liver by compensatory hyperplasia after partial resection. Each hepatocyte produces additional cells and then reenters quiescence.

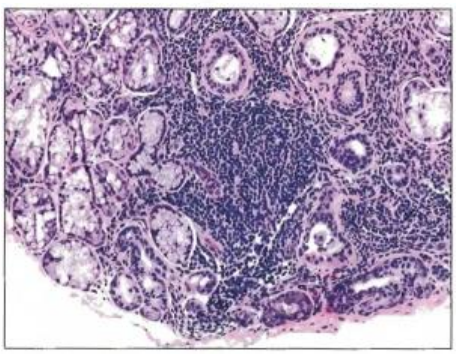


**Fig. 2.4C** Lymphocytic sialadenitis, Sjögren syndrome.

**Fig. 2.4D** Sclerodactyly, scleroderma.

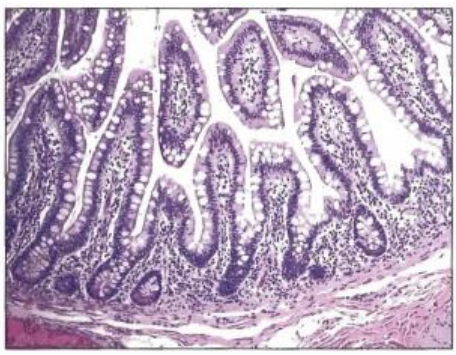


**Fig. 2.5** Intestinal crypts.

E. Permanent tissues lack significant regenerative potential (e.g., myocardium, skeletal muscle, and neurons).

## III. REPAIR

A. Replacement of damaged tissue with fibrous scar
B. Occurs when regenerative stem cells are lost (e.g., deep skin cut) or when a tissue lacks regenerative capacity (e.g., healing after a myocardial infarction, Fig. 2.7)
C. Granulation tissue formation is the initial phase of repair (Fig. 2.8).
   1. Consists of fibroblasts (deposit type III collagen), capillaries (provide nutrients), and myofibroblasts (contract wound)
D. Eventually results in scar formation, in which type III collagen is replaced with type I collagen
   1. Type III collagen is pliable and present in granulation tissue, embryonic tissue, uterus, and keloids.
   2. Type I collagen has high tensile strength and is present in skin, bone, tendons, and most organs.
   3. Collagenase removes type III collagen and requires zinc as a cofactor.

## IV. MECHANISMS OF TISSUE REGENERATION AND REPAIR

A. Mediated by paracrine signaling via growth factors (e.g., macrophages secrete growth factors that target fibroblasts)
B. Interaction of growth factors with receptors (e.g., epidermal growth factor with growth factor receptor) results in gene expression and cellular growth.
C. Examples of mediators include
   1. TGF-α - epithelial and fibroblast growth factor
   2. TGF-β - important fibroblast growth factor; also inhibits inflammation
   3. Platelet-derived growth factor - growth factor for endothelium, smooth muscle, and fibroblasts
   4. Fibroblast growth factor - important for angiogenesis; also mediates skeletal development
   5. Vascular endothelial growth factor (VEGF) - important for angiogenesis

## V. NORMAL AND ABERRANT WOUND HEALING

A. Cutaneous healing occurs via primary or secondary intention.
   1. Primary intention-Wound edges are brought together (e.g., suturing of a surgical incision); leads to minimal scar formation
   2. Secondary intention-Edges are not approximated. Granulation tissue fills the defect; myofibroblasts then contract the wound, forming a scar.
B. Delayed wound healing occurs in
   1. Infection (most common cause; ***S aureus*** is the most common offender)

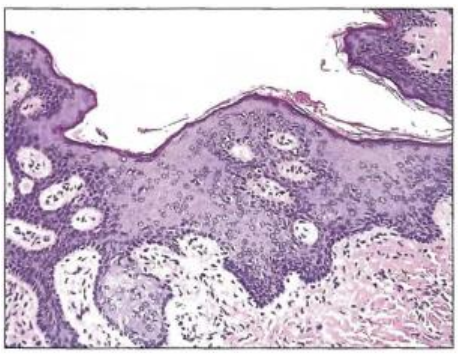


**Fig. 2.6** Basal layer of skin.

**Fig. 2.7** Myocardial scarring. (Courtesy of Aliya Husain.MD)

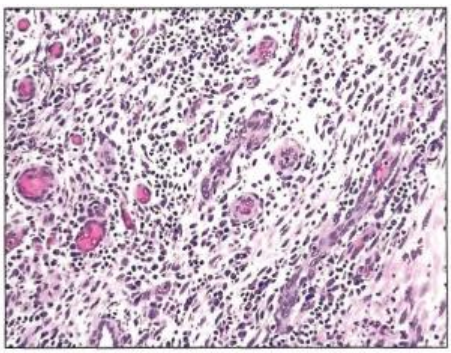


**Fig. 2.8** Granulation tissue.

2. Vitamin C, copper, or zinc deficiency
    i. Vitamin C is an important cofactor in the hydroxylation of proline and lysine procollagen residues; hydroxylation is necessary for eventual collagen cross-linking.
    ii. Copper is a cofactor for lysyl oxidase, which cross-links lysine and hydroxylysine to form stable collagen.
    iii. Zinc is a cofactor for collagenase, which replaces the type III collagen of granulation tissue with stronger type I collagen.
3. Other causes include foreign body, ischemia, diabetes, and malnutrition.

C. Dehiscence is rupture of a wound; most commonly seen after abdominal surgery

D. Hypertrophic scar is excess production of scar tissue that is localized to the wound (Fig. 2.9).

E. Keloid is excess production of scar tissue that is out of proportion to the wound (Fig. 2.10).
1. Characterized by excess type III collagen
2. Genetic predisposition (more common in African Americans)
3. Classically affects earlobes, face, and upper extremities

**Fig. 2.9** Hypertrophic scar. (Reprinted with permission, http://emedicine.medscape.com/article/1128404-overview)

**Fig. 2.10** Keloid.