2 INFLAMMATION

LEARNING OBJECTIVES OF CHAPTER

After completing this Chapter, reader should be able to:

- To understand the concept of inflammation.
- To study clinical signs of inflammation.
- To classify inflammation.
- To study chemical and cellular mediators of inflammation.
- To study process and mechanism of inflammation.
- To understand healing of skin wounds.

2.1 INTRODUCTION

Inflammation is a defensive response to the injury or infection, which induces physiological changes to control tissue damage and to remove the pathogenic infections. Diseases caused by inflammation are an important factor of morbidity and mortality in humans. It is a fundamental pathologic process consisting of cytologic and chemical reactions that occur in the affected blood vessels and adjacent tissues, in response to an injury caused by a physical, chemical or biological agents resulting in:

(i) The local reactions and resulting morphologic changes.

(ii) The destruction or removal of the injurious material.

(iii) The responses that lead to repair and healing.

The inflammatory response (Paul & Diana 1988) has many mediators, these include circulating cells and plasma proteins, vascular cells and extracellular matrix of the surrounding connective tissue. The circulating cells include bone marrow-derived polymorph nuclear leukocytes (neutrophils), eosinophils and basophils, lymphocytes and monocytes, and platelets, the circulating proteins include clotting factors, kininogens, and complement components, largely synthesized by the liver. The vascular wall cells include the endothelial cells in direct contact with the blood. The connective tissue cells include sentinels to invasion such as mast cells, macrophages and lymphocytes. The extra cellular matrix (ECM) consists of fibrous structural proteins (e.g., collagen and elastin).

2.2 CLINICAL SIGNS OF INFLAMMATION

The four principal effects of inflammation (rubor, tumor, caloret dolor) were described nearly 2,000 years ago by the Roman **Aulus Cornelius Celsus**, more commonly known as Celsus.

Redness (rubor)

The affected tissue appears red, due to dilation of small blood vessels within the damaged area (hyperemia).

Swelling (tumor)

Swelling occurs due to accumulation of fluid in the extravascular space (edema) as part of the inflammatory fluid exudate. It may contain cellular part of inflammatory cells.

Heat (calor)

The inflamed area of the skin appears warmer than healthy area. It is due to increased blood flow (hyperemia) through the region, resulting in vascular dilation and the delivery of warm blood to the area.

Pain (dolor)

Pain results from the distortion of tissues due to inflammation chemical mediators like bradykinin and some prostaglandins.

Loss of function (functiolaesa)

Loss of function was added by Virchow (1821-1902) to the list of features described by Celsus. Movement of an inflamed area is inhibited due to pain, either consciously or by reflexes, while severe swelling may physically immobilize the affected area.

2.3 CLASSIFICATION OF INFLAMMATION

The inflammation can be classified on the basis of duration as peracute, acute, subacute and chronic inflammation.

2.3.1 PERACUTE INFLAMMATION

It is very acute (short duration and intense reactions) form of inflammation. It occurs due to potent stimulus. It is less common form than acute inflammation. The duration of inflammation varies between 0 – 4 hours. The signs may include hyperaemia, slight edema, haemorrhage etc. at the site of inflammation other inflammatory cells may not be found but few leucocytes may be present. This may result in Shock and sudden death.

2.3.2 ACUTE INFLAMMATION

It occurs for short duration and inflammatory response is severe. It begins within 4-6 hours can last for 3-5 days. The vascular changes include active hyperaemia, edema, Occasional

fibrin thrombi within vessels. This shows all common clinical signs of inflammation. Neutrophils, lymphocytes and other plasma cells may found at affected area. Lymphatic vessel moves away the exudate. The transportation of the exudate can lead to acute regional lymphadenitis.

This process shows two major components.

(1) Vascular changes

Alterations in vessel caliber resulting in increased blood flow (vasodilatation) and structural changes that permits plasma proteins to leave the circulation (increased vascular permeability).

(2) Cellular events

Emigration of the leukocytes from the microcirculation and accumulation in the focus of injury (cellular recruitment and activation); these events includes

(a) Migration and rolling of leukocytes.

(b) Adhesion and transmigration between endothelial cells.

(c) Migration in interstitial tissues toward a chemotactic stimulus.

2.3.3 SUBACUTE INFLAMMATION

It shows duration between acute and chronic inflammation.Evidence of hyperaemia and edema is regressing but evidence of repair such as fibroplasia and angiogenesis is lacking. Duration varies from a few days to a few weeks. There is a decline in the magnitude of vascular changes, compared to acute inflammation (less haemorrhage, hyperaemia and edema). Characterised by a "mixed" or "pleocellular" inflammatory infiltrate. This means that the inflammatory cell typestill may be primarily neutrophilic but usually it is also associated with an infiltration by lymphocytes, macrophages and plasma cells. Fibrosis and neovascularization is not a feature of subacute inflammation.

2.3.4 CHRONIC INFLAMMATION

These type of inflammation of prolonged duration (weeks to months to years) and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and scarring chronic inflammation is characterized by the:

(a) Infiltration with mononuclear (chronic inflammatory) cells including macrophages, lymphocytes, and plasma cells.

(b) Tissue destruction largely directed by the inflammatory cells.

(c) Repair involving new vessel proliferation (angiogenesis) and fibrosis.

2.4 CELLULAR MEDIATORS (INFLAMMATORY CELLS)

2.4.1 MACROPHAGES

Macrophages constituting the critical mainstay and heart of chronic inflammation, and are tissue cells that derive from circulating blood monocytes after their emigration from the bloodstream. Their product include acid and neutral proteases, complement components and coagulation factors, reactive oxygen species and nitric oxide (NO), eicosanoids (Arachidonic Acid metabolites) and cytokines (IL-1, TNF).

2.4.2 LYMPHOCYTES, PLASMA CELLS, EOSINOPHILS AND MAST CELLS

Both *T* and *B* lymphocytes migrate into inflammatory sites; Lymphocytes are mobilized in the setting of any specific immune stimulus as well as in non-immune mediated inflammation. Plasma cells are the terminally differentiated end product of B-cell activation. Eosinophils are characteristically found in inflammatory sites around parasitic infection or as part of immune reactions mediated by immunoglobulin E (*Ig*E), typically associated with allergies. Mast cells are sentinel cells widely distributed in connective tissues throughout the body and can participate in both acute and chronic inflammatory responses.

2.5 CHEMICAL MEDIATORS OF INFLAMMATION

Mediators may be circulating in the plasma (typically synthesized by the liver), or they may be produced locally by cells at the site of inflammation. Plasma derived mediators (complement, kinins, coagulation factors) circulate as inactive precursors that must undergo proteolytic cleavage to acquire their biologic properties. Cell-derived mediators are normally sequestered in intracellular granules that are secreted upon activation (e.g., histamine in mast cells) or are synthesized de novo in response to a stimulus (e.g., prostaglandins).

2.5.1 VASOACTIVE AMINES

a) Histamine

It is stored in platelets, mast cells and basophils. The main actions of histamine are vasodilation, increased vascular permeability, itching and pain.

Histamine is released in response to a variety of stimuli.

- Physical injury such as trauma or heat.
- Immune reactions involving binding of IgE antibodies to Fc receptors onmast cells.
- C3a and C5a fragments of complement, the so called anaphylatoxins.
- Leukocyte derived histamine-releasing proteins.
- Neuropeptides (e.g., substance P).
- Certain cytokines (e.g., IL-1 and IL-8).

b) Serotonin (5-hydroxytryptamine)

Serotonin is vasoactive mediator with effects similar to those of histamine with less potency. It is found primarily within platelet dense body granules and releases during platelet aggregation.

2.5.2 NEUROPEPTIDES

Like the vasoactive amines, neuropeptides can initiate inflammatory responses; these 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 gastrointestinal tract.

2.5.3 PLATELET ACTIVATING FACTOR

It is released from IgE – sensitized basophils or mast cells, other leucocytes, endothelium and platelets. Apart from its action on platelet aggregation and release reaction, the actions of PAF as mediator of inflammation are:

- Increased vascular permeability.
- Vasodilation in low concentration and vasoconstriction at high concentration.
- Bronchoconstriction.
- Adhesion of leucocytes to endothelium.
- Chemotaxis.

2.5.4 CYTOKINES

Cytokines, including interleukins 1–10, tumor necrosis factor α (TNF-α), and interferon γ (INF-γ) are produced predominantly by macrophages and lymphocytes but can be synthesized by other cell types as well. Their role in inflammation is complex. These polypeptides modulate the activity and function of other cells to coordinate and control the inflammatory response. Two of the more important cytokines, interleukin-1 (IL-1) and TNF-α, mobilize and activate leukocytes, enhance proliferation of B and T cells and natural killer cell cytotoxicity, and are involved in the biologic response to endotoxins. IL-1, IL-6, and TNF-α mediate the acute phase response and pyrexia that may accompany infection and can induce systemic clinical signs, including sleep and anorexia. In the acute phase response, interleukins stimulate the liver to synthesize acute-phase proteins, including complement components, coagulation factors, protease inhibitors, and metal-binding proteins. By increasing intracellular Ca^{2+} concentrations in leukocytes, cytokines are also important in the induction of PLA2. Colony-stimulating factors (GM-CSF, G-CSF, and M-CSF) are cytokines that promote expansion of neutrophil, eosinophil, and macrophage colonies in bone marrow. In chronic inflammation, cytokines IL-1, IL-6, and TNF-α contribute to the activation of fibroblasts and osteoblasts and to the release of enzymes such as collagenase

and stromelysin that can cause cartilage and bone resorption. Cytokines also stimulate synovial cells and chondrocytes to release pain-inducing mediators.

2.5.5 NITRIC OXIDE

The role of the free radical gas nitric oxide (NO) in inflammation is well established. NO is an important cell-signaling messenger in a wide range of physiologic and pathophysiologic processes. Small amounts of NO play a role in maintaining resting vascular tone, vasodilation, and antiaggregation of platelets. In response to certain cytokines (TNF-α, IL-1) and other inflammatory mediators, the production of relatively large quantities of NO is stimulated. In larger quantities, NO is a potent vasodilator, facilitates macrophage-induced cytotoxicity, and may contribute to joint destruction in some types of arthritis.

2.5.6 PLASMA DERIVED FACTORS

Many of the effects of inflammation are mediated by three interrelated plasma derived factors like the kinins, the clotting system and complement.

a) Kinin, clotting and fibrinolytic system

The Hageman factor (factor XII) is activated on contact with collagen, basement membrane and platelets to produce prekallikrein. The prekallikrein is converted to kallikrein which will be converted to bradykinin that induces vascular permeability, pain and smooth muscle contraction.

Kallikrein also mediates plasminogen, vascular permeability and vascular dilatation

Activated Hageman factor is also involved in conversion of prothrombin to thrombin which in turn aids in conversion of fibrinogrn to fibrin. The fibrinolytic peptides and split products of fibrin can induces vascular permeability and chemotaxis.

b) Complement system

Complement represented as C consists of 20 proteins in an inactive form in plasma and body fluids. Complement is mainly synthesised by liver. Complement system may be activated in one of the two ways, classic or alternate pathway. But both the pathways converge to produce a membrane attack complex (MAC) which is responsible for lysis of bacterial cell membrane and also results in mediating inflammation. e.g. chemotaxis, histamine release from mast cells (C3a) and procoagulant from platelets. C3b is a major opsonin protein which adheres to bacteria (opsonisation). It is recognised, phagocytosed and destroyed by neutrophils and monocytes.

c) Kinin system

The vasoactive polypeptide (kinins) are derived from kininogen (plasma globulins). The kinins are potent mediator of vasodilatation, pain, increased capillary permeability. The bradykinin induces vascular leakage from post capillary venules. It is 10 times more active than histamine but short lived.

d) Clotting system

Coagulation is seen following damage of endothelium in inflammation through fibrinolytic system the initiated by activated Hageman factor.

Table 2.1: Chemical Mediators Classified by Effect

Effect	Mediator
Vasodilation	Histamine Nitric Oxide Prostaglandins: PGI2, PGE2, PGD2
Increased Vascular Permeability	Histamine Complement: C3a & C5a (anaphylatoxins) Bradykinin Oxygen metabolites (ROS) Leukotrienes: LTC4, LTD4, LTE4 Platelet-activating factor (PAF)
Chemotaxis	Complement: C5a Leukotrienes: LTB4 & LTC4 Chemokines such as TNF, IL-1, IL-8 Bacterial products such as LPS
Fever	IL-1, TNF, IL-6 Prostaglandins
Pain	Bradykinin Substance P Prostaglandin (PGF2)
Tissue Damage	Oxygen metabolites (ROS) Nitric Oxide Lysosomal Enzymes

2.6 PROCESS OF INFLAMMATION

The series of events in the process of inflammation are:

1. Vasodilation: leads to greater blood flow to the area of inflammation, resulting in redness and heat.
2. Vascular permeability: endothelial cells become "leaky" from either direct endothelial cell injury or via chemical mediators.

3. Exudation: fluid, proteins, red blood cells, and white blood cells escape from the intravascular space as a result of increased osmotic pressure extravascularly and increased hydrostatic pressure intravascularly.
4. Vascular stasis: slowing of the blood in the bloodstream with vasodilation and fluid exudation to allow chemical mediators and inflammatory cells to collect and respond to the stimulus.

2.6.1 MECHANISM

The process of inflammation is initiated by immune cells like macrophages, dendritic cells, histiocytes, Kupffer cells and mast cells which are already present in the affected tissue. These cells possess surface receptors known as pattern recognition receptors (PRRs), which recognize (i.e., bind) two subclasses of molecules: pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are compounds that are associated with various pathogens, but which are distinguishable from host molecules. DAMPs are compounds that are associated with host-related injury and cell damage.

After an injury, these receptors recognize PAMP or DAMP, then immune cells undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and increased blood flow causes the redness and increased heat. Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumor). Some of the released mediators such as bradykinin induces the sensitivity to pain. The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils and macrophages, outside of the blood vessels (extravasation) into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. The loss of function (functiolaesa) is probably the result of a neurological reflex in response to pain.

In addition to cell-derived mediators, several acellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria and the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma.

a) Vasodilation and increased permeability

Acute inflammation is an immunovascular response to an inflammatory stimulus. This means acute inflammation can be broadly divided into a vascular phase that occurs first, followed by a cellular phase involving immune cells. The vascular component of acute inflammation involves the movement of plasma fluid, containing important proteins such as fibrin and immunoglobulins (antibodies), into inflamed tissue.

Upon contact with PAMPs, tissue macrophages and mast cells release vasoactive amines such as histamine and serotonin, as well as eicosanoids such as prostaglandin E2 and leukotriene B4 to remodel the local vasculature.

Macrophages and endothelial cells release nitric oxide. These mediators cause vasodilation and increase their permeability, which results in distribution of blood plasma from the vessel into the tissue space. The increased collection of fluid into the tissue causes edema. This exuded tissue fluid contain various antimicrobial mediators from the plasma such as complement, lysozyme, antibodies, which kills the microbes. If the inflammatory stimulus is a lacerating (cutting or tearing) wound, exuded platelets, coagulants, plasmin and kinins can clot the wounded area and provide haemostasis in the first instance. The haemostasis is starts from the vasoconstriction due to release of serotonin from platelets. This decreases the blood flow from wound. Some of the exuded tissue fluid is also funnelled by lymphatics to the regional lymph nodes, flushing bacteria along to start the recognition and attack phase of the adaptive immune system.

Acute inflammation is characterized by marked vascular changes, including vasodilation, increased permeability and increased blood flow, which are induced by the actions of various inflammatory mediators. Vasodilation occurs first at the arteriole level, progressing to the capillary level, and brings about a net increase in the amount of blood present, causing the redness and heat of inflammation. Increased permeability of the vessels results in the movement of plasma into the tissues, with resultant stasis due to the increase in the concentration of the cells within blood (a condition characterized by enlarged vessels packed with cells). Stasis allows leukocytes to marginate (move) along the endothelium, a process critical to their recruitment into the tissues. Normal flowing blood prevents this, as the shearing forcealong the periphery of the vessels moves cells in the blood into the middle of the vessel.

b) Leucocytic events during inflammation

Accumulation of leukocytes is the most important feature of the inflammatory reaction. Leukocytes engulf and degrade bacteria, immune complexes, and the debris of necrotic cells. Leukocytes get to sites of inflammation by adhesion to vascular walls and transmigration through them.

This process is regulated by the **"Leukocyte Adhesion Cascade"**, characterized by binding of complementary adhesion molecules on membranes of leukocytes and endothelial cells.Chemical mediators such as chemoattractants and cytokines affect these processes.

1) Margination.
2) Rolling & Adhesion Leukocyte adhesion cascade.
3) Emigration.
4) Chemotaxis.
5) Phagocytosis and intracellular killing / degradation.

6) Extracellular release of leukocyte products.

7) Synthesis of chemical mediators of inflammation.

1) Margination

Slowing and stagnation of the flow occurs due to vasodilation and increased vascular permeability. Leukocytes fall out of the central column and tumble slowly to the periphery of the vascular lumen, until they come in contact with the surface of endothelial cells of capillaries and post-capillary venules.

2) Rolling & Adhesion

Marginated leukocytes line the endothelium. Leukocytes start to become adhered to the surface of endothelial cells through various adhesion molecules.

Adhesion to the endothelium is at first loose, allowing the leukocytes to roll along the endothelial surface. As adhesion becomes firmer, the leukocytes become stationary and can then begin to migrate through the endothelium and into the site of inflammation.

Adhesion occurs through adhesion molecules of which there are 4 main groups:

i. **Selectins** (P-selectin and E-selectin on endothelium and L-selectin on leukocytes)

ii. **Mucin-like ligands** (Sialyl-Lewis X, etc. on leukocytes)

iii. **Integrins**(CD11/CD18, etc. on leukocytes)

iv. **Immunoglobulin superfamily adhesion molecules** – IgSAM's (ICAM, VCAM, MadCAM, etc on endothelium, and PECAM on endothelium and leukocytes)

a) Rolling

P-selectin is first to become activated due to release of histamine, thrombin & Platelet Activating Factor (PAF).

E-selectin follows in 1-2 hours, stimulated by the secretion of TNF-alpha and IL-1 by macrophages, mast cells and/or damaged endothelial cells.

b) Arrest and adhesion

L-selectin on leukocytes binds toMadCAM (Mucosal addressin Cell Adhesion Molecule) on endothelial cells.

c) Firm adhesion

Leukocytes become activated and express integrins which bind to endothelial IgSAM's, ie ICAM (InterCellular Adhesion Molecule) and VCAM (Vascular Cell Adhesion Molecule).

In addition to E-selectin, other adhesion molecules (L-selectin, IgSAM's and Integrins) are activated by TNF-alpha & IL-1 secreted mainly by macrophages &/or damaged endothelial cells, as well as by IL-6, C5a, PAF, etc.

3) Emigration

It is the process by which leukocytes escape from the blood to perivascular tissues; moving to the site of inflammation. After firm adhesion, the leukocytes insert large cytoplasmic

extensions (pseudopodia) into endothelial gaps. Gaps have been created by actions of histamine and other chemical mediators as well as by the leukocytes themselves.

PECAM(Platelet Endothelial Cell Adhesion Molecule), an IgSAM expressed on both endothelial and leukocyte surfaces, is the adhesion molecule most directly responsible for this process.

To pass through the basement membrane of the vessel, the leukocyte must also secrete collagenases (this produces gaps of less than 1 micron in diameter). As the leukocyte leaves the vessel, it expresses β1 integrins that help it to bind with extracellular matrix (ECM) proteins in the perivascular tissue.

Emigration occurs in the postcapillary venule because it is there that adequate numbers of inter-endothelial gaps and receptors are found (particularly histamine receptors). Neutrophils are usually the first to emigrate; they predominate for the first 6-24 hrs, peaking at 4-6 hrs. Monocytes usually follow neutrophils, peaking at 18-24 hrs and becoming predominant in 24-48 hrs.

4) Chemotaxis

It is the directional migration in response to a chemical gradient of chemotaxins. The process is receptor-mediated and allows leukocytes to travel from the perivascular space to the site of injury / infection.

All leukocytes respond to chemotactic stimuli; neutrophils are the fastest (within 90 minutes), followed by monocytes / macrophages (several hours) and then lymphocytes.

Chemoattractants (chemotaxins) can be exogenous or endogenous.

Exogenous chemoattractants

These are not synthesized or secreted by our body these agents come from environment. For example, LPS in the wall of Gram-negative bacteria attract neutrophils, eosinophils, monocytes / macrophages.

Endogenous chemoattractants

These are from plasma and / or necrotic tissues.

Histamine - attracts eosinophils.

Complement (particularly **C5a**) - attracts neutrophils, eosinophils, monocytes, basophils.

Fibrin-degradation products **(FDPs)**- attracts neutrophils.

Leukotrienes (e.g. **LTB4**) from arachidonic acid metabolism - attract neutrophils and eosinophils.

Chemokines– these are type of cytokine (signal molecule produced by leukocytes) which main function is to attract leukocytes; i.e. make them migrate across capillaries and post capillary venules. Chemokines not only stimulate locomotion (chemotaxis) but also activate

leukocytes to produce inflammatory mediators, engage in phagocytosis, initiate the oxidative burst, etc.

Mechanisms of Chemotaxis

Leukocytes have receptors on their membrane that bind the chemoattractant initiates chain of biochemical reactions that cause increased intracellular calcium leads to assembly of contractile elements responsible for cell movement towards the highest concentration of chemoattractant.

i. Microtubules allow the cell to orient toward the chemotactic gradient while microfilaments (actin and myosin) are actually responsible for the movement (cytoskeletal movement or re-organization).

ii. Movement is achieved by formation of a pseudopod that pulls the remainder of the cell in its direction.

5) Phagocytosis, Intracellular Killing / Degradation

a) Phagocytosis

Phagocytosis means to engulf the foreign materials. This process kills or degrades the foreign materials/ pathogens. Cellular mechanisms are similar to those of chemotaxis (cytoskeletal re-organization) but aimed at engulfing an injurious agent; steps include:

- Recognition and attachment of agent.
- Engulfment (Pseudopods wrap around the attached particle until it is engulfed forming a phagosome.
- Phagolysosome formation (Fusion of lysosomal granules with phagosome, bacteria is killed and digested.

b) Intracellular Killing

Oxygen-dependent and independent mechanisms of bactericidal activity occur in the phagolysosome:

1. Oxygen-dependent mechanisms

These are the most common, and are based on the production of reactive oxygen species in the "respiratory burst" part of phagocytosis. These species include superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$).

2. Oxygen-independent mechanisms

It occurs due to substances within leukocyte granules such as:

Lysozyme

These are produced and stored in lysosomes, mostly neutrophils and macrophages. They attacks bacterial cell walls (especially gram +ve bacteria).

Lactoferrin

Iron-binding glycoprotein sequesters iron so that it is unavailable for use by bacteria.

Other agents include trypsin and chymase, Neutral proteases in mast cell granules, major basic protein,Cathepsin G.

c) Degradation

After the microorganism has been phagocytised, the pH in the phagolysosome drops to 4-5. This acid pH is optimal for the action of degradative enzymes within lysosomes.

6) Extracellular Release of Leukocyte Products

Leukocytes do not only release toxic metabolites or enzymes into phagolysosomes, they also release them into the site of inflammation. This extracellular release helps to kill microorganisms and enhances the inflammatory reaction, but can also cause tissue necrosis.

7) Synthesis of Chemical Mediators of Inflammation

A chemical mediator is any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response. These mediators originate from plasma or cells; when they are in plasma, remain inactive and must be activated and need to be secreted or they are synthesized in response to a stimulus. Some have direct enzymatic activity; most require binding to specific receptors on target cells for biologic activity. One mediator can stimulate the release of other mediators by target cells (i.e. provide amplification).

2.7 PRINCIPLES OF WOUND HEALING IN SKIN

Many key signaling pathways are activated during cutaneous wound repair like the Wnt/β-catenin, Notch, Hedgehog, and various growth factor/cytokine pathways. Furthermore, several 'embryonic' extracellular matrix (ECM) components, such as Extra-Domain-A (EDA) fibronectin, are also synthesized during skin wound repair. Most of skin repair process is similar as occurs in case of embryonic development but some differencesare responsible for the inability of repaired skin to achieve its original uninjured state.

Repaired skin, which usually heals as a scar, is weaker than intact skin. Cutaneous wounds do not normally show regeneration of hair follicles, although an exception has been documented in the case of large cutaneous wounds. The reason for differences between original skin and skin after healing is the inflammatory response, which is unique to postnatal wound healing. While the inflammatory response is crucial to protect the body from invading foreign organisms at the injury site, many of the inflammatory cytokines and growth factors released during this process promote fibrosis and scar formation.

Here, we discuss the role of developmental signaling pathways in cutaneous wound repair, with an emphasis on keratinocyte and fibroblast behavior, and compare and contrast this with their roles in skin development. We also outline the varying responses to injury across the taxa, ranging from complete regeneration to scar tissue formation. Finally, we discuss current clinical applications that may improve wound healing via the modulation of

developmental pathways, and map out future areas of research which remain to be addressed.

Stages of cutaneous wound healing

The skin is composed of two main layers: the superficial layer, the epidermis, which functions as a barrier to the external environment, and the deeper layer, the dermis, which is composed of connective tissue, and provides the skin with its mechanical properties. The epidermis consists of a stratified keratinized epithelium that is interspersed with hair follicles and glands. Underlying the epidermis is the dermis, subdivided into the upper 'papillary' dermis, and the lower 'reticular' dermis, which differ in the density of their collagen fibers. During cutaneous wound healing, the barrier and mechanical properties of skin are restored by the actions of numerous cell types which undergo proliferation, differentiation, migration and apoptosis to rebuild the skin. Normal cutaneous wound repair is characterized by three overlapping phases of healing termed the inflammatory, proliferative, and remodeling phases

a) Hemostasis and inflammation

Hemostasis is the immediate response to injury and itprevent the loss of blood at the wound site. Vascular injury initiates a cascade of events that terminates in coagulation, and encompasses vascular constriction, platelet aggregation and degranulation, and finally the formation of a fibrin clot. The fibrin clot also acts as a provisional matrix for the initial migration of inflammatory cells to the wound site. Inflammatory cells, such as neutrophils and monocytes, are attracted to the site of injury by cytokines, including TGF-β and platelet-derived growth factor (PDGF), which are released by platelets and from sites of sequestration in the disrupted ECM. Neutrophils remove bacteria and/or foreign objects from the wound and are followed by monocytes, which subsequently differentiate into macrophages. While macrophages phagocytose foreign organisms, particles, and dead neutrophils, they also release TGF-β and other cytokines, and thereby stimulate the movement of fibroblasts and epithelial cells into the wound.

b) Proliferation

The proliferative phase of wound healing is characterized by re-epithelialization of the epidermis, and by repair of the underlying dermal or mesenchymal layer. This is accompanied by neovascularization. The dermis is restored by invading and proliferating fibroblasts that synthesize and secrete ECM proteins and also release activating growth factors such as TGF-β1. During the proliferative phase of wound repair, fibroblasts produce immature or 'embryonic' ECM variants such as EDA fibronectin and type III collagen, as well as the collagen type I that is normally found in adult skin. The invasion of fibroblasts into the wound is facilitated by their secretion of ECM-cleaving matrix metalloproteinases (MMPs). Epidermal keratinocytes express various integrin receptors during wound repair and are thought to use the provisional matrix as a substrate for re-epithelialization. Closure of the epithelial gap and restoration of the epithelium is important as a barrier function, and

this is achieved by a combination of keratinocyte migration, proliferation and differentiation. Different populations of hair follicle stem cells, both inside and outside of the hair follicle bulge region, contribute to re-epithelialization during wound repair. Intermolecular epidermal stem cells may also participate in this process.

c) Remodeling

The remodeling of the wound site, following deposition of sufficient ECM and the closure of the epithelial gap, changes the properties of the tissue. Wound fibroblasts at this stage of the repair process tend to adopt a contractile myofibroblast phenotype. Reorganization of the wound tissue involves the degradation and replacement of immature ECM such as EDA fibronectin and collagen type III with collagen I, the organization of collagen I fibers into bundles, and the apoptosis of a variety of cell types at the wound site. Together, these changes result in the contraction of the wound and the formation of acellular scar tissue. The remodeling process can continue indefinitely, and scar tissue does not achieve the strength of intact uninjured skin.

Review Questions

MULTIPLE CHOICE QUESTIONS

1. **..... is a defensive response to the injury by body.**
   a) Infection b) Pain
   c) Inflammation d) Bleeding
2. **Inflammation process involves .....**
   a) Chemical and morphological changes at affected site
   b) Removal or destruction of cause
   c) Healing
   d) All of the above
3. **4 clinical signs of inflammation was first described by .....**
   a) Virchow b) Celsus
   c) Arrhenius d) Walter Cannon
4. **During inflammation redness occurs due to .....**
   a) Due to dilation of small blood vessels b) Due to accumulation
   c) Due to increased blood flow d) Due to inflammation chemical mediators
5. **During inflammation pain occurs due to .....**
   a) Due to dilation of small blood vessels b) Due to accumulation
   c) Due to increased blood flow d) Due to inflammatory chemicals

6. Duration of acute inflammation is .....
a) 0 – 4 hours
b) 3 – 5 Days
c) Months to years
d) None of the above

7. ..... is the effect of histamine during inflammatory reaction.
a) Vasodilation
b) Increased vascular permeability
c) Itching
d) All of the above

8. Following is the example of vasoactive chemical mediator of inflammation.
a) Serotonin
b) Histamine
c) NO_2
d) All of the above

9. ..... is the directional migration of WBC's in response to a chemical gradient of mediator
a) Chemotaxis
b) Emigration
c) Margination
d) Phagocytosis

Answers

1- c, 2- d, 3- b, 4- a, 5- d, 6- b, 7- d, 8-d, 9-a, 10-a

SHORT ANSWER QUESTIONS

Q.1. What do you mean by inflammation? Give clinical signs of inflammation.

Ans.: Refer 2.1, 2.2.

Q.2. What is inflammation? Give classification of inflammation.

Ans.: Refer 2.1, 2.3.

Q.3. Explain cellular and chemical mediators of inflammation.

Ans.: Refer 2.4, 2.5.

Q.4. Explain wound healing process in skin.

Ans.: Refer 2.7.

Q.5. Explain vasodilation and increase in vascular permeability during inflammation.

Ans.: Refer 2.6.1 a.

Q.6. Explain leucocyte events during inflammation.

Ans.: Refer 2.6.1 b.

LONG ANSWER QUESTIONS

Q.1. Explain mechanism of inflammation with vascular and leucocyte events.

Ans.: Refer 2.6.1.

Q.2. What is inflammation? Give clinical signs, classification and mediators of inflammation

Ans.: Refer 2.1, 2.2, 2.3, 2.4, 2.5.