BASIC MECHANISM INVOLVED IN THE PROCESS OF INFLAMMATION AND REPAIR

For Class- B.Pharmacy 2nd Semester

Subject- Pathophysiology (BP204T)

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2.1 INTRODUCTION OF INFLAMMATION

Inflammation is the dynamic process by which living tissues react to injury. The exogenous and endogenous stimulus is lead to a cell injury, cause complex reactions in vascularised connective tissue leading to inflammation. The inflammation is a protective response to eliminate the initial cause of cell injury as well as necrotic cells and tissue, resulting from triggered stimuli. It is a local physiological response to tissue injury. It is not, in itself, a disease, but is usually a manifestation of disease. Inflammation may have beneficial effects, it works by diluting, destroying or neutralizing harmful agents i.e. microbes and toxins. It set in motion that events that eventually heal and reconstitute the site of injury. Hence the inflammation help in the repair processes whereby the damaged tissue is replaced by regeneration of parenchymal cells or by repair of the defects with fibrous scar tissue. Inflammation thus helps in clear infection and repairs and also does the healing of the wound.

The inflammation responses are life threatening anaphylactic reactions, due to the insect bite or drugs and also due to certain chronic diseases such as rheumatoid arthritis and atherosclerosis.

There are various other harmful causes of inflammation, which lead to fibrous bands and to intestinal obstruction or pericardial inflammation resulting in dense scars that impair cardiac function.

On the other hand it may produce disease; for example, an abscess in the brain would act as a space-occupying lesion compressing vital surrounding structures, or fibrosis resulting from chronic inflammation may distort the tissues and permanently alter their function.

Modulators of inflammation can be exogenous or endogenous. The exogenous modulators may promote or amplify the effects of the endogenous modulators during the process of inflammation. The endogenous modulators in the regulation of peritoneal inflammation

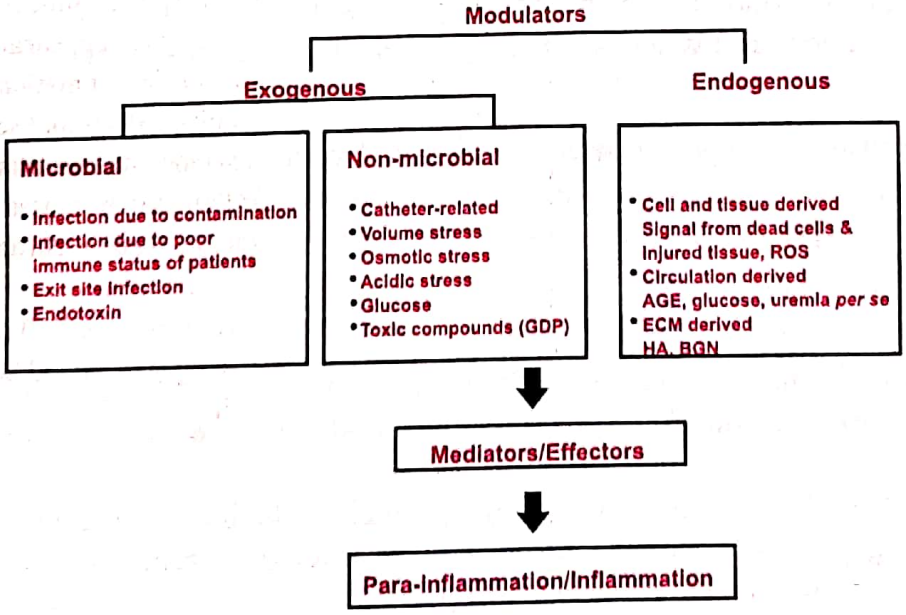

Fig. 2.1 The exogenous and endogenous modulators involves in inflammation

2.2 CAUSES OF INFLAMMATION

Various chemical and agent and any other circumstance cause the tissue damage. The initial stages are known as the acute inflammatory reaction. Where the process is prolonged duration the inflammation may be subacute or chronic

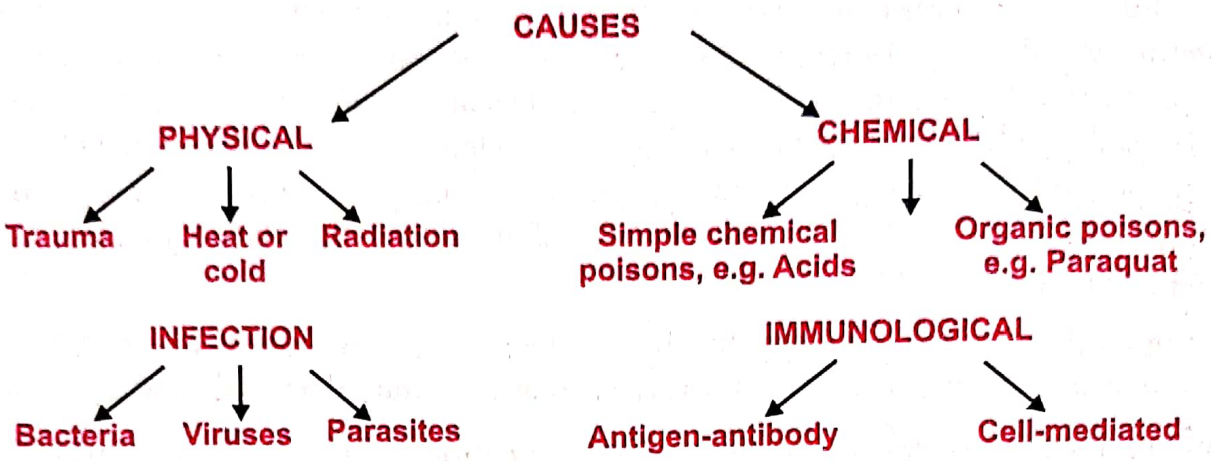

Fig. 2.2 Layout representation causes of inflammation.

Microbial infections: One of the commonest causes of inflammation is microbial infection. Viruses lead to the death of individual cells by intracellular multiplication. Bacteria release specific exotoxins which are chemicals synthesised by them, which specifically initiate inflammation associated with their cell walls. Additionally, some organisms cause immunologically-mediated inflammation through hypersensitivity reactions. Parasite infections and tuberculous inflammation are instances where hypersensitivity is important.

Hypersensitivity reactions: hypersensitivity reaction occurs when an altered state of immunological responsiveness causes an inappropriate or excessive immune reaction which damages the tissues. The types of reaction are due to chemical mediators similar to those involved in inflammation.

Physical agents: Tissue damage leading to inflammation may occur through physical trauma, ultraviolet or other ionising radiation, burns or excessive cooling (frostbite).

Irritant and corrosive chemicals: Corrosive chemicals (acids, alkalis, oxidising agents) provoke inflammation through gross tissue damage. However, the infecting agents may release specific chemical irritants, which lead directly to inflammation.

Tissue necrosis: Death of tissues from lack of oxygen or nutrients resulting from inadequate blood flow (infaction) is a potent inflammatory stimulus.

2.3 CLINICAL SIGNS OF INFLAMMATION

The physical signs of acute inflammation were described by using the Latin words rubor (Redness), calor (Heat), tumor (Swelling) and dolor (Pain). Loss of function is also a sign of inflammation.

Redness: An acutely inflamed tissue appears red, for example, skin affected by sunburn, cellulitis due to bacterial infection or acute conjunctivitis. This is due to dilatation of small blood vessels within the damaged area.

Heat: Increase in temperature is seen only in peripheral parts of the body, such as the skin. It is due to increased blood flow through the region, resulting in vascular dilatation and the delivery of warm blood to the area. Systemic fever, which results from some of the chemical mediators of inflammation, also contributes to the local temperature.

Swelling: Swelling results from oedema – the accumulation of fluid in the extravascular space as part of the fluid exudate – and, to a much lesser extent, from the physical mass of the inflammatory cells migrating into the area

Pain: Pain is one of the known features of acute inflammation. It results partly from the stretching and distortion of tissues due to inflammatory oedema and, in particular, from pus under pressure in an abscess cavity. Some of the chemical mediators of acute inflammation, including bradykinin, the prostaglandins and serotonin, are known to induce pain.

Loss of function: Loss of function, a well-known consequence of inflammation, was added by Virchow (1821–1902) to the list of features drawn up to Celsus. Movement of an inflamed area is consciously and reflexly inhibited by pain, while severe swelling may physically immo-bilise the tissues.

2.5 MECHANISM OF INFLAMMATION

The inflammatory responses are life threatening anaphylactic reactions, due to a insect bite or drugs and also due to certain chronic diseases such as rheumatoid arthritis, osteoarthritis and inflammatory lung disease, including inflammatory bowel disease, atherosclerosis, psoriasis and Chronic Obstructive Pulmonary Disease.

Inflammation is a process by which the body's white blood cells and chemicals protect the body infection and foreign substances such as bacteria and viruses. The body's defense mechanism (immune system) inappropriately triggers an inflammatory response when there are no foreign substances to fight off, these are called autoimmune diseases, where the body's normally protective immune system causes damage to its own tissues. When the tissue injury releases the inflammation mediators cause inflammation. These chemicals release from the site of injury fig.4 such as histamine, kinin, prostaglandins leukotrienes and white blood cells are released to protect or repair the body from foreign substances.

The inflammatory responses are due to circulating cells and plasma proteins, vascular cells and extracellular matrix of the surrounding connective tissue. *The circulating cell include* : neutrophils, eosinophils basophils, lymphocytes, monocytes and platelets. The circulating proteins includes : clotting factor, kininogen and complement components largely synthesized by the liver. *The vascular wall cells include:* endothelial cells, which come in direct contact with blood vessels. *The connective tissue cells includes* : mast cell macrophages, and lymphocytes and fibroblasts that synthesized the extracellular matrix, which can proliferate to fill in the wound. The extracellular matrix (ECM) consists of fibrous structural proteins i.e. collagen and elastin, gel forming proteoglycans and adhesive glycoproteins i.e. fibronectin that is the cell EMC and ECM-

ECM connectors. All these parameters interact to or resolve a local injury and restore normal tissue function.

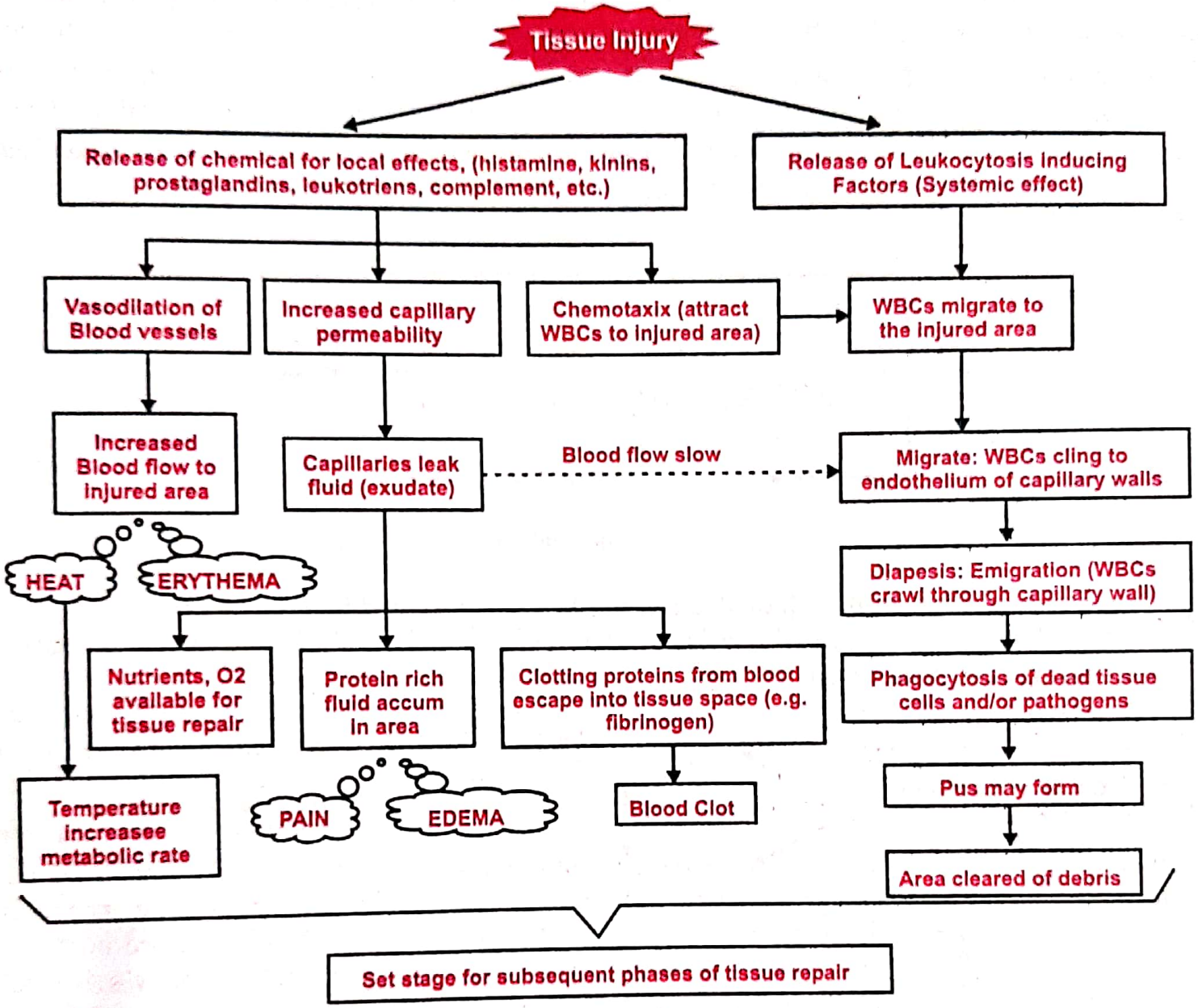

Fig. 2.3. The outline mechanism of inflammation is as follow: initial stimuli release of chemical mediators from plasma or connective tissue cells. These together or in sequence amplify the initial inflammatory response and by regulating the vascular responses. If the injurious stimuli of inflammation are removed and inflammediators are catalibolised or inhibited.

2.6 TYPE OF INFLAMMATION

I. Acute inflammation is a short duration lasting from a few minutes to few days which is characterized by fluid and plasma proteins exudation and predominantly neutrophilic leukocyte accumulation.

2. *Chronic inflammation* is of long duration, lasting from the days to years leads to an influx of lymphocytes and macrophages associated with vascular proliferation and scarring. Inflammation

is two types, it is characterized by differences in the cell types taking part in the inflammatory response.

Acute Inflammation: Acute inflammation is the initial tissue reaction to a wide range of injurious agents; it may last from a few hours to a few days.

(1) *Initial Phase:* The initial reaction of tissue to injury: It is an immediate and early response to injury caused by the accumulation of leukocytes to the site of injury. Once these leukocytes are there it can clear the way for any invading microbes and begins the process of breaking down necrotic tissue and release of chemical mediators.

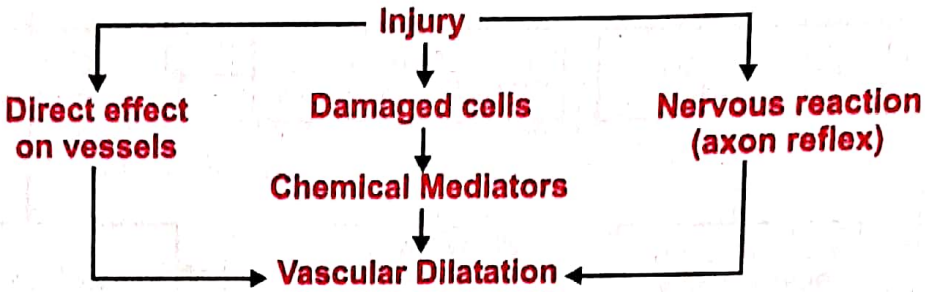

Fig. 2.4 Early response inflammation

(2) *Vascular Phase:* In this phase dilatation of blood vesicle and increased permeability. The vascular changes and cell recruitment are accounted for local signs of acute inflammation causing heat, redness and swelling.

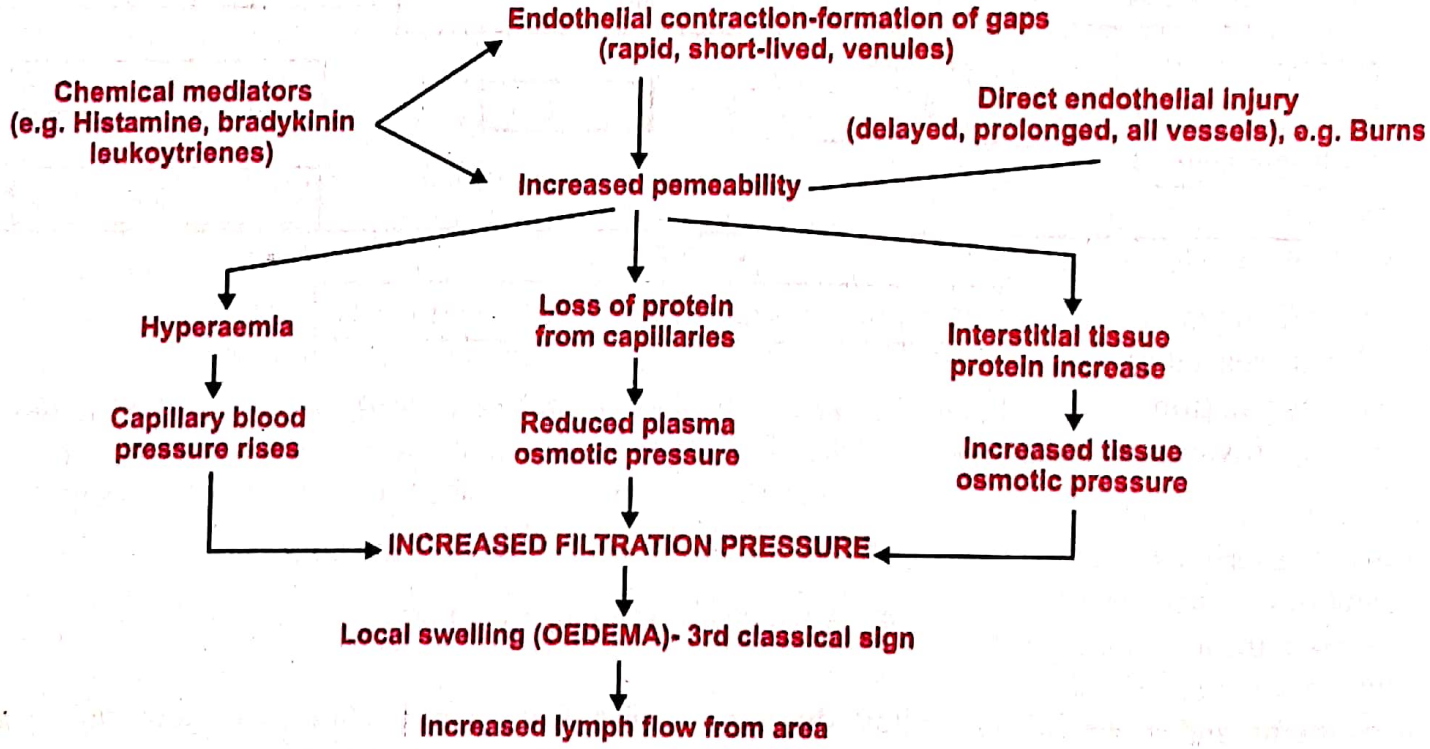

Fig. 2.5 Process of inflammation in vascular phase

(3) *Exudative Phase:* Fluid and cells escape from permeable venules. Exudation is the increased passage of protein-rich fluid through the vessel wall into the interstitial tissue. Neutrophils and mononuclears pass between the endothelial cell junctions by amoeboid

movement through the venule wall into the tissue spaces. In this process both neutrophils and endothelial cells are activated and both express cell adhesion molecules, is the predominant cell involved, but mast cells and macrophages are also important which is the progression of chronic inflammation. The features like pain and loss of function occur as consequences of mediator elaboration and leukocyte-mediated damage

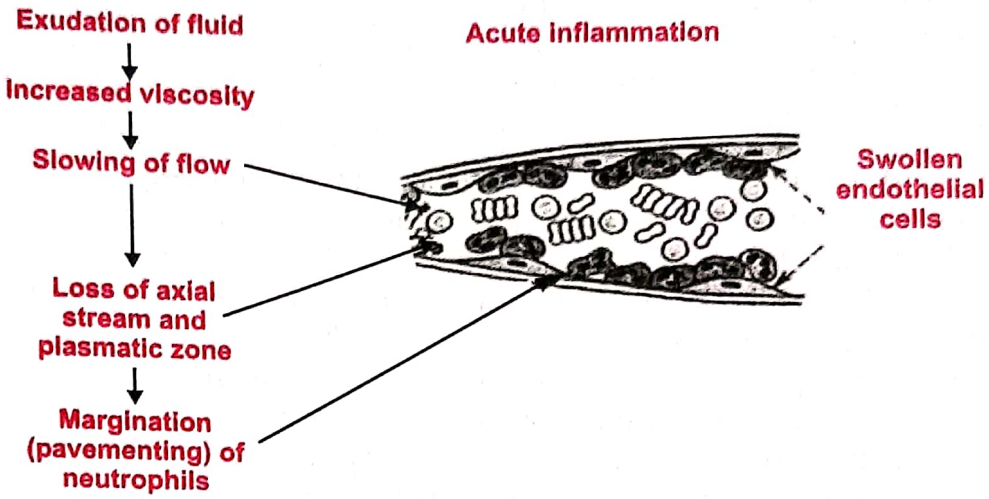

Fig. 2.6 Process of acute inflammation in *exudative phase*

During Organisation, new capillaries grow into the inert material (inflammatory exudate), macrophages migrate into the zone and fibroblasts proliferate, resulting in fibrosis. A good example is seen in the pleural spaces following acute lobar pneumonia. Resolution usually occurs in the lung parenchyma, but very extensive fibrinous exudate fills the pleural cavity. The fibrin is not easily removed and consequently capillaries grow into the fibrin, accompanied by macrophages and fibroblasts (the exudate becomes 'organised'). Eventually, fibrous adhesion occurs between the parietal and visceral pleura.

4. Progression to Chronic Inflammation: If the agent causing acute inflammation is not removed, the acute inflammation may progress to the chronic stage. In addition to Organisation of the tissue the character of the cellular exudate changes, with Lymphocytes, plasma cells and macrophages (sometimes including multi nucleate giant cells) replacing the neutrophil polymorphs. Often chronic inflammation will occur as a primary event, further with no preceding period of acute inflammation.

2.10 CHRONIC INFLAMMATION

Chronic inflammation is an inflammatory response of prolonged duration: weeks, months or even indefinitely by a continuous causative stimulus to inflammation in the tissue. The inflammatory process causes tissue damage and is accompanied by simultaneous healing and repair. The exact nature and extent time course of chronic inflammation is variable and depend on the balance between the causative agent and the attempts of the body to remove it. The Characteristics of chronic Inflammation are (1). Mononuclear inflammatory cells are the most numerous leukocytes and plasma cells. (2) Tissue destruction is present largely induced by the inflammatory cells. (3) Repair is underway through the proliferation of fibroblasts and endothelial cells (angiogenesis and fibrosis).

2.10.1 Etiology of chronic inflammation

May progress from acute inflammation, if the acute process cannot be recovered because of a persistence of the agent or interference with normal healing. However, Some specific injurious agents which typically cause chronic inflammation, such as (a) Persistent viral infections (eg. caprine arthritis encephalitis virus). **(2)** Persistent microbial infections (eg. Mycobacterium spp. and fungi). **(3)** Prolonged exposure to some toxic agents (eg. asbestos). **(4)** Few autoimmune diseases (eg. lupus erythematosus).

1. Infectious organisms that can avoid or resist host defences and so persist in the tissue for a prolonged period. Examples include Mycobacterium tuberculosis, Actinomycetes, and numerous fungi, protozoa and metazoal parasites. Such organisms are able to avoid phagocytosis or survive within phagocytic cells, and do not produce toxins causing acute tissue damage. Infectious organisms, which are not resistant, but lives in damaged regions where they are protected by the host defences. The common example is of bacteria, which grow in the pus within an undrained abscess cavity, where they are protected both from host immunity and from blood-borne therapeutic agents, *e.g.* antibiotics. Some locations are particularly prone to chronic abscess formation, *e.g.* bone, pleural cavities.

2.10.5 Pathogenesis of Chronic Inflammation

Chronic inflammation, also known as systemic inflammation, is delayed and ongoing inflammation that can occur internally and externally chronic arthritis is an example of chronic inflammation. Persistent release of chemical mediators induces, that is abundant the tissue destruction and increase the blood flow & increased vascular permeability. The recruitment of inflammatory cells are macrophages, lymphocytes and plasma cells. These inflammatory cell proliferation the B. cell, macrophases and leukocytes. The entire process occurs in repetitive cycle.

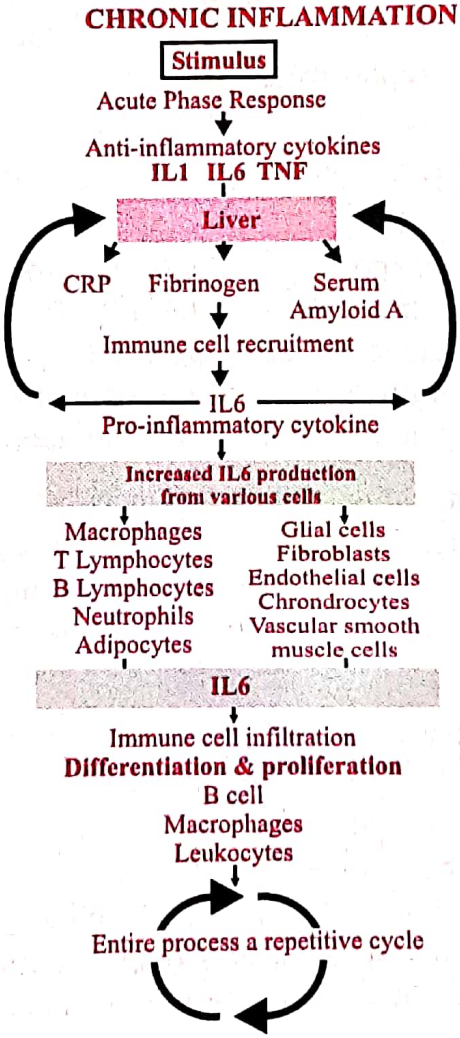

Fig.2.10 Chronic inflammation

2.6.1 ALTERATION IN VASCULAR PERMEABILITY AND BLOOD FLOW

In the early stages, oedema fluid, fibrin and neutrophil polymorphs accumulate in the extracellular spaces of the damaged tissue. The presence of the cellular component, neutrophil polymorph, is essential for a histological diagnosis of acute inflammation. The acute inflammatory response involves three processes:

1. Changes in vessel caliber and consequently flow
2. Increased vascular permeability and formation of the fluid exudates
3. Formation of the cellular exudate – emigration of the neutrophil polymorphs into the extravascular space.

Changes in vessel caliber: The microcirculation consists of the network of small capillaries lying between arterioles, which have a thick muscular wall, and thin-walled venules. Capillaries have no smooth muscle in their walls to control their caliber and are so narrow that red blood cells must pass through them in single file. The smooth muscle of arteriolar walls forms precapillary sphincters which regulate blood flow through the capillary bed. Flow through the capillaries is intermittent, and some form preferential channels for flow while others are usually shut down.

In blood vessels larger than capillaries, blood cells flow mainly in the center of the lumen (axial low), while the area near the vessel wall carries only plasma (plasmatic zone). This feature of normal blood flow keeps blood cells away from the vessel wall.

Changes in the microcirculation occur as a physio-logical response; for example, there is hyperaemia in exercising muscle and active endocrine glands. The changes following injury which make up the vascular component of the acute inflammatory reaction were described by Lewis in 1927 as 'the triple response to injury': a flush, a flare and a wheal. If a blunt instrument is drawn firmly across the skin, the following sequential changes take place:

A momentary white line follows the stroke: this is due to arteriolar vasoconstriction, the smooth muscle of arterioles contracting as a direct response to injury; *The flush*: a dull red line follows due to capillary dilatation; *The flare*: a red, irregular, surrounding zone then develops, due to arteriolar dilatation. Both nervous and chemical factors are involved in these vascular changes; and *The wheal*: a zone of oedema develops due to fluid exudation into the extravascular space.

The initial phase of arteriolar constriction is transient and probably of little importance in acute inflammation. The subsequent phase of vasodilatation (active hyperaemia) may last from 15 mins to several hours, depending upon the severity of the injury. There is experimental evidence that blood flow to the injured area may increase up to tenfold. As blood flow begins to slow again, blood cells begin to flow nearer to the vessel wall, in the plasmatic zone rather than the axial stream. This allows 'pavementing' of leukocytes (their adhesion to the vascular epithelium) to occur, which is the first step in leukocyte emigration into the extravascular space. The slowing of blood flow, which follows the phase of hyperaemia is due to increased vascular permeability, allowing plasma to escape into the tissues while blood cells are retained within the vessels. The blood viscosity is, therefore, increased.

Increased vascular permeability: Small blood vessels are lined by a single layer of endothelial cells. In some tissues, these form a complete layer of uniform thickness around the vessel wall, while in other tissues there are areas of endothelial cell thinning, known as fenestrations. The walls of small blood vessels act as a microfilter, allowing the passage of water and solutes but blocking that of large molecules and cells. Oxygen, carbon dioxide and some nutrients transfer across the wall by diffusion, but the main transfer of fluid and solutes is by ultrafiltration, as described by Starling.

The high colloid osmotic pressure inside the vessel, due to plasma proteins, favours the fluid return to the vascular compartment. Under normal circumstances, high hydrostatic pressure at the arteriolar end of capillaries forces fluid out into the extravascular space, but this fluid returns into the capillaries at their venous end, where hydrostatic pressure is low. In acute inflammation, however, not only is capillary hydrostatic pressure increased, but there is also escape of plasma proteins into the extravascular space, increasing the colloid osmotic pressure there. Consequently, much more fluid leaves the vessels than is returned to them. The net escape of protein-rich fluid is called *exudation*; hence, the fluid is called the fluid exudate.

2.7 MIGRATION OF WBC'S OR LEUKOCYTES

The main inflammatory cells are polymorphonuclear leukocytes (neutrophils/heterophils, eosinophils, basophils), mast cells, mononuclear cells (monocytes/macrophages, lymphocytes, plasma cells), and platelets. Most cells, except for plasma cells, macrophages & mast cells, are normal inhabitants of the circulating blood. The total leukocyte (WBC) count in peripheral blood and the relative proportions of different white blood cells may be greatly modified in the systemic response to inflammation and can, therefore, be used as a diagnostic tool.The migration of leukocytes involves in following Steps. These are

1) Margination, (2) Rolling & Adhesion, (3) Emigration (4) Chemotaxis (5) Phagocytosis

1). Margination: In the normal blood circulation, White blood cells are traveled generally to the central (axial) stream in blood vessels, and do not flow in the peripheral (plasmatic) zone near to the endothelium. However, loss of intravascular fluid and increase in plasma viscosity with slowing and stagnation of the flow occurs due to vasodilatation and increased vascular permeability. Thereafter the 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.

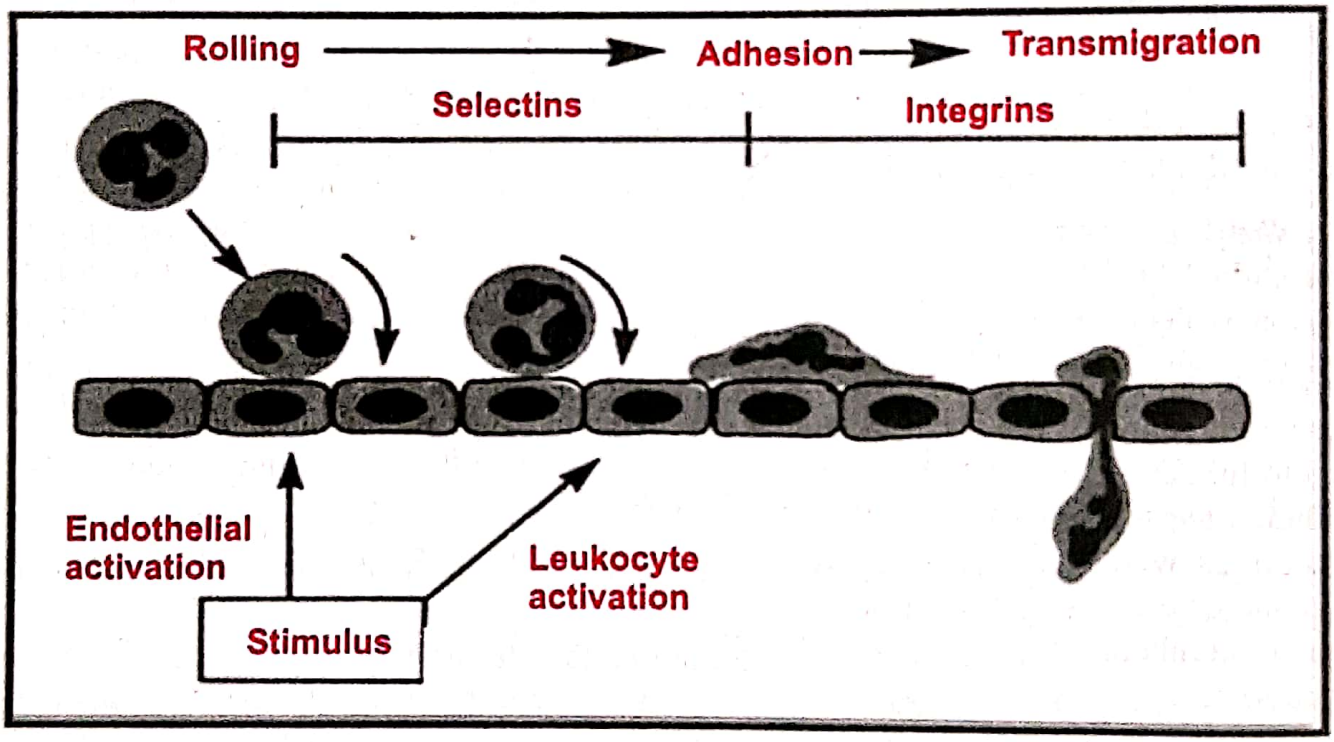

Fig. 2.7 Systematic representation of migration of Leukocyte

(2) Rolling & Adhesion: Marginated leukocytes line of the endothelium. Leukocytes start to become adhered to the surface of endothelial cells through various adhesion molecules. The adhesion of leukocyte to vascular endothelium is at first loose, allowing the leukocytes to roll along the endothelial surface. The adhesion becomes firmer, the leukocytes become stationary and can then begin to migrate through the endothelium and into the site of injury. Leukocyte randomly contacts the endothelium in normal tissues, but do not adhere to it. The process of adhesion occurs through adhesion molecules of which there are 4 main groups:

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

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

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

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

Increased leukocyte adhesion results from the interaction between paired *adhesion molecules* on leukocyte and endothelial surfaces. There are several classes of such adhesion molecules: some of them act as lectins which bind to carbohydrates on the partner cell. Leukocyte surface adhesion molecule expression is increased by: complement component C5a, leukotriene B4; and tumour necrosis factor. Endothelial cell expression of endothelial-leukocyte adhesion molecule-1 (ELAM-1) and intercellular adhesion molecule-1 (ICAM-1), to which the leukocytes' surface adhesion molecules bond, is increased by interleukin-1, endotoxins; and tumour necrosis factor.

In this way, a variety of chemical inflammatory mediators promote leukocyte-endothelial adhesion as a prelude to leukocyte emigration.

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.

Arrest and adhesion: L-selectin on leukocytes binds MadCAM (Mucosal addressin Cell Adhesion Molecule) on endothelial cells.

Firm adhesion: Leukocytes become activated and express integrins (eg CD11/CD18) which bind to endothelial IgSAM's, ie ICAM (InterCellular Adhesion Molecule) and VCAM (Vascular Cell Adhesion Molecule).

(3) Emigration of leukocyte: The process by which leukocytes escape from the blood to perivascular tissues; moving to the site of inflammation. After firm adhesion (using integrins bound to IgSAM's like ICAM or VCAM), the leukocytes insert large cytoplasmic extensions into endothelial gaps. The vascular gaps have been created by actions of histamine and other chemical mediators as well as by the leukocytes themselves. The leukocyte pass through the basement membrane of the vessel, the emigration occurs in the postcapillary venule because it is there that adequate numbers of inter-endothelial gaps and receptors are found (particularly histamine receptors).

In viral infections, lymphocytes are the first to arrive and in some hypersensitivity reactions, eosinophils arrive first.

(4) Chemotoxins: The initial margination of neutrophils and mononuclears is potentiated by slowing of blood flow and by increased 'stickiness' of the endothelial surface. After penetration of the vessel wall, the subsequent movement of the leucocytes is controlled by chemotaxis. The cell moves in response to an increasing concentration gradient of the particular chemotactic agent, usually a protein or polypeptide.

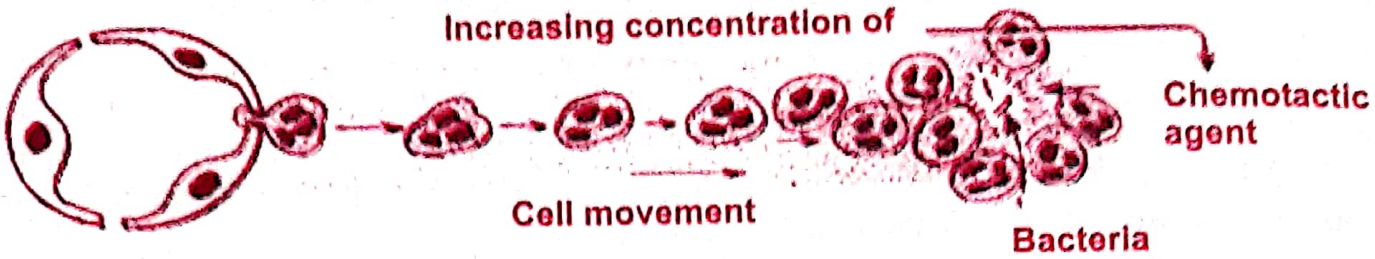

Fig. 2.8 Process of Chemoattractants (chemotaxins).

The process of chemotaxins can be exogenous or endogenous

Exogenous chemoattractants: LPS in the wall of Gram-negative bacteria; attract neutrophils, eosinophils, monocytes/macrophages. Foreign material (eg wood splinter).

Endogenous chemoattractants are from plasma and/or necrotic tissues, *Histamine* 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* are a type of cytokine (signal molecule produced by leukocytes) which main function is to attract leukocytes; ie make them migrate across capillaries and post- capillary venules. *IL-8* attracts mostly neutrophils, but may also attract macrophages and eosinophils. Chemokines not only stimulate locomotion (chemotaxis) but also activate leukocytes to: produce inflammatory mediators, engage in phagocytosis, initiate the oxidative burst.

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.

1. 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).
2. The movement is achieved by formation of a pseudopod that pulls the remainder of the cell in its direction.

5) Phagocytosis : Phagocytosis is a process of engulf, kill and degrade foreign material; most commonly bacteria. In this process, the neutrophils and macrophages clear the injurious agent. The aimed at engulfing an injurious agent include following steps.

Recognition and attachment of agent (in case of bacteria): Mannose on the bacterial wall is recognized directly by the leukocyte's mannose receptor or bacteria are opsonized by antibodies and complement (C3b) fragments that are then recognized by specific receptors on leukocytes.

Engulfment: Small cytoplasmic extensions (pseudopods) project from the leukocyte. Pseudopods wrap around the attached particle until it is engulfed. Pseudopods meet and fuse, forming a phagosome.

Phagolysosome formation: The fusion of lysosomal granules with phagosome to forms the phagolysosome. The phagolysome killed and digested the bacteria.

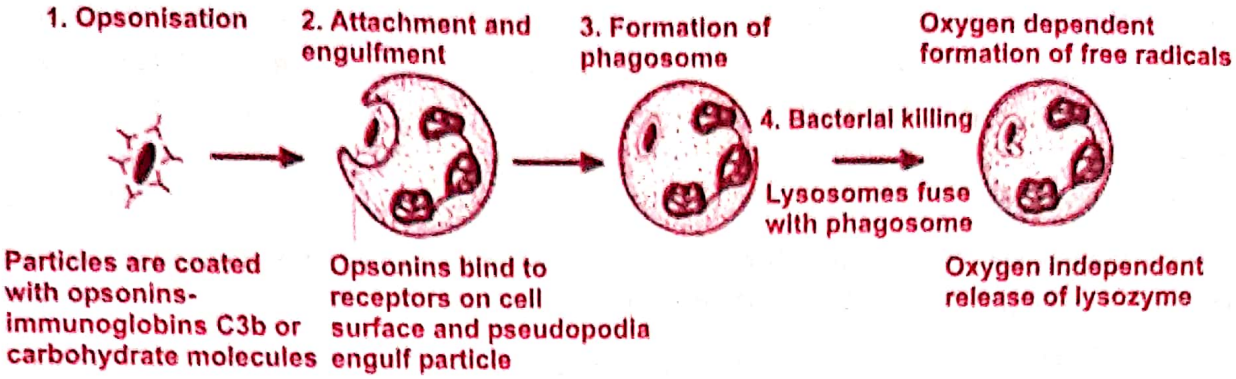

Fig. 2.9 Stages of phagocytosis process

2.8 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. Mediator of inflammation originates from plasma or cells. When mediators in plasma, are in an inactive state and must be activated and when in cells, they are often within granules and need to be secreted or they are synthesized in response to a stimulus. The production of active mediators is triggered by microbial products or host proteins (eg cytokines). However, some inflammatory mediators have direct enzymatic activity; most require binding to specific receptors on target cells for biologic activity. Some mediator can stimulate the release of other mediators by target cells (ie provide amplification).

List of Mediators is involved in inflammation

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

Few inflammation mediators details have been given:

Histamine: This is the best-known chemical mediator of acute inflammation. It causes vascular dilatation and the immediate transient phase of increased vascular permeability. It is stored in mast cells, basophil and eosinophil leukocytes, and platelets. Histamine release from these sites (for example, mast cell degranulation) is stimulated by complement components C3a and C5a, and by lysosomal proteins released from neutrophils.

Lysosomal compounds: These are released from neutrophils and include cationic proteins, which may increase vascular permeability, and neutral proteases, which may activate complement.

Prostaglandins: These are a group of long-chain fatty acids derived from arachidonic acid and synthesised by many cell types. Some prostaglandins potentiate the increase in vascular permeability caused by other compounds. Others include platelet aggregation (prostaglandin I_2 is inhibitory while prostaglandin A_2 is stimulatory). Part of the anti-inflammatory activity of drugs such as aspirin and the non-steroidal anti-inflammatory drugs are attributable to inhibition of one of the enzymes involved in prostaglandin synthesis.

Leukotrienes: These are also synthesised from arachidonic acid, especially in neutrophils, and appear to have vasoactive properties. SRS-A (slow reacting substance of anaphylaxis), involved in type I hypersensitivity is a mixture of leukotrienes.

5-hydroxytryptamine (serotonin): This is present in high concentration in mast cells and platelets. It is a potent vasoconstrictor.

Chemokines: This large family of 8–10 proteins selectively attract various types of leukocytes to the site of inflammation. Some chemokines such as IL-8 are mainly specific for neutrophil polymorphs and to a lesser extent lymphocytes whereas other types of chemokines are chemotactic for monocytes, natural killer (NK) cells, basophils and eosinophils. The various chemokines bind to extracellular matrix components such as heparin and heparan sulphate glycosaminoglycans, setting up a gradient of chemotactic molecules fixed in the extracellular matrix.

Plasma factors: The plasma contains four enzymatic cascade systems – complement, the kinins, the coagulation factors and the fibrinolytic system – which are inter-related and produce various inflammatory mediators.

Complement system: The complement system is a cascade system of enzymatic proteins. It can be activated during the acute inflammatory reaction in various ways:

1. Tissue necrosis, enzymes capable of activating complement are released from dying cells;
2. During infection, the formation of antigen-antibody complexes can activate complement via the *classical pathway*, while the endotoxins of Gram-negative bacteria activate complement via the *alternative pathway.*

complement factors) attached to their surfaces. Nonimmune phagocytosis is directed against foreign nonantigenic particles.

3. **Necrosis:** Commonly there is some degree of necrosis that may affect only scattered individual cells or may be extensive.
4. **Repair:** Repair of tissues damaged by persistent injury is characterized by new blood vessel formation, fibroblastic proliferation, and collagen deposition (fibrosis).

2.12 PRINCIPLE OF WOUND HEALING IN THE SKIN

Wound healing is a complex cellular and biochemical process that leads to restitution of integrity and function. Although individual tissues may have unique healing characteristics, all tissues heal by similar mechanisms and the process undergoes phases of inflammation.

Healing by First Intention (primary union): This occurs where the tissue surfaces have been approximated (closed). This can be with stitches, or staples, or skin glue (like Derma bond), or even with tapes (like steri-strips). This kind of closure is used when there has been very little tissue loss. It is also called "primary union" or "first intention healing." An example of wound healing by primary intention is a surgical incision. A primary union where epithelial regeneration predominates over fibrosis.

The process of wound healing progress as per timeline:

Hours/Dys/Week	Wound healing Process
24 hours:	Neutrophils at the incision margin migrate toward the fibrin clot. Basal epidermal cells at edges of incision increase mitotic activity.
24-48 hours:	Basal epidermal cells start to migrate and proliferate with deposition of basement membrane components and formed the continuous epithelial layer.
Day 3:	In this stage the neutrophils are replaced by macrophages and granulation tissue are invaded in the incision space. Fibroblasts & collagen fibres are evident at incision margins (at first are vertically oriented and do not bridge the incision). Epithelial cells continue to proliferate and formed the epidermal covering layer.
Day 5:	In this duration the neovascularization its peaks as granulation tissue fills incision space. Collagen fibrils become more abundant & begin to bridge the incision and epidermis recovery its normal thicness. The surface cells differentiation and formed mature epidermal architecture along surface keratinization.
Week 2:	In this period the accumulation of collagen and fibroblast proliferation. The edema and leukocytes are suppressed. In long duration Blanching occurs and vascular channels regression.

Week 4:	The composition of scar is composed of fibrous connective tissue with few inflammatory cells. Permanently lost the line incision due to destroy the dermal appendages and the tensile strength continues to increase with time.

Healing by the Second intention: A wound is extensive and involves considerable tissue loss and in which the edges cannot be brought together heals in this manner. Secondary intention healing differs from primary intention healing in three ways: **(I)** The repair time is longer. **(II)** The scarring is greater. **(III)** The chances of infection are far greater. The mechanism of second-intention healing is by granulation, eventual reepithelialization, and wound contraction which occurs due to myofibroblasts, it is rather than with suturing closed by first intention. The wound heals from the bottom up; it heals spontaneously if the dermal base is preserved.

Healing by the third intention: This type of wound healing is also known as "delayed" or "secondary closure" and is indicated where there is a reason to delay suturing or closing a wound some other way, for example when there is poor circulation to the injured area. The edges are closed 4 to 6 days postoperatively after meticulous debridement.

Mechanism of wound healing: It is a complex process of wound healing normally proceeds from coagulation and inflammation through fibroplasia, matrix deposition, angiogenesis, epithelialization, collagen maturation, and finally wound contraction. The wound healing signals include peptide growth factors, complement, cytokine inflammatory mediators, and metabolic signals such as hypoxia and accumulated lactate. Many of these cellular signaling pathways are redundant and pleiotropic.

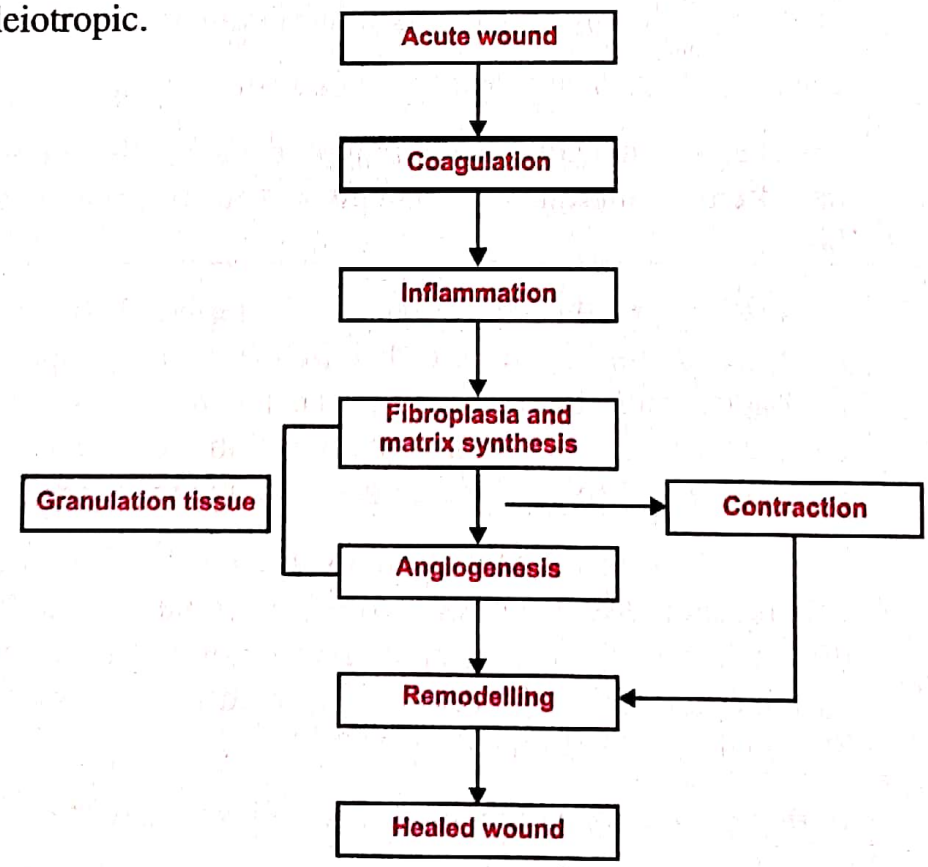

Fig. 2.11 Process of wound healing

1. **Hemostasis Phase:** in this phase stops the bleeding of an incision. The blood vessels in the sub-dermal plexus and deeper in the subcutaneous planes are cut, and bleeding occurs. The cellular and molecular elements involved in hemostasis which is a signal of tissue repair. In injury release, the fibrin, fibrinopeptides, thrombin split products for coagulation the blood, and complement components attract inflammatory cells into the wound. The thrombin activates the platelets and release the insulin like growth factor 1 (IGF-1), transforming growth factor α (TGF-α), transforming growth factor β (TGF-β), and platelet-derived growth factor (PDGF), all these growth factors attract leukocytes, particularly macrophages, and fibroblasts into the wound. The endothelial cells are damaged which respond to involving the complement products C5a, tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and interleukin-8 (IL-8), which express the integrin molecules on the cell membranes of leukocytes. The leukocytes circulate in the blood, which adhere to the endothelium and migrate into the wounded tissue. Interleukins and other inflammatory components, such as histamine, serotonin, and bradykinin, cause vessels initial to constrict for hemostasis and later to dilate. The permeability of the blood vessels is increased. Thereafter, blood plasma and leukocytes can migrate into the injured area.

2. **Inflammation:** In is the second phase of healing which involves inflammation, which starts at the moment of tissue disruption. Inflammatory cells are the presence during the phase of healing is polymorphonuclear leukocytes (PMNs) and macrophages. The PMNs are initial to appear and clear the devitalized tissue, blood clot, foreign material, and bacteria from the wound. PMNs are also a part of host defenses that destroy bacteria by phagocytosis mechanism and secreting oxygen free radicals. large numbers of macrophages appear within 48 hours of wounding and play a central role in the inflammatory phase.
3. **Migration and Proliferation:** Migration of PMNs from the intravascular compartment (the lumen) to the ECM and to the wound site is controlled by several biochemical agents, including selectin, cytokines, and integrins that act in series to activate, tether, and facilitate extravascular escape. The proliferative phase begins with formation of a provisional ECM composed of fibrin and fibronectin precipitated from blood extravasated into the wound at the time of the initial injury. The provisional matrix is a protein scaffold that stabilizes the wound edges and provides a framework for migration of PMNs, macrophages, fibroblasts, and other cells into the wound from surrounding tissues. The fibroblasts replace macrophages as the most numerous cell type such as macrophages, fibroblasts that are multifunctional. These are responsible for new tissue formation, collagen production, and the laying down of the extracellular matrix.
4. **Angiogenesis:** In this stage, the bleeding is under control, the body then starts the process of rebuilding tissue is called Angiogenesis. It involves the formation of new blood vessels. This process occurs when the cells begin to replace the veins and arteries that were damaged, either creating new sections or adding to existing portions. In the process of angiogenesis or neovascularization is stimulated by VEGF, basic fibroblast growth factor (bFGF), and TGFb. These factors are secreted by several cell types including vascular endothelial cells, epidermal cells, fibroblasts, and macrophages.
5. **Reepithelialization:** During the reepithelialization process, forging the chemical components. In this duration, the veins and damage skin began to regrow. The epidermis are comprised of cells called keratinocytes and it involves the creation of several layers, each working in tandem to offer protection and prevent fluid loss.

6. **Remodling**: In this process, involves certain proteins form blood clots, which helps further prevent bleeding as new skin and veins are formed. The remodeling phase is characterized by continued synthesis and degradation of the ECM components. There are various cells are secreted the specific collagenases such as fibroblasts, neutrophils, and macrophages each of which can cleave the collagen molecule. Several molecules, including TGF play an important role in the remodeling phase. The cellular molecules TGF-β-induced intracellular signaling acts through a set of proteins called the SMAD proteins, which act as direct links between the cell surface and the nucleus. In particular, the precise spatiotemporal role of each TGF-β/SMAD pathway component during the development of excessive ECM deposition leading to tissue fibrosis remains to be ascertained. As the scar matures, late remodeling occurs (that takes up to 1 year); the scar contracts and thins out.