OVERVIEW OF INFLAMMATION

The body is exposed to a potentially wide range of causes of injury e.g. trauma, infection, heat/cold, ischaemia, radiation, chemical, particulate.

How does the body protect itself against injury?

Non-specific mechanisms: e.g. neural reflexes, fight/flight responses, acid in stomach, skin barrier, mucus and cilia in lungs and if invasion/injury occurs - acute inflammation.

Specific mechanisms: i.e. body develops specific immune response directed against specific antigens. Two types of immune responses: humoral (antibody) mediated and cell-mediated. The antigens can be ‘remembered’ for improved responses on repeat exposure.

Tissue response to injury

Inflammation is a protective response of living vascularized tissues whose function is to rid the body of the cause of the injury (if still present) and the consequences of that injury.

Inflammatory responses involve an interaction of blood cells, blood vessels, blood proteins, various chemical mediators and cellular and extracellular components of connective tissue.

Inflammation is divided into acute and chronic patterns, each being mediated by chemical factors derived from plasma or cells. Acute inflammation is characterized by vasodilatation and increased vascular permeability with oedematous inflammatory exudate generally including neutrophils and some macrophages, whereas macrophages, lymphocytes and often plasma cells and scarring characterize chronic inflammatory responses. Acute and chronic inflammation are not mutually exclusive, they may occur together (active chronic inflammation).

Inflammatory responses are closely associated with the process of repair which involves regeneration of parenchymal cells and/or formation of fibrous scar tissue via granulation tissue (organization).

Whilst normally beneficial, inflammation and repair may have potentially harmful outcomes.

There are many factors that can interfere with normal inflammatory and immune responses including certain diseases and medications, that make persons susceptible to certain infections and sometimes malignancies, and that impair healing.

ACUTE INFLAMMATION

Acute inflammation is a non-specific early response to tissue damage, beginning within seconds and lasting hours to several days, or longer if the cause persists. Causes include certain infections, chemicals, burns, infarction, trauma. It aims to mediate local defences, destroy any infective agents and remove debris. Numerous mediators activate and drive the acute inflammatory response.

- Mediators are released from the offending agent, damaged cells (parenchymal, endothelial, mast cells, tissue macrophages, fibroblasts) and extracellular matrix
- Vascular response: Inflammatory agents induce changes in small blood vessels
  - Transient vasoconstriction of arterioles followed by vasodilatation, vasodilatation also of venules and capillaries -> hyperemia
  - Increased endothelial permeability via endothelial cell swelling and retraction, particularly in capillaries and postcapillary venules -> passage of protein rich fluid and inflammatory cells into the damaged area (exudate), resulting in slowing of the circulation due to increased blood viscosity
  - Direct damage to vessels may allow leakage of red blood cells
  - Endothelial cells are important in inflammation.
    - Expression of adhesion molecules for leucocytes e.g. ICAM-1
    - Retraction
    - Production of mediators e.g. nitric oxide and prostacyclin which induce vasodilation and inhibit platelet aggregation
- Exudation
  - Fluid and plasma proteins including immunoglobulins, complement and fibrinogen
    - Fibrinogen is converted via coagulation cascade into fibrin – ?provides scaffold for migration of inflammatory cells, limits spread of microorganisms
Cells

- Neutrophils
  - Most numerous white cell in blood; short life (hours-days); not normally present in tissues
  - Due to changes in blood flow, neutrophils assume a peripheral position along the endothelium (margination) along which they roll then more firmly adhere. Attachment is mediated via the expression of complementary adhesion molecules on leukocytes and endothelium.
  - They then migrate into tissues (emigration or diapedesis)
  - Attracted to damaged area (chemotaxis) via chemical chemoattractants such as complement, bacterial products
  - Predominant cell from 6-72 hours
  - Stored neutrophils are released from the bone marrow and increased numbers are also produced -> neutrophilia
  - Role of neutrophils
    - Phagocytosis and killing of bacteria
    - Debridement (release proteases and toxic oxygen metabolites from lysosomes that breakdown damaged tissues) and phagocytosis of dead tissue
    - Short life span

- Macrophages:
  - Derived from blood monocytes
  - Some present in certain tissues normally e.g. liver, spleen, lung, GIT
  - Recruited by chemotactic factors, arrive a little later than neutrophils (day 2-3)
  - Phagocytosis of dead tissue and microorganisms
  - Longer lived than neutrophils
  - Release a variety of mediators
    - Chemotactic factors
    - Mediators of tissue destruction e.g. proteases
    - Mediators of bacterial killing e.g. toxic oxygen metabolites
    - Cytokines and growth factors (e.g. PDGF, FGF) which stimulate repair: fibroblast migration, proliferation, angiogenesis etc
    - Lymphocyte activating factors
    - Mediators of acute phase response in certain situations – fever etc

(N.B. Exudate arises in inflammation due to increased vascular permeability and has a high protein content. Transudate results from imbalances in hydrostatic or osmotic pressures between blood in vessel lumen and extravascular fluid, vascular permeability is normal, has a lower protein content.)

Phagocytosis

- Main phagocytic cells are neutrophils and macrophages
- Phagocytosis promoted by opsonins (e.g. complement components, immunoglobulin) bound to antigen
- Antigen/opsonin attaches to receptors on phagocyte and are internalized into cell forming the phagosome
- Phagosome combines with lysosome forming phagolysosome
- Antigen degraded/killed by lysosomal contents: reactive oxygen species, enzymes
- Neutrophils die following phagocytosis
- During the process of phagocytosis, lysosomal contents can be released into the tissues contributing to tissue damage

Local signs/symptoms of acute inflammation

- Redness
- Heat
- Pain
- Swelling
- +/- impaired movement

Types of acute inflammatory exudates

The components of the acute inflammatory exudate may vary in their relative proportions depending on the site and cause.

Suppurative or purulent

- Characterised by a large amount of neutrophil rich (purulent) exudate (e.g. acute appendicitis, certain bacterial causes of meningitis). Necrosis develops due to the action of lysosomal enzymes and oxygen derived free radicals from neutrophils +/- bacterial products resulting in the formation of pus.
- May occur in a body cavity (e.g. pleural space)
- When localised, i.e. buried in a tissue, organ or confined space, may get formation of an abscess
- Abscess: characterised by central liquefactive tissue necrosis with purulent exudate. If not drained becomes surrounded by granulation tissue and fibrosis. Caused by specific types of bacteria, described as pyogenic (e.g. *Staphylococci*). Necrosis is related to bacterial products and large numbers of neutrophils releasing toxic oxygen species and proteases from lysosomes

Serous

- Outpouring of thin fluid, emigration of white blood cells is minimal e.g. blisters, pleural effusion
Fibrinous
- On serosal surfaces of body cavities when there is underlying acute inflammation
- Generally heals by organization
- Examples: in acute appendicitis, fibrinous pericarditis post myocardial infarction, pleuritis with underlying pneumonia. In peritoneal cavity, healed fibrinous exudates (adhesions) following surgery are an important cause of bowel obstruction

Systemic effects of inflammation
Acute phase response: mediated by numerous circulating chemical mediators e.g IL1 and TNF produced by macrophages
- Fever: IL1, IL6 and TNF stimulate prostaglandin synthesis in vessels in the hypothalamus. The prostaglandins stimulate production of neurotransmitters that act to reset the body temperature thermostat at a higher level. Mechanisms activated to raise temperature include activation of the sympathetic nervous system leading to vasoconstriction in the skin and shivering to conserve heat, and alteration of the basal metabolic rate.
- Malaise
- Decreased appetite
- Tachycardia
- Increased acute phase proteins, primarily from the liver: can be detected in blood
- Neutrophilia in acute inflammation: circulating cytokines including IL1 and TNF released from macrophages stimulate increased neutrophil release and later, increased production of neutrophils, in bone marrow. The extra neutrophils released from the bone marrow are often immature, giving a “shift to the left” i.e. they have fewer lobes in their nucleus compared to mature neutrophils.

Mediators of acute inflammation
- Acute inflammation is mediated by a complex interaction of chemical substances derived from cells (neutrophils, platelets, mast cells, endothelial cells, macrophages) and plasma. Plasma derived mediators must first be activated.
- Gain entry via inflammatory exudates
- Have short lives
- Bring about vasodilation, increased vascular permeability, chemotaxis, fever, pain etc
- Various anti-inflammatory drugs have been developed to inhibit specific mediators

Mediators include:
- Vasodilatation histamine, prostaglandins, nitric oxide, bradykinin, platelet activating factor (PAF)
- Increased permeability histamine, C3a, C5a, bradykinin, leukotrienes, nitric oxide, PAF
- Neutrophil adhesion IL-1, TNF-alpha, PAF, leukotriene B4, C5a, chemokines
- Neutrophil chemotaxis C5a, leukotriene B4, bacterial components, chemokines
- Fever IL-1, TNF, prostaglandins
- Pain prostaglandins, bradykinin
- Tissue necrosis neutrophil lysosomal granule contents and free radicals generated by neutrophils

Outcomes of acute inflammation
- Depend on extent of tissue damage, the tissue affected and the type and duration of injury
- Neutrophils die, macrophages phagocytose debris, fluid and macrophages drain via lymphatics to local lymph nodes and lymphoid tissues (where an inflammatory response may also arise and specific immune responses develop)
- Variable regeneration (proliferation of parenchymal cells), organization (process of scar tissue formation via granulation tissue) and scarring, or progression to chronic inflammation
- If tissue damage is mild, connective tissue framework is intact, in sites where regeneration is possible -> resolution (complete restoration of normal structure)
- Chronic abscess formation with certain infections if not treated

HEALING
Healing is a complex phenomenon involving a number of well-orchestrated processes, including proliferation and migration of both parenchymal and connective tissue cells, synthesis of extracellular matrix and remodelling of connective tissue and parenchymal components.

Repair/healing: combination of regeneration and organization in varying proportions. May result in complete resolution, replacement of whole area by fibrous tissue (scar) or varying combinations of regeneration and scarring.

Regeneration: replacement of injured/dead parenchymal cells by cells of the same type.

Different cells have differing abilities to regenerate
- Labile: those that are continually dividing i.e. are continually in the cell cycle e.g. haematopoietic, basal GIT and epidermal epithelial cells
- Stable: parenchymal cells of most glands and organs, don't normally divide (in G0 phase of cell cycle) or divide only slowly but can enter cell cycle/divide more rapidly if necessary
- Permanent: cells that can’t divide e.g. nerve cells and cardiac muscle cells

Labile and stable cell containing tissues can regenerate. However, they can only do so in an orderly fashion if the connective tissue framework is intact. Permanent cells cannot regenerate.

If regeneration is not possible, or damage is too extensive, injured tissue and cells will be replaced by granulation tissue followed by a scar (a process called organization).

Resolution: complete restoration of normal structure without scarring. Usually occurs where there has been only mild tissue damage with intact connective tissue framework, rapid elimination of the causal agent, where local conditions favour removal of fluid and debris, in tissues able to regenerate fully. A promptly treated uncomplicated case of lobar pneumonia is an example of a condition which can completely resolve since the only cells damaged are the alveolar epithelial cells which can
regenerate. However, if there is any degree of alveolar wall destruction, the connective tissue framework will be lost and some scarring will take place.

**Organization** occurs when tissue which has been damaged is too specialized to regenerate, when there has been excessive necrosis or exudation and/or when the local conditions are unfavourable for removal of the necrotic and inflammatory material. Involves the replacement of the necrotic material and/or inflammatory exudate by granulation tissue with subsequent scar formation. This happens to some extent in most situations of tissue damage e.g. skin wounds, infarcts, abscesses, chronic ulcers.

The steps of healing take place in an orderly sequence. When there has been recent tissue damage e.g. a skin wound or an infarct in a solid organ, the initial response is acute inflammation, which begins immediately and continues for several days. If there are no complicating factors, granulation tissue then starts to form (3 or 4 days after injury) with progressive deposition of collagen and scar formation over weeks-months. Healing of a clean narrow incision of the skin is referred to as healing by primary or first intention and that of a larger defect e.g. large skin defect or burn, infarct or ulcer, is referred to as healing by secondary intention. The latter takes longer, requires more granulation tissue and a larger scar is formed. There is often also wound contraction via the action of myofibroblasts.

Granulation tissue, involved in healing, replaces areas of dead tissue with scar tissue (except in the brain). Scar tissue is dense, collagen rich connective tissue. Components of granulation tissue include newly formed thin-walled blood vessels to provide oxygen and nutrients, macrophages to remove debris and produce growth factors, lymphocytes, sometimes other chronic inflammatory cells and fibroblasts. As progressively more collagen and other connective tissue components are deposited, the initial vascular granulation tissue becomes more fibrous (fibrous granulation tissue). Eventually the inflammatory cells drain away via the lymphatics and many of the blood vessels degenerate leaving paucicellular and paucivascular scar tissue (6-8 weeks). Remodelling and strengthening may continue for months but the tissue is never as strong as it once was. Scarring may also occur as a component of chronic inflammation.

In the healing of skin by primary intention, basal cells of the epidermis begin to proliferate within 24 hours, migrate under the scab, depositing basement membrane as they move, and form a continuous but thin layer after a few days. The epidermis is completely regenerated after about 7-8 days.

With a larger wound (healing by secondary intention) a larger area of epidermis needs to be reconstituted. Epidermal cells migrate from the edge of the wound, also cells from hair follicles or sweat glands can migrate upwards to aid in epidermal regeneration. Epidermal appendages are not regenerated.

Healing is orchestrated by a variety of growth factors including epidermal growth factor, transforming growth factor alpha, vascular endothelial growth factor, platelet derived growth factor and fibroblast growth factor, and cytokines. These are produced by inflammatory cells, endothelial cells, platelets, parenchymal cells and mesenchymal cells. Actions include stimulation of angiogenesis, “chemotaxis” of fibroblasts and macrophages, promotion of connective tissue formation and stimulation of epithelial proliferation.

**Complications of wound healing**
- Hypertrophic scar
- Keloid
- Excessive contraction

**Factors modifying the inflammatory-reparative response**

**Local**
- Blood supply
- Infection
- Foreign material
- Excessive blood clot
- Movement
- Tissue in which the injury has occurred
- Size of wound
- Previous radiotherapy

**Systemic**
- Nutrition: deficiency of vitamin C, certain amino acids, zinc
- Immunosuppressant drugs e.g. corticosteroid therapy, chemotherapy
- Old age
- Diabetes - increased susceptibility to infection, narrowing of blood vessels

**Fracture healing**

Immediately following a fracture, there is a variable amount of bleeding from torn vessels. The extravasated blood coagulates into a clot. Interrupted blood supply will result in death of bone cells either side of the fracture line. Dead bone can be recognised histologically by empty lacunae. In a fracture of bone there is often also some tearing of the periosteum and the fragments can be displaced relative to each other such that ‘reduction’ of the fracture is required. To maintain the appropriate position for proper healing the bone is often either stabilized externally, usually by a plaster cast, or internally, using metal rods (placed at operation).

**Inflammatory phase**. Tissue damage excites an acute inflammatory response. Fibrin in the blood clot and acute inflammatory exudate acts as a framework for the influx of inflammatory cells, new vessels, fibroblasts and osteoprogenitor cells. Macrophages invade and remove blood clot and necrotic tissue over several weeks. Large blood clots may organise initially. Dead bone fragments are removed by osteoclasts. Degranulated platelets and inflammatory cells release interleukins and growth factors.
including PDGF, TGF-beta and FGF, which activate osteoprogenitor cells and stimulate osteoclasts and osteoblasts. By the end of the first week, osteoprogenitor cells from the periosteum differentiate into osteoblasts and chondroblasts and start forming osteoid and cartilage. Fracture repair tissue is termed callus. The callus forms both around (external callus) and within (internal callus) the fracture site.

**Reparative phase.** Callus continues to form over several weeks. The initial osteoid (non-mineralized bone) that is deposited by the osteoblasts comprises irregularly arranged collagen, which calcifies to become immature woven bone. Much of the new bone forms directly, but in some fractures, some develops through the process of endochondral ossification of cartilage, formed by osteoprogenitor cells that have differentiated into chondroblasts. More mobile fractures develop more cartilage, and more callus. Callus helps stabilize the fracture site but it is still not very strong e.g. for weight bearing. Callus can be seen on x-ray.

**Remodelling phase.** Gradually, mature lamellar bone forms in the fracture gap reconstituting the dense cortical bone, and the no longer needed external callus is gradually removed. Medullary callus is remodelled and the marrow cavity restored. New bone is organised along lines of stress and mechanical forces. Remodelling and strengthening can continue for months or even years. The latter describes the healing of a simple fracture of a long bone. There may be slight variations in the healing of other types of fracture.

**Complications of fracture include**

Haemorrhage, soft tissue (muscles, tendons, ligaments etc) and nerve injury (stretching or tearing), bone marrow or fat embolism, infection, complications of surgery, complications related to plaster cast, DVT.

**Pathological fracture**

A pathological fracture is one which occurs in diseased bone. It often requires less than normal stresses for fracturing. Causes include: osteoporosis, primary or secondary bone tumours, Paget’s Disease of bone, hyperparathyroidism, osteogenesis imperfecta.

**Factors influencing fracture healing**

Local: movement of bone ends, poor alignment, poor blood supply (including damage to vessels during the traumatic event), large haematoma, multiple bone fragments (i.e. comminuted fracture), infection, underlying bone disease. Significant disruption of the periosteum or of the blood supply to the medullary cavity lead to impaired formation of external and internal callus respectively.

**General:** old age, poor nutrition

### CHRONIC INFLAMMATION

Chronic inflammation is a process in which ongoing inflammation and tissue damage proceed at the same time as attempts at healing, seen as scar tissue. It aims to eradicate and/or contain the harmful agent and heal areas of tissue damage. It can persist, sometimes for years, until the damaging stimulus is eradicated. Chronic inflammation may follow acute inflammation because of persistence of the injurious agent or interference with the normal process of healing. Changes are superimposed upon and/or replace those of acute inflammation. However, probably more commonly it has an insidious onset e.g. with certain infections (e.g. TB), in autoimmune disease (e.g. rheumatoid arthritis) or from repeated or prolonged exposure to potentially toxic (e.g. asbestos) and sometimes unknown agents (e.g. atherosclerosis).

The cells of chronic inflammation are ‘mononuclear’ (compared to the polymorphonuclear neutrophils of acute inflammation) i.e. T and B lymphocytes, plasma cells, macrophages. A variable amount of scar tissue is present and in some situations ongoing acute inflammation is seen.

**Features of chronic inflammation**

**Cells**

- Lymphocytes
  - Made in the bone marrow
  - Long-lived
  - Some present normally in certain tissues
  - Others attracted by chemotactic factors
  - T and B lymphocytes become activated/differentiated following antigen recognition in lymphoid tissues or areas of chronic inflammation
  - Types:
    - T cells
      - CD4 helper cells: activated and secrete cytokines when presented with processed antigen by antigen presenting cells (e.g. macrophages and dendritic cells) in association with MHC class II molecules, involved in regulation of immune responses.
      - CD8 cytotoxic cells: can directly destroy cells (e.g. tumour and virus infected) following presentation with antigen in association with MHC class I molecules.
    - B cells: differentiate into plasma cells (in areas of lymphoid tissue known as germinal centers) in appropriate circumstances on binding of antigen to surface IgM
  - Natural killer cells: able to destroy a variety of cells in a non-specific manner

- Plasma cells: produce antibody

- Macrophages:
  - Derived from blood monocytes
  - Many present normally in tissues: mononuclear phagocyte system
• Recruited by chemotactic factors
• Activated by interferon (IFN)-gamma, bacterial toxins, tissue factors
• May form multinucleated giant cells
• Phagocytic
  • Clear up debris following acute inflammation and in healing
  • Involved in effector phase of humoral immunity
• Role in delayed type hypersensitivity
• Present antigens to CD4 T cells
• Secrete mediators of inflammation, tissue damage and repair

Variable granulation tissue
• Involved in repair and results in scarring (dense, collagen rich connective tissue)
• Contains newly formed thin-walled blood vessels, macrophages (which remove debris), lymphocytes, other chronic inflammatory cells and fibroblasts which make collagen and other connective tissue components.

Variable scarring
Sometimes necrosis
Sometimes persisting acute inflammation

Mediators of chronic inflammation include:
From CD4 T lymphocytes
• Interferon-gamma: activates macrophages
• Interleukin-2: stimulates T lymphocyte proliferation
From macrophages
• Chemotactic factors
• Mediators of tissue destruction e.g. proteases
• Mediators of bacterial killing e.g. toxic oxygen metabolites
• Cytokines and growth factors (e.g. PDGF, FGF which stimulate repair: fibroblast proliferation, angiogenesis etc
• Lymphocyte activating factors
• Mediators of acute phase response – fever etc

Granulomatous inflammation
Is a specific type/pattern of chronic inflammation characterised by the presence of activated macrophages that have become either ‘epithelioid’ (resembling epithelial cells) in appearance or multinucleate. Necrosis is sometimes present. The specific histologic features of granulomatous inflammation vary depending on the cause e.g. there is no necrosis in sarcoidosis. Recognition of this pattern of inflammation in a biopsy is important diagnostically. This inflammation is driven by CD4 T lymphocytes (delayed type hypersensitivity) in response to certain persistent or non-degradable antigens. Granulomatous inflammation is initiated by a variety of infectious (e.g. TB, leprosy, certain fungal and parasitic infections) and non-infectious stimuli (e.g. in sarcoidosis, Crohn’s disease, reactions to endogenous or exogenous irritants (e.g. suture, keratin, urate crystals) and certain malignancies (e.g. lymphoma)). A granuloma is a small discrete well-defined aggregate of these modified macrophages seen histologically.

EXCEPTIONS TO TYPICAL ACUTE AND CHRONIC INFLAMMATORY RESPONSES
Viral infections: lymphocytes predominate from early on
Hypersensitivity reactions and parasitic infections: many eosinophils

Resources used include:
Robbins and Cotran Pathologic Basis of Disease (7th ed.) by Kumar, Abbas and Fausto (Elsevier Saunders).
Rubin’s Pathology, Clinicopathologic Foundations of Medicine (5th ed., 2008) by Rubin and Strayer (Lippincott Williams and Wilkins)