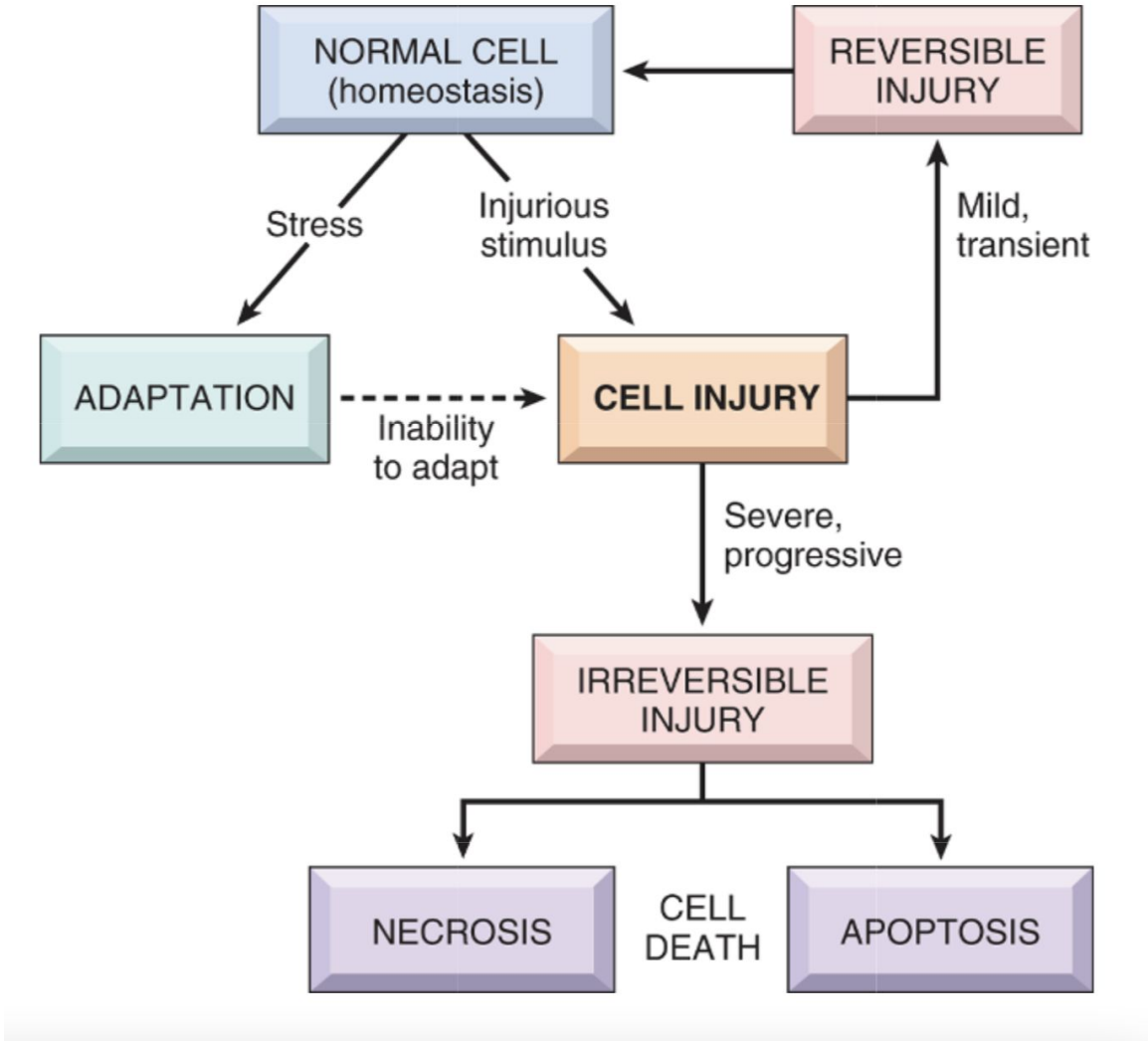
Primary Cast Episode 6 - Cellular Injury and Inflammation

Host - Dr. Charlotte Durand
Guest - Dr. Janet Boromeo



Great diagram from Robbins that Janet referred to for setting up your ‘structure’

1. **Apoptosis**
   What is apoptosis?
   - Programmed cell death
   - A process that removes degraded or unneeded cells, prevents excess growth
   - Tightly controlled
   - Cells activate degradation enzymes, chromatin is condensed, the cell contents are degraded within cytoplasmic blebs forming apoptotic bodies, and the cell shrinks
   - Cell membrane remains intact
   - Not an inflammatory process

List some important stimuli for apoptosis

Physiological

- Developmental atrophy (embryogenesis)
- Loss of growth stimulation (such as endometrial cells during menstruation)
- Cell death induced by cytotoxic T cells
- Elimination of potentially harmful self reactive lymphocytes

Pathological

- Excessive DNA damage (p53 build up)
- Unfolded protein build up
- Cell death secondary to radiation or cytotoxic injury
- Cells displaying harmful characteristics
- Viral infections such as hepatitis

2. **Necrosis**

Describe the cellular changes in necrosis

- Irreversible injury
- Swollen cells
- Myelin figures
- Nucleus may fade, shrink and fragment
- Organelle and cell membrane disruption with release of contents
- Adjacent or surrounding inflammation

What are the patterns of tissue necrosis?

- Coagulative - architecture of tissue preserved
- Liquefactive - digestion of tissue into a viscous liquid mass
- Fibrinoid - a microscopic feature of antigen/antibody complexes in vessel walls
- Caseous - friable white (such as in TB)
- Gangrenous - typically a type of coagulable necrosis applied to a limb, may have superimposed liquefactive necrosis
- Fat necrosis - focal area of fat destruction

TABLE 1–2 Features of Necrosis and Apoptosis

Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome-size fragments
Plasma membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic, means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage

Figure from Robbins that we talked about ↑

3. **Atrophy**

What is atrophy?

Decrease in the size of an organ or tissue resulting from a decrease in cell size and number. Cal be physiological or pathological.

What are the causes of atrophy?

- Decreased workload (such as immobilisation of a limb in plaster)
- Denervation
- Diminished blood supply (such as arterial occlusion)
- Inadequate nutrition (such as marasmus)
- Loss of endocrine stimulation
- Ageing
- Pressure

What are the mechanisms underlying atrophy?

- Decreased protein synthesis
- Increased protein degradation
- May be accompanied by increased autophagy, where a cell consumes its own components for energy and nutrients

4. **Calcification**

Please describe the 2 different forms of pathological calcification and give an example of each.

- **Dystrophic calcification**
    - Normal serum calcium
    - Occurs in necrotic or damages/dying tissue
    - Examples: atherosclerosis, calcific aortic stenosis, tuberculous nodes
- **Metastatic calcification**
    - Abnormally raised calcium
    - Occurs in normal tissues
    - Examples: nephrocalcinosis, pulmonary calcinosis, gastric mucosal calcification

What are the different causes of hypercalcaemia?

- Increased parathyroid hormone (PTH) secretion + bone resorption, seen in hyperparathyroidism
- Destruction of bone tissue - skeletal mets, myeloma, Paget's disease
- Vitamin D related disorders - sarcoidosis, hypervitaminosis D
- Renal failure causing secondary hyperparathyroidism and phosphate retention

5. **Hyperplasia**

   What is hyperplasia?

   The increase in the number of cells in an organ or tissue. Usually associated with increase mass.

   What are the different types of hyperplasia? Please give some examples.

   - Physiologic:
     - Hormonal i.e. breast tissue development during puberty and pregnancy
     - Compensatory: post partial hepatectomy, skeletal muscle with increased workload
   - Pathological:
     - Excess hormones i.e. Benign prostatic hyperplasia or dysfunctional uterine bleeding
     - Viral infection i.e. papillomavirus

6. **Hypertrophy**

   What is hypertrophy?

   Increased size of a tissue due to increased cell size. Arises from increased synthesis of cell structural components

   What are the types of hypertrophy?

   May be physiological or pathological depending on either increased functional demand or specific hormonal stimulation.
   Cell hypertrophy can occur in dividing or non dividing cells

   Give some examples of each type of hypertrophy

   Physiological - skeletal muscles with exercise, the uterus during pregnancy, breast tissue during lactation
   Pathological - heart in chronic hypertension

7. **Metaplasia**

   What is metaplasia?

   Replacement of one normal cell type with another normal cell type. May be adaptive or pathological. Can be reversible.

   Describe some examples

   Columnar to squamous due to chronic respiratory irritation i.e. smoking
   Squamous to columnar i.e. in Barretts oesophagus
   Connective tissue change in myositis ossificans - muscle to bone or cartilage

What are the potential outcomes of metaplasia?

- Malignant transformation
- Reversibility/resolution
- Ongoing change

What is the mechanism underlying metaplasia?

- Reprogramming of epithelial stem cells or undifferentiated mesenchymal cells
- Involves signals from cytokines, growth factors, cellular matrix components, genes and DNA methylation

8. **Steatosis**

What is steatosis?

Abnormal accumulation of triglycerides within parenchymal cells

Which organs are commonly involved?

Liver, kidneys, muscle

What are the causes of hepatic steatosis?

- Alcohol excess
- Toxins
- Protein malnutrition
- Obesity
- Anoxia
- Starvation

9. **Cell Injury**

Describe the sequence of events that occur in reversible ischaemic cellular injury

- ATP depletion due to decreased oxidative phosphorylation
- Failure of the sodium/potassium pump, leading to K efflux and Na influx
- Cell swelling, leads to
- Calcium influx
- Detachment of ribosomes from the ER
- Cytoskeleton changes: loss of microvilli, bleb formation, myelin figures from degenerating cell membrane
- Mitochondrial swelling

List the morphological changes of irreversible cell injury

- Severe mitochondrial swelling
- Extensive damage to plasma membrane
- DNA/protein damage and leakage of proteins such as AST/ALT or troponin
- Lysosomal swelling and rupture
- Necrosis or apoptosis

Describe reperfusion injury

Further cell death in ischaemic tissues following restoration of blood flow. Occurs during MI and stroke with reperfusion therapy, as well as in ischaemic bowel due to collateral blood supply.

4 major mechanisms

- Reactive oxygen species
    - Generated from the incomplete reduction of O2 by damaged mitochondria in affected tissue, PLUS action of oxidases from damaged cells and incoming leukocytes.
- Inflammation
    - Via cells and cytokines.
    - Damaged cells release adhesion molecules that attract neutrophils.
    - This inflammation causes additional injury.
- Complement Activation
    - IgM deposits in ischaemic tissues and when reperfusion occurs, complement proteins bind, causing inflammation
- Mitochondrial permeability transition pore
    - Pore in mitochondria that opens after reperfusion.
    - Stimulated by oxidative stress via ROS
    - Decreases mitochondrial function
    - Uncoupling of oxidative phosphorylation
    - Matrix swelling
    - Prevents recovery of ATP generation
    - Pivotal point in cell death

What is a free radical?

Chemical species with a single unpaired electron in outer orbit e.g. reactive oxygen species: super oxide, hydrogen peroxide, hydroxyls

What are the pathological effects of free radicals?

- Overall cause necrosis or apoptosis and can stimulate production of degrading enzymes.
- Directly cause:
    - Lipid peroxidation (plasma membrane or organelle damage)
    - Oxidation of proteins (affecting the protein structure i.e. enzymes)
    - DNA lesions (breaks in DNA or cross linkages)

10. Acute inflammation

Please describe the major components of acute inflammation

- Small vessel dilatation - leading to increased blood flow
- Increased vascular permeability - enabling plasma proteins and leukocytes to leave the circulation

- Leukocyte emigration - from the microcirculation towards the focus of injury, and activation to eliminate the offending agent

What are the mechanisms responsible for increased vascular permeability?

- Contraction of endothelial cells - resulting in increased inter-endothelial spaces (most common)
- Direct endothelial injury - resulting in endothelial cell necrosis and detachment (such as in burns, as a result of toxins or by direct action of neutrophils)
- Transcytosis - increased transport of fluids and proteins through the endothelial cell itself.

What are the different types of acute inflammation?

- *Serous:* thin fluid from plasma or mesothelial lining cells e.g. burns, effusions
- *Fibrinous:* more severe injuries and greater vascular permeability allows larger molecules, such as fibrin, to pass into the space.
- *Suppurative/purulent:* large amounts of pus or purulent exudates from neutrophils, necrotic cells and oedema. Depends on organism type and location.
- *Ulcerative:* local defect in an organ or tissue

What are the outcomes of acute inflammation?

- Complete resolution with or without scarring
- Abscess formation
- Fibrosis
- Chronic inflammation

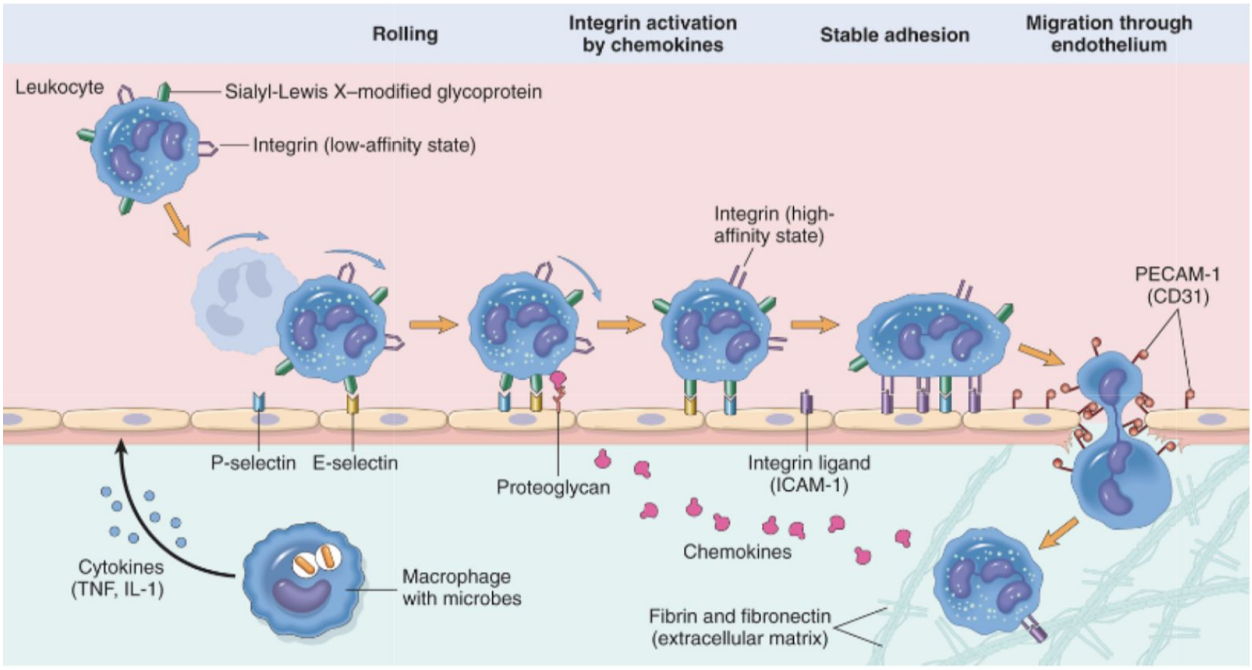
11. Leukocytes in inflammation

- What leukocytes are involved in acute inflammation?
- Neutrophils in first 6-24 hours - may last longer in pseudomonas infections
- Monocytes at 24-48 hours
- Lymphocytes in viral
- Eosinophils in hypersensitivity reactions

How are leukocytes delivered to the site of injury?

This is a multistep process, mediated and controlled by adhesion molecules and chemokines.

- *Margination* - occurs when leukocytes adopt a peripheral position along the vessel wall. Rolling (transient adherence mediated by selectins), activation and attachment (mediated by integrins) to the epithelium.
- *Transmigration or diapedesis* - occurs when leukocytes cross the endothelium. Migration occurs through the interendothelial spaces, typically in the post capillary venules.
- *Chemotaxis* - leukocytes move towards the site of injury along a chemical gradient.



What are the mediators that aid chemotaxis?

Common chemo-attractants include

- Exogenous - mostly bacterial products/proteins/peptides
- Endogenous - cytokines (IL-8) complement (C5a) arachidonic acid metabolites (leukotriene B4)
- They all bind to specific receptors and promote polymerisation of actin

12. Mediators

What stimuli cause production of mediators of inflammation?

Substances released from necrotic cells, microbial products, cell injury, mechanical irritation

What are the chemical mediators of acute inflammation and what are their actions?

- Histamine: vasodilation, increased vascular permeability, endothelial activation
- Prostaglandins: vasodilation, increased vascular permeability
- Leukotrienes: increased vascular permeability, chemotaxis, WBC adhesion and activation
- Platelet activating factor: vasodilation, increased vascular permeability, chemotaxis, WBC adhesion, degranulation
- Complement: WBC chemotaxis, activation and vasodilation
- Cytokines (TNF, IL-1): endothelial activation, fever, pain, hypotension, decreased vascular resistance

- Chemokines : chemotaxis, WBC activation
- Kinins: vascular permeability, vasodilation, paion, smooth muscle contraction

13. Chronic Inflammation

What are the characteristics of chronic inflammation?

- Inflammation for a prolonged period of time
- Characterised by macrophages, lymphocytes and plasma cells
- Simultaneous active inflammation/tissue destruction and attempts at repair by connective tissue fibrosis

What cell types are present in chronic inflammation?

- Macrophages
- Lymphocytes
- Eosinophils
- Plasma cells
- Mast cells
- Multinucleate giant cells

What processes mediate the persistent accumulation of macrophages seen in chronic inflammation?

- Continued recruitment of monocytes - due to continued expression of adhesion molecules and chemotactic factors
- Local proliferation of macrophages
- Immobilisation of macrophages at the site of injury (via migration inhibition factor)

What products are released by activated macrophages in chronic inflammation?

- Products associated with tissue injury
    - Toxic O2 metabolites
    - Proteases
    - Neutrophil chemotactic factors
    - Coagulation factors
    - AA metabolites
    - Nitric oxide
- Products associated with fibrosis
    - Growth factors (PDGF, FGF, TGF)
    - Fibrogenic cytokines
    - Angiogenesis factors
    - Remodelling collagenases

What clinical conditions cause chronic inflammation?

- Persistent infection i.e. tuberculosis, abscess, syphilis, empyema, osteomyelitis
- Prolonged exposure to an agent i.e. exogenous foreign body, persistent trauma, silica leading to silicosis or lipid accumulation leading to atherosclerosis
- Autoimmune i.e. rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, SLE.

14. Complement System

What is the complement system?

- A plasma protein system involved in immunity against microbes.
- Complement proteins numbered C1- C9 are present in plasma in inactive forms

What are the pathways by which complement activation occurs?

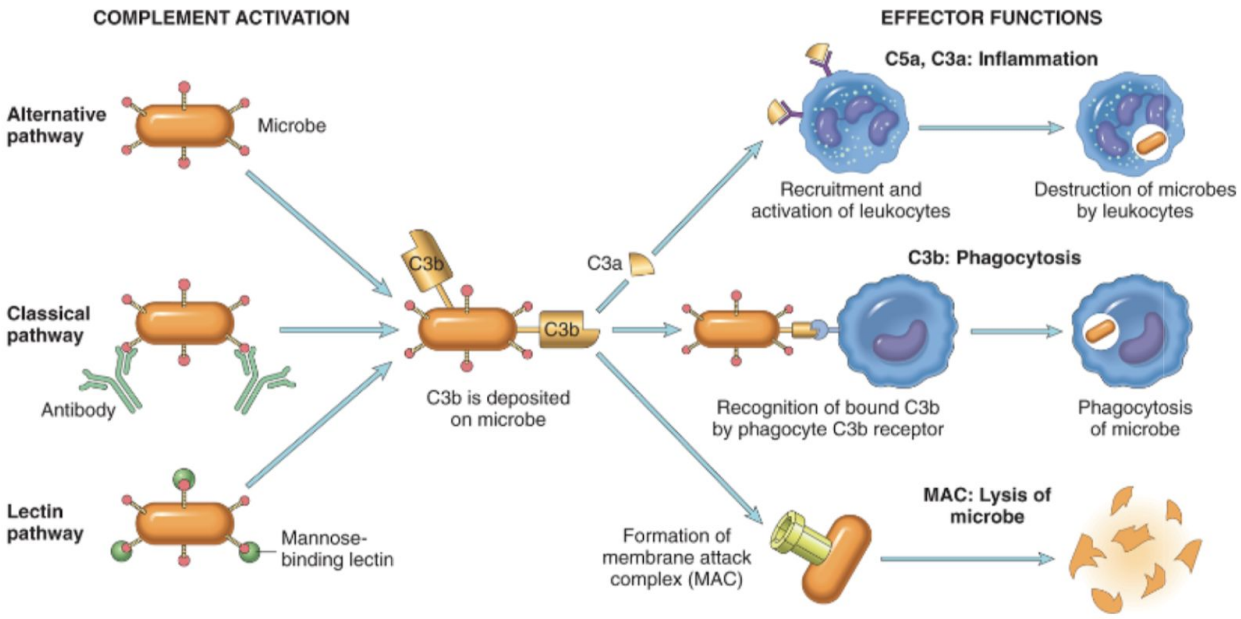
- There are 3 pathways
- *Classical pathway:* involving antigen-antibody complex
- *Alternate pathway:* triggered by microbial surface molecules e.g. endotoxin. No antibody involvement
- *Lectin pathway:* plasma mannose binding lectin binds to carbohydrate on microbe
- All pathways result in cleavage and activartion of C3 (the most important and abundant complement component)

What are the most important complement components?

- C3 and C5

How do activated complement products mediate acute inflammation?

- Vascular effects - increased permeability, vasodilation via C3a and C5a mediated histamine release from mast cells
- Leukocyte adhesion chemotaxis and activation via C5a
- Phagocytosis vis C3b which acts as an opsonin on the microbe and leads to phagocytosis
- Cell lysis by the membrane attack complex (MAC) composed multiple C9 molecules



The complement system diagram from Robbins that we referenced towards the end