
Lecture 1
Cellular injury and responses
Causes of cell injury

- Oxygen deprivation
- Chemical agents
- Infectious agents
- Immunologic reactions
- Genetic factors
- Nutritional imbalances
- Physical agents
- Aging
Oxygen deprivation

• Hypoxia – most common cause of cell injury

Hypoxia causes a loss of ATP production secondary to oxygen deficiency and can be caused by ischemia, cardiopulmonary failure, or decreased oxygen-carrying capacity of the blood.
Causes of cell injury

Oxygen deprivation in tetralogy of Fallot
Causes of cell injury

Oxygen deprivation in tetralogy of Fallot
Chemical agents

- Hypertonic concentration of salt – deranging electrolyte homeostasis
- Poisons – arsenic, cyanide, or mercuric salts
- Insecticides and Herbicides
- Air pollutant – carbon monoxide
- Occupational hazard – asbestos
- Alcohol and Narcotic drugs
Infectious agents

can injure cells directly, or indirectly, via toxin production or host inflammatory response

- Parasites
- Fungi
- Bacteria
- Rickettsiae
- Viruses
Immunologic reactions

• Anaphylactic reaction to foreign protein or drug
• Reactions to endogenous self-antigens – autoimmune diseases
Immunologic reactions

Causes of cell injury
Genetic factors

- Congenital malformation – Down syndrome
- Decreased life of red blood cell – Thalassemia, Sickle cell anemia
- Inborn errors of metabolism
Genetic factors

Causes of cell injury

A Normal red blood cells

- Normal red blood cell (RBC)
- RBCs flow freely within blood vessel

B Abnormal, sickled, red blood cells (sickle cells)

- Sickle cells blocking blood flow
- Sticky sickle cells

- Cross-section of RBC
- Normal hemoglobin

- Cross-section of sickle cell
- Abnormal hemoglobin form strands that cause sickle shape
Nutritional imbalances

- Protein-calorie deficiencies
- Vitamin deficiencies
- Anorexia nervosa
- Excesses of lipids – Obesity, Atherosclerosis
- Metabolic diseases – Diabetes
Causes of cell injury

Nutritional imbalances
Physical agents

- Mechanical trauma
- Extremes of temperature – burns, deep cold
- Radiation
- Electric shock
Physical agents

Electrical burn of the skin
Aging

“Individuals age because their cells age”
Factors that affect cell injury

1. Type, duration and severity of injury.
2. Type of injured tissue, its adaptability and genetic makeup e.g.:
   - Brain tissue is very sensitive to hypoxia (2-5 min.)
   - Myocardium 1-2 hours
   - Skeletal muscles can adapt hypoxia for 2-6 hours
   - Fibroblasts – hours
Important targets of cell injury

• Aerobic respiration –  
  – ATP depletion or decreased synthesis.
• Cell membranes - plasma membranes, mitochondrial, lysosomal and other organelle membranes.
• Protein synthesis.
• Cytoskeleton.
• Genetic apparatus.
Mechanisms of cell injury

MITOCHONDRIAL DAMAGE

- ↓ ATP
  - Multiple downstream effects
- ↑ ROS
  - Damage to lipids, proteins, DNA

ENTRY OF Ca²⁺

- Mitochondrial permeability
- Activation of multiple cellular enzymes

MEMBRANE DAMAGE

- Plasma membrane
  - Loss of cellular components
- Lysosomal membrane
  - Enzymatic digestion of cellular components

PROTEIN MISFOLDING, DNA DAMAGE

- Activation of pro-apoptotic proteins
Oxidative phosphorylation
Mitochondrial damage

Mechanisms of cell injury

- Reduced O₂ supply
- Toxins
- Radiation

Mitochondrial damage or dysfunction

- Reduced ATP generation
- Increased production of ROS

Multiple cellular abnormalities

Necrosis

Survival signals
- DNA, protein damage

Pro-apoptotic proteins
- Anti-apoptotic proteins

Leakage of mitochondrial proteins

Apoptosis
Mitochondrial damage: depletion of ATP
Mitochondrial damage: depletion of ATP

- **Mitochondria** - reduced oxidative phosphorylation.
- **Cell membrane** - reduced sodium pump.
- **Sodium** and **water** enter the cell; **potassium** exits.
- **Endoplasmic reticulum** dilates, the cell swells, blebs appear.
- **Anaerobic glycolysis** occurs with loss of glycogen, accumulation of lactic acid, acid pH which interferes with enzymes.
- Failure of the **calcium pump** leads to influx of Ca++ into the cell, activate various enzymes to the detriment of the cell.
- **ER** loses ribosomes and protein synthesis falls - structural proteins (membranes, cytoskeleton) and enzymes.
- **Misfolded proteins** lead to the unfolded protein response which may further injure the cell.
Mitochondrial damage: oxidative stress

Mechanisms of cell injury

Production of ROS:
- Superoxide ($O_2^-$)
- Hydrogen peroxide ($H_2O_2$)
- Hydroxyl radical (•OH)

Conversion to $H_2O_2$ by SOD
Decomposition to $H_2O$ by glutathione peroxidase, catalase

Pathologic effects:
- Lipid peroxidation → Membrane damage
- Protein modifications
- DNA damage → Mutations
- Breakdown, misfolding

Radiation
Toxins
Reperfusion
Mitochondrial damage: oxidative stress

- Free radicals have a **single unpaired electron** in the outer orbit. They are **highly reactive** with adjacent molecules.
- Are usually derived from oxygen to produce reactive oxygen species, superoxide, hydroxyl radicals, H2O2, etc.
- Are normally produced during cellular respiration. Protective molecules include superoxide dismutase, glutathione peroxidase, vitamin E, vitamin C, catalase.
- Produced in excess, they react with, and damage proteins, lipids, carbohydrates, nucleic acids.
- These damaged molecules may themselves be reactive species with a chain reaction being set up with widespread damage.
Mitochondrial damage: oxidative stress

- In addition to oxygen-derived free radicals, nitric oxide (NO) can act as a free radical and be converted to an even more reactive anion.
- Iron and copper catalyze free radical formation and are thus important in the generation of reactive oxygen species.

**Fenton reaction**

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \]

- Binding to molecules such as transferrin, ferritin and ceruloplasmin is protective.
- Free radicals cause lipid peroxidation in cell membranes, oxidation of amino acids and proteins resulting in fragmentation, and protein-protein cross linkages. Altered proteins are acted on by the proteosomes with further cell damage.
Mitochondrial damage: oxidative stress

- Free radicals may be a common pathway for most types of cell damage, particularly oxygen-derived free radicals (oxidative stress).

Some examples are:

- oxygen toxicity, ischaemia/reperfusion injury, radiation injury (hydrolyses H2O to OH & H), metabolism of drugs, toxins, pollutants (eg Paracetamol to reactive metabolite; CCl4 to CCl3, cigarette smoke);
- leukocyte killing of bacteria or in non-bacterial inflammations, release of iron in haemorrhages enhances oxidative stress (important in CNS),
- lipid peroxidation of low-density lipoproteins in atherosclerosis, cancer production (damage to DNA), ageing.

Therapies for combating oxidative stress are available for prevention or treatment with antioxidants and/or free-radical scavengers.
Entry of Ca\(^{2+}\)

Mechanisms of cell injury

- Injurious agent
  - Ca\(^{2+}\) Extracellular Ca\(^{2+}\)
  - Mitochondrion
  - Smooth ER
  - Increased cytosolic Ca\(^{2+}\)
  - Activation of cellular enzymes
    - Phospholipase
      - ↓ Phospholipids
      - MEMBRANE DAMAGE
    - Protease
      - Disruption of membrane and cytoskeletal proteins
      - NUCLEAR DAMAGE
    - Endonuclease
    - ATPase
  - ↑ Mitochondrial permeability transition
  - ↓ ATP
Entry of Ca\(^{2+}\)

Influx of calcium to the cytosol comes from the extracellular fluid and stores in mitochondria and endoplasmic reticulum.

Ca\(^{++}\) activates phospholipases (damages cell membranes), proteases (damages cell membranes and cytoskeleton) and endonucleases (damages DNA).

This is one of the main mechanisms of cell death, either through severe damage to membranes of lysosomes and leakage of lysosomal enzymes or triggering apoptosis.

Occurs particularly in hypoxia and ischaemia and with certain toxins. Preventing the rise in Ca\(^{++}\) or restoring to normal levels prevents cell death.
Membrane damage

Mechanisms of cell injury

- Reactive oxygen species
- Lipid peroxidation
- Phospholipid reacylation/synthesis
- Phospholipase activation
- Phospholipid degradation
- Protease activation
- Cytoskeletal damage
- Lipid breakdown products
- ATP
- O₂
- Cytosolic Ca²⁺
Membrane damage

- **Mitochondria** –
  - mitochondrial permeability transition;
  - this non-selective pore may be reversible or become permanent leading to cell death.
  - Leakage of cytochrome c can trigger apoptosis.

- **Plasma membrane** –
  - mechanisms include those occurring with hypoxia/ischaemia and free radicals, but also
  - immune mechanisms as with complement activation and
  - perforin from lymphocyte attack on cells infected with a virus.

- **All membranes may be damaged and ruptured by**
  - mechanical force as in trauma, or by
  - ice crystals as in extreme cold.

Damage to lysosomal membranes can lead to cell death by necrosis.
Protein misfolding, DNA damage

Mechanisms of cell injury

Figure 1–24 The unfolded protein response and ER stress. A, In healthy cells, newly synthesized proteins are folded with the help of chaperones and are then incorporated into the cell or secreted. B, Various external stresses or mutations induce a state called ER stress, in which the cell is unable to cope with the load of misfolded proteins. Accumulation of these proteins in the ER triggers the unfolded protein response, which tries to restore protein homeostasis; if this response is inadequate, the cell dies by apoptosis.
Characteristics of reversible cell injury

- Decreased synthesis of ATP by oxidative phosphorylation.
- Decreased function of Na+K+ ATPase membrane pumps, which in turn causes influx of Na+ and water, efflux of K+, cellular swelling (hydropic swelling), and swelling of the endoplasmic reticulum.
- The switch to glycolysis results in depletion of cytoplasmic glycogen, increased lactic acid production, and decreased intracellular pH.
- Decreased protein synthesis leads to detachment of ribosomes from the rough endoplasmic reticulum.
- Plasma-membrane blebs and myelin figures may be seen
Characteristics of irreversible cell injury

• Severe membrane damage plays a critical role in irreversible injury, allows a massive influx of calcium into the cell, and allows efflux of intracellular enzymes and proteins into the circulation.

• Marked mitochondrial dysfunction produces mitochondrial swelling, large densities seen within the mitochondrial matrix, irreparable damage of the oxidative phosphorylation pathway, and an inability to produce ATP.

• Rupture of the lysosomes causes release of lysosomal digestive enzymes into the cytosol and activation of acid hydrolases followed by autolysis.
REVERSIBLE \rightarrow IRREVERSIBLE \rightarrow DEATH \rightarrow EM \rightarrow LIGHT MICROSCOPY \rightarrow GROSS APPEARANCES