General Pathology
Overview of Cell Injury and Cell Death

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Overview of Cell Injury and Cell Death

Injury may progress through a reversible stage and culminate in cell death.
1- Reversible cell injury. In early stages or mild forms of injury, the functional and morphologic changes are reversible if the damaging stimulus is removed.

The hallmarks of reversible injury are reduced oxidative phosphorylation with resultant depletion of energy stores (ATP), and cellular swelling caused by changes in ion concentrations and water influx. Various intracellular organelles, may also show alterations.
2- Cell death:

When damage to membranes is severe, lysosomal enzymes enter the cytoplasm and digest the cell, and cellular contents leak out, resulting in **necrosis**. In situations when the cell's DNA or proteins are damaged beyond repair, the cell kills itself by **apoptosis**, which is characterized by nuclear dissolution, fragmentation of the cell without complete loss of membrane integrity, and rapid removal of the cellular debris.
Whereas necrosis is always a pathologic process, apoptosis serves many normal functions and is not necessarily associated with cell injury. Cell death is also sometimes the end result of autophagy.

Both apoptosis and necrosis may be seen in response to the same insult, such as ischemia, perhaps at different stages. Apoptosis can progress to necrosis, and cell death during autophagy may show many of the biochemical characteristics of apoptosis.
Causes of Cell Injury

Oxygen Deprivation

**Hypoxia**: is a deficiency of oxygen, which causes cell injury by reducing aerobic oxidative respiration. Causes of **hypoxia** include: Ischemia, Anemia, Hypoxemia, Carbone monoxide poisoning and severe blood loss.

Depending on the severity of the hypoxic state, cells may adapt, undergo injury, or die.
Physical Agents.

Capable of causing cell injury include mechanical trauma, extremes of temperature (burns and deep cold), sudden changes in atmospheric pressure, radiation, and electric shock.

Chemical Agents and Drugs.

The list of chemicals that may produce cell injury defies compilation. Simple chemicals such as glucose or salt in hypertonic concentrations may cause cell injury directly or by deranging electrolyte balance in cells. Even oxygen at high concentrations is toxic.
**Infectious Agents.**
These agents range from the submicroscopic viruses to the large tapeworms. In between are the rickettsiae, bacteria, fungi, and higher forms of parasites.

**Immunologic Reactions.**
Injurious reactions to endogenous self-antigens are responsible for several autoimmune diseases. Immune reactions to many external agents, such as microbes and environmental substances, are also important causes of cell and tissue injury.
Genetic Derangements.
Genetic abnormalities may result in a defect as severe as the congenital malformations associated with Down syndrome, caused by a chromosomal anomaly, or as subtle as the decreased life span of red blood cells caused by a single amino acid substitution in hemoglobin in sickle cell anemia.

Nutritional Imbalances.
Protein-calorie deficiencies cause a large number of deaths, chiefly among underprivileged populations. Deficiencies of specific vitamins are found throughout the world. Ironically, nutritional excesses have also become important causes of cell injury.
Cells may become rapidly nonfunctional after the onset of injury, although they are still viable, with potentially reversible damage; a longer duration of injury may eventually lead to irreversible injury and cell death. Note that irreversible biochemical alterations may cause cell death, and typically this precedes ultrastructural, light microscopic, and grossly visible morphologic changes.
**Reversible injury** is characterized by generalized swelling of the cell and its organelles; blebbing of the plasma membrane; detachment of ribosomes from the ER; and clumping of nuclear chromatin, associated with decreased generation of **ATP**, loss of cell membrane integrity, defects in protein synthesis, cytoskeletal, and DNA damage.

**Within limits, the cell can repair these derangements and, if the injurious stimulus abates, will return to normalcy.**
Persistent or excessive injury, however, causes cells to pass the rather nebulous "point of no return" into irreversible injury and cell death.

Different injurious stimuli may induce death by necrosis or apoptosis. Severe mitochondrial damage with depletion of ATP and rupture of lysosomal and plasma membranes are typically associated with necrosis.
## Features of Necrosis and Apoptosis

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

Two features of reversible cell injury can be recognized under the light microscope: cellular swelling and fatty change. Cellular swelling appears whenever cells are incapable of maintaining ionic and fluid homeostasis and is the result of failure of energy-dependent ion pumps in the plasma membrane.
Fatty change occurs in hypoxic injury and various forms of toxic or metabolic injury. It is manifested by the appearance of lipid vacuoles in the cytoplasm. It is seen mainly in cells involved in and dependent on fat metabolism, such as hepatocytes and myocardial cells.
Morphology

Cellular swelling is the first manifestation of almost all forms of injury to cells. This pattern of nonlethal injury is sometimes called **hydropic change or vacuolar degeneration**.

A- Normal kidney tubules with viable epithelial cells.

B- Early (reversible) ischemic injury showing surface blebs, increased eosinophilia of cytoplasm, and swelling of occasional cells.
Morphologic changes in **reversible** cell injury and **necrosis**

**A-** Normal kidney tubules with viable epithelial cells.

**B-** Early (reversible) ischemic injury showing surface blebs, increased eosinophilia of cytoplasm, and swelling of occasional cells.

**C-** Necrosis (irreversible injury) of epithelial cells, with loss of nuclei, fragmentation of cells, and leakage of contents.

(Courtesy of Drs. Neal Pinckard and M.A. Venkatachalam, University of Texas Health Sciences Center, San Antonio, TX.)
Ultrastructural features of reversible and irreversible cell injury (necrosis) in a rabbit kidney.

A- Electron micrograph of a normal epithelial cell of the proximal kidney tubule. **Note abundant microvilli (mv)** (lining the luminal surface (L).

B- Epithelial cell of the proximal tubule showing early cell injury resulting from reperfusion following ischemia. The microvilli are lost; blebs have formed. **Mitochondria** would have been swollen during ischemia; with reperfusion, they rapidly **undergo condensation and become electron-dense**.
C- Proximal tubular cell showing **late injury**, expected to be irreversible.

Note the markedly swollen mitochondria containing electron-dense deposits, expected to contain precipitated calcium and proteins. **Higher magnification micrographs of the cell would show disrupted plasma membrane and swelling and fragmentation of organelles.**

(A, Courtesy of Dr. Brigitte Kaisslin, Institute of Anatomy, University of Zurich, Switzerland. B, C, Courtesy of Dr. M.A. Venkatachalam, University of Texas Health Sciences Center, San Antonio, TX.)
The morphologic appearance of necrosis is the result of denaturation of intracellular proteins and enzymatic digestion of the lethally injured cell.

The contents of necrotic cells are often leak out, a process that may elicit inflammation in the surrounding tissue.

The enzymes that digest the necrotic cell are derived from the lysosomes of the dying cells themselves and from the lysosomes of leukocytes that are called in as part of the inflammatory reaction.
Morphology.

Necrotic cells show increased eosinophilia in H & E stains, attributable in part to the loss of cytoplasmic RNA (which binds the blue dye, hematoxylin) and in part to denatured cytoplasmic proteins (which bind the red dye, eosin).

The necrotic cell may have a more glassy homogeneous appearance than do normal cells, mainly as a result of the loss of glycogen particles.
Necrotic cells are unable to maintain membrane integrity, and the subsequent leakage of intracellular proteins through the damaged cell membrane, into the circulation provides a means of detecting tissue-specific necrosis using blood or serum samples.

Examples:
(1) Cardiac muscle, for example, contains a unique isoform of the enzyme creatine kinases and of the contractile protein troponin, whereas…
(2) hepatic bile duct epithelium contains a temperature-resistant isoform of the enzyme alkaline phosphatase,&
(3) hepatocytes contain transaminases.
Irreversible injury & cell death in these tissues are reflected in increased serum levels of such proteins, and measurement of their serum levels is used clinically to assess damage to these tissues.

These leakage processes require hours to develop, and so, there are no detectable changes in cells if, for example, a MI causes immediate sudden death.
When enzymes have digested the cytoplasmic organelles, the cytoplasm becomes vacuolated and appears moth-eaten.

**Dead cells** may be replaced by large, whorled phospholipid masses called *myelin figures* that are derived from damaged cell membranes, which then either phagocytosed by other cells or further degraded into fatty acids; calcification of such fatty acid residues results in the generation of calcium soaps. Thus, the dead cells may ultimately become calcified.
Nuclear changes appear in one of three patterns, all due to nonspecific breakdown of DNA.

The **basophilia** of the chromatin may fade (*karyolysis*), a change that presumably reflects loss of DNA because of enzymatic degradation by endonucleases.

A second pattern (which is also seen in apoptotic cell death) is **pyknosis**, characterized by nuclear shrinkage and increased basophilia. **Here the chromatin condenses into a solid, shrunken basophilic mass.**
In the third pattern, known as **karyorrhexis**, the pyknotic nucleus undergoes fragmentation. With the passage of time (a day or two), the nucleus in the necrotic cell totally disappears.
When large numbers of cells die the tissue or organ is said to be necrotic.

Necrosis of tissues has several morphologically distinct patterns, which are important to recognize because they may provide clues about the underlying cause.
Morphology

Coagulative necrosis: is a form of necrosis in which the architecture of dead tissues is preserved for a span of at least some days. The affected tissues exhibit a firm texture. Presumably, the injury denatures not only structural proteins but also enzymes and so blocks the proteolysis of the dead cells; as a result, eosinophilic, anucleate cells may persist for days or weeks.
Coagulative necrosis.
A- A wedge-shaped kidney infarct (yellow).
B- Microscopic view of the edge of the infarct, with normal kidney (N) and necrotic cells in the infarct (I) showing preserved cellular outlines with loss of nuclei and an inflammatory infiltrate (which is difficult to discern at this magnification).
Ultimately the necrotic cells are removed by phagocytosis of the cellular debris by infiltrating leukocytes and by digestion of the dead cells by the action of lysosomal enzymes of the leukocytes.

Ischemia caused by obstruction in a vessel may lead to coagulative necrosis of the supplied tissue in all organs except the brain.

A localized area of coagulative necrosis is called an infarct.
Liquefactive necrosis

Is characterized by digestion of the dead cells, resulting in transformation of the tissue into a liquid viscous mass.

It is seen in focal bacterial or, occasionally, fungal infections, because microbes stimulate the accumulation of leukocytes and the liberation of enzymes from these cells.
Liquefactive necrosis.
An infarct in the brain, showing dissolution of the tissue.

The necrotic material is frequently **creamy yellow** because of the presence of dead leukocytes and is called pus. For unknown reasons, hypoxic death of cells within the central nervous system often manifests as liquefactive necrosis.
Gangrenous necrosis:

Is not a specific pattern of cell death, but the term is commonly used in clinical practice. It is usually applied to a limb, generally the lower leg, that has lost its blood supply and has undergone necrosis (typically coagulative necrosis) involving multiple tissue planes.

When bacterial infection is superimposed there is more liquefactive necrosis because of the actions of degradative enzymes in the bacteria and the attracted leukocytes (giving rise to so-called wet gangrene).
Gangrenous infarction of small intestine

As a result of volvulus, i.e., twisting of a loop (or loops) of intestine upon itself through 180 degree or more, a process which obstructs the intestine and interferes with its blood supply.

The gangrenous small intestinal coils are greatly distended and blue-black.
**Caseous necrosis:** is encountered most often in foci of tuberculous (TB) infection. The term “caseous” (cheese like) is derived from the friable white appearance of the area of necrosis.

On microscopic examination, the necrotic area appears as a collection of fragmented or lysed cells and amorphous granular debris enclosed within a distinctive inflammatory border; this appearance is characteristic of a focus of inflammation known as a granuloma.
**Tuberculosis** of the lung, with a large area of **caseous necrosis** containing **yellow-white and cheesy debris**.

**Caseous tuberculosis (TB): Lymph node**. Several enlarged discrete (separated from each other) lymph nodes are present within fat and fibrous tissue. The two largest nodes contain white areas of **caseation necrosis**.
Fat necrosis

It refers to **focal areas** of **fat destruction**, typically resulting from **release** of activated **pancreatic lipases** into the substance of the pancreas and the peritoneal cavity.

This occurs in the very serious severe abdominal emergency known as **acute pancreatitis**. In this disorder pancreatic enzymes leak out of acinar cells and **liquefy the membranes** of fat cells in the peritoneum.

The released lipases split the triglyceride esters contained within fat cells.
The fatty acids, so derived, combine with calcium to produce grossly visible chalky-white areas (fat saponification).

On histologic examination the necrosis takes the form of foci of shadowy outlines of necrotic fat cells, with basophilic calcium deposits, surrounded by an inflammatory reaction.
Fibrinoid necrosis

Is a special form of necrosis usually seen in immune reactions involving blood vessels.

This pattern of necrosis typically occurs when complexes of antigens and antibodies are deposited in the walls of arteries.

Deposits of these “immune complexes,” together with fibrin that has leaked out of vessels, result in a bright pink and amorphous appearance in H&E stains, called “fibrinoid” (fibrin-like) by pathologists.
Fibrinoid necrosis in an artery.
The wall of the artery shows a circumferential bright pink area of necrosis with inflammation (neutrophils with dark nuclei).
Ultimately, in the living patient most necrotic cells and their contents disappear by phagocytosis of the debris and enzymatic digestion by leukocytes. If necrotic cells and cellular debris are not promptly destroyed and reabsorbed, they tend to attract calcium salts and other minerals and to become calcified.

This phenomenon, called dystrophic calcification.
Next lecture

Mechanisms of Cell Injury