General Pathology

Clinico-Pathologic Correlations: Selected Examples of Cell Injury and Necrosis and Apoptosis

Dr. Al-Saghibini M. S.
MD. PhD. Pathology
Cyto/Histopathology Consultant
Assistant Prof.
Ischemic and Hypoxic Injury

In ischemia, the supply of oxygen and nutrients is decreased most often because of reduced blood flow as a consequence of a mechanical obstruction in the arterial system. It can also be caused by reduced venous drainage.

In contrast to hypoxia, during which energy production by anaerobic glycolysis can continue, ischemia compromises the delivery of substrates for glycolysis.
In ischemic tissues, not only is aerobic metabolism compromised but anaerobic energy generation also stops after glycolytic substrates are exhausted, or glycolysis is inhibited by the accumulation of metabolites that would have been removed otherwise by blood flow. For this reason, ischemia tends to cause more rapid and severe cell and tissue injury than does hypoxia in the absence of ischemia.
Mechanisms of Ischemic Cell Injury

As the \textbf{O}_2 tension within the cell decreases, there is decreased generation of \textbf{ATP} which results in failure of the sodium pump, with loss of potassium, influx of sodium and water, and cell swelling.

There is also influx of Ca$^{2+}$, with its deleterious effects.

There is progressive loss of glycogen and decreased protein synthesis.
If oxygen is restored, all disturbances are reversible. If ischemia persists, irreversible injury and necrosis ensue. Irreversible injury is associated morphologically with severe swelling of mitochondria, extensive damage to plasma membranes (giving rise to myelin figures) and swelling of lysosomes.

**Death** is mainly by necrosis, but apoptosis also contributes; the apoptotic pathway is probably activated by release of pro-apoptotic molecules from leaky mitochondria.
The cell's components are progressively degraded, and there is widespread leakage of cellular enzymes into the extracellular space and, conversely, entry of extracellular macromolecules from the interstitial space into the dying cells. Finally, the dead cells may become replaced by large masses composed of phospholipids in the form of myelin figures.

These are then either phagocytosed by leukocytes or degraded further into fatty acids. Calcification of such fatty acid residues may occur, with the formation of calcium soaps.
Mammalian cells have developed protective responses to hypoxic stress. The best-defined of these is induction of a transcription factor called \textit{hypoxia-inducible factor-1}, which promotes new blood vessel formation, stimulates cell survival pathways, and enhances anaerobic glycolysis.
Ischemia-Reperfusion Injury

As a consequence, reperfused tissues may sustain loss of cells in addition to the cells that are irreversibly damaged at the end of ischemia.

This process, called ischemia-reperfusion injury, is clinically important because it contributes to tissue damage during myocardial and cerebral infarction and following therapies to restore blood flow.
**How does reperfusion injury occur?**

1- New damage may be initiated during re-oxygenation by increased generation of reactive oxygen and nitrogen species from parenchymal and endothelial cells and from infiltrating leukocytes.

2- Ischemic injury is associated with inflammation as a result of the production of cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells, which recruit circulating neutrophils to reperfused tissue.

The inflammation causes additional tissue injury.
3- Activation of the complement system may contribute to ischemia-reperfusion injury. The complement system is involved in host defense and is an important mechanism of immune injury. Some IgM antibodies have a propensity to deposit in ischemic tissues, for unknown reasons, and when blood flow is resumed, complement proteins bind to the deposited antibodies, are activated, and cause more cell injury and inflammation.
Because many drugs are metabolized in the liver, this organ is a frequent target of drug toxicity. Chemicals induce cell injury by one of two general mechanisms.

1- Some chemicals can injure cells directly by combining with critical molecular components.

For example, in mercuric chloride poisoning, mercury binds to the sulfhydryl groups of cell membrane proteins, causing increased membrane permeability and inhibition of ion transport. In such instances, the greatest damage is usually to the cells that use, absorb, excrete, or concentrate the chemicals (The cells of GIT & kidney).
Most toxic chemicals are not biologically active in their native form but must be converted to reactive toxic metabolites, which then act on target molecules. This modification is usually accomplished by the cytochrome P-450 mixed-function oxidases in the smooth ER of the liver and other organs. The toxic metabolites cause membrane damage and cell injury mainly by formation of free radicals and subsequent lipid peroxidation; direct covalent binding to membrane proteins and lipids may also contribute.
Apoptosis: is a pathway of cell death that is induced by a tightly regulated suicide program in which cells destined to die activate enzymes that degrade their own nuclear DNA and nuclear and cytoplasmic proteins.
Apoptotic cells break up into fragments, called apoptotic bodies, contain portions of the cytoplasm and nucleus.

The plasma membrane of the apoptotic cell and bodies remains intact, but its structure is altered and become “tasty” targets for phagocytes.

The dead cell and its fragments are rapidly devoured, before the contents have leaked out, and therefore cell death by this pathway does not elicit an inflammatory reaction in the host.
Causes of Apoptosis

Apoptosis occurs normally both during development and throughout adulthood, and serves to eliminate unwanted, aged or potentially harmful cells.
It is also a pathologic event when diseased cells become damaged beyond repair and are eliminated.

Apoptosis in Physiologic Situations

1- The programmed destruction of cells during embryogenesis, including implantation, organogenesis, developmental involution, and metamorphosis.
2- Involution of hormone-dependent tissues upon hormone withdrawal, such as endometrial cell breakdown during the menstrual cycle, ovarian follicular atresia in menopause, the regression of the lactating breast after weaning, and prostatic atrophy after castration.

3- Cell loss in proliferating cell populations, such as immature lymphocytes in the bone marrow and thymus that fail to express useful antigen receptors, B lymphocytes in germinal centers, and epithelial cells in intestinal crypts, so as to maintain a constant number (homeostasis).
4- Elimination of potentially harmful self-reactive lymphocytes, either before or after they have completed their maturation, so as to prevent reactions against one's own tissues.

5- Death of host cells that have served their useful purpose, such as neutrophils in an acute inflammatory response, and lymphocytes at the end of an immune response. In these situations cells undergo apoptosis because they are deprived of necessary survival signals, such as growth factors.
Apoptosis in Pathologic Conditions

1- **DNA damage**. Radiation, cytotoxic anticancer drugs, and hypoxia can damage DNA, either directly or via production of free radicals.

If repair mechanisms cannot cope with the injury, the cell triggers intrinsic mechanisms that induce apoptosis. **Larger doses of the same stimuli may result in necrotic cell death.**

2- **Pathologic atrophy in parenchymal organs after duct obstruction**, such as occurs in the pancreas, parotid gland, and kidney.
3- **Accumulation of misfolded proteins** (may arise because of mutations in the genes encoding these proteins or because of extrinsic factors, such as damage caused by free radicals). Excessive accumulation of these proteins in the ER leads to a condition called **ER stress**, which culminates in **apoptotic cell death**.

4- **Cell death in certain infections**, particularly viral infections, in which loss of infected cells is largely due to apoptosis that may be induced by the virus (as in adenovirus and HIV infections) or by the host immune response (as in viral hepatitis).
Morphologic and Biochemical Changes in Apoptosis

Morphology

Cell shrinkage. The cell is smaller in size; the cytoplasm is dense; and the organelles, though relatively normal, are more tightly packed. (Recall that in other forms of cell injury, an early feature is cell swelling, not shrinkage.)

- A) Low power --cell shrinkage in apoptotic cell
- B) High power --apoptotic cell nucleus showing chromatin condensation
- In both surrounding cells are normal
Chromatin condensation. This is the most characteristic feature of apoptosis.

The chromatin aggregates peripherally, under the nuclear membrane, into dense masses of various shapes and sizes. The nucleus itself may break up, producing two or more fragments.

This electron micrograph of cultured cells undergoing apoptosis shows some nuclei with peripheral crescents of compacted chromatin, and others that are uniformly dense or fragmented.
Formation of cytoplasmic blebs and apoptotic bodies.

The apoptotic cell first shows extensive surface blebbing, then undergoes fragmentation into membrane-bound apoptotic bodies composed of cytoplasm and tightly packed organelles, with or without nuclear fragments.

These images of cultured cells undergoing apoptosis show:

- Blebbing and formation of apoptotic bodies (phase contrast micrograph),
- Stain for DNA showing nuclear fragmentation,
- Activation of caspase-3 immunofluorescence stain with an antibody specific for the active form of caspase-3, revealed as red color.)
Plasma membranes are thought to remain intact during apoptosis, until the last stages, when they become permeable to normally retained solutes. This classical description is accurate with respect to apoptosis during physiologic conditions such as embryogenesis and deletion of immune cells.

Phagocytosis of apoptotic cells or cell bodies, usually by macrophages

The apoptotic bodies are rapidly ingested by phagocytes and degraded by the phagocyte's lysosomal enzymes.

Plasma membranes are thought to remain intact during apoptosis, until the last stages, when they become permeable to normally retained solutes. This classical description is accurate with respect to apoptosis during physiologic conditions such as embryogenesis and deletion of immune cells.
Biochemical Features of Apoptosis

Activation of Caspases.

A specific feature of apoptosis is the activation of several members of a family of cysteine proteases named caspases.

The term caspase is based on two properties of this family of enzymes: the “c” refers to a cysteine protease (i.e., an enzyme with cysteine in its active site), and “aspase” refers to the unique ability of these enzymes to cleave after aspartic acid residues.

The caspase family, now including more than 10 members, can be divided functionally into two groups—initiator and executioner—depending on the order in which they are activated during apoptosis.
DNA and Protein Breakdown

Apoptotic cells exhibit a characteristic breakdown of DNA into large 50- to 300-kilobase pieces. Subsequently, there is cleavage of DNA by Ca$^{2+}$- and Mg$^{2+}$- dependent endonucleases into fragments whose sizes are multiples of 180 to 200 base pairs, reflecting cleavage between nucleosomal subunits.
The fragments may be visualized by electrophoresis as DNA “ladders”

**Lane A**- Viable cells in culture.
**Lane B**- Culture of cells exposed to heat showing extensive apoptosis; note ladder pattern of DNA fragments, which represent multiples of oligonucleosomes.
**Lane C**- Culture showing cell necrosis; note diffuse smearing of DNA.

Agarose gel electrophoresis of DNA extracted from culture cells. Ethidium bromide stain; photographed under ultraviolet illumination.
One of these changes is the movement of some **phospholipids** from the inner leaflet to the outer leaflet of the membrane, where they are recognized by a number of receptors on phagocytes.

These lipids are also detectable by binding of a protein called annexin V; thus, **annexin V** staining is commonly used to identify apoptotic cells.
Mechanisms of Apoptosis

All cells contain intrinsic mechanisms that signal death or survival, and apoptosis results from an imbalance in these signals.

The process of apoptosis may be divided into an initiation phase, during which some caspases become catalytically active, and an execution phase, during which other caspases trigger the degradation of critical cellular components.
Initiation of apoptosis occurs principally by signals from two distinct pathways:
1- The intrinsic, or mitochondrial, pathway, and
2- The extrinsic, or death receptor–initiated, pathway.

These pathways are induced by distinct stimuli and involve different sets of proteins, and both converge to activate caspases, which are the actual mediators of cell death.
**Mechanisms of apoptosis.** The induction of apoptosis by the **mitochondrial** pathway involves the action of sensors and effectors of the Bcl-2 family, which induce leakage of mitochondrial proteins.

Also shown are some of the anti-apoptotic proteins ("regulators") that inhibit mitochondrial leakiness and **cytochrome c** dependent caspase activation in the mitochondrial pathway.

In the **death** receptor pathway engagement of death receptors leads directly to caspase activation.
The Intrinsic (Mitochondrial) Pathway of Apoptosis

This pathway is the result of increased mitochondrial permeability and release of pro-apoptotic molecules (death inducers) into the cytoplasm such as cytochrome *c* that are essential for life, but some of the same proteins, when released into the cytoplasm (an indication that the cell is not healthy), initiate the suicide program of apoptosis.
A- Cell viability is maintained by the induction of anti-apoptotic proteins such as Bcl-2 by survival signals. These proteins maintain the integrity of mitochondrial membranes and prevent leakage of mitochondrial proteins. The release of these mitochondrial proteins is controlled by a finely orchestrated balance between pro- and anti-apoptotic members of the Bcl family of proteins.
B- Loss of survival signals, DNA damage, and other insults activate sensors that antagonize the anti-apoptotic proteins and activate the pro-apoptotic proteins Bax and Bak, which form channels in the mitochondrial membrane. The subsequent leakage of cytochrome c (and other proteins, not shown) leads to caspase activation and apoptosis.
Cytochrome c, well known for its role in mitochondrial respiration. Once released into the cytosol, cytochrome c binds to a protein called Apaf-1 (apoptosis-activating factor-1, homologous to Ced-4 in *C. elegans*), which forms a wheel-like hexamer that has been called the *apoptosome*. This complex is able to bind caspase-9, the critical initiator caspase of the mitochondrial pathway, and the enzyme cleaves adjacent caspase-9 molecules, thus setting up an auto-amplification process.
The Extrinsic (Death Receptor–Initiated) Pathway of Apoptosis

This pathway is initiated by engagement of plasma membrane death receptors on a variety of cells. Death receptors are members of the TNF receptor family that contain a cytoplasmic domain involved in protein-protein interactions that is called the death domain because it is essential for delivering apoptotic signals.
The best-known death receptors are the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95), but several others have been described.

The extrinsic (death receptor–initiated) pathway of apoptosis, illustrated by the events following Fas engagement. FAAD, Fas-associated death domain; FasL, Fas ligand.
FasL is expressed on T cells that recognize self antigens (and functions to eliminate self-reactive lymphocytes), and on some cytotoxic T lymphocytes (which kill virus-infected and tumor cells).
When FasL binds to Fas, three or more molecules of Fas are brought together, and their cytoplasmic death domains form a binding site for an adapter protein that also contains a death domain and is called **FADD** (Fas-associated death domain).
FADD that is attached to the death receptors in turn binds an inactive form of caspase-8 in mice and, in humans, caspase-10, again via a death domain. Multiple pro-caspase-8 molecules are thus brought into proximity, and they cleave one another to generate active caspase-8. The enzyme then triggers a cascade of caspase activation by cleaving and thereby activating other pro-caspases, and the active enzymes mediate the execution phase of apoptosis.
This pathway of apoptosis can be inhibited by a protein called FLIP (Fas ligand inhibitor protein), which binds to pro-caspase-8 but cannot cleave and activate the caspase because it lacks a protease domain.

Some viruses and normal cells produce FLIP and use this inhibitor to protect themselves from Fas-mediated apoptosis.
The Execution Phase of Apoptosis

The two initiating pathways converge to a cascade of caspase activation, which mediates the final phase of apoptosis.

The mitochondrial pathway leads to activation of the initiator **caspase-9**, and the death receptor pathway to the initiators **caspase-8 and -10**.

After an initiator caspase is cleaved to generate its active form, the enzymatic death program is set in motion by rapid and sequential activation of the **executioner caspases**.
Executioner caspases, such as caspase-3 and -6, act on many cellular components, also degrade structural components of the nuclear matrix, and thus promote fragmentation of nuclei.

Some of the steps in apoptosis are not fully defined.

For instance, we do not know how the structure of the plasma membrane is changed in apoptotic cells, or how membrane blebs and apoptotic bodies are formed.
Removal of Dead Cells

The formation of apoptotic bodies breaks cells up into “bite-sized” fragments that are edible for phagocytes. Apoptotic cells and their fragments also undergo several changes in their membranes that actively promote their phagocytosis so they are cleared before they undergo secondary necrosis and release their cellular contents (which can result in injurious inflammation).
Phosphatidylserine (PS) is present on the inner leaflet of the plasma membrane, but in apoptotic cells this phospholipid “flips” out and is expressed on the outer layer of the membrane, where it is recognized by several macrophage receptors.

Some apoptotic bodies express thrombospondin, an adhesive glycoprotein that is recognized by phagocytes, and macrophages themselves may produce proteins that bind to apoptotic cells (but not to live cells) and thus target the dead cells for engulfment.

Apoptotic bodies may also become coated with natural antibodies and proteins of the complement system, notably C1q, which are recognized by phagocytes.
Clinico-Pathologic Correlations: Apoptosis in Health and Disease

**Growth Factor Deprivation.**

Hormone-sensitive cells deprived of the relevant hormone, lymphocytes that are not stimulated by antigens and cytokines, and neurons deprived of nerve growth factor die by apoptosis. In all these situations, apoptosis is triggered by the intrinsic (mitochondrial) pathway and is attributable to decreased synthesis of Bcl-2 and Bcl-x and activation of Bim and other pro-apoptotic members of the Bcl family.
DNA Damage.
Exposure of cells to radiation or chemotherapeutic agents induces apoptosis by a mechanism that is initiated by DNA damage (genotoxic stress) and that involves the tumor-suppressor gene p53.

p53 protein accumulates in cells when DNA is damaged, and it arrests the cell cycle (at the G₁ phase) to allow time for repair.

However, if the damage is too great to be repaired successfully, p53 triggers apoptosis.
Protein Misfolding.

Chaperones in the ER control the proper folding of newly synthesized proteins. Misfolded polypeptides are ubiquitinated and targeted for proteolysis in proteasomes.

A- Chaperones, such as heat shock proteins (Hsp), protect unfolded or partially folded proteins from degradation and guide proteins into organelles.
If, however, unfolded or misfolded proteins accumulate in the ER, because of inherited mutations or stresses, they trigger a number of cellular responses, collectively called the **unfolded protein response**. If cytoprotective response is unable to cope with the accumulation of misfolded proteins, the cell activates caspases and induces apoptosis. **This process is called ER stress.**

B- Misfolded proteins trigger a protective unfolded protein response (UPR). If this response is inadequate to cope with the level of misfolded proteins, it induces apoptosis.
Apoptosis Induced By the TNF Receptor Family.

FasL on T cells binds to Fas on the same or neighboring lymphocytes. This interaction plays a role in the elimination of lymphocytes that recognize self-antigens, and mutations affecting Fas or FasL result in autoimmune diseases.

The cytokine TNF is an important mediator of the inflammatory reaction, but it is also capable of inducing apoptosis.

(The name “tumor necrosis factor” arose not because the cytokine kills tumor cells directly but because it induces thrombosis of tumor blood vessels, resulting in ischemic death of the tumor.)
Cytotoxic T Lymphocyte–Mediated Apoptosis. (CTLs)

Upon activation, CTLs secrete **perforin**, a transmembrane pore-forming molecule, which promotes entry of the CTL granule serine proteases called **granzymes**, which cleave proteins at aspartate residues and thus activate a variety of cellular caspases.

In this way the CTL kills target cells by directly inducing the **effector phase of apoptosis**.

CTLs also **express FasL** on their surface and may kill target cells by **ligation of Fas receptors**.
Disorders Associated with Dysregulated Apoptosis

1- Disorders associated with defective apoptosis and increased cell survival.

An inappropriately low rate of apoptosis may permit the survival of abnormal cells, which may have a variety of consequences.

For instance, if cells that carry mutations in \( p53 \) are subjected to DNA damage, the cells not only fail to die but are susceptible to the accumulation of mutations because of defective DNA repair, and these abnormalities can give rise to cancer.
2- Disorders associated with increased apoptosis and excessive cell death.

These diseases are characterized by a loss of cells and include:

(1) **neurodegenerative diseases**, manifested by loss of specific sets of neurons, in which apoptosis is caused by mutations and misfolded proteins;

(2) **ischemic injury**, as in myocardial infarction and stroke; and

(3) **death of virus-infected cells**, in many viral infections.
Next lecture

Autophagy, Intracellular Accumulations, Pathologic Calcification and Cellular Aging