Apoptosis: Programmed Cell Death

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Currently there is much interest and excitement in the understanding of how cells undergo the process of apoptosis or programmed cell death. Understanding how, why, and when cells are instructed to die may provide insight into the aging process, autoimmune syndromes, degenerative diseases, and malignant transformation. This review focuses on the development of apoptosis and describes the process of programmed cell death, some of the factors that incite or prevent its occurrence, and finally some of the diseases in which it may play a role. The hope is that in the not too distant future we may be able to modify or thwart the apoptotic process for therapeutic benefit.

The notion that cells are eliminated or absorbed in an orderly manner is not new. What is new is the recognition that this is an important physiologic process.[1] More than 40 years ago embryologists noted that during morphogenesis cells and tissues were being deleted in a predictable fashion. During human development as ontogeny recapitulates phylogeny, there is the loss of branchial arches, the tail, the cloaca, and webbing between fingers. Pediatric surgery is replete with examples where there has been failure of this process, with double aortic arch, sacrococcygeal teratomas, extrophy of the bladder, and syndactyly to name but a few. It is now recognized that apoptosis continues on after development, while the organism is undergoing growth and maturation. In fact the process is life long.

An excellent example of the importance of programmed cell death during both development and maturation is provided by the vertebrate nervous system. Here again there was long-standing recognition that during development the death of neurons is a regular feature, but only recently has the extent of the neuronal death (50%) and its importance become understood.[2] The seminal observations include the discovery of a nerve growth factor (NGF) produced by the target cell that is required for the sustenance of the neuron responsible for making contact with that target cell. In experiments, the death or survival of neurons could be modulated by the loss of NGF, by antibodies, or by the addition of exogenous NGF. During development and maturation, many types of neurons are being produced in excess. This seemingly extravagant waste of excessive neurons has several survival advantages for the organism. For example neurons that have found their way to the wrong target cell do not survive. In turn, the large excess of neurons make it unlikely that any target cell will not make contact with the appropriate neuron. The result is that an excellent balance is achieved between the surviving neurons and target cells.

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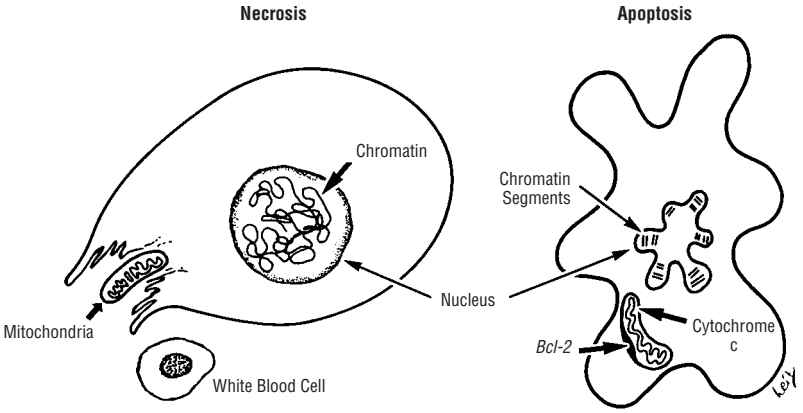
APOPTOSIS VS NECROSIS

Apoptosis stands in sharp contrast to necrosis—a condition familiar to surgeons (**Figure**). Necrosis is caused by an external noxious stimulus and involves contiguous portions of tissue. The process is passive and pathologic in that injury is produced. The insult causes lysis or rupture of cell membranes and the leakage of cytosol into the surroundings. Organelles such as mitochondria are easily injured whereas the nucleus, protected by a strong membrane, may remain intact. The escape of the cytosol releases kinins into the tissue that incite inflammation. Edema, capillary dilation, and macrophage aggregation ensue. The inflammatory response takes hours to days to both occur and subside and leaves traces of its presence by scar formation.

Apoptosis (literally a "falling off" as with leaves) occurs because of a preprogrammed process contained within the cell that can be either activated by the cell itself or by external stimuli. Specific genes have been identified that regulate the process. It is an active and physiologic process that requires the cell to expend energy in causing its own demise. Initially, an individual cell becomes loose or detached from its neighbors and morphologically shrinks. The cell membrane becomes contorted and folded into blebs. Intracellular proteases (interleukin converting enzymes) activate enzymes that begin to cleave the cytoskeletal framework.[3] The chromatin becomes condensed near the nuclear membrane and is cut into regular repeating lengths. The blebs of the intact cell membrane, many of which now contain condensed nuclear fragments (apoptotic bodies), pinch off as separate packets. Although these packets may undergo phagocytosis by macrophages, there is no inflammatory response. The process is relatively rapid, reaching completion in approximately 2 hours. Apoptotic bodies are the only visible remains. For these reasons the process and its magnitude went undetected for a long time.



	Necrosis	Apoptosis
Process	Pathological	Physiologic
Energy	Passive	Active
Stimulus	External	Internal
Host response	Inflammation	None
Cell membrane	Ruptured	Intact Blebs 36+9
Mitochondria	Loss	Intact (Release Cytochrome c)
Duration	Days	Hours
Result	Scar	None/apoptotic bodies

Differences between apoptosis and necrosis.

APOPTOSIS AND HOMEOSTASIS

Activation of apoptosis is only partly understood and the object of much investigation. The process may be initiated by agents such as bacterial toxins, viral infections, and cytolytic T lymphocytes.[4] This pathway can be thought of as serving a "housekeeping function" to ensure that cells that have become injured, infected, altered or incompetent are removed in an expeditious and efficient manner. For example, a lymphocyte infected with the Epstein-Barr virus, a virus with a potential to cause malignant transformation of the cell, would be stimulated to undergo apoptosis and thereby lessen the risk to the organism. The virus or other noxious agents activate specific receptors and genes for apoptosis resulting in prompt elimination of the affected lymphocytes.

The other major pathway can be conceived as a homeostatic process. For example, in both the developing and mature intestine the survival of an individual cell can be dependent on contact with an extracellular matrix, or a threshold level of a growth or a stimulatory substance.[5] The loss of either can cause the activation of the cell's intrinsic destruction or suicide mechanism. During the development of the gut, the solid tube of cells develops a lumen by apoptosis of the centrally located cells because of a loss of contact with the peripheral extracellular matrix. In the fully developed bowel, as enterocytes migrate toward the tip of the villus they lose contact with the basement membrane and become apoptotic.[6] Hence the bowel does not slough off viable cells into its lumen, but rather cells that are dead. This is similar to the skin that sloughs off dead cells at its surface. The death of cells that have lost contact with the extracellular matrix or with each other is termed *anoias* ("homelessness"). Similarly, neurons that fail to receive sufficient NGF, for whatever reason, undergo apoptosis.[7]

Programmed cell death is important in many physiologic processes. The most evident example is the skin. Epidermal cells grow from the basal germinal layer and migrate to the surface. En route they undergo apoptosis, resulting in flattened dead squamous cells on the surface. Similarly, during menstruation the sloughed uterine lining quickly undergoes apoptosis as the cells are deprived of hormones necessary for survival. Above all, maintaining the appropriate cell mass of an organ depends on a balance between production of new cells and the loss of old cells by programmed cell death.

MECHANISM OF APOPTOSIS

Two opposing mechanisms that maintain homeostatic control of apoptosis are the Fas ligand[8] and the protein expressed by the *Bcl-2* gene.[9] The Fas ligand promotes cell death by binding to Fas, a cell membrane receptor, initiating a series of intracellular events leading to apoptosis. Fas expression can be induced by encountering an antigen, as occurs with T cells, or it may be expressed at some low level by the tissue itself. The Fas ligand can be induced and up-regulated by the presence of kinins such as interleukins. The complexing of Fas to the Fas ligand, whether on the surface of the same cell or with another cell, initiates the apoptotic process.

The *Bcl-2* gene is an interesting and important gene involved in human malignancies such as B-cell lymphomas from which it derives its

name. It has been highly conserved from primitive worms up to humans and, therefore, thought to be important. It is the best characterized gene of a family of genes that may affect programmed cell death. Protein expressed by *Bcl-2* inhibits the apoptotic process and is powerful enough to block the process in the early phases.[10] *Bcl-2* is intimately associated with the outer mitochondrial membrane. *Bcl-2* inhibits early apoptotic events by preventing the release of cytochrome c from the mitochondrial membrane into the cytosolic space.[11] If cytochrome is released into the mitochondria, it will proceed to activate various apoptotic pathways, leading to the depolarization of the mitochondrial membrane, condensation of chromatin, and fragmentation of DNA. These 2 important mechanisms, Fas ligand and *Bcl-2*, modulate or control apoptosis to a large degree and help maintain the equilibrium in normal cell populations. Disorders of this homeostasis can be broadly classified into those characterized by excessive cell loss from increased apoptosis and those characterized by excessive cell numbers from thwarted apoptosis.

APOPTOSIS AND DISEASE

In patients with acquired immunodeficiency syndrome (AIDS) the human immunodeficiency virus causes the death of T-helper cells.[12] Ordinarily the T-helper cells prevent apoptosis of cytotoxic T cells by elaborating kinins that are necessary for maintaining the cytotoxic T cells. Loss of the sustaining kinins causes widespread death of cytotoxic T cells by apoptosis. Without adequate numbers of cytotoxic T cells, patients with AIDS are prone to develop the infections and malignancies characteristic of AIDS.

Recently Hashimoto thyroiditis has been shown to involve abnormal rates of programmed cell death.[13] Increased quantities of interleukin-1B produced by infiltrating macrophages induce Fas expression by the thyrocytes. Normal thyrocytes do not express Fas. Since Fas ligand is ordinarily produced by the thyroid cells, it complexes readily with the thyrocytes that overexpress Fas and induces apoptosis in them. The result is the loss of most of the secreting thyroid. This is not the result of attacking monocytes and macrophages, but rather of excessive activation of programmed cell death. This is an important modification of our paradigm of some autoimmune diseases such as Hashimoto thyroiditis.

However, other chronic autoimmune diseases such as systemic lupus erythematosus appear to result from the unbridled attack of reactive lymphocytes on otherwise healthy tissue.[14] Both in experimental animals and in patients with this disease, it can be shown that the reactive lymphocytes are living too long. It is thought that there is competitive inhibition of the Fas—Fas ligand complex, thereby preventing the normal prompt induction of apoptosis in these reactive lymphocytes. The result is that their numbers increase and the disease progresses inexorably.

Clinically the most important and common manifestation of excessive and progressive apoptosis is the loss of tissue from myocardial infarctions and strokes.[15] In both instances though there is a loss of tissue from ischemic necrosis, the larger loss of tissue results from the extension of the original vascular insult by apoptosis. As the cells surrounding those undergoing necrosis are exposed to kinins from the dying tissue, apoptosis is activated in otherwise healthy tissue. Efforts are being made to intervene early in both conditions so as to abrogate or modulate the progressive spread of programmed cell death.

Finally, cancer represents a failure in the processes that regulate cell survival and/or growth. Cells with DNA defects are detected by intranuclear monitoring processes that activate the *p53* gene, whose protein in turn initiates apoptosis.[16] Derangement in the *p53* mechanism and inactivation of apoptosis are the most common defects noted to date in human solid tumors and they permit survival of cells that should otherwise undergo programmed cell death.[17]

The response of tumors to radiation therapy and chemotherapy was thought to be a direct one on the tumor cells producing tumor necrosis. Newer studies suggest, however, that these cancer therapies rely on inducing apoptosis in cells that have suffered DNA damage.[18] Similarly tumors with *p53* deficiencies are much less responsive to anticancer therapies.

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