Apoptosis: Molecular mechanisms, regulation and role in pathogenesis

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OBJECTIVE: To review the current state of knowledge of apoptosis, with an emphasis on identifying potential and established roles for apoptosis in the pathogenesis of infectious diseases.

DATA SOURCES: MEDLINE and the University of British Columbia library system were searched using the search subject, “apoptosis”, for the years 1992 to 1996. Further search terms (eg, “pathogenesis”) were used to narrow the results. These review articles and reference books were used as the basis for locating original articles on particular studies.

DATA SELECTION: Approximately 40 studies were reviewed, with the criterion for selection being the relevance to either the molecular mechanisms behind apoptosis or roles for apoptosis in the pathogenesis of infectious diseases.

DATA EXTRACTION: Relevant information from each study was collated into categories specific to morphological and biochemical characterization, and the regulation and molecular mechanisms of apoptosis and its role in the pathogenesis of infectious diseases.

DATA SYNTHESIS AND CONCLUSIONS: Apoptosis is characterized by distinct morphological and biochemical changes that distinguish it from cell necrosis. Different signal transduction events and transcription factors can promote or inhibit apoptosis, although where and how these tie into the cell death pathway is still poorly understood. Apoptosis has been implicated in the pathogenesis of infectious diseases in two distinct ways: first, multicellular organisms use apoptosis to combat viral infections; and second, pathogens can alter the normal process of apoptosis in host cells by abnormal upregulation or downregulation. Many diseases have been shown to implicate apoptosis in their pathogenesis, raising the possibility of novel treatments for some disorders by therapeutically altering the occurrence and course of apoptosis. Therefore, further study of apoptosis in both health and disease needs to be rigorously pursued.

Key Words: Apoptosis, Cell death, Pathogenesis, Regulation

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Five years ago, few biologists could have defined the term apoptosis. Today apoptosis, or programmed cell death, is recognized as so fundamentally important to almost all organisms that most researchers in the life sciences are familiar with it. Every week, new information is added to the mountain of research already published on apoptosis, with implications for a great number of fields: immunology, embryogenesis, oncology, pathogenesis and others (1). It is evident that a review of this scale cannot possibly cover in any depth the hundreds of research already published on apoptosis, with implications for a great number of fields: immunology, embryogenesis, oncology, pathogenesis and others (1). It is evident that a review of this scale cannot possibly cover in any depth the most important and relevant topics of apoptosis are presented, with a particular emphasis on its role in the pathogenesis of infectious diseases.

Apoptosis is not a new discovery – it can be traced to the 1970s when pathologists first described apoptosis as being a morphologically distinct process from necrosis, the other major form of cell death. However, after the initial characterization in 1972 (2), the scientific community set apoptosis on the backburner for almost 10 years. There were several reasons for this apparent lack of interest. First, while most biologists knew that cells had to die naturally for a multicellular organism to maintain homeostasis, few could fathom why learning the exact mechanisms of cell death would be important. Second, cell death in itself seemed somewhat counter-intuitive: why should multicellular organisms use the luxury of wasting cells? Many believed cell death to be a relatively uncommon process, associated more with unusual or abnormal circumstances than with normalcy. Finally, apoptosis itself, by virtue of its morphology and quickness of completion, was difficult to observe directly. Hence, pathologists studying cells in the light microscope for half a century failed to notice apoptotic cells.

In the 10 year hiatus from the initial characterization to the major developments in apoptosis research, many scientists considered it to be a relatively rare process in organisms. In fact, for another 10 years after this, the scientific validity of apoptosis, in terms of whether it existed and what its importance was, engendered considerable controversy.

CHARACTERIZATION

The first characteristics of apoptosis identified were morphological (3). The term apoptosis is derived from Greek to describe its morphological features, meaning ‘falling off’, in reference to leaves falling off a tree. Apoptosis usually affects individual, scattered cells, making it difficult to observe by light microscopy, even though a large number of cells may be dying as in embryogenesis.

Under the light microscope, one observes two phases of apoptosis. First, an apoptotic cell forms apoptotic bodies, small membrane-bound fragments of the original cell. Second, the apoptotic bodies are rapidly phagocytized by surrounding cells, either neighbouring cells or by macrophages (4). In the initial stage, the first visible indication of apoptosis is cell shrinkage. This is attributed to water loss, and hence implicates the membrane ion channels. The nuclear chromatin becomes aggregated in well-defined crescent-shaped clumps on the nuclear membrane. The cell membrane loses microvilli and becomes convoluted from both cell shrinkage around organelles and cytoskeletal reorganization (5). The convolution causes membrane blebbing that eventually leads to the budding off of apoptotic bodies. The apoptotic bodies usually contain one or a few organelles or condensed nuclear chromatin. Changes to the cell membrane of apoptotic cells apparently play a role in promoting phagocytosis of the apoptotic bodies by neighbouring cells. As well, these changes allow macrophages to phagocytose without apparent activation.

In mammalian cells, the molecular hallmark used today for the characterization of apoptosis is the nucleosomal ladder. Chromosomal DNA is degraded during apoptosis into single and multiple oligonucleosomes by cleavage in the internucleosomal linker region of the DNA. A calcium-dependent endonuclease is responsible for this specific cleavage. Electrophoretic separation of the DNA extracted from apoptotic cells show a characteristic banding pattern corresponding to fragments which are multiples of 180 to 200 base pairs, the length of the nucleosomes (6). However, caution must be exercised with this approach because apoptosis has been described in the absence of DNA ladder (7-9). Therefore, the nucleosomal ladder cannot be used as the sole criterion for apoptosis.

The entire process of apoptosis is very quick, with only minutes passing from the first visible signs to the formation of apoptotic bodies. However, phagocytosis of the apoptotic bodies may take up to 24 h. The entire process, when occurring in vivo, leads to no release of inflammatory mediators and no induction of an inflammatory response.

Both apoptosis and necrosis lead to cell death. However, there are significant differences between these two types of
cell death in terms of context, morphology and biochemistry. While apoptosis appears to be an innate process of virtually all cells of multicellular organisms and necessary for maintaining homeostasis, necrosis is a pathological form of cell death associated with an insult or injury to the cell. Necrosis is characterized by an initial cell swelling, indicating a loss of cell membrane function that results in increased permeability and intracellular edema. Nuclear chromatin breaks down into ill-defined clumps. Finally, organelle and lysosomal degradation cause cell lysis, releasing cytoplasmic and nuclear macromolecules and leading to an inflammatory response. In contrast, cells undergoing apoptosis retain membrane integrity, and inflammation is prevented. At the molecular level, the apoptotic process consists of the orderly implementation of the cell death program of biochemical events. It is, therefore, a biologically active process requiring protein synthesis (6,10).

An important characteristic of this is the specific DNA cleavage that occurs between nucleosomes. Necrosis, by contrast, is more an effect of injury than an actual orderly biochemical process. In necrosis, DNA cleavage and degradation is random.

MOLECULAR MECHANISMS AND REGULATION

While apoptosis is increasingly recognized as complementary to mitosis, used by organisms to remove unwanted, undesirable or redundant cells, very little is known about the basic biochemical processes and mechanisms of this form of cell death or its effect on the overall well-being of the organism. Much of the current knowledge of the molecular mechanisms and biochemistry of apoptosis stems from studies of the nematode Caenorhabditis elegans. C. elegans is a very useful
model system for the study of apoptosis because it has a very precise developmental pattern, with 131 of 1090 total cells undergoing apoptosis. Mutant nematodes have allowed the cell death pathways and the role of various genes important to apoptosis to be pieced together. These genes can be divided into several classes: those that are required for the induction of apoptosis, those required for carrying out the cell death program and those involved in the disintegration of the dead cells. This model of cell death has been found to translate well into mammalian systems. While the actual genes differ, a great number of the cell death genes in C elegans have parallels in mammalian cells. In particular, the Ced-9 suppressor of apoptosis in C elegans is homologous to the Bcl-2 family of proteins in mammalian cells, and the Ced-3 protein is homologous to the interleukin-1 beta-converting enzyme (ICE) family of proteases. There are, as expected, some deficiencies in the model because certain parallels are absent.

A vastly simplified scheme of the current understanding of the molecular mechanisms, some triggering factors and regulatory components of the cell death pathway are depicted in Figure 1. It is now recognized that the cells of multicellular organisms require signals to remain alive. In the absence of these factors, most cells seem to initiate apoptosis by default. The factors involved and their modes of action are extremely diverse and differ among cell types. The induction of apoptosis can be brought about by either the removal of growth factors or the addition of some triggering factor. A combination of the two allows for precise control of the populations of cell types and allows for targeting of particular cells. Some fairly ubiquitous triggering factors include DNA damage, release of tumour necrosis factor (TNF) or glucocorticoids, and Fas/Apo-1 activation. Other triggering factors are more specific to the cell types involved, such as the deletion of immature thymocytes by binding of the T cell receptor (TCR) and death of macrophages following certain bacterial infections. Fas (also called CD95) is a receptor of the TNF receptor family. Fas-mediated apoptosis can be used by cytotoxic T lymphocytes and is also important for negative selection in T cell development. Another interesting triggering factor allows cells to detect changes in its extracellular structure and environment. For example, endothelial cells that lose contact with their extracellular matrix detect this via integrins and are induced to die by apoptosis.

A number of signal transduction pathways are used to communicate to the cell either a positive or negative signal for apoptosis. The pathways may be activated either through cell-surface receptors or through direct stimulation by agents which can traverse the cell membrane. Such signaling is usually context-dependent: intracellular signals involved in apoptosis are often also involved in differentiation and proliferation under different situations or conditions. In the case of the Fas/TNF receptor 1 (TNFR1) family of cell-surface receptors, ligation of certain members will promote cell survival, while ligation of others such as Fas and TNFR1 tends to cause apoptosis. The cell death signal in the case of Fas and TNFR1 is transduced by the activation of proteins associated with these receptors called FADD and TRADD, respectively. These, in turn, function as adapter molecules linking the death signal downstream by causing the activation of various ICE-family proteases, which activate other ICE-family proteases and finally result in apoptosis.

The protease apopain and its proenzyme CPP32 have been recently identified and shown to be necessary for apoptosis, but are not sufficient to induce apoptosis. CPP32, unlike many proteases, is unable autocatalytically to cleave and activate itself into apopain. It requires the proteolytic activity of other proteases. Possible proteases include ICE, granzyme B (secreted by cytotoxic T cells to induce apoptosis in a target cell) and fragmentin-2 (secreted by natural killer cells). As mentioned earlier, ICE-family proteases are involved in Fas/TNFR1 family receptor-mediated apoptosis. After activation of CPP32 to apopain by other proteases, apopain cleaves several other cellular substrates. One that has been identified is poly(adenosine diphosphate-ribose) polymerase (PARP). PARP is involved in DNA repair and maintenance and its proteolytic inactivation by apopain has been shown to be an initial event in apoptosis.

One of the earliest changes observed after a cell is committed to death is an increase in the intracellular calcium levels. Many cell-surface receptors signal through the Ca^{2+} pathway by activating phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate into inositol trisphosphate and diacylglycerol. Inositol trisphosphate causes an increase in calcium levels by opening calcium regulating channels in the endoplasmic reticulum and plasma membrane. Diacylglycerol exerts its effect by activating protein kinase C (PKC). However, because of crosstalk between different signaling pathways, PKC activation has been reported to both induce apoptosis and proliferation. Furthermore, many studies of PKC activation and apoptosis use inhibitors of PKC that may themselves induce apoptosis, thus complicating the interpretation of the studies’ results.

Protein tyrosine kinases (PTK) are often the intermediate molecules involved in transducing either positive or negative signals for apoptosis from cell-surface receptors and cell-surface associated proteins to the nucleus. Certain cell-surface receptors possess intrinsic PTK activity, while others are associated with other proteins that are PTKs. A number of signaling pathways promoting cell survival involve PTKs; for example, activation of certain PTKs is required for growth factor promoted survival, and cells that overproduce some of the PTKs may become resistant to apoptosis induced by growth factor withdrawal.

Another important signalling pathway in apoptosis involves Ras, the GTPase that links upstream receptor and non-receptor associated PTKs to downstream serine/threonine kinases. Ras is switched off by GTPase-activating proteins and is switched on by guanine nucleotide-releasing proteins. Activated Ras binds to and activates Raf, a serine/threonine kinase that activates other serine/threonine kinases. This cascade continues down to the mitogen-activated protein (MAP) kinases. The Ras/Raf/MAP kinase cascade appears to be involved in the inhibition of apoptosis, either directly or through the regulation of other apoptosis inhibitors (eg, Bcl-2).
The tumour repressor gene p53 is known to promote differentiation or apoptosis depending on the context and is one of the most common genes inactivated in human cancers. p53 is important, allowing cells to respond to DNA damage (eg, by irradiation) by causing growth arrest at the G1 phase. Cells with mutant p53 lack this checkpoint and arrest in G2 (21). This system allows the cell to assess the damage and to repair itself, if possible, or to undergo apoptosis if the DNA damage is too great. The ability of p53 to induce G1 arrest following DNA damage lies in its function as a transcription factor. Increased levels of p53 following DNA damage upregulate a number of proteins that, in turn, inhibit cyclin-dependent kinases, causing cell cycle arrest. p53 appears to be necessary for the induction of apoptosis following DNA damage (22).

However, p53-independent apoptosis also appears because certain cells in p53-deficient mice are resistant to radiation-induced apoptosis, but still undergo apoptosis in response to other stimuli.

A large number of oncogenes mediate and regulate apoptosis. A typical example of a transcription factor that is an oncogene affecting apoptosis is Myc. Myc is important in both cell proliferation and apoptosis. It interacts with the retinoblastoma protein (pRB), so that in the presence of certain growth-promoting cytokines, Myc can override the pRB-induced cell cycle arrest. However, during growth arrest (eg, during the absence of a growth factor), the presence of Myc induces p53-dependent apoptosis, which is suppressed by Bcl-2 (see below). Thus, overexpression of Myc acts as a two-way controlling mechanism that forces cells to either proliferate or die, depending on the immediate environment and presence of signals (23). Many viruses also produce oncoproteins that can act by binding to proteins that normally restrict cell growth, such as pRB (which controls cell cycle progression and suppresses apoptosis) or p53 (described below), or by suppressing apoptosis of the host cell, thus preventing the important immune defense mechanism of inducing apoptosis in virally infected cells.

Bcl-2 (first isolated from a B-cell lymphoma) has an inhibitory effect on apoptosis and was the first gene to be shown to regulate apoptosis (24). In adult organisms, Bcl-2 is primarily expressed in tissues that are constantly renewed, are proliferative or are long-lived. Bcl-2 derives its oncogenic potential not from any inherent ability to induce proliferation, but rather by its ability to prevent apoptosis in cycling cells, to promote the survival of noncycling cells, thus acting in conjunction with oncogenes like c-Myc, which can disregulate the cell cycle. Bcl-2 accomplishes these effects, in part, by forming high affinity heterodimers with Bax. Because Bax homodimers promote apoptosis (overexpression speeds apoptosis during growth factor withdrawal for some cells, but does not induce apoptosis in the presence of growth factor), either raising the transcription level of the Bax gene or decreasing the amount of Bcl-2 has the effect of increasing the susceptibility of the cell to apoptosis. The tumour suppressor p53 also plays a role because it is both a negative regulator of Bcl-2 expression and a transcription factor for the Bax gene (the Bax promoter has four p53 binding regions) (25, 26). The scope of Bcl-2 protection against apoptosis appears quite large, including protection of many factor-dependent cells in G0 until growth factor is restored; delay of p53-dependent apoptosis; resistance to killing by various chemotherapeutic agents; and blocking of apoptosis induced by glucocorticoids in immature lymphocytes. While much speculation about the exact mechanism of Bcl-2 action exists, little is known about how it actually prevents the induction of apoptosis.

**SIGNIFICANCE OF APOPTOSIS**

The significance of apoptosis is two-fold: first, it is essential for the normal health of multicellular organisms; and second, it has been implicated in both the pathogenesis and battle against many diseases.

From the earliest stages of the development of an organism, apoptosis plays a role in the maturation of tissues by altering their structure and function. During embryogenesis of humans, for example, the interdigital webs and other vestigial organs are removed by apoptosis. In the fetal lung, specific cells are programmed to die in order to enlarge the alveoli.

Throughout a human’s life, cell turnover occurs in many locations in the body. The hemopoietic system constantly produces an enormous number of cells per day, which must be eliminated to maintain a constant level of blood cells. This is accomplished by apoptosis. Even neutrophils, which are packed with highly toxic chemicals, die by apoptosis. This indicates how inconspicuously apoptosis is integrated into the everyday existence of organisms. Tissue turnover also occurs on a variety of epithelial surfaces, including the skin and the intestinal lining (27). During the menstrual cycle, many types of cells proliferate and then must be eliminated. The process includes the shedding of the uterine lining and the turnover of mammary epithelial cells.

Apoptosis also plays a vital role in developmental immunology and host defense against certain viral infections. Both B cell and T cell development and selection involve apoptosis. Cells that recognize self-antigens must be eliminated so that immune responses are not targeted to the body’s own cells. During T cell maturation, cells differentiate into different types of T cells. One type, the activated effector T killer cells, uses apoptosis to kill virally infected cells. Cytotoxic T cells recognize viral antigens presented on the cell surface and signal to them (either by degranulation or by Fas activation) to undergo apoptosis.

The improper activation or the deactivation of apoptosis has been implicated in many disorders. It has been associated with the etiology of several neurodegenerative disorders, including Alzheimer’s, Parkinson’s and Huntington’s diseases. As well, increased apoptosis has been associated with ischemic injury (28).

As one might expect, a sizable number of cancers have been shown to be caused not so much by an increase in growth and response to growth signals, but by a decrease in responsiveness to apoptotic signals, making cells less likely to die. For example, the B cell lymphoma from which Bcl-2 was first isolated, overexpressed Bcl-2, thus inhibiting apoptosis. Other tumours modify or suppress p53, making them resistant to...
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death from DNA damage. This is an initial step allowing an otherwise normal cell to accumulate further mutations, and thus adapt readily for metastasis to other tissues.

A variety of viruses have been found that can modify the host cells’ behaviour by expressing viral copies of some of the genes involved in apoptosis. For example, viruses such as the herpes viruses, pox viruses and adenoviruses encode apoptosis-inhibitors in their genomes (29, 30), increasing the probability that the host cells they infect will survive to produce more viral particles. It is by this mechanism that some viruses ultimately induce the formation of tumour cells in the infected host cells. A virus that acts not by inhibiting apoptosis, but by inducing it, is human immunodeficiency virus (HIV). Virally infected CD4+ T cells undergo activation-induced cell death (31). This contributes to the depletion of CD4+ T cells during AIDS.

Apoptosis has also been implicated in many superantigen (sAg)-mediated diseases, such as staphylococcal and streptococcal toxic shock, certain autoimmune and neoplastic diseases, and both murine and human retroviral infections. While conventional antigens bind within the antigen-presenting groove of the major histocompatibility complex molecules and are recognized by TCR in that context, sAgs bind outside of the antigen-presenting groove and interact with the V-beta region of the TCR. sAgs bypass the normal selectivity of TCR and can stimulate entire V-beta subsets of the T cell repertoire present within the host organism. In the case of staphylococcal and group A streptococcal toxic shock syndrome, this causes a massive proliferation of the sAg-reactive T cells, along with a pronounced release of various pro-inflammatory cytokines. For staphylococcal toxic shock syndrome, several sAgs produced by *Staphylococcus aureus*, including toxic shock syndrome toxin-1 and staphylococcal enterotoxins A, B, C1, C2, D and E, have been implicated as the primary cause of this illness. For streptococcal toxic shock syndrome, the streptococcal pyrogenic exotoxins A and C as well as several other sAgs produced by *Streptococcus pyogenes* are the major causes (32). Some other known sAg-producing microorganisms include *Versinia enterocolitica*, *Mycoplasma arthritidis* and the mouse mammary tumour virus. While the most obvious effect of sAgs is the polyclonal proliferation of T cells, the other important effect of sAgs is apoptosis that appears to occur simultaneously, along with a downregulation of the TCR that results in anergy (33). The net result of these two contrary effects is to produce an initial rapid proliferation of sAg-reactive T cells, followed by either a profound deletion or anergy of these T cells. Therefore, while patients might survive staphylococcal or streptococcal toxic shock, they may still be in danger because of immunosuppression from the loss of up to 20% of their T cells. With viral sAgs, the picture is somewhat different. Instead, some viruses use sAgs to compel the host cells to proliferate, thereby promoting their own survival, replication and spread. In regard to HIV, there have been reports of Vbeta specific deletion of CD4+ T cells (34). This suggests that sAgs may be a cause of CD4+ T cell depletion in AIDS patients, with the sAg in question being either an unidentified HIV product, or more likely, a sAg from another pathogen that opportunistically coinfects the immunocompromised AIDS patient. Recently, evidence has also emerged to suggest that coinfection of host cells with cytomegalovirus may result in the expression of a herpes viral sAg that drives HIV replication preferentially in selected sAg-responsive CD4+ V-beta12 T cell subsets (35). Hence, in AIDS retroviral sAgs may exert their effect primarily through inducing apoptosis and depleting HIV-infected CD4+ T cells, while sAgs from coexisting infections may actively promote HIV replication.

**CONCLUSION**

Within the past few years, apoptosis has been recognized to be fundamentally important in both the health and disease of multicellular organisms. Despite the enormous explosion of investigation in this area, little is known about the underlying mechanisms and pathways of apoptosis, and much of its regulation still remains a mystery. Future applications from an improved knowledge base of apoptosis include the possibility of treating many disorders and diseases by therapeutically altering the occurrence of apoptosis. As well, greater knowledge of apoptosis will improve the understanding of the complex balance that exists between life and death in multicellular organisms.

**REFERENCES**
