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Apoptosis in the Pathogenesis and Treatment of Disease

Craig B. Thompson

In multicellular organisms, homeostasis is maintained through a balance between cell proliferation and cell death. Although much is known about the control of cell proliferation, less is known about the control of cell death. Physiologic cell death occurs primarily through an evolutionarily conserved form of cell suicide termed apoptosis. The decision of a cell to undergo apoptosis can be influenced by a wide variety of regulatory stimuli. Recent evidence suggests that alterations in cell survival contribute to the pathogenesis of a number of human diseases, including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and AIDS (acquired immunodeficiency syndrome). Treatments designed to specifically alter the apoptotic threshold may have the potential to change the natural progression of some of these diseases.

The survival of multicellular organisms depends on the function of a diverse set of differentiated cell types. Once development is complete, the viability of the organism depends on the maintenance and renewal of these diverse lineages. Within vertebrates, different cell types vary widely in the mechanisms by which they maintain themselves over the life of the organism. Blood cells, for instance, undergo constant renewal from hematopoietic progenitor cells. In addition, lymphocytes and cells within the reproductive organs undergo cyclical expansions and contractions as they participate in host defense and reproduction, respectively. In contrast, neural cells have at best a limited capacity for self-renewal, and most neurons survive for the life of the organism.

Within each lineage, the control of cell number is determined by a balance between cell proliferation and cell death (Fig. 1). Cell proliferation is a highly regulated process with numerous checks and balances. For example, growth factors and proto-oncogenes are positive regulators of cell cycle progression (*1*). In contrast, tumor suppressor genes act to oppose uncontrolled cell proliferation (*1*, *2*). Tumor suppressors can prevent cell cycle progression by inhibiting the activity of proto-oncogenes. In the last 15 years there has been a rapid increase in our understanding of the mechanisms that control cell proliferation.

Biologists are now beginning to appreciate that the regulation of cell death is just as complex as the regulation of cell proliferation (*3*). The differentiated cells of multicellular organisms all appear to share the ability to carry out their own death through activation of an internally encoded suicide program (*4*). When activated, this suicide program initiates a characteristic form of cell death called apoptosis (*5*, *6*). Apoptosis can be triggered by a variety of extrinsic and intrinsic signals (*7*) (Fig. 2). This type of regulation allows for the elimination of cells that have been produced in excess, that have developed improperly, or that have sustained genetic damage. Although diverse signals can induce apoptosis in a wide variety of cell types, a number of evolutionarily conserved genes regulate a final common cell death pathway that is conserved from worms to humans (*8*) (Fig. 3).

Apoptotic cell death can be distinguished from necrotic cell death (*4*–*6*). Necrotic cell death is a pathologic form of cell death resulting from acute cellular injury, which is typified by rapid cell swelling and lysis. In contrast, apoptotic cell death is

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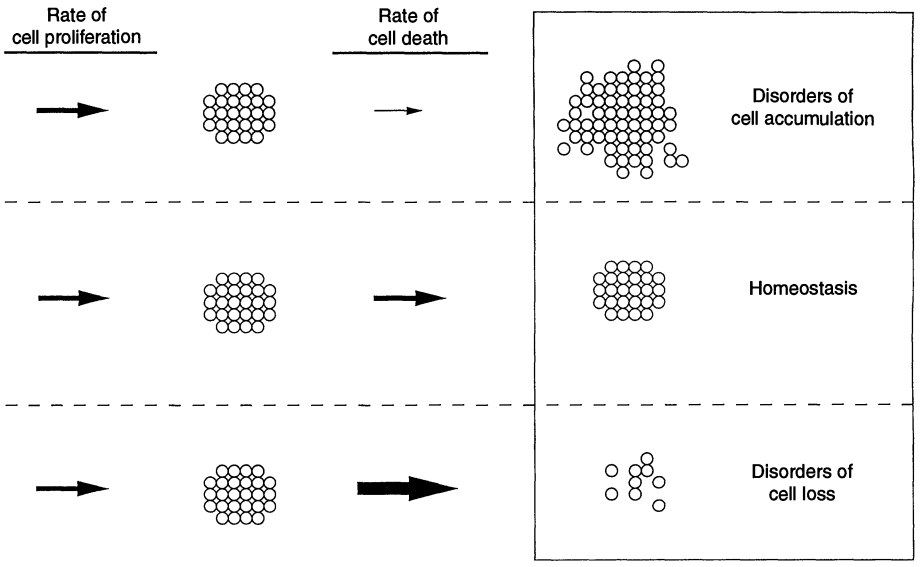

Fig. 1. The effect of different rates of cell death on homeostasis. In mature organisms, cell number is controlled as a result of the net effects of cell proliferation and cell death. Here, the rates of cell proliferation and cell death are indicated by the size of the arrows. In the absence of compensatory changes in the rate of cell proliferation, changes in the rate of cell death can result in either cell accumulation or cell loss.

Inhibitors of Apoptosis

Physiologic inhibitors
1. Growth factors
2. Extracellular matrix
3. CD40 ligand
4. Neutral amino acids
5. Zinc
6. Estrogen
7. Androgens

Viral genes
1. Adenovirus *E1B*
2. Baculovirus *p35*
3. Baculovirus *IAP*
4. Cowpox virus *crmA*
5. Epstein-Barr virus *BHRF1, LMP-1*
6. African swine fever virus *LMW5-HL*
7. Herpesvirus γ1 34.5

Pharmacological agents
1. Calpain inhibitors
2. Cysteine protease inhibitors
3. Tumor promoters
   PMA
   Phenobarbital
   α-Hexachlorocyclohexane

Inducers of Apoptosis

Physiologic activators
1. TNF family
   Fas ligand
   TNF
2. Transforming growth factor β
3. Neurotransmitters
   Glutamate
   Dopamine
   N-methyl-D-aspartate
4. Growth factor withdrawal
5. Loss of matrix attachment
6. Calcium
7. Glucocorticoids

Damage-related inducers
1. Heat shock
2. Viral infection
3. Bacterial toxins
4. Oncogenes
   myc, rel, E1A
5. Tumor suppressors
   p53
6. Cytolytic T cells
7. Oxidants
8. Free radicals
9. Nutrient deprivation—antimetabolites

Therapy-associated agents
1. Chemotherapeutic drugs
   Cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate, vincristine
2. Gamma radiation
3. UV radiation

Toxins
1. Ethanol
2. β-amyloid peptide

Fig. 2. A partial list of the agents that have been reported to induce or inhibit apoptosis.

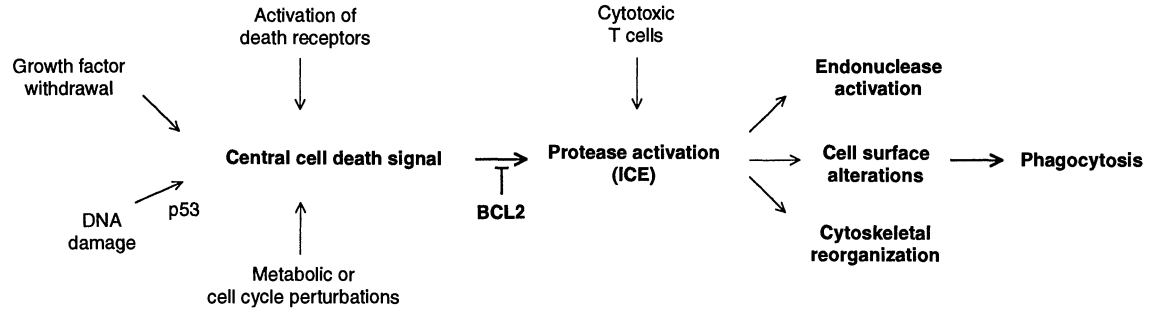

Fig. 3. A hypothetical model for the regulation of apoptotic cell death. As diagrammed, the major end point of apoptotic cell death is the removal of the dying cell by phagocytosis. One of the difficulties in determining the contribution of apoptosis to the pathogenesis of disease is the rapidity with which the phagocytosis of apoptotic cells occurs in vivo. Both the death repressor *BCL2* and ICE are members of larger gene families.

characterized by controlled autodigestion of the cell. Cells appear to initiate their own apoptotic death through the activation of endogenous proteases. This results in cytoskeletal disruption, cell shrinkage, and membrane blebbing. Apoptosis also involves characteristic changes within the nucleus. The nucleus undergoes condensation as endonucleases are activated and begin to degrade nuclear DNA. In many cell types, DNA is degraded into DNA fragments the size of oligonucleosomes, whereas in others larger DNA fragments are produced. Apoptosis is also characterized by a loss of mitochondrial function. This has led to speculation that mitochondria may have an important function in regulating apoptosis; however, data to support this hypothesis are currently unavailable. The dying cell maintains its plasma membrane integrity. However, alterations in the plasma membrane of apoptotic cells signal neighboring phagocytic cells to engulf them and thus to complete the degradation process (*9*). Cells not immediately phagocytosed break down into smaller membrane-bound fragments called apoptotic bodies. An important feature of apoptosis is that it results in the elimination of the dying cell without induction of an inflammatory response. In contrast, necrotic cell death is associated with an early loss of cell membrane integrity, resulting in leakage of cytoplasmic contents and the induction of an inflammatory response.

In most tissues, cell survival appears to depend on the constant supply of survival signals provided by neighboring cells and the extracellular matrix (*10*). Cells from most organs will undergo apoptosis if cultured individually in the absence of exogenous survival factors. Death in these cases appears to occur even in the absence of new protein synthesis, which suggests that the proteins that mediate apoptosis are constitutively expressed in many cell types (*4*). One interpretation of these results is that most cells are programmed to commit suicide if survival signals are not received from the environment, either constantly or at regular intervals (*4*). Viewed in this way, apoptotic cell death can be thought of as a

default pathway. However, some types of physiologic cell death involve the induction of apoptosis through mechanisms that require new protein synthesis.

Recent evidence suggests that the failure of cells to undergo apoptotic cell death might be involved in the pathogenesis of a variety of human diseases, including cancer, autoimmune diseases, and viral infections (*6*). In addition, a wide number of diseases characterized by cell loss, such as neurodegenerative disorders, AIDS (acquired immunodeficiency syndrome), and osteoporosis, may result from accelerated rates of physiologic cell death (Fig. 4). Specific therapies designed to enhance or decrease the susceptibility of individual cell types to undergo apoptosis could form the basis for treatment of a variety of human diseases.

Disorders Associated with Increased Cell Survival

Diseases characterized by the accumulation of cells include cancer, autoimmune diseases, and certain viral illnesses. Cell accumulation can result from either increased proliferation or the failure of cells to undergo apoptosis in response to appropriate stimuli (Fig. 1). Although much attention has focused on the potential role of cell proliferation in these disorders, increasing evidence suggests that alterations in the control of cell survival are important in the pathogenesis of these so-called proliferative disorders.

Cell death in cancer. Cells from a wide variety of human malignancies have a decreased ability to undergo apoptosis in response to at least some physiologic stimuli (*11*). This is most apparent in metastatic tumors. Most normal cells depend on environment-specific factors to maintain their viability (*10*). This dependence may serve to prevent normal cells from surviving in nonphysiologic sites. Metastatic tumor cells have circumvented this homeostatic mechanism and can survive at sites distinct from the tissue in which they arose. To do this, tumor cells must develop some degree of independence from the survival factors that restrict the distribution of their nontransformed counterparts. Recent advances are beginning to shed some light on the molecular bases for the increased resistance of tumor cells to undergo apoptosis, and several genes that are critical in the regulation of apoptosis have been defined.

The gene *BCL2* was first discovered as a result of its location at the site of a translocation between chromosomes 14 and 18 and is present in most human follicular lymphomas (*12*). Initially viewed as an oncogene, *BCL2* was found to have little or no ability to promote cell cycle progression or cell proliferation. Instead, overexpression of *BCL2* specifically prevents cells from initiating apoptosis in response to a number of stimuli (*13*). Furthermore, the introduction of genes that inhibit *BCL2* can induce apoptosis in a wide variety of tumor types, which suggests that many tumors continually rely on *BCL2* or related gene products to prevent cell death (*14*). Consistent with this hypothesis, *BCL2* expression has been associated with a poor prognosis in prostatic cancer, colon cancer, and neuroblastoma (*15*).

Recently, it has been demonstrated that *BCL2* is only one member of a family of genes that can control the apoptotic threshold of a cell (*16*). The roles of these other *BCL2* family members in the pathogenesis of human malignancies are just beginning to be examined. In tumor cell lines, overexpression of *BCL2* or of the related gene *BCLx* has been found to confer resistance to cell death in response to chemotherapeutic agents such as cytosine arabinoside, methotrexate, vincristine, and cisplatin (*17*). These results are surprising because chemotherapy was previously thought to kill cells by inducing irreversible metabolic damage that results in target cell necrosis. It now appears that the primary mechanism by which most chemotherapeutic agents induce cell death is through creating aberrations in cellular physiology that result in the induction of apoptosis. Consistent with this hypothesis, overexpression of *BCL2* or related genes can result in a multidrug resistance phenotype in vitro. The role of the *BCL2* family in the development of multidrug resistance in vivo has not yet been evaluated.

A wide variety of chemotherapeutic agents work by initiating DNA damage. Cell death in response to DNA damage in most instances has been shown to result from apoptosis. The p53 gene product is required for cells to initiate apoptosis in response to genotoxic damage (*18*). It is also fundamental in the pathway that leads from the sensing of DNA damage to the initiation of apoptosis. The inability of cells to undergo apoptosis in response to DNA damage may underlie the enhanced resistance to chemotherapeutic agents and radiation observed in tumors that are deficient in p53 (*19*). The failure of cells to die in response to DNA damage may also underlie the high rate of gene amplification observed in p53-deficient cells (*20*). Cells unable to undergo apoptosis in response to DNA damage may be more prone to acquire genetic alterations than normal cells. Errors in the repair of DNA damage that might otherwise induce apoptosis could contribute to the high mutation rate observed in many human cancers.

For years investigators have used chemicals referred to as tumor promoters to induce experimental malignancies. The exact role of tumor promoters in the pathogenesis of cancer is not clear. Most tumor promoters cannot induce cell proliferation on their own. Recently, it has been shown that tumor promoters such as phorbol myristate acetate (PMA) and α-hexachlorocyclohexane can act as specific survival factors for the cells in which they promote tumor development (*21*). These data suggest that inhibition of apoptosis is more important in the development of malignancy than previously believed.

A critical question in cancer biology is why two or more cooperative transforming events are usually required to induce a full neoplastic lesion. One potential explanation is that oncogene transformation leads to a dysregulation of genes that control cell division, such as cyclins and cyclin-dependent kinases (*22*). Cells normally appear to be able to detect such an imbalance and respond by undergoing apoptosis. Apoptosis may represent a mechanism for protecting the organism from cells that have acquired genetic alterations that predispose them to cell proliferation. For example, in the absence of other transforming events, dysregulation of the *myc* oncogene leads to the induction of apoptosis in serum-starved fibroblasts (*23*). However, simultaneous overexpression of *BCL2* prevents this apoptotic

Diseases Associated with the Inhibition of Apoptosis

1. Cancer
   - Follicular lymphomas
   - Carcinomas with *p53* mutations
   - Hormone-dependent tumors
     - Breast cancer
     - Prostate cancer
     - Ovarian cancer
2. Autoimmune disorders
   - Systemic lupus erythematosus
   - Immune-mediated glomerulonephritis
3. Viral infections
   - Herpesviruses
   - Poxviruses
   - Adenoviruses

Diseases Associated with Increased Apoptosis

1. AIDS
2. Neurodegenerative disorders
   - Alzheimer's disease
   - Parkinson's disease
   - Amyotrophic lateral sclerosis
   - Retinitis pigmentosa
   - Cerebellar degeneration
3. Myelodysplastic syndromes
   - Aplastic anemia
4. Ischemic injury
   - Myocardial infarction
   - Stroke
   - Reperfusion injury
5. Toxin-induced liver disease
   - Alcohol

Fig. 4. Diseases associated with the induction or inhibition of apoptotic cell death.

response. Cooperation between *myc* and *BCL2* in the pathogenesis of experimental tumors has also been demonstrated (*24*).

Cell death and autoimmunity. Physiologic regulation of cell death is essential for the removal of potentially autoreactive lymphocytes during development and for the removal of excess cells after the completion of an immune response. Failure to remove autoimmune cells that arise during development or that develop as a result of somatic mutation during an immune response can result in autoimmune disease. Recent work in animal model systems has clearly demonstrated the importance of dysregulated apoptosis in the etiology of autoimmune diseases. For example, one molecule critical in regulating cell death in lymphocytes is the cell surface receptor Fas, a member of the tumor necrosis factor (TNF) receptor family (*25*). Stimulation of Fas on activated lymphocytes can induce apoptosis. Two forms of hereditary autoimmune disease have been attributed to alterations in Fas-mediated apoptosis (*25, 26*). MRL-lpr mice, which develop fatal systemic lupus erythematosus by 6 months of age, have a mutation in the Fas receptor. In contrast, the GLD mouse, which develops a similar illness, has a mutation in the Fas ligand (*26*). In humans, a secreted form of Fas has been identified (*27*). Patients with systemic lupus erythematosus have elevated levels of soluble Fas, which may competitively inhibit Fas ligand–Fas interactions. The resulting decrease in Fas-mediated apoptosis may contribute to the accumulation of autoimmune cells in this disorder. A lupuslike autoimmune disease has also been reported in transgenic mice constitutively overexpressing *BCL2* in their B cells (*28*). Finally, linkage analysis has established an association between the *BCL2* locus and autoimmune diabetes in nonobese diabetic (NOD) mice (*29*).

To date, no autoimmune diseases in humans have been directly linked to genes involved in the control of apoptosis. However, investigations into the role of apoptosis in the development of autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and autoimmune diabetes mellitus are just beginning. Alterations in the susceptibility of lymphocytes to die by apoptosis in vitro have been reported in several of these diseases (*30*).

Cell death in viral infection. The disruption of cell physiology as a result of viral infection can cause an infected cell to undergo apoptosis (*31*). The suicide of an infected cell may be viewed as a cellular defense mechanism to prevent viral propagation. Cytotoxic T cells also act to prevent viral spread (*32*) by recognizing and killing cells that present viral peptides in association with cell surface major histocompatibility complex (MHC) class I molecules. Recent evidence has demonstrated that T cells can induce cell death by activating the target cell's endogenous cell death program. Cytotoxic T cells induce apoptosis either by activation of the Fas receptor on the surface of the target cell or by introduction of proteases, such as granzyme B, which activate the cell death program from within the cytoplasm (*33*).

To circumvent these host defenses, a number of viruses have developed mechanisms to disrupt the normal regulation of apoptosis within the infected cell. For example, establishment of an effective adenoviral infection depends on the function of the E1B 19-kD protein (*34*). The E1B 19-kD protein has been shown to block apoptosis directly, and its function can be replaced in adenovirus by *BCL2*. Primary sequence and mutational analyses suggest that there may also be structural similarity between these two genes (*35*). The *BHRF1* gene of Epstein-Barr virus and the *LMW5-HL* gene of African swine fever virus both have sequence and functional similarity to *BCL2* (*36*).

Other viral genes that can inhibit apoptosis have been reported that show no similarity to *BCL2*. For example, both the p35 gene and the inhibitor of apoptosis gene (*IAP*) found in baculoviruses can inhibit apoptosis in response to a wide variety of stimuli (*37*). The ability of p35 to inhibit apoptosis is not dependent on the expression of any other viral proteins. Poxviruses appear to inhibit apoptosis by producing an inhibitor of the death effector molecule interleukin-1β (IL-1β)–converting enzyme (ICE). ICE is a cysteine protease closely related to the protein encoded by the *Caenorhabditis elegans* cell death gene, *ced-3*. The *ced-3* product is required for cells to undergo programmed cell death during development in *C. elegans* (*38*). The cowpox gene *crmA* is a member of the serpin family of protease inhibitors and acts as a specific inhibitor of ICE (*39*). The *crmA* gene can inhibit apoptosis in response to a number of stimuli and has also been shown to be required to inhibit the development of an inflammatory response to virally infected cells (*39, 40*).

The prevention of apoptosis is also important for the establishment of viral latency. Epstein-Barr virus establishes a latent infection in B cells. The viral gene *LMP-1*, which is produced during latency, specifically up-regulates the expression of *BCL2*, potentially providing a survival advantage to latently infected cells (*41*). Chronic Sindbis virus infection has also been reported to be dependent on the host cell's expression of *BCL2* (*31*).

Disorders Associated with Excess Cell Death

Excessive cell death can result from acquired or genetic conditions that enhance the accumulation of signals that induce apoptosis or that decrease the threshold at which such events induce apoptosis. Although increased apoptotic cell death has been observed in many of the diseases discussed below, in most degenerative disorders an underlying defect in cell death control has not been defined.

Virus-induced lymphocyte depletion: AIDS. Perhaps the most dramatic example of virus-associated cell depletion is AIDS, which is induced by the human immunodeficiency virus (HIV) (*42*). The development of AIDS has been directly correlated with the depletion of $CD4^+$ T cells, the cellular targets of viral infection. It has been shown that CD4 acts as a receptor for viral attachment, thus facilitating HIV infection of $CD4^+$ T cells. Surprisingly, most T cells that die during HIV infections do not appear to be infected with HIV. Although a number of explanations have been proposed, recent evidence suggests that stimulation of the CD4 receptor, by its binding to the soluble viral product gp120, results in the enhanced susceptibility of uninfected T cells to undergo apoptosis (*43*). In several models of cell death, it has been suggested that disordered signal transduction can result in the induction of apoptosis, perhaps by inducing an abortive cell cycle. $CD4^+$ T cells are normally activated by simultaneous engagement of the T cell receptor and CD4 by antigen–MHC class II complexes on the surface of an antigen-presenting cell. Both $CD4^+$ T cells from normal individuals as well as from individuals with HIV undergo apoptosis in vitro if cell-surface CD4 is crosslinked before engagement of T cell receptors (*44*). Thus, it is possible that gp120 promotes apoptotic cell death by its interaction with CD4.

One important question concerning this model is why the virus would develop a mechanism to selectively deplete its host cell. The answer perhaps reflects the fact that $CD4^+$ T cells have an important function in establishing protective immunity against a wide variety of viral infections. The establishment of a chronic HIV infection may therefore depend on virally mediated depletion of $CD4^+$ T cells and the concomitant loss of a protective cell-mediated immune response. Viral replication itself may not be limited by this form of CD4-dependent cell death because the viral protein Nef specifically down-regulates the CD4 receptor on infected T cells (*45*). This down-regulation has been thought to be involved in preventing viral reinfections. However, it may also help to prevent apop-

tosis in response to dysregulated CD4 stimulation. Immunodepletion as a result of viral infection is not restricted to HIV. Viruses in birds, cats, and mice have all been reported to induce immune cell depletion during systemic infection (*46*).

Cell death in neurodegenerative disorders. A wide variety of neurological diseases are characterized by the gradual loss of specific sets of neurons (*47*). Such disorders include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), retinitis pigmentosa, spinal muscular atrophy, and various forms of cerebellar degeneration. In these diseases, cell death results in specific disorders of movement and central nervous system function. The cell loss in these diseases does not induce an inflammatory response, and apoptosis appears to be the mechanism of cell death. Oxidative stress, calcium toxicity, mitochondrial defects, excitatory toxicity, and deficiency of survival factors have all been postulated to contribute to the pathogenesis of these disorders (*48*). Each of these pathways predisposes neurons to apoptosis, either in vitro or in vivo. Overexpression of *BCL2* decreases the neurotoxicity of each of these potential inducers of cell death (*49*). Neurotrophic growth factors and the extracellular matrix also alter the apoptotic threshold of neural cells (*50*). Together, these data suggest a model in which the threshold for cell death is dynamically regulated. The apoptotic threshold of a cell is thus determined by the combined effects of external and internal survival factors.

One form of hereditary ALS results from mutations in the gene encoding copper-zinc superoxide dismutase (*51*). Patients with this form of ALS have a mutation that results in a decrease in the ability of a cell to detoxify free radicals. The cell injury caused by free radicals has been shown to induce cells to undergo apoptosis in vitro. Superoxide-induced death can be specifically inhibited by treatment with survival growth factors or antioxidants (*52*).

Retinal degeneration associated with retinitis pigmentosa occurs as a result of mutations in any one of three photoreceptor-specific genes: rhodopsin, the β subunit of cyclic guanosine monophosphate phosphodiesterase, and the peripherin gene. All three mutations lead to photoreceptor apoptosis (*53*). Apoptosis may be initiated in response to the accumulation of mutant proteins or as a result of the altered functional properties of the mutant proteins (*54*). In a hereditary rat model of light-induced retinal degeneration, intraocular injections of eight distinct neurotrophic and growth factors enhanced photoreceptor survival, whereas seven others displayed no beneficial effects (*55*). These data suggest that treatment of such disorders may be possible with specific neurotrophic factors either alone or in combination.

Alzheimer's disease is associated with the progressive accumulation of β-amyloid peptide in plaques. Mutations in the β-amyloid precursor protein are associated with some forms of familial Alzheimer's disease. Recently, several groups have shown that β-amyloid peptide induces neurons to undergo apoptosis (*56*). This effect can be reversed by antioxidants (*57*).

The spinal muscular atrophies are a group of recessive neurodegenerative disorders of childhood. These disorders are characterized by progressive spinal cord motor neuron depletion. One of the genes recently linked to these disorders, neuronal apoptosis inhibitory protein (*NAIP*), is homologous to the baculovirus inhibitor of apoptosis protein (IAP) (*58*). Baculovirus *IAP* genes have been shown to inhibit apoptosis in insect cells independent of other viral proteins. These data suggest that mutations in the *NAIP* gene may result in motor neurons being more susceptible to apoptosis in patients with spinal muscular atrophy.

Cell death in blood cell disorders. Mature blood cells are constantly being produced from hematopoietic stem cells located in the bone marrow. The regulation of hematopoiesis is influenced by a number of growth factors, including stem cell factor, erythropoietin, colony-stimulating factors, and thrombopoietin (*59*). In addition to stimulating the proliferation of hematopoietic progenitors, hematopoietic growth factors are required to support the survival of their target cells (*60*). Hematopoietic progenitors rapidly undergo apoptosis in vitro if deprived of growth factors. In fact, hematopoietic growth factors are also important in regulating the survival of postmitotic blood cells such as neutrophils.

It has been suggested that hematopoietic differentiation is primarily determined intrinsically within the precursor cell rather than as a result of the inductive effects of hematopoietic growth factors (*61*). Consistent with this view, hematopoietic stem cells in which apoptosis is suppressed by overproduction of *BCL2* can differentiate in the absence of extracellular growth factors or cell division. Together, these data suggest that hematopoietic growth factors control blood cell production at least in part by inhibiting apoptosis during the expansion and differentiation of intrinsically committed progenitors.

A number of hematologic diseases are associated with a decreased production of blood cells. Such disorders include anemia associated with chronic disease, aplastic anemia, chronic neutropenia, and the myelodysplastic syndromes. Disorders of blood cell production, such as myelodysplastic syndrome and some forms of aplastic anemia, are associated with increased apoptotic cell death within the bone marrow (*62*). These disorders could result from the activation of genes that promote apoptosis, acquired deficiencies in stromal cells or hematopoietic survival factors, or the direct effects of toxins and mediators of immune responses.

Several hematopoietic growth factors are now in widespread clinical use because of their ability to increase the net production of individual types of blood cells (*59*). For example, erythropoietin can be used to augment red blood cell production in patients with anemia secondary to renal failure and other chronic illnesses. Granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage CSF, and granulocyte CSF have all been used to promote the recovery of granulocytes and macrophages after systemic chemotherapy for cancer.

Additional Disorders in Which Apoptosis May Function

Two common disorders associated with cell death are myocardial infarctions and stroke. These diseases arise primarily as a result of an acute loss of blood flow (ischemia). In both disorders, cells within the central area of ischemia appear to die rapidly as a result of necrosis. However, outside the central ischemic zone, cells die over a more protracted time period and morphologically appear to die by apoptosis (*63*). Ischemia of both neurons and cardiac myocytes in culture results in the induction of apoptosis (*64*). Agents known to be inhibitors of apoptosis in vitro have been shown to limit infarct size in these disorders (*65*). However, the most effective method of limiting infarct size is restoration of blood flow. Advances in medicine have allowed for the development of a number of techniques to restore blood flow rapidly in acutely occluded blood vessels. Unfortunately, further tissue injury frequently occurs during establishment of reperfusion. Reperfusion is associated with acute increases in free radical production and increases in intracellular calcium, both potent inducers of apoptosis. The death of cardiomyocytes that occurs during reperfusion bears all the hallmarks of apoptosis (*66*). Whether agents that alter the apoptotic threshold can prevent reperfusion injury remains to be determined.

Relatively little is known about the mechanisms underlying the two major degenerative disorders of the musculoskeletal system: osteoporosis and degenerative arthritis. To date, no evidence exists that the genes involved in the control of apoptosis are involved in the pathogenesis of these disorders. Nevertheless, the progressive cell death of chondrocytes in osteoarthritis and osteocyte cell death in osteoporosis have morphological features suggestive of apoptosis (*67*).

Liver cells also undergo programmed cell death. In fact, the term apoptosis was originally used to describe the cell death that occurred outside the zone of central necrosis resulting from ligation of the portal vein (5). Since that time, various toxins (including alcohol) that are associated with acute fatty degeneration of the liver have been shown to induce apoptosis in hepatocytes (68).

Regulation of Cell Death: Therapeutic Potential

A number of effective methods that induce target cells to undergo apoptosis already exist. Both chemotherapeutic agents and radiation induce tumor cell death primarily by causing damage that induces the cell to commit suicide (*17, 69*). In addition, many tumors retain some of the same physiologic controls for cell death as the cells from which they arise. For example, tumors arising in reproductive organs are responsive to hormonal manipulation that results in apoptosis (*6, 70*). Prostate cancers can be treated with androgen-ablation therapy. Moreover, breast cancers often undergo regression when treated with estrogen receptor antagonists. Treatments that restore the ability to properly regulate apoptosis could also be of considerable benefit in some malignancies. For example, it has recently been shown that the growth of human B cell lymphomas bearing *BCL2* translocations can be specifically inhibited in vitro by antisense oligonucleotides targeted against the *BCL2* gene (*71*).

Autoimmune diseases are characterized by the proliferative expansion of lymphocytes reactive to self-antigens. Several groups have been exploring methods to induce selective apoptosis in the autoreactive cells that cause disease. Recently, it has been shown that repetitive treatment with antigen can result in the selective death of antigen-reactive lymphocytes in vivo. Although the exact mechanisms by which such treatments induce apoptosis are unclear, the treatments may prime cells for Fas-mediated death (*72*). Specific deletion of lymphocytes by repetitive treatment with a disease-associated autoantigen has been shown to be effective in the treatment of experimental autoimmune encephalitis in mice (*73*). Similar treatment strategies may prove effective in human autoimmune disease if the specific antigens involved in the autoimmune reaction can be identified.

Conversely, treatments that increase a cell's resistance to undergo apoptosis may be of benefit in degenerative disorders. These treatments may be of benefit even in the absence of specific alterations in the genes involved in cell death regulation. Current evidence suggests that the susceptibility of cells to undergo apoptosis is regulated continuously. For example, enhancing the expression of *BCL2* can increase the resistance of cells to almost all apoptotic stimuli (*13, 17*). Thus, treatments that can increase the apoptotic thresholds of specific cells may be beneficial in the treatment of disorders associated with cell loss. Such treatments include the use of growth factors to promote hematopoietic cell recovery after cancer chemotherapy (*56*), trials of neurotrophic survival factors to enhance the survival of neurons in neurodegenerative disorders or trauma (*50, 55*), and treatments with antioxidants such as *n*-acetyl cysteine to prevent the death of $CD4^+$ T cells in response to HIV infection (*74*). Agents that alter calcium metabolism are currently being tested for the treatment of ischemic injuries (*75*).

It may also be possible to alter the apoptotic threshold by inhibiting the action of cell surface death effectors. For example, treatment with soluble Fas receptor or TNF antibodies may limit cell death in vivo by preventing deaths induced by Fas ligand and TNF, respectively (*27, 76*). Although the use of cell surface receptors and second messenger systems to regulate cell death responses in vivo has much appeal, it should be reemphasized that these agents can have pleiotropic effects. Under some circumstances Fas activation has been reported to stimulate lymphocyte proliferation instead of cell death (*77*). IL-12 treatment in vivo has recently been shown to protect bone marrow cells from gamma irradiation; however, it also potentiates cell death within the gastrointestinal tract in response to the same stimulus (*78*).

In principle, the genes involved in central cell death control, such as members of the *BCL2* and ICE families, could provide ideal targets for therapeutic intervention. However, most diseases are not characterized by a generalized increase in the susceptibility or resistance to apoptosis. There may be relatively little benefit in a therapy that enhances the survival of all neural cells at the expense of an increase in autoimmune disease or the enhancement of tumor progression through the prevention of apoptotic cell death.

Several observations suggest that the central mediators of apoptosis may still be pharmacologically manipulated in a cell-specific fashion. Individual tissues in the body can vary significantly in the expression of individual members of the *BCL2* and *ICE* gene families (*79*). Furthermore, specific inhibitors of individual members of the ICE family exist. The gene *crmA* inhibits ICE but not the related Ich-1 cysteine protease (*80*). This suggests that cysteine protease inhibitors may be developed that are specific for individual members of the ICE family. There is also evidence that alternatively spliced forms of members of both the *BCL2* family and cysteine protease family of death effectors can act as dominant inhibitors, perhaps by forming multimers that inhibit the function of active family members (*80, 81*). Pharmacologic agents that mimic the inhibitors or prevent specific protein-protein interactions within the *BCL2* or ICE families may have relative specificity for individual family members or heteromers formed from them. Finally, there is evidence that both the expression and function of individual members of the *BCL2* and ICE families are themselves regulated by lineage-specific signal transduction events. *BCL2* has been shown to undergo specific modulation in response to cytokines, cell-cell contact, or cell contact with the extracellular matrix (*82*). In addition, certain growth factors may function by inducing posttranslational modification of *BCL2* (*83*). Recent data analyzing mice deficient in *BCL2* expression suggest that the function of *BCL2* is absolutely necessary only in a limited number of normal cell types or, alternatively, that there is significant redundancy within the *BCL2* family such that specific loss of *BCL2* is relatively well tolerated outside of the immune and renal systems (*84*). Therefore, systemic inhibition of *BCL2* may not be as toxic as might have been expected initially.

One future application of our knowledge concerning cell death may be in designing safer, more effective vectors for gene therapy or vaccination. That many DNA viruses require survival genes to establish persistent viral infection may allow for the generation of vaccines consisting of attenuated viruses that do not have the ability to establish latent infections. Similarly, engineering gene therapy vectors to incorporate specifically regulated cell survival genes may help facilitate the establishment and removal of genetically altered cells in vivo.

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