REVIEW ARTICLE

Life and Death in Otolaryngology

Mechanisms of Apoptosis and Its Role in the Pathology and Treatment of Disease

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Objectives: To review recent advances in our understanding of programmed cell death, or apoptosis, and discuss implications of these basic science advances in our understanding of causes and potential treatments of a variety of diseases of the head and neck.

Data Sources: Basic science literature relevant to the study of apoptosis and its clinical implications.

Conclusions: Apoptosis is now understood to be important in the normal development and survival of all multicellular organisms. Deregulation of this normally tightly controlled process underlies a variety of disease states, including neoplasia, autoimmune disease, and disorders of the central nervous system. A better understanding of this process and its regulation may help otolaryngologists better understand diseases relevant to this specialty and will lead to improved therapeutic interventions.

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The accelerated rate of scientific progress demands not only an expanded vocabulary but also a willingness to adopt new ideas and strategies and to readjust basic biologic concepts. Without this approach, future surgeons would be in danger of becoming surgical technicians and would fail their heritage as major contributors to medical progress.

Lazar J. Greenfield, MD[1]

THE RAPID expansion in clinical science that has occurred in otolaryngology, and medicine in general, has been fueled by a similar expansion in basic science. Basic and clinical research have now progressed to the cellular and molecular levels. As Greenfield implies in the above quotation, these developments are changing the fundamental ways we think about injury and disease. One example of such a fundamental change is our new understanding of the control of apoptosis and the mechanisms by which cells die. This is demonstrated by the observation that many articles in the recent otolaryngological literature directly address the regulation of cell death in a variety of systems, including the auditory system and epithelial neoplasia. The purpose of this review is to provide a framework for otolaryngologists to understand the rapid advances in our understanding of the mechanisms of cell death. We hope that with this will come an understanding of how fundamental this process is, and how its deregulation can result in diseases that affect all areas of medicine, and otolaryngology in particular.

Apoptosis, or programmed cell death, represents a highly conserved pathway by which unwanted or unneeded cells are removed efficiently from multicellular organisms. Apoptosis plays a critical role in the normal development of all multicellular organisms and is largely responsible for the homeostasis of cell populations in the adult organism. Deregulation of apoptosis may result in excessive cell loss, as in the loss of peripheral or central auditory neurons, or cell accumulation, as occurs in various neoplasias, including carcinomas of the head and neck. The role of deregulation of apoptosis in several diseases related to otolaryngology is an active area of investigation. A better understanding of the molecular mechanisms of apoptosis may lead to interventions aimed at preventing cell death in cases of its excess, as in autoimmune disease, or accelerating cell death, as in the case of neoplastic cell accumulations.

In the following review, we will, first, discuss the concept of control of cell number as an essential homeostatic mechanism; second, define and compare ne-

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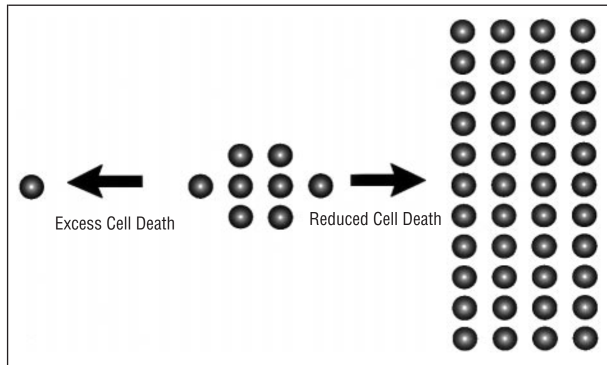

Figure 1. *Regulation of cell number by multicellular organsisms can be thought of as a homeostatic process. Deregulation of the normally tight control of cell death can affect this homeostasis. Disorders of excess cell death have been shown to underlie disorders of development or autoimmune disease. Disorders of decreased cell death may result in neoplastic accumulations.*

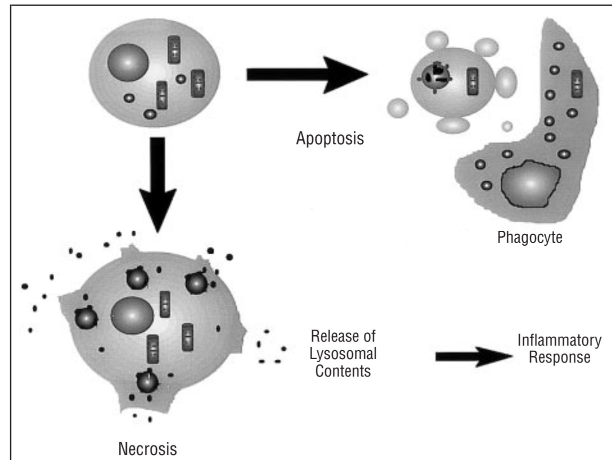

Figure 2. *Necrotic cell death (left) results in cellular and organellar swelling and lysis. Release of lysosomal contents into the extracellular milieu results in the activation of inflammatory mediators. Apoptosis, on the other hand, is characterized by cellular shrinkage, condensation of nuclear DNA, and cytoplasmic blebbing with the formation of apoptotic bodies. These apoptotic bodies are phagocytosed by macrophages (or other phagocytic cells) and the extracellular release of cytotoxic compounds is averted. Thus, apoptosis can be thought of as a controlled autodigestive process.*

crotic and apoptotic cell death; third, discuss recent advances in understanding the genetic control of programmed cell death; fourth, examine a model for programmed cell death under study in the auditory system; and, finally, discuss implications of these recent basic science advances in our understanding of the causes and potential treatments of a variety of diseases.

CONTROL OF CELL NUMBER: PROLIFERATION VS CELL DEATH

The concept of homeostasis has been traditionally applied to the physiologic regulation of the cellular environment. We now know that the tight regulation of cell number can be thought of as similarly essential to the normal growth and development of multicellular organisms. One model proposes that the control of cell populations may be thought of as a balance between cell proliferation and cell death.[2] As depicted in **Figure 1**, we see that for a given group of cells, changes in the rate of cell death can have profound effects on cell population size. One can easily imagine that for a given rate of cell proliferation, a decreased rate of cell death would lead to the accumulation of cells and neoplasia. This has been described as the molecular basis for several tumors, including follicular lymphoma (see below) and carcinomas with mutations of *p53*, including squamous cell carcinomas of the head and neck,[3-5] On the other hand, abnormal activation of the cell death program may lead to excessive cell loss. Since we now know that this is the basis of action of many chemotherapeutic agents as well as radiotherapy, a better understanding of the cell death pathway may also lead to more efficacious treatments for cancer, as discussed below.

The importance of the control of cell proliferation is indicated by its normally strict regulation in development and in the adult organism. A number of regulators of this process have been described. These include the positive regulators of cell proliferation, such as various growth factors and proto-oncogenes (eg, platelet-derived growth factor and *c-myc*, respectively). Disruptions in proto-oncogenes have been well documented in human cancers.[6] Negative regulators include tumor suppressor genes such as the prototypical tumor suppressor gene involved in the pathogenesis of retinoblastoma, *Rb-1*.[6] In this review, however, we will concentrate on the molecular control of cell death.

The differentiated cells of multicellular organisms all share the ability to undergo apoptosis through the activation of an internally coded "suicide" program. The central importance of this process is underscored by its conservation throughout metazoan evolution.[7] This program is so well conserved that some of the genes involved in its execution may function interchangeably among species vastly separated on the evolutionary timescale. For example, the human antiapoptotic gene *bcl-2* (discussed below) may substitute for its counterpart in the nematode *Caenorhabditis elegans*.[8,9] The conservation of this cell death program is also evident in the presence of genes homologous to known cell death regulators in some DNA viruses,[2] allowing the virus to use the host's cell death machinery to its advantage.

It seems that in many or most cells, the machinery required to cause the death of a given cell is present at all times, awaiting the proper signal for self-destruction. Why should cells be so ready to die? To understand the importance of controlled cell death, it is instructive to consider the differences between it and uncontrolled or necrotic cell death.

APOPTOSIS VS NECROSIS

In general, 2 types of cell death occur in multicellular organisms: necrosis and apoptosis. Necrotic cell death is a pathologic form of cell death resulting from acute cellular injury, which is usually manifest ultrastructurally by rapid cellular and organellar swelling and eventual cellular disintegration (**Figure 2**). Cellular lysis results in the uncontrolled release of lysosomal enzymes, which incites a robust inflammatory response in vivo. Apoptosis, in contrast, can be characterized as a

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controlled autodigestive process.[10] Through the activation of the well-conserved cell death program, a number of characteristic morphologic changes occur (Figure 2). These include cell shrinkage, condensation of nuclear chromatin into sharply circumscribed masses, and in the final stages, membrane blebbing with the formation of so-called apoptotic bodies. These apoptotic bodies are phagocytosed by macrophages and thus no cytosolic contents are released. The inflammatory response typical of necrosis is thereby averted.

It may be useful to consider necrotic cell death an uncontrolled process that is not directly related to normal homeostasis and development but rather represents a direct effect of a pathophysiological insult. On the other hand, apoptosis represents a controlled process that allows a multicellular organism to efficiently remove unwanted, unneeded, or abnormal cells without inciting an inflammatory response. This process is normally under tight control; the genes and cytoplasmic events responsible for its control are the subject of intense investigation.[11-13]

MOLECULAR MECHANISMS OF PROGRAMMED CELL DEATH

The complexity of higher-order vertebrates makes the study of cells and cell fate during development quite challenging. The nematode *C elegans* has provided an exemplary model in which to investigate programmed cell death. Much of the work done during the past 20 years to characterize the molecular mechanisms and genetics of programmed cell death was begun in *C elegans* by Robert Horvitz and his group at Massachusetts Institute of Technology in Cambridge. The diminutive size and translucency of this organism facilitates accurate tracking of each cell's fate during the organism's development. To be precise, 131 cells die during the development of the adult organism of 959 cells.[14] Much work that has been done on cloning the genes involved in the cell death pathway in *C elegans* seems directly relevant to mammalian cells. In particular, the genes *ced-3*, *ced-4*, and *ced-9* in the nematode have been shown to be critical to understanding programmed cell death in normal mammalian development as well as pathologic states.

Programmed cell death in *C elegans* can be conceptualized as occurring in 3 stages. Stage 1 (signal) is generally characterized as the presentation of a signal to the cell to trigger the cell death program (**Figure 3**). This signal may be intracellular or extracellular. Extracellular signals include ionizing radiation, hormonal signals, growth factor withdrawal, or removal of afferent input in the neonatal nervous system.

In stage 2 (execution), the cell death program is actually executed (Figure 3) by activation of a family of cysteine proteases with aspartate specificity, or caspases; formerly known as interleukin 1β–converting enzymelike proteases.[15] These represent a novel class of cell death regulators. Ten different caspases have been shown to exist in humans.[15]

In stage 3 (morphologic changes), caspase activation leads to the characteristic apoptotic morphologic changes described above, such as cell shrinkage, condensation of nuclear chromatin, and the formation of "apoptotic bodies." These morphologic and structural changes represent the final stage of the programmed cell death cascade (Figure 3).

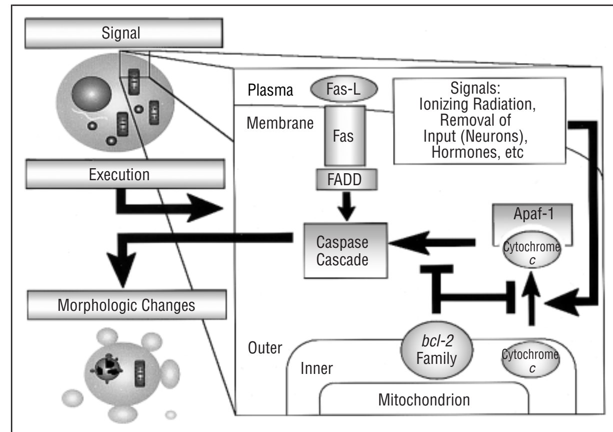

Figure 3. *Molecular control of apoptosis: a current 3-stage model. Fas-L is an extracellular cell death protein; Fas, its receptor. FADD indicates Fas-associated death domain protein; Apaf-1, apoptotic protease-activating factor 1.*

In the nematode, the protein product of *ced-3* has been shown to be a caspase.[16,17] The protein product of *ced-4* is necessary to activate this caspase, and is homologous to the human protein apoptotic protease-activating factor 1 (Apaf-1).[18] Thus, *ced-4* acts as an upstream activating factor for *ced-3*. The protein coded by *ced-9* has been shown to bind to *ced-4* and prevent its activation of *ced-3*.[19] The human homolog of *ced-9* is bcl-2,[7] which is the prototypical member of a family of cell death regulators.

THE *bcl*-2 FAMILY OF PROGRAMMED CELL DEATH REGULATORS

The *bcl*-2 gene represents the first described member of a family of genes that has been found to be important in controlling programmed cell death. It was identified as the cellular oncogene activated in follicular lymphoma, a type of non-Hodgkin lymphoma.[20] A t(14;18) translocation in most follicular lymphomas indicated the presence of a then novel oncogene, designated *bcl*-2 (B-cell lymphoma/leukemia 2), that was activated by the translocation.[21-23] Subsequently it was discovered that *bcl*-2 on chromosome 18q and the immunoglobulin heavy-chain locus on 14q are recombined in a balanced translocation that places the *bcl*-2 gene under the transcriptional regulation of the heavy-chain locus. This transcriptional deregulation resulted in the overexpression of wild-type *bcl*-2 RNA and protein.[24]

The function of *bcl*-2, inhibition of the apoptotic cell death program, was first demonstrated in 1988 by Vaux et al[25] with the use of a transgenic mouse. By transgenic we mean a mouse in which the genome has been altered in some way. For example, a given gene may be manipulated to be placed under a different promoter, as in this example, resulting in altered expression of its messenger RNA and thus its protein product. Another example

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is the "knockout" (see below), in which a given gene is inactivated or knocked out.

Vaux and colleagues produced a transgenic mouse in which manipulation of the mouse genome resulted in the placement of the native *bcl-2* gene under transcriptional regulation of the immunoglobulin heavy-chain enhancer, similar to the effect of the t(14;18) translocation in human lymphomas (above). This was found to cause polyclonal expansion of mature B lymphocytes, confirming the suspected mechanism of B-cell accumulation in human follicular lymphoma. Ten percent to 15% of these animals eventually developed a diffuse immunoblastic B-cell lymphoma.[26,27] This suggested that overexpression of *bcl-2* resulted in immortalization of a clone of lymphocytes, resulting in lymphoma. At the time, this was quite important as it was the first demonstration that a single gene could block cell death, effectively immortalizing a cell. Could this gene be solely responsible for the regulation of apoptosis?

Evidence of the presence of other genes involved in apoptosis, particularly in development, again came from studies on transgenic mice. Interestingly, mice with homozygous deletions (knockout mice) of *bcl-2* develop normally with the exception of growth retardation and 3 tissue-specific phenotypes[28]: (1) massive lymphoid involution within 2 months of birth; (2) graying of the hair during the second follicle cycle; and (3) polycystic kidney lesions resulting in the animal's death (typically during the third month of life). The otherwise relatively normal development of *bcl-2* knockout mice suggested that other genes may be involved in regulating apoptosis during development.

Subsequently, several genes with varying homology to *bcl-2* have been cloned. Together, the *bcl-2* family of genes and their respective protein products seem to act as a complex, functionally redundant system by which cells regulate their number. These proteins have opposing function; some promote cell death while others inhibit it. For example, BAX, a protein that is found to colocalize with bcl-2 in cells, promotes cell death.[29] Current evidence suggests that BAX and bcl-2, positive and negative regulators of apoptosis, respectively, compete with one another to alter cell fate. These proteins bind to one another to form pairs of either the same (homodimers) or opposing proteins (heterodimers). In this model, an excess of BAX homodimers promotes activation of the cell death program, while an excess of bcl-2 homodimers inhibits it.[29] The heterodimers are presumably neutral.

To make matters more complicated, other members of the family exist, as shown in the tabulation below. For example, another protein, called BAD, has been shown to be active in promoting cell death in a similar, competitive fashion.[30] Alternative splicing of another family member, bcl-x, into long (L) or short (S) forms results in either promotion of cell survival (*bcl-x*$_L$) or cell death (bcl-x$_S$).[31] All of these proteins may form heterodimers or homodimers with one another to tip the scale in favor of or against initiation of the apoptotic pathway. Analysis of the sequences of the protein products of members of this gene family reveals that 2 regions are conserved among most family members. These regions are called BH1 and BH2.[32] The functionality of these regions on the molecular level is still unclear.

Members and functions of the bcl-2 family of proteins in humans are as follows:

Gene and/or Protein Product	Function
bcl-2	Antiapoptotic
bcl-x$_L$	Antiapoptotic
bcl-x$_S$	Proapoptotic
BAX	Proapoptotic
BAK	Proapoptotic
BAD	Proapoptotic
BIK	Proapoptotic

How the bcl-2 family of cell death regulators acts to control programmed cell death remains under intense investigation. Recent work indicates that members of this family act indirectly to either initiate or suppress activation of caspases. It seems that members of this protein family interact with themselves in the form of heterodimers or homodimers (as described above). These protein complexes, or nondimerized members of the bcl-2 family, may then act by binding with Apaf-1, thus influencing caspase activation, or by influencing mitochondrial release of cytochrome *c* into the cytosol (required for caspase activation).[13,33,34] (Cytochrome *c* is a catalytic protein [enzyme] that is essential to production of adenosine triphosphate in aerobic metabolism [oxidative phosphorylation].) Thus the bcl-2 family of cell death regulators modulates the cell death program, as shown schematically in Figure 3 and reviewed in detail by Adams and Cory.[35] The localization of members of this family to the mitochondria and the role of cytochrome *c* in apoptosis have pointed toward a central role for the mitochondria in this process.

MITOCHONDRIA AND CALCIUM IN APOPTOSIS

Cytochrome *c* is synthesized in the nucleus in inactive form (apocytochrome *c*) and transported across the outer mitochondrial membrane to the space between this and the inner mitochondrial membrane, where it combines with heme to form the active protein, or holoenzyme.[13] How cytochrome *c* is extruded from mitochondria in apoptosis remains a mystery. Two models for its release have been proposed: In the first model, mitochondrial rupture results in release of the enzyme. Evidence for this is that mitochondrial membrane potential has been shown to drop from its normally highly negative state in apoptosis.[36] This is thought to be due to a change in mitochondrial membrane permeability[37] (called the permeability transition), caused by the opening of a cyclosporin-inhibitable channel or pore in the inner mitochondrial membrane.[37,38] It is possible that the resultant swelling of the inner membrane causes rupture of the outer membrane, allowing cytochrome *c* to escape into the cytosol. This theory has been supplanted by evidence that the release of cytochrome *c* can occur before the mitochondrial permeability transition occurs.[33,34]

A second model proposes that a specific channel allows the release of cytochrome *c*. In support of this model, members of the bcl-2 family of proteins share some structural homologous features with the pore-forming do-

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mains of certain bacterial toxins, such as diphtheria toxin,[39] and bcl-x_L and BAX have been shown to have pore-forming activity.[40,41] Furthermore, bcl-2 has been shown to block the pore-forming activity of BAX and the mitochondrial permeability transition.[41,42]

Another feature noteworthy of apoptotic cells is often a rise in cytosolic calcium.[43] Rises in intracellular calcium are thought to play a primary role in excitatory neurotoxic effects.[44] Mitochondria seem to play an important role in the regulation of calcium in a variety of cell types, including neurons.[45-48] Interestingly, overexpression of bcl-2 can affect both apoptosis and rises in intracellular calcium in some cell types.[49,50] Taken together, these data appear to indicate that bcl-2 (and related proteins) act at the level of the mitochondria to regulate cell death (or survival). For a detailed review of the relationship between bcl-2, cytochrome *c*, and mitochondria in apoptosis, please see Green and Reed.[51]

EXTRACELLULAR CELL DEATH LIGANDS

Other mechanisms for induction of cell death exist. The non-*bcl-2*–dependent activation of caspases and thus induction of apoptosis has been demonstrated, and this may be related to autoimmune disorders.[12] In the immune system, expression of the extracellular cell death protein, Fas-L, occurs on the surface of cytotoxic T cells in response to presentation of foreign peptides by major histocompatibility complex class I on antigen-presenting cells. Fas-L then binds its receptor, Fas, on the antigen-presenting cell plasma membrane. This, in turn, causes its association with the Fas-associated death domain protein (FADD), resulting in activation of the caspase cascade.[52] This mechanism is responsible for cytotoxic immune-mediated cell death as well as removal of self-reactive lymphocytes. Disorders of this system may thus lead to diseases of autoimmunity at 2 levels: First, failure to remove self-reactive lymphocytes results in the persistence of cytotoxic cells directed toward self-antigens. Second, these cells use cytotoxic immune-mediated apoptotic cell death (via the Fas-L-Fas-FADD pathway) to kill normal host cells. Ashkenazi and Dixit[53] have recently published a detailed review of the extracellular cell death ligand pathways.

p53 AND APOPTOSIS

A number of other oncogenes may act via the apoptotic pathway to influence neoplastic processes. For example, *p53* is believed to function as an oncosuppressor and plays an important role in repair of DNA damage.[6] Mutations of this gene are the most common in human malignancy.[54,55] The *p53* gene encodes a transcriptional activator that is thought to play a role in the cellular response to DNA damage and cell cycle progression.[56] As with *bcl-2*, important insights regarding its function have come from the analysis of transgenic *p53*-deficient mice. These mice show normal development into adulthood, indicating that the *p53* gene is not responsible for regulating apoptosis in development. However, thymocytes from these mice are resistant to ionizing radiation, which is usually an apoptotic stimulus in thymocytes possessing the wild-type *p53* allele.[57,58] Interestingly, *p53*-deficient thymocytes show normal apoptosis when exposed to glucocorticoids, T-cell receptor activation, or high calcium loads.[57,58]

In the current model, *p53* seems to induce cell cycle arrest and apoptosis in response to DNA damage, as occurs with ionizing radiation,[59] by activating genes whose products generate free radicals, causing damage to mitochondria and release of mitochondrial contents.[60] As indicated earlier, release of cytochrome *c* into the cytosol from the mitochondria may be necessary for the activation of caspases and the execution of the cell death program.

The clinical implication is that mutations in *p53* are responsible not only for induction of neoplasia, but also for resistance to radiotherapy. Mutations in *p53* have been demonstrated in carcinomas of the head and neck, and some have used this as a molecular biologic staging tool.[4,5] In fact, overexpression of p53 (a typical response to a mutation of the allele) has been shown to predict poor response to radiotherapy in at least 1 recent study.[61] Investigation into the possibility of introduction of wild-type *p53* into tumors of the head and neck via viral-mediated gene transfer look promising.[62]

THE AUDITORY SYSTEM AS A MODEL FOR APOPTOTIC CELL DEATH: MITOCHONDRIA AND CALCIUM REVISITED

While programmed cell death is believed to play a role in the development of virtually all tissue types, it has been studied most extensively in the developing nervous system. It was within this context that trophic factors such as nerve growth factor and brain-derived neurotrophic factor were discovered.[14,63] More recently, the importance of afferent innervation in regulating neuronal survival has been recognized. Removal of peripheral sense organs is known to cause degeneration of neurons in the corresponding central nuclei.[63] The auditory system is no exception, as removal of afferent input via the eighth cranial nerve has been shown to cause transneuronal degeneration in the cochlear nucleus.[64,65] Such transneuronal degeneration occurs in humans after the onset of acquired profound deafness, and may ultimately limit the benefit obtainable from auditory prostheses.[66] It is important to understand the mechanisms involved in transneuronal degeneration of cochlear nucleus neurons not only for this reason, but also because this serves as a good model for understanding the mechanisms underlying neural control of apoptosis throughout the central nervous system. Furthermore, since the basic mechanisms of apoptosis are so well conserved, information gathered from this model may be applicable to other systems.

Neurons of the anteroventral cochlear nucleus (or its homolog in the avian brain, nucleus magnocellularis [NM]) receive unilateral input from the ipsilateral auditory nerve. Ipsilateral cochlear removal or action potential blockade of the auditory nerve triggers a series of intracellular events leading to the death of 30% to 80% of these neurons, depending on the age and species.[64,67,68] During this process, these neurons undergo characteristic metabolic and morphologic changes similar to apoptosis.[69,70] These changes include (but are not limited to) an increase in mitochondrial density, an increase in in-

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tracellular calcium, decreased RNA and protein synthesis, and changes in oxidative metabolism.[67,71-73]

Once again, it appears that mitochondria are of central importance in determining cell survival in this model. Multiple lines of evidence support this hypothesis. After deafferentation in the chicken, NM neurons show an increased number of mitochondria when examined using electron microscopy.[71] When investigated further, it was found that inhibition of mitochondrial but not cytoplasmic protein synthesis specifically potentiates cell death in NM after afferent deprivation.[69,70,74] These observations indicate a protective role for mitochondria in determining cell fate after deafferentation.

A second line of evidence comes from examination of the role of mitochondria in regulating cytosolic calcium. Rises in cytosolic calcium levels are a primary event in excitatory neurotoxic effects,[44] and rises in cytosolic calcium levels are also a common finding in apoptotic cells.[43] Recently, several groups have found that mitochondrial function is critical to the normal regulation of cytosolic calcium levels in various cell types.[46,75,76] It has been demonstrated that mitochondria are important in regulating intracellular free calcium in NM neurons in 2 ways: (1) mitochondria may buffer calcium via direct uptake; and (2) mitochondrial oxidative phosphorylation provides adenosine triphosphate for adenosine triphosphate–dependent cytosolic calcium clearance mechanisms present in the plasma membrane or elsewhere.[47]

These independent lines of evidence seem to indicate that mitochondrial function is critical to cochlear nucleus neuronal survival after deafferentation. Interestingly, changes in mitochondrial function are a primary event in cell death in other cell types.[11,37] Furthermore, mutations in mitochondrial DNA have been found in a number of cases, including some families with hereditary susceptibility to aminoglycoside toxic effects and some forms of deafness (for reviews, see Jacobs[77] and Suomalainen[78]). Whether such mutations place cells at an increased risk for abnormal triggering of the apoptotic cell death program remains to be shown.

Transneuronal degeneration of mammalian central auditory neurons has been studied most extensively in the gerbil and mouse.[65,79,80] The sensitivity of cochlear nucleus neurons to deafferentation changes significantly as a function of the animal's age. There is a critical period around the time of onset of hearing in the gerbil and mouse during which a dramatic drop in sensitivity to deafferentation-induced cell death occurs.[81,82] While the molecular basis for this change remains unknown, the existence of numerous transgenic mutant mice has made this model for studying the critical period particularly powerful. Preliminary studies on homozygous *bcl-2* knockout mice has indicated that this gene product may have a protective role in these neurons after removal of afferent input.[81]

CLINICAL IMPLICATIONS

Cancer

As discussed above, disrupted regulation of cell number can lead to neoplastic cell accumulations. Nearly all chemotherapeutic drugs kill tumor cells by activation of the apoptotic cell death program.[83] Altered expression of cell death regulators may underlie some forms of chemoresistance.[83,84] For example, increased expression of bcl-2 has been found to occur in multiple myeloma cells that survive exposure to the chemotherapeutic agent, doxorubicin.[85] This may be a mechanism for selection of chemoresistant cells in vivo. Increased expression of bcl-2 has been found in patients with chemoresistant forms of breast cancer.[86] Oncologists hope to use information about the biochemical mechanisms of action of the bcl-2 protein and other members of the bcl-2 family to overcome the cytoprotective effects of bcl-2 overexpression in chemoresistant cancers. A study on the expression of members of the bcl-2 family in squamous cell carcinomas of the larynx found altered expression of these proteins but did not evaluate the prognostic significance of these alterations.[87] Future studies will presumably more closely examine the prognostic value of studying the expression of these cell death regulators in determining response to chemotherapy.

Radiation also induces apoptosis in tumor cells. Mutations in regulators of apoptosis may underlie radioresistance. For example, p53 is thought to induce apoptosis in response to DNA damage from ionizing radiation (as described above). Therefore, tumors with mutations in *p53* may be less sensitive to radiotherapy. One preliminary study has indicated that p53 may be a useful predictor of outcome in potential radiotherapy candidates with squamous cell carcinomas of the head and neck.[61] Recently, Spafford et al[88] examined the expression of p53 and bcl-2 in patients with squamous cell carcinoma of the larynx. They found that increased expression of p53 correlated with poor survival, while bcl-2 was not a prognostic discriminator. However, the detection of p53 by immunochemical means (as in this study) has its limitations. For example, this technique does not necessarily discriminate mutant (nonfunctional) from wild-type (functional) proteins. Detection of mutation by polymerase chain reaction is both expensive and time-consuming. Since it has been shown that mutations in *p53* result in accumulation of the protein, the presence of increased levels of the protein detected by immunohistochemical analysis has been interpreted as synonymous with mutation in the gene. However, recent work (reviewed in Prives[89] and Steele et al[90]) has demonstrated that increased levels of p53 may not necessarily represent mutations in the *p53* gene, but instead in other, related proteins. Second, even increased expression of this cell death regulator in its wild-type form may be unable to overcome the effects of disrupted expression of other members of the apoptotic cell death pathway, such as bcl-2 or caspase (see Figure 3). This redundancy in the cell death machinery has been shown in head and neck tumor cell lines in vitro.[91] Thus, a basic knowledge of the cell death pathway is requisite to critical reading of such literature.

Autoimmune Disease

Defective regulation of apoptosis may play a role in the development of autoimmune diseases. As discussed above, expression of the extracellular cell death ligand, Fas-L,

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occurs on the surface of cytotoxic T cells in response to presentation of foreign peptides by major histocompatibility complex class I on antigen-presenting cells. This pathway is responsible for cytotoxic immune-mediated cell death as well as removal of self-reactive lymphocytes. Disorders of this system may thus lead to diseases of autoimmunity at 2 levels: First, failure to remove self-reactive lymphocytes results in the persistence of cytotoxic cells directed toward self-antigens. In this case, lymphocytes recognize a particular self-antigen as foreign, and incite an inflammatory response. Second, these cells use cytotoxic immune-mediated apoptotic cell death (via the Fas-L-Fas-FADD pathway) to kill normal host cells.

Autoimmune diseases are often systemic and have a number of manifestations in the head and neck. For example, dysregulation of Fas and Fas-L and *bcl-2* has been found to underlie development of Hashimoto thyroiditis.[92,93] Furthermore, inhibition of Fas-mediated removal of thyrocytes may underlie the development of goiter in patients with Graves disease.[94] Peripheral blood cells from patients with a variety of vasculitides (such as Wegener granulomatosis) demonstrate accelerated rates of apoptosis when compared with controls.[95] As we learn more about the role of cell death regulators in the pathogenesis of autoimmune diseases, novel drug therapies may be devised.

Olfaction

The olfactory epithelium is unique in that continual nerve cell turnover occurs. Neuronal precursor cells differentiate into olfactory receptor neurons from embryonic development through adult life.[96,97] This continual turnover has made the olfactory system a valuable model for studying how neurogenesis and apoptosis interact to regulate neuron number during development and regeneration.[98] For example, unilateral nasal occlusion results in a significant reduction of olfactory bulb size in neonatal rats.[99] This loss of central neurons is reminiscent of the loss of cochlear nucleus neurons after removal of eighth-nerve input, as discussed above. While not examined in humans, it implies that prolonged loss of sensory input to the olfactory epithelium may result in a reduction of central olfactory neurons in humans as well. Whether this mechanism is responsible for the hyposmia or anosmia observed in some patients after correction of chronic nasal obstruction remains to be investigated.

Auditory and Vestibular Systems

Disorders in the regulation of apoptosis may affect the auditory and vestibular systems in a variety of ways, from anomalies of development to hair-cell death. For example, expression of cell death regulators and apoptosis are thought to be critical to normal development of the inner ear.[100,101] Overexpression of bcl-2 has been shown to result in morphologic abnormalities of the otic capsule.[102] Disordered craniofacial development is seen in *bcl-2* knockout mice, including abnormal development of the external ear.[28] The inner ear has not been examined in these mice.

Examination of normal human inner ear sensory epithelia has revealed little to no apoptosis.[103] This finding is not surprising, given that this epithelium is nonregenerative. Gentamicin has been shown to induce apoptosis and hair-cell degeneration in mammalian vestibular hair cells.[104,105] These findings suggest the potential for therapeutic (or preventative) intervention since specific inhibition of apoptosis within the inner ear may prevent chemotherapeutic or antibiotic-associated ototoxic effects.

Removal of peripheral sense organs is known to cause degeneration of neurons in the corresponding central nuclei.[63] Within the auditory system, removal of afferent input via the eighth cranial nerve has been shown to cause transneuronal degeneration in the cochlear nucleus in animal models.[64,65] Such transneuronal degeneration occurs in humans after onset of acquired profound deafness.[66] Clinically, this becomes particularly important as a variety of therapies to compensate for loss of cochlear input are on the horizon. For example, the loss of central auditory neurons may limit the benefit obtained from cochlear prosthesis implantation. A better understanding of the timing, extent, and functional consequences of central auditory neuronal cell death after deafferentation is critical to the future of such therapies.

CONCLUSIONS

Apoptosis represents a highly conserved pathway by which unwanted or unneeded cells are removed efficiently from multicellular organisms. Apoptosis plays a critical role in the normal development of all multicellular organisms and is largely responsible for homeostasis of cell populations in the adult organism. Deregulation of apoptosis may result in excessive cell loss, as in the loss of central auditory neurons in sudden-onset deafness in humans, or cell accumulation, as occurs in various neoplasias, including carcinomas of the head and neck. A better understanding of the molecular mechanisms of apoptosis may lead to interventions aimed at preventing cell death in cases of its excess, or accelerating cell death in the case of neoplastic cell accumulations.

We have attempted to provide a brief overview of the current state of knowledge in the study of apoptosis in a manner that is valuable to otolaryngologists, including both those with primarily clinical interests and those with an investment in basic science. We hope we have provided some insight into the potential clinical applications of this information. It is only by continuing to update our armamentarium of knowledge that we will be able to keep up with the rapid expansion in fundamental cellular science and use this to the benefit of patients.

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