Mechanisms of Neurodegenerative Disorders

Part 2: Control of Cell Death

Benjamin Wolozin, MD, PhD; Christian Behl, PhD

Recent research into mechanisms of neurodegeneration in Alzheimer disease (AD), Parkinson disease, and other neurodegenerative disorders has lead to a dramatic increase in our understanding of the mechanisms of cell death and neuroprotection. Apoptosis is an active form of cell death that is carried out by proteins that are designed to kill the cell. Necrosis tends to occur as a by-product of excessive oxidative stress, which can be induced by agents such as β-amloid, or excessive calcium influx induced by agents such as glutamate. The neuron also has strong homeostatic mechanisms that can delay or prevent activation of apoptosis and necrosis. The balance between neurotoxic and neuroprotective mechanisms determines whether a neuron lives or dies.

CELL DEATH AS NATURALLY OCCURRING MECHANISM IN THE DEVELOPMENT OF THE NERVOUS SYSTEM AND DURING NEURODEGENERATIVE EVENTS

Much of our knowledge of cell death comes from studies of physiological “programmed cell death.” The term programmed cell death refers to the physiological suicide program that is critical for the development and maintenance of healthy tissues. There is another term, “apoptosis,” that includes programmed cell death and refers to the process of cell suicide under any condition when carried out by a cascade of executioner proteases. The studies of programmed cell death have identified many of the key proteins that carry out apoptosis. However, as we will discuss later, we are learning that in neurons the boundary between apoptosis and necrosis is much less distinct.

During the development of the nervous system a large amount of neurons degenerate owing to the competition for neurotrophic molecules such as the nerve growth factor. This is a paradigm of physiological neuronal apoptosis that allows the nervous system to eliminate excess neurons. The importance of apoptosis for development of the nervous system is exemplified by the phenotype of a knockout mouse lacking caspase 3, the critical effector protease in apoptosis. This mouse has deficient apoptosis and develops a brain that is hypertrophic because of the extra neurons that were not killed off by apoptosis.

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The mechanism by which apoptosis most commonly occurs in the nervous system seems to differ from that occurring in the immune system, where mechanisms of apoptosis were first studied. Apoptosis in the immune system commonly occurs through engagement of death receptors, such as Fas. Ligand binding leads to trimerization that activates a death domain on the intracellular portion. The activated death domain forms a large complex that ultimately engages caspases inside the cells. In contrast, in the nervous system apoptosis seems to occur most commonly through loss of trophic signaling (Figure 1). Neurons deprived of growth factors become committed to apoptosis approximately 10 hours after trophic withdrawal. Thus, neuronal apoptosis is commonly initiated by a loss of signaling rather than a gain of signaling, which is more common in the immune system.

From the Department of Pharmacology, Loyola University Medical Center, Maywood, Ill (Dr Wolozin); and the Max-Planck-Institute of Psychiatry, Munich, Germany (Dr Behl).
Apoptotic pathways. In neurons, the main pathway regulating apoptosis seems to be mediated by tyrosine kinases. In this pathway, growth factors stimulate tyrosine kinases, which stimulate PI3 kinase, which activates protein kinase B (PKB), which phosphorylates and inactivates BAD. Loss of growth factor stimulation increases the level of phosphorylated-BAD (BAD-P), which inactivates Bcl-2 and leads to activation of caspase 3 and, thence, apoptosis. Immune cells, on the other hand, have receptors that can directly activate caspases. Hence, apoptosis in immune cells can occur rapidly after receptor activation. IGF-1 indicates insulinlike growth factor-1; NGF, nerve growth factor; TNF-RI, tumor necrosis factor receptor; YP, tyrosine phosphate; P13K, phosphoinositide 3 kinase; Cyt C, cytochrome C; APAF, apoptosis-initiating factor; PARP, polyadenosine ribose phosphatase; and PAK2, p21-activated kinase.

Caspases are the actual enzymes that carry out apoptosis. While there are many particular events that occur during apoptosis, such as DNA cleavage, nuclear collapse, or membrane ruffling, the only event that is universal to all forms of apoptosis is caspase activation. Unfortunately, caspase activation can be a complicated process because there are at least 9 caspases, and many of these caspases exist as inactive zymogens that must be cleaved for activation. The most common caspases cited in the literature are caspases 3, 8, and 9. Caspase 8 is activated by binding to the signaling proteins, like FADD, that interact with death receptors (Figure 1). Activated caspase 8 cleaves a series of other caspases that are sequentially activated until caspase 3, the final executioner, is activated. It is caspase 3 that seems to carry out many of the lethal cleavages that produce apoptosis. Caspase 3 can also be activated by another mechanism in which dephosphorylated BAD binds to Bcl-2, which leads to release of cytochrome C and apoptosis activating factor, activation of caspase 3, and apoptosis.

Growth factors prevent apoptosis by a pathway that sequentially stimulates receptor tyrosine kinases, phosphoinositide 3 kinase and protein kinase B (also known as Akt, Figure 1). Protein kinase B phosphorylates BAD, which is a proapoptotic Bcl-2 homologue. Loss of growth factor stimulation leads to dephosphorylation of BAD, which binds to Bcl-2, inducing release of cytochrome C and activation of caspase 3. Thus, caspases are proteases that degrade many proteins critical for cell viability and kill the neuron.

Because caspases are the proteins that actually kill the cell, inhibitors of caspases may be of therapeutic value to stop disease-related apoptotic processes. Viruses have developed physiological inhibitors of apoptosis (IAP), such as p35 and CrmA, that suppress the host cell death response to viral infections. The specificity of the IAP vary among IAPs. CrmA blocks caspase 3, while p35 exhibits a much broader spectrum of activity and inhibits caspase 1 to 4 and 7 to 10. Recent evidence points out that the mechanisms used by IAPs are conserved among diverse species. Inhibitors of apoptosis might also affect the signaling of the stress-kinase pathways mediated by nuclear factor (NF)-κB or JNK. Peptide inhibitors of apoptosis have also been developed. The most commonly used peptide inhibitor is the caspase 3 inhibitor DEVD. Cell permeable analogs of DEVD, such as DEVD-fluoromethylketone, have been shown to block apoptosis induced by trophic deprivation in cell culture and ischemia in the brain. Recently, smaller peptide inhibitors of apoptosis have also been developed, such as the dimer bocasparyl(OMe)-fluoromethylketone, which also block apoptosis in culture and in ischemic neurons. Very likely, more and more molecular players in the induction and inhibition of apoptotic events will be identified in the future. As our ability to inhibit apoptosis improves, particularly as nonpeptide inhibitors that readily cross the blood-brain barrier are developed, the potential applications to neurodegenerative disease will also increase.

**APOTOPSIS IN NEOURODEGENERATION**

Apoptosis occurs in many pathological situations in the brain, including ischemia, Huntington disease, and AD. In the ischemic brain, apoptotic neurons appear about 4 days after the injury. Although not the main form of cell death in ischemia, the lag time between injury and apoptosis offers a window for therapeutic intervention to block this mode of cell death. The role of apoptosis has not been proven in Huntington disease; however, transgenic mice overexpressing the mutant form of Huntingtin show extensive apoptosis in striatal neurons. The ability of the mutant Huntingtin protein to induce apoptosis suggests that apoptosis could play an important role in the pathology of Huntington disease. Apoptosis has also been implicated in AD. Neuropathologic studies of AD-affected brain tissue show that the rate of apoptosis is increased 30- to 50-fold over that of age matched controls. Since apoptotic cells appeared to be cleared rapidly by the body, rapid clearance of apoptotic neurons in the AD-affected brain might lead to an underestimate of the total amount of apoptosis occurring in the disease. However, the significance of the increased apoptosis is unclear because the apoptosis could be one of many harmful processes occurring in the AD-affected brain.

**PRESENILINS AND APOPTOSIS**

One of the strongest lines of evidence implicating apoptosis in AD comes from the putative role of presenilins in apoptosis. Presenilins are proteins that cause early-onset familial AD. The mutations in presenilins that cause familial AD have been shown to render neurons more vulnerable to apoptosis. The exact mechanism through which presenilins affect apoptosis is unknown, but given the pivotal role presenilins seem to play in protein pro-

Figure 1. Apoptotic pathways. In neurons, the main pathway regulating apoptosis seems to be mediated by tyrosine kinases. This pathway, growth factors stimulate tyrosine kinases, which stimulate PI3 kinase, which activates protein kinase B (PKB), which phosphorylates and inactivates BAD. Loss of growth factor stimulation increases the level of phosphorylated-BAD (BAD-P), which inactivates Bcl-2 and leads to activation of caspase 3 and, thence, apoptosis. Immune cells, on the other hand, have receptors that can directly activate caspases. Hence, apoptosis in immune cells can occur rapidly after receptor activation. IGF-1 indicates insulinlike growth factor-1; NGF, nerve growth factor; TNF-RI, tumor necrosis factor receptor; YP, tyrosine phosphate; P13K, phosphoinositide 3 kinase; Cyt C, cytochrome C; APAF, apoptosis-initiating factor; PARP, polyadenosine ribose phosphatase; and PAK2, p21-activated kinase.
cessing, there are many possible mechanisms by which abnormalities in presenilin function could affect apoptosis. The presenilins interact with Bcl-XL, Bcl-2, β-catenin, and Notch, all of which regulate apoptosis and cell death. The carboxy terminus of presenilin 2 (PS2) is the region that binds to Bcl-XL, which could explain why the carboxy terminal PS2 fragment, Alg-3, is neuroprotective. Because Bcl-XL plays a pivotal role in apoptosis, the interaction between presenilins and Bcl-XL could account for much of the effects of presenilins on apoptosis. The interaction of PS1 with β-catenin might affect presenilin-induced apoptosis because β-catenin also can regulate apoptosis under some circumstances. Altering presenilin activity might be an important regulatory step in the apoptotic process because both PS1 and PS2 have been shown to be substrates of caspases. Cleavage of the presenilins might reflect a homeostatic, antiapoptotic function, because the C-terminal fragment generated by the PS2–caspase 3 cleavage inhibits apoptosis. Thus, the AD-associated presenilin proteins are intimately involved in the process of apoptosis.

The significant role of presenilins in apoptosis does not imply that activation of apoptotic pathways drives the pathophysiology of AD. Presenilins seem to regulate the processing of multiple proteins that affect apoptosis (such as Notch, β-catenin, Bcl-X, and amyloid precursor protein). On the other hand, many general observations argue strongly against a primary role of apoptosis in AD. Although cell death is an important feature of the AD-affected brain, synaptic loss is thought to be the most important feature of AD. In addition, most studies suggest that the amyloid-β (Aβ) peptide, whose accumulation is thought to drive AD, causes toxicity by inducing free radical production rather than caspase activation. Finally, although the mutations in presenilins increase apoptosis, they also increase production of Aβ42, which is a form of Aβ that is particularly pernicious because it aggregates rapidly. Because the ability of presenilins to increase Aβ42 production is sufficient to explain familial AD, the increases in apoptosis associated with these mutations might be an epiphenomenon. The increases in apoptosis associated with mutant presenilins might occur as a by-product of the dual roles of presenilins in signal transduction (such as Notch and β-catenin signaling) and protein processing. We have shown that presenilins couple the processing of amyloid precursor protein to signal transduction. Mutations in presenilins that affect the processing of amyloid precursor protein would be likely to also affect signal transduction enzymes. Any change in the regulation of signal transduction enzymes could easily affect apoptosis, even if this is not the major reason that the mutations cause AD. Thus, given all the questions about the potential role of apoptosis in AD, caution argues against invoking a major role for apoptosis in AD.

NECROSIS IN NEURODEGENERATION

One of the major reasons that the importance of apoptosis in neurodegeneration is questioned is the overlap between apoptosis and necrosis in neuronal biology. Necrotic cells have swollen nuclei, swollen mitochondria, and loss of plasma membrane integrity. Necrosis is a passive form of cell death that typically results from injury or from excess calcium influx during excitotoxicity. Excitotoxicity occurs in multiple pathological situations including ischemia, seizures, and head trauma. The dividing line that separates necrosis from apoptosis has been emphasized for years owing to the clear distinct features that classifies both events. However, death in neurons can be biphasic, beginning with necrosis and then showing delayed apoptosis. Moreover, if apoptosis is blocked, for instance by overexpressing the neuroprotective transcription factor NF-κB, the mode of cell death often simply switches from apoptosis to necrosis. The ability of neurons to switch from apoptotic death to necrotic death raises the possibility that antiapoptotic treatments, such as caspase inhibitors, will block apoptosis but not prevent cell death. Moreover, in neurodegenerative disorders such as AD, Aβ is able to kill via multifaceted pathways, which raises the possibility that both types of cell death contribute to the neurodegenerative events.

NEUROPROTECTION: EXOGENOUS NEUROPROTECTION AND INTRACELLULAR NEUROPROTECTION—BASIC RESEARCH AND CLINICAL PERSPECTIVES

A variety of endogenous neuroprotective programs exist that protect nerve cells against degenerative insults (Figure 2). Several such triggers of endogenous neuroprotective programs have been already identified. One important endogenous neuroprotective agent is the tran-
NF-kB is a protein that contains 2 subunits and represents a family of homologous proteins (Figure 2). NF-kB protects neuronal cells against oxidative stress. Activated NF-kB can be found in so-called early senile plaques consisting of low-molecular-weight aggregates of AD that may indicate the compensatory activation of self-defense genes in nerve cells that encounter Aβ-aggregates in the brain tissue. NF-kB may be induced by various stimuli including neurotrophic factors such as nerve growth factor and insulin-like growth factor 1, which are potential neuroprotective agents. Insulin-like growth factor 1 has been reported to protect cultured hippocampal neurons against Aβ toxicity via a mechanism involving NF-kB and the kinase Akt. Therefore, NF-kB turns out to be a central modulator of neuroprotective mechanisms, which may be activated by various trophic input.

In addition to NF-kB, other important proteins such as Bcl-2 and Bcl-XL mediate endogenous neuroprotective programs. Bcl-2 and Bcl-XL are mitochondrial proteins that inhibit activation of apoptosis by binding critical proteins such as apoptosis activating factor, which is important for the activation of caspase 3 and induction of chromatin condensation (Figure 1). Although Bcl-2 and Bcl-XL have not been used directly for preventing apoptosis, a number of caspase inhibitors have been developed that can prevent apoptosis. Caspase 3 is induced during stroke, so inhibiting its activity has been hypothesized to reduce cell death. In fact, small caspase inhibitors, such as boc-Asp-CH2F, reduce the amount of apoptosis in the penumbra of area damaged by the stroke. The difficulty is that in these types of paradigms, preventing apoptosis or excitotoxicity does not prevent cell death. Since, in stroke for instance, cell injury rather than programmed cell death (apoptosis) is the primary stimulus for death, blocking apoptosis causes neurons to switch to a necrotic mode of cell death. Antioxidants have proven to be among the best tools identified to date for protecting neurons against toxicity owing to oxidative stress. Antioxidants are powerful neuroprotectants and may prevent oxidative nerve cell death in vitro and in vivo. The classical free radical scavenger α-tocopherol (vitamin E) prevents Aβ- and glutamate-induced cell death. Recently, we identified the female sex hormone estrogen as another phenolic antioxidant that may serve as an antioxidant nerve cell shield. Vitamin E is also one of the few agents that has been demonstrated to slow the progression of AD in a clinical setting. Many antioxidants suffer from poor central nervous system penetration, but newer antioxidants, based on the structures of estrogen or melatonin, might overcome this limitation. Thus, antioxidants serve as a general neuroprotective shield, which reduce the damage caused by production of free radicals. Some potential limitations of antioxidants are not commonly discussed in the literature. Production of free radicals is an important part of our immune defense system. When a macrophage engulfs a bacterium, it kills by generating a burst of free radicals. Since antioxidants reduce the amount of oxidation, it is possible that excessive use of antioxidants might increase morbidity owing to infection in some patients. Thus, antioxidants need to be used with caution.

**OUTLOOK**

With increasing knowledge of specific mechanisms mediating cell death comes increasing hope of developing specific tools that can prevent neurodegeneration without inducing unacceptable side effects. As in many parts of medicine, different medications might be valuable in different clinical settings. For instance, inhibiting protein aggregation might be useful as a preventive strategy; however, some patients will likely be initially seen with clinical disease, despite the availability of preventive strategies. In these cases, use of antioxidants, anti-inflammatory drugs, apoptosis inhibitors, and/or other therapeutic modalities, such as the neurotransmitter based strategies (which were not covered in this review) might be indicated.

**REFERENCES**