

## REVIEW ARTICLE

## MECHANISMS OF DISEASE

# Apoptosis and Caspases in Neurodegenerative Diseases

Robert M. Friedlander, M.D.

**A**CUTE AND CHRONIC NEURODEGENERATIVE DISEASES ARE ILLNESSES associated with high morbidity and mortality, and few or no effective options are available for their treatment. A characteristic of many neurodegenerative diseases — which include stroke, brain trauma, spinal cord injury, amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, and Parkinson's disease — is neuronal-cell death.<sup>1</sup> Given that central nervous system tissue has very limited, if any, regenerative capacity, it is of utmost importance to limit the damage caused by neuronal death.<sup>2-5</sup> During the past decade, considerable progress has been made in understanding the process of cell death.<sup>6</sup> In this article, I review the causes and mechanisms of neuronal-cell death, especially as it pertains to the caspase family of proteases associated with cell death. I will review evidence linking specific cell-death pathways to neurologic diseases and discuss how knowledge of the mechanisms of cell death has led to novel therapeutic strategies.

From the Neuroapoptosis Laboratory, Division of Cerebrovascular Surgery, Department of Neurosurgery, Brigham and Women's Hospital and Harvard Medical School, Boston. Address reprint requests to Dr. Friedlander at the Department of Neurosurgery, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115, or at rfriedlander@rics.bwh.harvard.edu.

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## TYPES OF CELL DEATH

Cell death occurs by necrosis or apoptosis.<sup>7-9</sup> These two mechanisms have distinct histologic and biochemical signatures. In necrosis, the stimulus of death (e.g., ischemia) is itself often the direct cause of the demise of the cell. In apoptosis, by contrast, the stimulus of death activates a cascade of events that orchestrate the destruction of the cell. Unlike necrosis, which is a pathologic process, apoptosis is part of normal development (physiologic apoptosis); however, it also occurs in a variety of diseases (aberrant apoptosis).

**NECROSIS**

Necrotic cell death in the central nervous system follows acute ischemia or traumatic injury to the brain or spinal cord.<sup>10,11</sup> It occurs in areas that are most severely affected by abrupt biochemical collapse, which leads to the generation of free radicals and excitotoxins (e.g., glutamate, cytotoxic cytokines, and calcium). The histologic features of necrotic cell death are mitochondrial and nuclear swelling, dissolution of organelles, and condensation of chromatin around the nucleus. These events are followed by the rupture of nuclear and cytoplasmic membranes and the degradation of DNA by random enzymatic cuts in the molecule.<sup>9,12</sup> Given these mechanisms and the rapidity with which the process occurs, necrotic cell death is extremely difficult to treat or prevent.

**APOPTOSIS**

Apoptotic cell death, also known as programmed cell death, can be a feature of both acute and chronic neurologic diseases.<sup>1,9,13</sup> After acute insults, apoptosis occurs in areas that are not severely affected by the injury. For example, after ischemia, there is ne-

crotic cell death in the core of the lesion, where hypoxia is most severe, and apoptosis occurs in the penumbra, where collateral blood flow reduces the degree of hypoxia (Fig. 1).<sup>10,14-16</sup> Apoptotic death is also a component of the lesion that appears after brain or spinal cord injury.<sup>11,17-20</sup> In chronic neurodegenerative diseases, it is the predominant form of cell death.<sup>21-23</sup>

In apoptosis, a biochemical cascade activates proteases that destroy molecules that are required for cell survival and others that mediate a program of cell suicide. During the process, the cytoplasm condenses, mitochondria and ribosomes aggregate, the nucleus condenses, and chromatin aggregates. After its death, the cell fragments into “apoptotic bodies,” and chromosomal DNA is enzymatically cleaved to 180-bp internucleosomal fragments. Other features of apoptosis are a reduction in the membrane potential of the mitochondria, intracellular

acidification, generation of free radicals, and externalization of phosphatidylserine residues.<sup>6,7,12,24,25</sup>

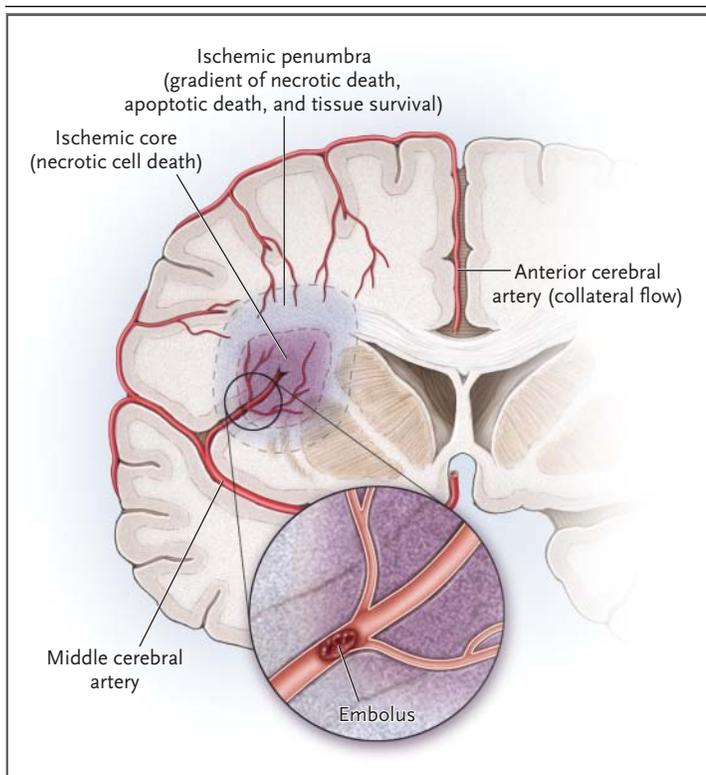
#### MECHANISMS OF PROGRAMMED CELL DEATH

The rational development of target-based strategies for the treatment of diseases in which apoptosis is prominent requires an understanding of the molecular mechanisms of programmed cell death. As recently as 10 years ago, the mediators of this process were for the most part unknown. Beginning in 1993, a series of seminal studies of the nematode *Caenorhabditis elegans* identified several genes that control cell death.<sup>26</sup> In this worm, four genes are required for the orderly execution of the developmental apoptotic program. The *ced-3*, *ced-4*, and *egl-1* genes mediate cell death, and worms that have lost the function of these genes harbor extra cells.<sup>27,28</sup> By contrast, *ced-9*-deficient worms have diffuse apoptotic cell death, indicating that this gene functions as an inhibitor of apoptosis. Metazoan homologues of *ced-3* (caspases), *ced-4* (Apaf-1), *ced-9* (Bcl-2), and *egl-1* (BH3-only proteins) have been identified.<sup>27,29-32</sup>

#### CASPASE FAMILY

The major executioners in the apoptotic program are proteases known as caspases (cysteine-dependent, aspartate-specific proteases).<sup>6,33</sup> Caspases are cysteine proteases that are homologous to the nematode *ced-3* gene product. The interleukin-1 $\beta$ -converting enzyme (also known as caspase 1), the founding member of the caspase family in vertebrates, was identified by its homology to *ced-3*.<sup>27,29</sup> Thus far, 14 members of the caspase family have been identified, 11 of which are present in humans.<sup>27</sup> Caspases directly and indirectly orchestrate the morphologic changes of the cell during apoptosis.

Caspases exist as latent precursors, which, when activated, initiate the death program by destroying key components of the cellular infrastructure and activating factors that mediate damage to the cells. Pro-caspases are composed of p10 and p20 subunits and an N-terminal recruitment domain. Active caspases are heterotetramers consisting of two p10 and two p20 subunits derived from two pro-caspase molecules (Fig. 2). Caspases have been categorized into upstream initiators and downstream executioners. Upstream caspases are activated by the cell-death signal (e.g., tumor necrosis factor  $\alpha$



**Figure 1. An Embolus in the Bifurcation of the Middle Cerebral Artery.**

The territory perfused by this artery and areas with little or no collateral flow are subjected to extreme hypoxia and necrotic cell death. In the penumbra, where there is some degree of collateral blood flow, a gradient of tissue perfusion establishes a threshold among necrotic cell death, apoptotic cell death, and tissue survival.

[TNF- $\alpha$ ]) and have a long N-terminal prodomain that regulates their activation.<sup>6,34</sup> These upstream caspases activate downstream caspases, which directly mediate the events leading to the demise of the cell. Downstream caspases have a short N-terminal prodomain.

A critical aspect of caspase-mediated cell death lies in the events regulating the activation of initiator caspases. Upstream caspases may be subclassified into two groups, according to the molecules modulating their activation. Procaspases 1, 2, 4, 5, 9, 11, 12, and 13 have a long N-terminal prodomain called the caspase-recruiting domain (CARD). Caspases 8 and 10 have a long N-terminal prodomain called the death-effector domain (DED). A regulating molecule is required for specific binding to the CARD/DED domain, which results in caspase activation. These molecules are caspase-specific and trigger-specific. For example, after the binding of TNF- $\alpha$  to its receptor, the TNF receptor binds to the DED molecule that mediates caspase 8 activation. Of the caspases with a long prodomain, caspases 2, 8, 9, and 10 are initiators of apoptosis and caspases 1, 4, 5, 11, 12, and 13 are involved in cytokine activation.<sup>34</sup> There is mounting evidence that in addition to its role in inflammation, caspase 1 is also an important upstream caspase.<sup>18,35-45</sup>

Once upstream caspases are activated in an amplifying cascade, they activate the executioner caspases downstream.<sup>6,34,46</sup> Of these caspases with a short prodomain, caspases 3, 6, and 7 are effectors of apoptosis and caspase 14 is involved in cytokine maturation. Executioner caspases mediate cell death by two main mechanisms: destruction and activation. The systematic destruction of key cellular substrates is crucial. The death process begins its terminal phase when executioner caspases activate the machinery that degrades DNA.<sup>25,47-49</sup>

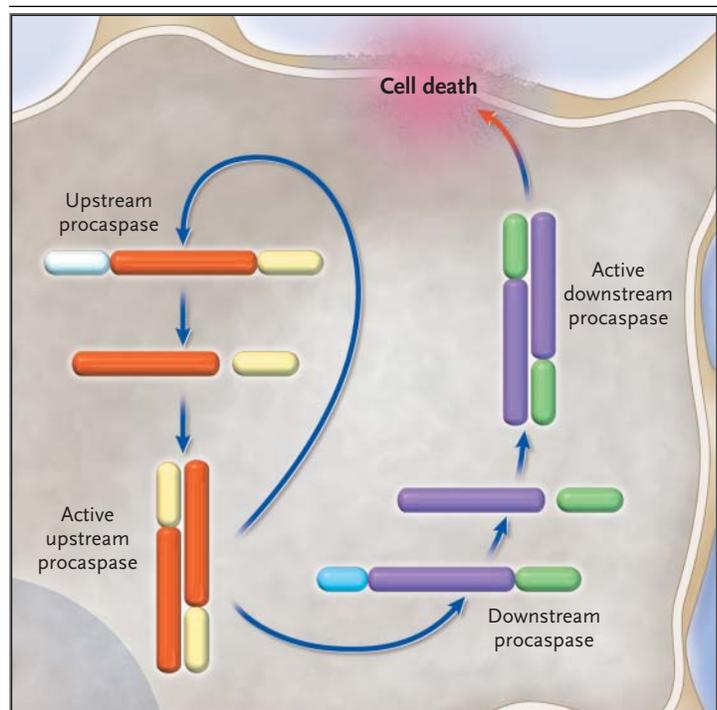
Caspases are also regulated at the transcriptional level. Transcriptional up-regulation of caspases occurs in chronic neurologic diseases such as ALS and Huntington's disease, as well as in acute neurologic diseases such as stroke,<sup>35,38,50,51</sup> which indicates that the degree of activation and the number of caspase molecules within the cell determine the level of caspase activity.

#### ROLE OF THE Bcl-2 FAMILY IN REGULATING RELEASE OF MITOCHONDRIAL CYTOCHROME c

Cytochrome *c* is a member of the mitochondrial electron-transport chain that is required for the generation of ATP. In addition to its role in cellular

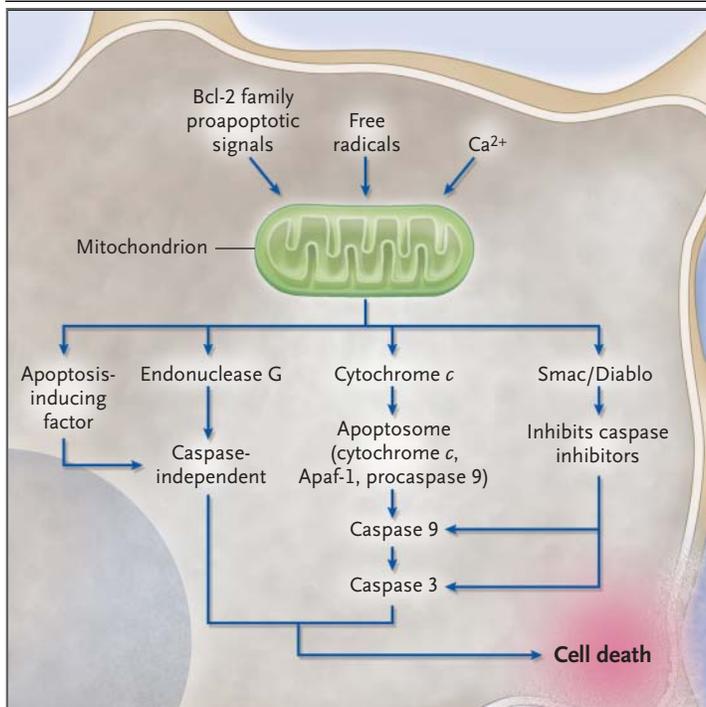
energetics, cytochrome *c* is an important trigger of the caspase cascade. Cytochrome *c*-mediated activation of cell-death pathways occurs if cytochrome *c* is released from the mitochondria into the cytoplasm. In the cytoplasm, cytochrome *c* binds to Apaf-1 to form the apoptosome—a molecular complex consisting of cytochrome *c*, Apaf-1, ATP, and procaspase 9. The apoptosome activates caspase 9,<sup>30,52</sup> an upstream initiator of apoptosis. This mechanism makes regulation of the release of cytochrome *c* a key step in the initiation of apoptosis (Fig. 3).<sup>6,53</sup>

Members of the Bcl-2 family are proapoptotic or antiapoptotic. The balance between proapoptotic and antiapoptotic signals from the Bcl-2 family has a crucial role in the release of cytochrome *c*.<sup>6,54,55</sup> Moreover, members of the caspase family can influence the balance of proapoptotic and antiapoptotic signals from the Bcl-2 family. For example, caspase 8 and caspase 1 cleave Bid, a member of



**Figure 2. Mechanisms of Caspase Activation.**

Upstream initiator caspases are activated during the initiation of the cell-death cascade. They contain an activation or binding prodomain (white), a large subunit (orange), and a small subunit (yellow). Activated upstream caspases have autocatalytic activity and activate downstream effector caspases, which have a short prodomain (blue), as well as a large subunit (purple) and a short subunit (green). Downstream caspases mediate many of the classic phenomena of apoptotic cell death.



**Figure 3. Key Mediators of the Caspase Pathway in the Mitochondria.**

Three main signals cause the release of apoptogenic mitochondrial mediators: proapoptotic members of the Bcl-2 family, elevated levels of intracellular calcium, and reactive oxygen species. Four mitochondrial molecules mediating downstream cell-death pathways have been identified: cytochrome *c*, Smac/Diablo, apoptosis-inducing factor, and endonuclease G. Cytochrome *c* binds to Apaf-1, which, together with procaspase 9, forms the “apoptosome,” which activates caspase 9. In turn, caspase 9 activates caspase 3. Smac/Diablo binds to inhibitors of activated caspases and causes further caspase activation. Apoptosis-inducing factor and endonuclease G mediate caspase-independent cell-death pathways.

the Bcl-2 family, generating a truncated fragment with proapoptotic activity.<sup>56</sup> In addition to cytochrome *c*, other modulators of cell death within mitochondria are released during the apoptotic process.<sup>53</sup>

#### INHIBITORS OF APOPTOSIS

To control aberrant caspase activation, which can kill the cell, additional molecules inhibit caspase-mediated pathways. Among these are proteins known as inhibitors of apoptosis. These inhibitors interact directly with modulators of cell death. For example, the X-linked inhibitor of apoptosis and the neuronal inhibitor of apoptosis are proteins in neurons that directly inhibit caspase 3 activity and protect neurons from ischemic injury.<sup>34,55,57</sup>

#### CASPASES IN NEUROLOGIC DISEASES

Caspases have a pivotal role in the progression of a variety of neurologic disorders. Despite the various causes of such disorders, the mechanism of cell death is similar in a broad spectrum of neurologic diseases.<sup>1,37,58</sup> However, the trigger of aberrant caspase activation in most of these diseases is not well understood. In acute neurologic diseases, both necrosis and caspase-mediated apoptotic cell death occur.<sup>11,17,36,59,60</sup> By contrast, in chronic neurodegenerative diseases, caspase-mediated apoptotic pathways have the dominant role in mediating cell dysfunction and cell death.<sup>38,39,61,62</sup> A primary difference between acute and chronic neurologic diseases is the magnitude of the stimulus causing cell death. The greater stimulus in acute diseases results in both necrotic and apoptotic cell death, whereas the milder insults in chronic diseases initiate apoptotic cell death.

#### ACUTE NEUROLOGIC DISEASES

Ischemic stroke was the first neurologic disease in which the activation of a caspase (caspase 1) was documented.<sup>44</sup> Moreover, inhibition of caspases reduces tissue damage and allows remarkable neurologic improvement.<sup>44,63,64</sup> Activation of caspases 1, 3, 8, 9, and 11 and release of cytochrome *c* have been demonstrated in cerebral ischemia,<sup>41,65-67</sup> and the Bcl-2 family has also been incriminated.<sup>68,69</sup> Mice that express a dominant-negative caspase 1 construct or that are deficient in caspase 1 or caspase 11 have significant protection from ischemic injury.<sup>44,65,70</sup> Pharmacologic pretreatment of mice with a broad caspase inhibitor or with semi-selective inhibitors of caspase 1 and caspase 3 or delayed treatment with a caspase 3 inhibitor protect the brain from ischemic injury.<sup>64,71</sup>

There is a pattern of combined necrotic and apoptotic cell death after ischemic or traumatic injury.<sup>15,18-20,36,59</sup> In ischemia, necrotic cell death occurs in the core of the infarction, where hypoxia is most severe, and leads to abrupt cessation of energy supply and acute cellular collapse. Conversely, in the ischemic penumbra, the degree of energy deprivation is not as severe, because collateral vessels supply the region with oxygenated blood. In this case, the cell must reach a critical threshold of injury to activate the caspase cascade. Before this threshold is reached, however, a compromise in neuronal energetics can cause cell dysfunction before cell death. What determines the threshold in a

particular cell is unknown. Nevertheless, the existence of the threshold offers an opportunity to rescue cells in the penumbra by reversing the initial neurologic deficit caused by cell dysfunction. Factors that promote survival can raise the threshold, as evidenced in the experiments with caspase inhibition described above and in studies in which the balance among members of the Bcl-2 family was transgenically manipulated.<sup>68,69</sup> The cerebral tissue protected by modulation of caspase activation is invariably the penumbra.<sup>44,64,66,68</sup>

#### CHRONIC NEURODEGENERATIVE DISEASES

Cell death in chronic neurodegenerative diseases often occurs as a result of a mutation in one or several genes. This genetic alteration changes the function of the gene product in a way that has a detrimental effect on the cell. Environmental factors have also been incriminated in chronic neurodegeneration, but the cause of many such disorders remains unknown. I will describe the key role of the caspase family in two diseases, ALS and Huntington's disease. There is evidence suggesting that caspases have a role in Alzheimer's disease, Parkinson's disease, and dementia associated with human immunodeficiency virus infection.<sup>62,72,73</sup> The cause of the selective death of motor neurons in ALS or of medium-sized spiny neurons in the striatum in Huntington's disease is, for the most part, not understood. This question is the focus of intense investigation.

#### ALS

ALS is characterized by the progressive and specific loss of motor neurons in the brain, brain stem, and spinal cord.<sup>74</sup> The average age at onset is 55 years, and the average life expectancy after the clinical onset is 4 years. The only recognized treatment for ALS is riluzole, whose use extends survival by only about three months. Familial and sporadic forms of the disease have been described. The natural history and histologic abnormalities in these two forms of ALS are not distinguishable.

A mutation in the gene encoding superoxide dismutase 1 (SOD1) has been identified in 10 percent of patients with familial ALS.<sup>75</sup> In transgenic mice expressing the human mutant SOD1 gene, a syndrome develops with many features of ALS, including specific cell death of motor neurons, progressive weakness, and early death.<sup>76</sup> These mouse models of ALS and other mice with additional ALS-linked mutations in SOD1 are effective tools for the

study of molecular mechanisms and pharmacotherapy for ALS.<sup>38,67,77</sup> The first evidence of a role of a caspase in a neurodegenerative disease came from experiments in which the "ALS mouse" was cross-bred with a mouse expressing a mutant caspase 1 gene that inhibited caspase 1 in neurons.<sup>61</sup> As compared with mice expressing only the mutant SOD1 transgene, mice expressing both the mutant SOD1 transgene and the mutant caspase 1 transgene had a duration of survival that was greater by 9 percent, and disease progression was slowed by more than 50 percent. Furthermore, intraventricular administration of a broad caspase inhibitor (zVAD-fmk) was neuroprotective and extended survival in the ALS mice by 22 percent.<sup>38</sup>

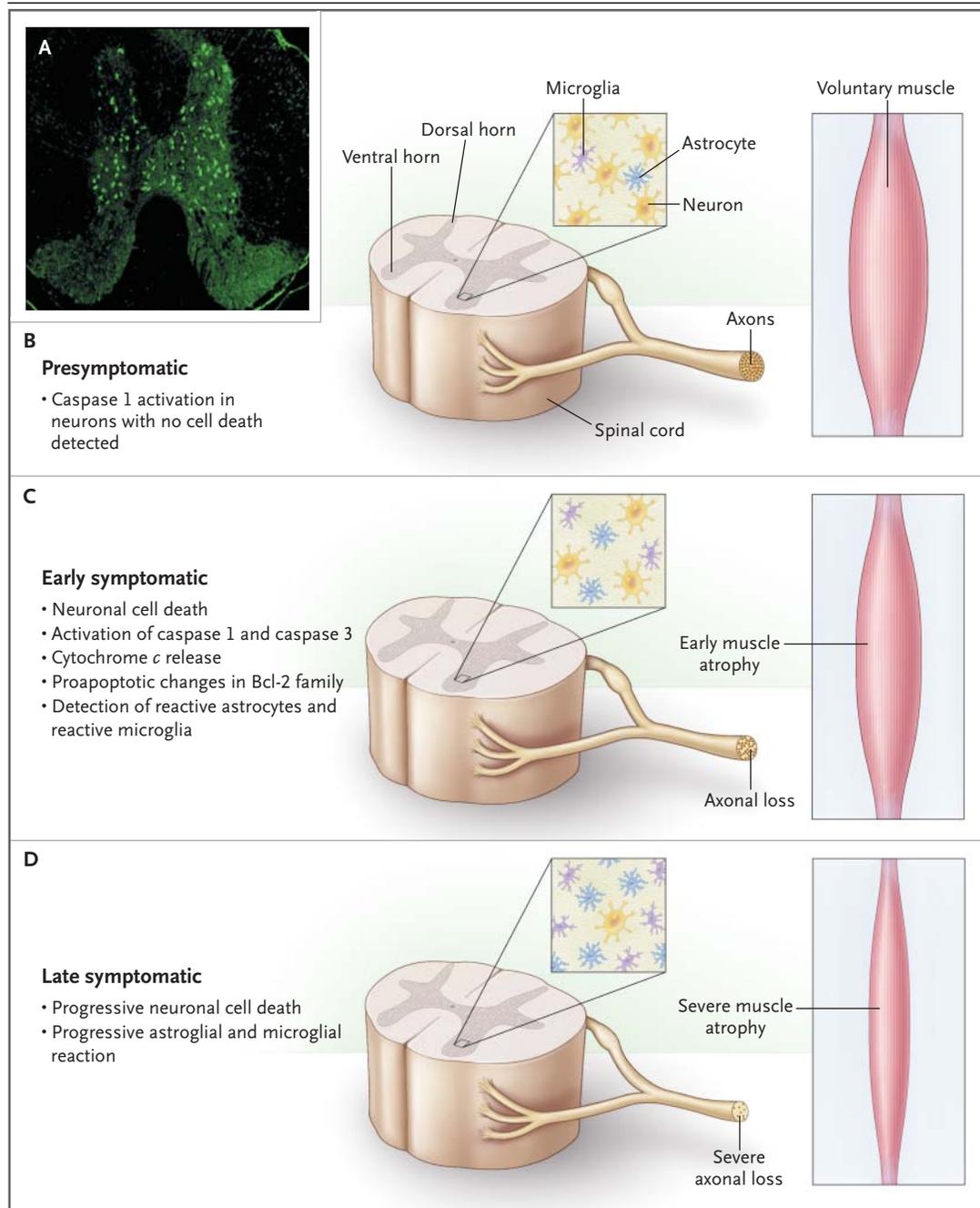
A prolonged period of neuronal caspase activation (especially of caspase 1) was detected in transgenic ALS mice (Fig. 4A).<sup>38,42,43</sup> As these mice aged, there was progressive transcriptional up-regulation of caspase 1 messenger RNA (mRNA), followed by up-regulation of caspase 3 mRNA (Fig. 4B). Despite treatment of ALS mice with the enzymatic caspase inhibitor zVAD-fmk, transcriptional up-regulation of caspase 1 and caspase 3 was delayed, suggesting that there is a non-cell-autonomous "contagious" apoptotic process in these mice (see below).<sup>38</sup> These sequential events are also detected at the level of enzymatic activity.<sup>38,40,43</sup> The finding of caspase 1 and caspase 3 activation in spinal cord samples from patients with ALS indicates the clinical relevance of these animal models of ALS.<sup>38,78</sup>

Caspase 9 activation, cytochrome *c* release, and proapoptotic changes in the Bcl-2 family have also been detected in spinal cords of ALS mice.<sup>67,79</sup> Moreover, ALS mice bearing a transgenic Bcl-2 gene survive longer than other ALS mice.<sup>80</sup>

#### Huntington's Disease

Huntington's disease is an autosomal dominant neurodegenerative disorder in which specific cell death occurs in the neostriatum and cortex.<sup>13,81</sup> Onset usually occurs during the fourth or fifth decade of life, with a mean survival after onset of 15 to 20 years. Huntington's disease is universally fatal, and there is no effective treatment. Symptoms include a characteristic movement disorder (Huntington's chorea), cognitive dysfunction, and psychiatric symptoms. The disease is caused by a mutation encoding an abnormal expansion of CAG-encoded polyglutamine repeats in a protein called huntingtin.<sup>82</sup>

The discovery of the mutant gene responsible for the disease made it possible to create transgenic



**Figure 4. Neurologic Lesions in Mice with ALS.**

Panel A shows activation of neuronal caspase 1 in an axial section of the spinal cord of a 90-day-old mouse with ALS (immunostained with a caspase 1 antibody). At this age, the mouse is at the beginning or the middle of the symptomatic stage. There is no caspase 1 activation in the dorsal horn or in the white matter. In the presymptomatic stage (Panel B), the earliest cell-death signal detected is the activation of neuronal caspase 1. At this stage, there are no overt signs of cell death or strong tissue reaction. In the early symptomatic stage (Panel C), there is widespread activation of caspase 1 and caspase 3, release of cytochrome *c*, and proapoptotic changes in Bcl-2 family members. Ventral motor neurons and axons die, and reactive microgliosis and astrocytosis are present. As the disease advances, the findings described above become more overt (Panel D) and are accompanied by progressive muscle atrophy.

mouse models of it.<sup>83</sup> In these mice, apoptotic pathways and newly described cell-death pathways that are neither apoptotic nor necrotic have been demonstrated.<sup>84,85</sup> One of the earliest events in the pre-symptomatic and early symptomatic stages of the disease is transcriptional up-regulation of the caspase 1 gene.<sup>39</sup> This event appears to result from nuclear translocation of N-terminal fragments of mutant huntingtin.<sup>86</sup> As the disease progresses, the caspase 3 gene is transcriptionally up-regulated, and the protein is activated.<sup>35</sup> Activation of caspase 8 and caspase 9 and release of cytochrome *c* have also been demonstrated in Huntington's disease.<sup>87,88</sup>

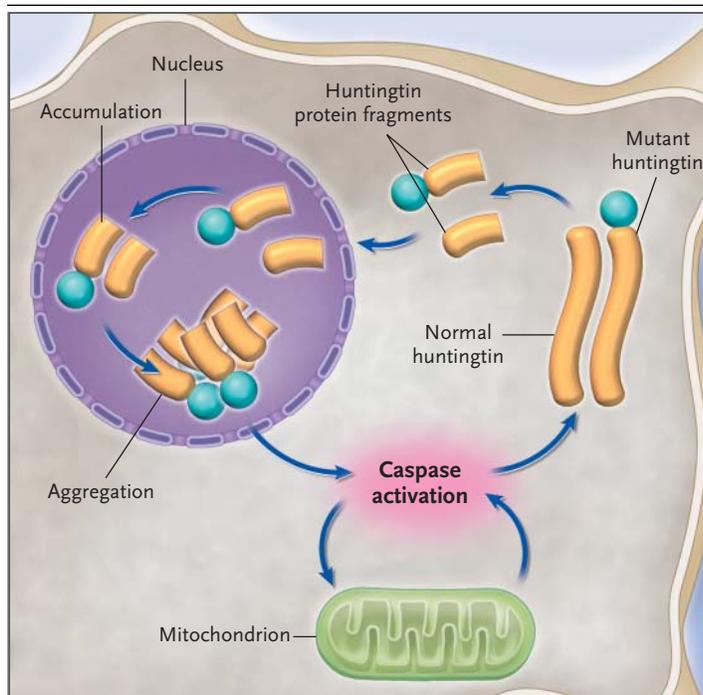
Evidence is beginning to accumulate of both a toxic effect of huntingtin fragments and depletion of huntingtin in Huntington's disease.<sup>35,39,89-91</sup> Huntingtin is a substrate for caspase 1 and caspase 3.<sup>92,93</sup> As the disease progresses, increased caspase-mediated cleavage of huntingtin increases the generation of huntingtin fragments and depletes wild-type huntingtin (Fig. 5).<sup>39</sup> It appears that some features of Huntington's disease result from the depletion of this protein.<sup>94</sup>

Neuronal dysfunction caused by the down-regulation of receptors that bind important neurotransmitters is another important feature of Huntington's disease.<sup>95</sup> We know that this down-regulation of receptors is, at least in part, a caspase-mediated event, since the inhibition of caspase also inhibits receptor down-regulation.<sup>39</sup> This evidence suggests that caspases are mediators not only of cell death but also of cell dysfunction.

Several of the findings in mouse models of Huntington's disease have also been demonstrated in human striatal brain tissue, including activation of caspases 1, 3, 8, and 9 and release of cytochrome *c*.<sup>39,87,88</sup> Transgenic mice have been used as a tool for evaluating and demonstrating the efficacy of caspase inhibitors, creatine, and minocycline in an animal model of Huntington's disease.<sup>35,39,85</sup>

#### MINOCYCLINE

Minocycline is a second-generation tetracycline with remarkable neuroprotective properties. Because it inhibits the production of nitric oxide by the inducible form of nitric oxide synthetase, minocycline was evaluated in experimental models of cerebral ischemia. Minocycline significantly reduced the severity of ischemia-induced tissue injury and neurologic dysfunction.<sup>50,51</sup> Along with the neuroprotection it provided, minocycline inhibited the



**Figure 5. Huntington's Disease.**

Neurons of patients with Huntington's disease contain one copy of the wild-type huntingtin allele (producing orange protein) and one copy of the mutant allele (producing orange and blue protein). Possibly as part of the normal proteolysis of huntingtin, an N-terminal fragment is generated. Mutant N-terminal fragments accumulate and aggregate, forming neuronal intranuclear inclusions. Nuclear translocation of mutant N-terminal fragments up-regulates transcription of caspase 1. As the disease progresses, caspase 1 activates caspase 3. Caspase 1 and caspase 3 cleave huntingtin, producing N-terminal fragments and resulting in the depletion of huntingtin. As the disease progresses further, Bid is activated, releasing cytochrome *c*. Assembly of the apoptosome activates caspase 9 and caspase 3. Progressive caspase activation leads to neuronal dysfunction and cell death.

ischemia-induced up-regulation of nitric oxide synthase, caspase 1, and reactive microgliosis.<sup>96</sup> Neuroprotection by minocycline has also been observed in mouse models of Huntington's disease, ALS, brain injury, Parkinson's disease, and multiple sclerosis.<sup>35,67,97</sup> The primary mechanism of action of minocycline is the direct inhibition of the release of cytochrome *c*; secondarily, it inhibits downstream events related to cell death — in particular, the activation of caspase 3.<sup>67</sup> It is not clear whether minocycline inhibits reactive microgliosis or the production of nitric oxide synthase directly or by a secondary process that follows the inhibition of cytochrome *c* release. Minocycline is orally bioavailable, crosses the blood-brain barrier, and has a proven safety record in humans. It is being evalu-

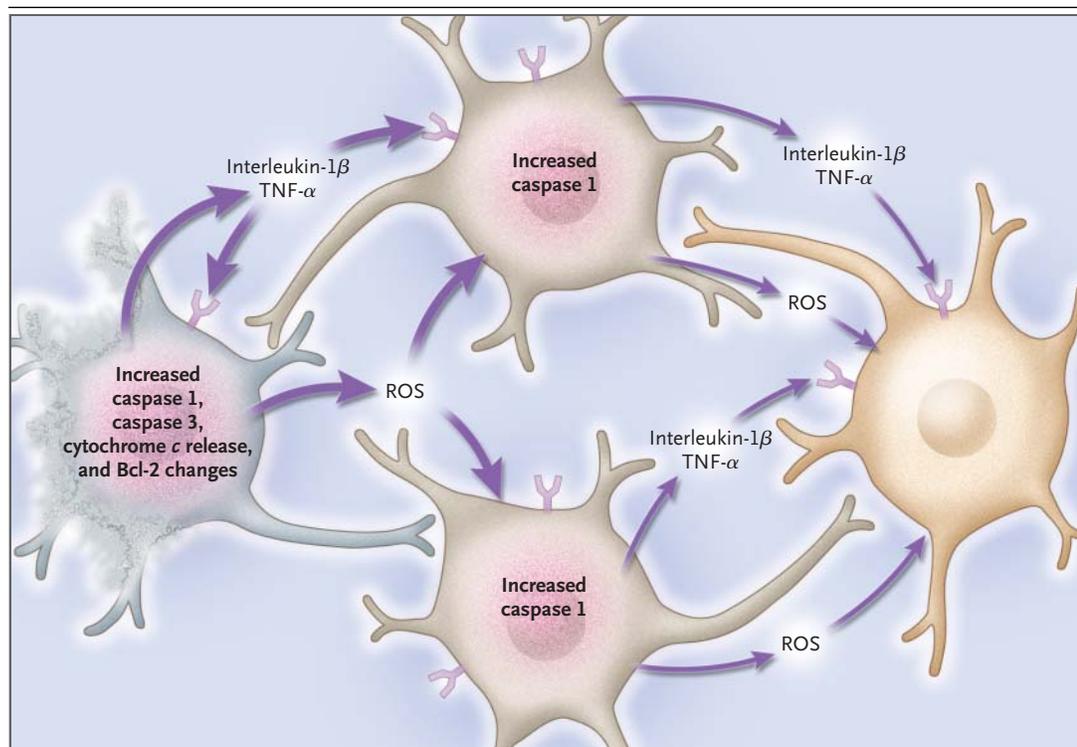
ated in clinical trials in patients with Huntington's disease and ALS.

CONTAGIOUS APOPTOSIS  
(“THE KINDERGARTEN EFFECT”)

The process of cell death in one cell can affect the dynamics of cell death in neighboring cells.<sup>38</sup> Factors generated by cells as they die and after they die are detrimental to neighboring cells. Neighboring cells are exposed to triggering factors that are similar to those that affect a cell that is dying. For example, during a stroke, a neuron exposed to an ischemic environment triggers the cell-death cascade and produces interleukin-1 $\beta$ , TNF- $\alpha$ , and free radicals that play a part in the cell's own demise.<sup>1</sup> These diffusible factors affect neighboring neurons that have been similarly exposed to ischemia. Since there

is a gradient of ischemia, neurons that might not have died as the result of the ischemic insult alone die from a combination of exposure to a sublethal ischemic environment and the diffusible toxic factors generated by their dying neighbors.

This phenomenon also occurs in chronic neurodegenerative diseases. For example, in ALS mediated by mutant SOD1, the mutant SOD1 protein initiates the cell-death cascade in one particular motor neuron. As the neuron progresses through the cascade and eventually dies, it releases proapoptotic factors that affect neighboring cells.<sup>38</sup> Since these cells have the same genetic predisposition as their dying neighbor, such factors might induce them, too, to initiate the cell-death cascade (Fig. 6). From a therapeutic standpoint, this concept is important, because an inhibitor of apoptosis not only will slow the process of cell death in one particular cell, but



**Figure 6. Contagious Apoptosis and Cell Dysfunction.**

As one initial neuron (gray) proceeds through the cell-death pathway, apoptotic cascades are activated and diffusible toxic factors (interleukin- $\beta$ , tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ], and reactive oxygen species [ROS]) are released. These factors induce neighboring cells (tan) to enter the cell-death cascade (“the kindergarten effect”), and the earliest detectable change is the up-regulation of caspase 1. As these neurons become dysfunctional, they begin to secrete the same toxic factors, which will, in turn, affect the surrounding healthy neurons (pink). Once a lethal threshold has been reached, the cell dies.

is also likely to inhibit the production of the diffusible toxic factors that might initiate the cell-death cascade in a neighboring cell.

en the chronic nature of caspase activation, caspase inhibition is a plausible approach to the treatment of neurologic diseases.

#### CHRONIC CASPASE ACTIVATION AND CELL DYSFUNCTION

Apoptotic cell death in the ischemic penumbra results from massive cytotoxic activation of cell-death pathways, whereas in chronic neurodegenerative diseases, weaker stimuli of cell death cause sublethal activation of caspase. Chronic, sublethal activation of caspase appears to mediate cell dysfunction, which precedes cell death.<sup>38,40</sup> Cell dysfunction of substantial magnitude, occurring before cell death, might result in symptomatic disease. Given that caspases may be active in individual neurons for a long period (potentially weeks to months), inhibition of caspase in these circumstances could reduce cell dysfunction and delay cell death.<sup>39</sup> In acute diseases, a delayed wave of cell death can be detected up to one month after the initial injury.<sup>19,98</sup> Given

#### CONCLUSIONS

During the past several years, our understanding of the mechanisms mediating cell death in neurologic diseases has improved considerably. The fact that activation of these pathways is a feature of a broad range of neurologic diseases makes them important and attractive therapeutic targets. Pharmaceutical companies are actively searching for compounds that inhibit these pathways. The first clinical trials of an inhibitor of apoptosis (minocycline) for neurodegenerative disorders (Huntington's disease and ALS) are in progress.<sup>35,38</sup> It is likely that in the next several years, additional inhibitors of apoptosis will become part of the everyday armamentarium of clinicians who are treating neurologic diseases that involve caspase-mediated cell dysfunction and cell death.

#### REFERENCES

1. Yuan J, Yankner BA. Apoptosis in the nervous system. *Nature* 2000;407:802-9.
2. Rossi F, Cattaneo E. Neural stem cell therapy for neurological diseases: dreams and reality. *Nat Rev Neurosci* 2002;3:401-9.
3. Kuhn HG, Palmer TD, Fuchs E. Adult neurogenesis: a compensatory mechanism for neuronal damage. *Eur Arch Psychiatry Clin Neurosci* 2001;251:152-8.
4. Eriksson PS, Perfilieva E, Bjork-Eriksson T, et al. Neurogenesis in the adult human hippocampus. *Nat Med* 1998;4:1313-7.
5. Liu J, Solway K, Messing RO, Sharp FR. Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. *J Neurosci* 1998;18:7768-78.
6. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770-6.
7. Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980;68:251-306.
8. Kanduc D, Mittelman A, Serpico R, et al. Cell death: apoptosis versus necrosis. *Int J Oncol* 2002;21:165-70.
9. Martin LJ. Neuronal cell death in nervous system development, disease, and injury. *Int J Mol Med* 2001;7:455-78.
10. Linnik MD, Zobrist RH, Hatfield MD. Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. *Stroke* 1993;24:2002-9.
11. Emery E, Aldana P, Bunge MB, et al. Apoptosis after traumatic human spinal cord injury. *J Neurosurg* 1998;89:911-20.
12. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972;26:239-57.
13. Martin JB. Molecular basis of the neurodegenerative disorders. *N Engl J Med* 1999;340:1970-80. [Erratum, *N Engl J Med* 1999;341:1407.]
14. Charriaud-Marlangue C, Margail I, Represa A, Popovici T, Plotkine M, Ben-Ari Y. Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. *J Cereb Blood Flow Metab* 1996;16:186-94.
15. Li Y, Chopp M, Jiang N, Zaloga C. In situ detection of DNA fragmentation after focal cerebral ischemia in mice. *Brain Res Mol Brain Res* 1995;28:164-8.
16. MacManus JP, Buchan AM, Hill IE, Rasquinha I, Preston E. Global ischemia can cause DNA fragmentation indicative of apoptosis in rat brain. *Neurosci Lett* 1993;164:89-92.
17. Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS. Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. *Nat Med* 1997;3:73-6. [Erratum, *Nat Med* 1997;3:240.]
18. Li M, Ona VO, Chen M, et al. Functional role and therapeutic implications of neuronal caspase-1 and -3 in a mouse model of traumatic spinal cord injury. *Neuroscience* 2000;99:333-42.
19. Liu XZ, Xu XM, Hu R, et al. Neuronal and glial apoptosis after traumatic spinal cord injury. *J Neurosci* 1997;17:5395-406.
20. Rink A, Fung KM, Trojanowski JQ, Lee VM, Neugebauer E, McIntosh TK. Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. *Am J Pathol* 1995;147:1575-83.
21. Thomas LB, Gates DJ, Richfield EK, O'Brien TF, Schweitzer JB, Steindler DA. DNA end labeling (TUNEL) in Huntington's disease and other neuropathological conditions. *Exp Neurol* 1995;133:265-72.
22. Troost D, Aten J, Morsink F, de Jong JM. Apoptosis in amyotrophic lateral sclerosis is not restricted to motor neurons: Bcl-2 expression is increased in unaffected post-central gyrus. *Neuropathol Appl Neurobiol* 1995;21:498-504.
23. Smale G, Nichols NR, Brady DR, Finch CE, Horton WE Jr. Evidence for apoptotic cell death in Alzheimer's disease. *Exp Neurol* 1995;133:225-30.
24. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 1980;284:555-6.
25. Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* 1997;89:175-84.
26. Horvitz HR. Genetic control of programmed cell death in the nematode *Caenorhabditis elegans*. *Cancer Res* 1999;59:Suppl:1701s-1706s.
27. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR. The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* 1993;75:641-52.
28. Yuan JY, Horvitz HR. The *Caenorhabditis elegans* genes *ced-3* and *ced-4* act cell autonomously to cause programmed cell death. *Dev Biol* 1990;138:33-41.
29. Miura M, Zhu H, Rotello R, Hartweg EA, Yuan J. Induction of apoptosis in fibroblasts

- by IL-1 beta-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* 1993;75:653-60.
30. Li P, Nijhawan D, Budihardjo I, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997;91:479-89.
31. Hengartner MO, Horvitz HR. *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* 1994;76:665-76.
32. Conradt B, Horvitz HR. The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* 1998;93:519-29.
33. Alnemri ES, Livingston DJ, Nicholson DW, et al. Human ICE/CED-3 protease nomenclature. *Cell* 1996;87:171.
34. Shi Y. Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell* 2002;9:459-70.
35. Chen M, Ona VO, Li M, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 2000;6:797-801.
36. Fink KB, Andrews LJ, Butler WE, et al. Reduction of post-traumatic brain injury and free radical production by inhibition of the caspase-1 cascade. *Neuroscience* 1999;94:1213-8.
37. Friedlander RM, Yuan J. ICE, neuronal apoptosis and neurodegeneration. *Cell Death Differ* 1998;5:823-31.
38. Li M, Ona VO, Guegan C, et al. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. *Science* 2000;288:335-9.
39. Ona VO, Li M, Vonsattel JP, et al. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 1999;399:263-7.
40. Pasinelli P, Houseweart MK, Brown RH Jr, Cleveland DW. Caspase-1 and -3 are sequentially activated in motor neuron death in Cu,Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 2000;97:13901-6.
41. Rabuffetti M, Sciorati C, Tarozzo G, Clementi E, Manfredi AA, Beltramo M. Inhibition of caspase-1-like activity by Ac-Tyr-Val-Ala-Asp-chloromethyl ketone induces long-lasting neuroprotection in cerebral ischemia through apoptosis reduction and decrease of proinflammatory cytokines. *J Neurosci* 2000;20:4398-404.
42. Sun W, Funakoshi H, Nakamura T. Overexpression of HGF retards disease progression and prolongs life span in a transgenic mouse model of ALS. *J Neurosci* 2002;22:6537-48.
43. Vukosavic S, Stefanis L, Jackson-Lewis V, et al. Delaying caspase activation by Bcl-2: a clue to disease retardation in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci* 2000;20:9119-25.
44. Friedlander RM, Gagliardini V, Hara H, et al. Expression of a dominant negative mutant of interleukin-1 beta converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. *J Exp Med* 1997;185:933-40.
45. Enari M, Talanian RV, Wong WW, Nagata S. Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis. *Nature* 1996;380:723-6.
46. Bouchier-Hayes L, Martin SJ. CARD games in apoptosis and immunity. *EMBO Rep* 2002;3:616-21.
47. Sakahira H, Enari M, Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* 1998;391:96-9.
48. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 1998;391:43-50. [Erratum, *Nature* 1998;393:396.]
49. Liu X, Li P, Widlak P, et al. The 40-kDa subunit of DNA fragmentation factor induces DNA fragmentation and chromatin condensation during apoptosis. *Proc Natl Acad Sci U S A* 1998;95:8461-6.
50. Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A* 1999;96:13496-500.
51. Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci U S A* 1998;95:15769-74.
52. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 1996;86:147-57.
53. Wang X. The expanding role of mitochondria in apoptosis. *Genes Dev* 2001;15:2922-33.
54. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 1999;13:1899-911.
55. Deveraux QL, Schendel SL, Reed JC. Antiapoptotic proteins: the bcl-2 and inhibitor of apoptosis protein families. *Cardiol Clin* 2001;19:57-74.
56. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 1998;94:491-501.
57. Xu D, Bureau Y, McIntyre DC, et al. Attenuation of ischemia-induced cellular and behavioral deficits by X chromosome-linked inhibitor of apoptosis protein overexpression in the rat hippocampus. *J Neurosci* 1999;19:5026-33.
58. Troy CM, Salvesen GS. Caspases on the brain. *J Neurosci Res* 2002;69:145-50.
59. Yakovlev AG, Knoblach SM, Fan L, Fox GB, Goodnight R, Faden AI. Activation of CPP32-like caspases contributes to neuronal apoptosis and neurological dysfunction after traumatic brain injury. *J Neurosci* 1997;17:7415-24.
60. Springer JE, Azbill RD, Knapp PE. Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. *Nat Med* 1999;5:943-6.
61. Friedlander RM, Brown RH, Gagliardini V, Wang J, Yuan J. Inhibition of ICE slows ALS in mice. *Nature* 1997;388:31. [Erratum, *Nature* 1998;392:560.]
62. Gervais FG, Xu D, Robertson GS, et al. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. *Cell* 1999;97:395-406.
63. Loddick SA, MacKenzie A, Rothwell NJ. An ICE inhibitor, z-VAD-DCB attenuates ischaemic brain damage in the rat. *Neuroreport* 1996;7:1465-8. [Erratum, *Neuroreport* 1999;10(9):inside back cover.]
64. Hara H, Friedlander RM, Gagliardini V, et al. Inhibition of interleukin 1 beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc Natl Acad Sci U S A* 1997;94:2007-12.
65. Kang SJ, Wang S, Hara H, et al. Dual role of caspase-11 in mediating activation of caspase-1 and caspase-3 under pathological conditions. *J Cell Biol* 2000;149:613-22.
66. Benchoua A, Guegan C, Couriaud C, et al. Specific caspase pathways are activated in the two stages of cerebral infarction. *J Neurosci* 2001;21:7127-34.
67. Zhu S, Stavrovskaya IG, Drozda M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 2002;417:74-8.
68. Martinou JC, Dubois-Dauphin M, Staple JK, et al. Overexpression of BCL-2 in transgenic mice protects neurons from naturally occurring cell death and experimental ischemia. *Neuron* 1994;13:1017-30.
69. Plesnila N, Zinkel S, Le DA, et al. BID mediates neuronal cell death after oxygen-glucose deprivation and focal cerebral ischemia. *Proc Natl Acad Sci U S A* 2001;98:15318-23.
70. Schielke GP, Yang GY, Shivers BD, Betz AL. Reduced ischemic brain injury in interleukin-1 beta converting enzyme-deficient mice. *J Cereb Blood Flow Metab* 1998;18:180-5.
71. Fink K, Zhu J, Namura S, et al. Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation. *J Cereb Blood Flow Metab* 1998;18:1071-6.
72. Garden GA, Budd SL, Tsai E, et al. Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *J Neurosci* 2002;22:4015-24.
73. Klivenyi P, Andreassen O, Ferrante RJ, Schleicher JR Jr, Friedlander RM, Beal MF. Transgenic mice expressing a dominant negative mutant interleukin-1 beta converting enzyme show resistance to MPTP neurotoxicity. *Neuroreport* 1999;10:635-8.
74. Rowland LP, Schneider NA. Amyotrophic lateral sclerosis. *N Engl J Med* 2001;344:1688-700.
75. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59-62. [Erratum, *Nature* 1993;364:362.]

76. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 1994;264:1772-5. [Erratum, *Science* 1995;269:149.]
77. Klivenyi P, Ferrante RJ, Matthews RT, et al. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med* 1999;5:347-50.
78. Martin LJ. Neuronal death in amyotrophic lateral sclerosis is apoptosis: possible contribution of a programmed cell death mechanism. *J Neuropathol Exp Neurol* 1999; 58:459-71.
79. Guegan C, Vila M, Rosoklija G, Hays AP, Przedborski S. Recruitment of the mitochondrial-dependent apoptotic pathway in amyotrophic lateral sclerosis. *J Neurosci* 2001;21:6569-76.
80. Kostic V, Jackson-Lewis V, de Bilbao F, Dubois-Dauphin M, Przedborski S. Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Science* 1997;277:559-62.
81. Harper PS. Huntington's disease. London: W.B. Saunders, 1991.
82. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993;72:971-83.
83. Mangiarini L, Sathasivam K, Seller M, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 1996;87:493-506.
84. Reddy PH, Williams M, Charles V, et al. Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nat Genet* 1998;20:198-202.
85. Ferrante RJ, Andreassen OA, Jenkins BG, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* 2000;20:4389-97.
86. Li SH, Lam S, Cheng AL, Li XJ. Intracellular huntingtin increases the expression of caspase-1 and induces apoptosis. *Hum Mol Genet* 2000;9:2859-67.
87. Kiechle T, Dedeoglu A, Kubilus J, et al. Cytochrome c and caspase-9 expression in Huntington's disease. *Neuromolecular Med* 2002;1:183-95.
88. Sanchez I, Xu CJ, Juo P, Kakizaka A, Bennis J, Yuan J. Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* 1999;22:623-33.
89. Zuccato C, Ciammola A, Rigamonti D, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 2001;293:493-8.
90. Rigamonti D, Bauer JH, De-Fraja C, et al. Wild-type huntingtin protects from apoptosis upstream of caspase-3. *J Neurosci* 2000;20:3705-13.
91. Rigamonti D, Sipione S, Goffredo D, Zuccato C, Fossale E, Cattaneo E. Huntingtin's neuroprotective activity occurs via inhibition of procaspase-9 processing. *J Biol Chem* 2001;276:14545-8.
92. Goldberg YP, Nicholson DW, Rasper DM, et al. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat Genet* 1996;13:442-9.
93. Wellington CL, Ellerby LM, Hackam AS, et al. Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J Biol Chem* 1998;273: 9158-67.
94. Nucifora FC Jr, Sasaki M, Peters MF, et al. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* 2001;291:2423-8.
95. Cha JH, Kosinski CM, Kerner JA, et al. Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human Huntington disease gene. *Proc Natl Acad Sci U S A* 1998;95:6480-5.
96. Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* 2001;166:7527-33.
97. Sanchez Mejia RO, Ona VO, Li M, Friedlander RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. *Neurosurgery* 2001;48:1393-401.
98. Shuman SL, Bresnahan JC, Beattie MS. Apoptosis of microglia and oligodendrocytes after spinal cord contusion in rats. *J Neurosci Res* 1997;50:798-808.

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