ABSTRACT

This article discusses the various mechanisms, biochemical pathways, and chemical substances involved in neuronal cell death and cell survival, in an attempt to put an order to the sequence of events that ultimately lead to a commitment to neurological cell death. An understanding of these pathways is crucial to discovering etiologies involved in the pathologic changes that occur in patients with multiple sclerosis and the development of therapeutic interventions. (Adv Stud Med. 2005;5(4D):S387-S391)

In order to effect a true cure for a neurological disease such as multiple sclerosis (MS) where there is damage to nerve cells, several fundamental issues will need to be addressed. It will probably be necessary to ascertain the cause of neuronal injury, what initiates the death cascade for the various mechanisms of nerve cell death that exist, and conversely, how might cell survival pathways be activated in order to attempt regeneration of surviving cells and/or replacement of lost cells. While the cause of injury is still unknown, researchers are beginning to gain a better understanding of various pathways of cell death as well as identifying genes and biochemical pathways involved in assuring survival. Nerve cell survival, and more specifically, the discovery of how scientists might accomplish the replacement of damaged tissue, is probably most critical to clinicians, because they generally manage patients with MS who already have significant disability due to loss of central nervous system function. This article discusses what is presently known about molecular mechanisms of neuronal and axonal apoptosis, necrosis, other avenues of neuronal cell death, as well as how cells survive.

Various theories exist regarding how MS begins. There is controversy as to whether MS is fundamentally a neurodegenerative disease causing neurologic and axonal injury that triggers inflammation and damage to oligodendrocytes, or whether oligodendrocytes are at the root of the disease, degenerating themselves and subsequently causing damage and inflammation to neurons, which then subsequently degenerate. Furthermore, even within the cell, the question arises as to whether cell death begins with the nucleus and cell body via some unknown interaction between surface receptors, the mitochondria and the nucleus, or at the terminals (dendrites and axons), in a dying back type of process (Figure 1). Because the nerves of the central nervous system are so varied and complex, it is a challenge to know where the cycle begins, yet it is very important to attempt to discover this, so that scientists and clinicians will be able to develop therapies that will arrest the degeneration at its inception.

MECHANISMS OF CELL DEATH—Necrosis, Apoptosis, Autophagy, and Exocytosis

The 2 most common forms of cell death that occur in the general cell population are necrosis and apopto-
With necrosis, there is swelling of the cytoplasm and mitochondria, disintegration and lysis of the organelles, enzymatic digestion, dissolution of nuclear chromatin, shrinkage of the nucleus, and loss of membrane integrity leading to cell death via an accidental process. Apoptosis, by contrast, is programmed cell death. The membrane of the cell remains intact, the cytoplasm and nucleus shrink, the mitochondria lose their integrity and leak, chromatin clumps with fragmentation of DNA, and the end result is the fragmentation of cells into smaller apoptotic bodies (Figure 2).2

However, in the nervous system, due to the tremendous numbers of different types of cells (estimated to be between 1500 and 5000), cell death cannot be classified as purely necrotic or apoptotic. Indeed, other types of cell death have been identified among nerve cells, and these include autophagy, or the self-digestion of cellular organelles mediated by exposure to various hydrolytic enzymes, and exocytosis, during which excitotoxins such as glutamate damage neurons.

Autophagy is a normal function of the cell, generally carried out to rid itself of defective organelles (such as mitochondria) and recycle the constituents, but it also can be instituted when a cell is faced with an inadequate supply of nutrients, especially proteins. Autophagy can be recruited into a cell’s death process by a variety of different stressors (such as oxidative stress or synthetic retinoids), using cathepsin as its protease, and then proceed to a variety of different forms of cell death, including apoptotic-like or necrotic cell death. In addition, autophagy may be a useful means of trimming off damaged portions of a neuron (dendritic pruning and axonal degeneration) prior to actual cell death.

Excitotoxicity is a form of cell death that occurs when glutamate is released from the presynaptic terminal, crosses the synaptic cleft, and activates a variety of receptors, both inotropic and metabotropic. The inotropic N-methyl-D-aspartate (NMDA) receptor...
allows calcium entry into the cell and elicits the majority of glutamate neurotoxicity via activation of calcium-dependent enzymes. Glial cells play an important role in removing glutamate from the synaptic cleft, and stressors such as oxidative stress may block this action, thus exacerbating excitotoxicity.

One family of calcium-dependent enzymes that play a role in glutamate release and hence excitotoxicity are the various isoforms of neuronal nitric oxide synthase (nNOS). Experimental data reveal their role in neuronal toxicity and cerebral ischemia. Nitric oxide (NO) is an important neural messenger molecule, but when generated in high concentrations in the presence of superoxide anion, peroxynitrite is generated. It is now appreciated that peroxynitrite mediates a large component of neurotoxicity under excitotoxic conditions and may contribute significantly to injury during inflammation.

By studying other diseases of the nervous system and specifically what occurs at the cellular level, scientists may begin to learn answers to what causes MS, and what to target in terms of therapeutic interventions. For example, in heroin-induced Parkinson’s disease, a chemical called N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the neurotoxin that destroys the dopamine-producing neurons of the substantia nigra, causing symptoms seen in Parkinson’s disease, such as tremors and rigidity. Studies reveal that MPTP (and another form, MPP), crosses the blood-brain barrier, is taken up by the glial cells and dopaminergic neurons where it targets the mitochondria, blocking the electron transport chain resulting in the production of superoxide anion. In this setting, NO has the opportunity to react with the elevated levels of superoxide anion and toxic peroxynitrite it forms. Consistent with this idea, studies of laboratory animals reveal that inhibition of nNOS protects against MPTP-induced dopaminergic cell death.

Oxidative stress and nNOS are also responsible for the disruption of a protein, parkin, which is involved in familial Parkinson’s disease and occurs when the gene becomes mutated. Parkin is important to neuronal survival, and if it is exposed to nitrosative stress, like exposure to oxidative stress, the function of parkin is inhibited while inappropriate interactions between other proteins are allowed to occur, somehow leading to cell death and disease symptoms. In summary, it would appear that parkin and other proteins present in nerve cells may have their function modified by oxidative stress, and this then plays a role in their chaperone activity, their ability to interact with proteasomes, and their targeting toward the Lewy bodies of Parkinson’s disease.

**SEQUENCES AND MEDIATORS OF CELL DEATH—PARP AND AIF**

Multiple mechanisms of neuronal cell death and overlap between them thus complicates the search for a cure for neurologic diseases like MS. One current avenue for research is attempting to discover the sequence of events, or, in other words, the order in which a cell dies, to examine various triggers, what amplifies the process, and the point of commitment after which death is certain and irreversible. From investigations of animal models and other neurological diseases, such as Parkinson’s disease, scientists are beginning to grasp the order in which events occur that trigger cell death, as well separate out those events that are consequences of events rather than true causes or contributors.

For example, it has been discovered when calcium passes through the NMDA receptor, NO is formed, and this leads to the formation of peroxynitrite. Peroxynitrite, the reaction product between NO and superoxide, has been found to be a mediator of cellular and tissue injury in various pathological situations because it is a potent oxidant. In fact, however, while one might expect peroxynitrite to simply destroy the neurons at this point, instead, it damages DNA without destroying the entire cell, and activates a nuclear enzyme, poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 catalyzes attachment of ADP ribose units from nicotinamide adenine dinucleotide (NAD) to nuclear proteins following DNA damage. Excessive activation of PARP-1 can deplete NAD and adenosine triphosphate, which are consumed in regeneration of NAD. Thus, PARP appears to be an important amplification and commitment step in the cell death process (Figure 3), as indicated by the resistance animals develop to experimentally induced strokes (via oxygen and glucose deprivation) when PARP is genetically deleted. Eliasson et al demonstrated that genetic disruption of PARP also provided profound protection against glutamate-NO-mediated ischemic insults in vitro (under conditions of oxygen and glucose deprivation), as evidenced by an 80% to 90% reduction in neuronal cell death in cultured cortical neurons. The authors describe these findings as “compelling evidence” for a primary involvement of PARP activation in neuronal
damage following focal ischemia, and this can be extrapolated to play a vital role in finding therapies for other neurological diseases, such as MS.\(^4\)

Likewise, in animals exposed to MPTP, the neurotoxin that causes parkinsonism in humans and some animal models, it has been shown that mice lacking the gene for PARP-1 are dramatically spared from MPTP neurotoxicity—there is no damage to the dopaminergic cells in the substantia nigra.\(^5\) Indeed, evidence from other scientific studies support the finding that the role of PARP extends beyond the nervous system to other organ systems and even to different forms of injury beside ischemia, such as inflammatory conditions. However, the question remains as to how PARP activation kills cells.

One theory of the role of PARP in cell death is that PARP consumes NAD, resulting in disruption of cellular respiration and energy failure. However, while this may be contributory, it is not entirely responsible. It is now believed that PARP triggers the release of a molecule called “apoptosis-inducing factor” (AIF) into the cell nucleus. AIF cleaves DNA, and that results in the commitment of the cell to death. This has been supported by experiments in which AIF has been blocked from being transported from the mitochondria, where it normally resides, to the nucleus, and the result has been an attenuation of excitotoxicity.

Furthermore, AIF may play a role in terms of axonal degeneration and dendritic dying back, as seen in diseases of the peripheral nervous system. In the presence of AIF, phosphatidylserine is exposed on the surface of the cell, and because phosphatidylserine traditionally triggers autophagy in cells, this may be a trigger for dendritic pruning and axonal dying back in neurons.

**Mechanisms of Wallerian Degeneration and Dendritic Pruning**

A study by Araki et al suggests that therapeutic strategies directed at increasing the supply of NAD and/or sirtuin (SIRT) 2 activation may be effective for treatment of diseases characterized by axonopathy and neurodegeneration, such as MS.\(^6\) In their experiments, it was found that certain genetic strains of mice have a delayed manifestation of Wallerian degeneration in response to axonal injury because of a mutation involving the NAD biosynthetic enzyme nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 and its associated product, SIRT1.\(^6\)

The exact mechanism of how this occurs has not been fully elucidated, but may have to do with the role of regulation of calcium flow between the mitochondria and the endoplasmic reticulum. Exposure to excitotoxins such as glutamate cause the mitochondria to undergo fission, and inhibits their movement and communication between these organelles and the endoplasmic reticulum.

**Mechanisms of Cell Survival—Preconditioning**

Another approach to the management of MS is to focus on cell survival rather than cell death, and within this context, scientists are exploring a phenomenon that induces profound cell survival called preconditioning. Cells are taken to the brink of death, but are not allowed to die. Preconditioning may be carried out with any stimulus capable of causing injury to a tissue or organ (such as subexcitotoxic level of NMDA), which can then also activate endogenous protective mechanisms. Interestingly, upon rechallenge with a usually lethal insult, the cells survive without any evidence of damage or disturbance. Upon performing genetic studies to determine which genes are changed following preconditioning, approximately 2% to 3% of the stress response genes serve to mediate cell survival. One such gene encodes a protein capable of protecting cortical neurons from such stressors as NMDA toxicity upon preconditioning, and has been dubbed Iduna. Iduna is a PARP-binding protein, which, if

![Figure 3. Cause, Contributor, Consequence?](image-url)

\[^{MAP} = \text{mitogen-activated protein; MEK} = \text{mitogen-activated extracellular signal-related kinase; Erk} = \text{extracellular signal-related kinase; NMDA} = \text{N}-\text{methyl-D-aspartate; nNOS} = \text{neuronal nitric oxide synthase; NO} = \text{nitric oxide; ONOO} = \text{peroxynitrite; PARP} = \text{poly(ADP-ribose) polymerase.}\]

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mutated so that it no longer binds PARP, is no longer neuroprotective. Iduna promises to be an interesting avenue of research in the ongoing quest to determine mechanisms of cell death and cell survival as well as mediators of the same processes, as these ultimately will hold the key to finding a treatment, and even a cure, for such neurodegenerative diseases as MS.

CONCLUSION

In summary, we have seen that “cross-talk” exists between various organelles within the cell, such as the mitochondria and the nucleus (Figure 4), and signals originating from the mitochondria may initiate DNA damage. Likewise, messages from the nucleus out to the cytosol may induce AIF release, which in turn fragments DNA and results in cell death. Events may follow a slightly different course in the peripheral nervous system where dendritic pruning and dying back of axons may precede cell death. In all of these cases, in the future, it will be important to determine what triggers the initiation of cell death (via mitochondrial stress, stress on the endoplasmic reticulum, nuclear injury, etc). Some of the triggers we know of at this juncture include ischemia, inflammation, oxidative stress, protein mutations, trauma, and aging. A variety of molecules (including cytochrome C and PARP, for example) serve to amplify the cell death “signal,” which then inevitably leads to cell death once substances such as AIF are activated. With regard to cell survival, there are novel survival proteins and pathways in the brain that need to be uncovered and understood as these have tremendous promise as future therapeutic targets. Perhaps genetic screening to reveal these not only will illuminate pathways for cell survival, but will also reveal death pathways in the nervous system, which if interrupted, may help those who suffer from MS and other similar conditions.

REFERENCES