Neuroprotection in the Peripheral Nervous System

Rationale for More Effective Therapies

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Most peripheral neuropathies are length dependent and result in distal axonal degeneration rather than loss of neuronal cell bodies. Available therapies for axonal peripheral neuropathies are designed to control painful symptoms and not to treat the underlying axonal degeneration. Many neuroprotective therapies are being developed, primarily for central nervous system disorders such as stroke or multiple sclerosis. However, strategies with the purpose of promoting survival of injured neurons (ie, preventing cell death) may not be applicable in many peripheral nervous system illnesses when the primary pathologic disorder that leads to symptoms is distal axonal degeneration. Neuronal cell death, if it occurs, is often a late event and may be untreatable in the near future. In contrast, distal axonal degeneration is an early event that may be amenable to treatment. Mechanistic studies that examine the axon-glia interaction and axonal biology are likely to yield novel therapeutic targets for peripheral neuropathies.

Peripheral neuropathies are common neurologic disorders, and effective therapies are limited. Most peripheral neuropathies are characterized by distal axonal loss that results from dying-back degeneration. This length-dependent dying-back phenomenon is not unique to diseases that affect the peripheral nervous system and likely has an important role in degenerative diseases that affect the central nervous system, such as Alzheimer disease, Parkinson disease, and motoneuron disease. In dying-back degeneration, the axon slowly degenerates centripetally from the distal end. However, the nature and site of initial injury to the neuron or the axon are unknown; in particular, it is unclear whether the process starts in the axon itself or in the neuronal cell body. The classical view of dying-back axonal degeneration has been that the distal portion of the axon degenerates first because it is the last field of irrigation and likely does not get adequate nutritional support from the neuronal cell body. However, this view has been questioned in light of new research that identified retrograde axonal transport problems in a variety of inherited disorders of the central and peripheral nervous systems (reviewed in Griffin et al). Griffin and colleagues proposed that the concept of the last field of irrigation is reversed and that the neuronal body is dependent on target-derived growth factors for survival and maintenance of the anterograde transport of materials to the distal axon. In addition to axonal transport defects that may lead to distal axonal degeneration, Raff et al proposed the intriguing idea that dying-back degeneration is a stereotyped response of axons that, under some circumstances, activate a self-destruct program similar to that which occurs in the cell bodies during apoptosis. Experimental data for this hypothesis are limited, but support is emerging.

Most of the current focus on developing neuroprotective therapies is aimed at preventing neuronal death. This is the case in stroke or spinal cord injury, but these approaches may be inapplicable to peripheral neuropathies because of lack of significant neuronal death in most peripher-
eral neuropathies. Even in stroke, current approaches to prevention of late stages of neuronal death have not been successful despite many years of clinical trials. In disorders that affect the peripheral nervous system, a new paradigm is needed in designing models and developing therapies that are directed at the axon rather than events occurring in the cell body. Figure 1 shows an important point about the peripheral nervous system. In a typical lumbar spinal motoneuron or dorsal root ganglion sensory neuron, the ratio of the cell body cytoplasm to axoplasm is miniscule. Many events occur at the axon that are not directly or immediately under the control of the neuronal body. Because a neuron is a functional unit only when it is connected to its target and the second-order neuron, even a small degree of distal axonal degeneration is, in practical terms, as bad as neuronal death. The difference is that, through better understanding of the mechanisms of distal axonal degeneration, we may be able to prevent further centripetal degeneration and, perhaps, enable axonal regeneration and connection with the target cells. This will require research in the following 2 areas: mechanisms underlying distal axonal degeneration and molecular events that occur in axons independent of the cell body, and axon-glia interactions that are important for neuroprotection.

**MECHANISMS OF DISTAL AXONAL DEGENERATION**

In Wallerian degeneration, the distal segment of a transected axon is separated and compartmentalized from the cell body and the proximal axon. Degeneration of the compartmentalized distal segment can continue without any harm to the proximal axon or the cell body. However, in the case of peripheral neuropathies, the distal segment of the axon has to degenerate without physical compartmentalization. The molecular mechanisms of such nonphysical compartmentalization and degeneration are unknown but may differ from the molecular mechanisms used in typical Wallerian degeneration. There are only a few experimental studies available from which to draw any conclusions on this matter. One experimental approach has been to use compartmentalized cell culture techniques in which the neuronal cell body and the axon can be manipulated independently. Campenot demonstrated that local withdrawal of nerve growth factor from the axonal chamber leads to degeneration of the axons only in that chamber, with no effect on survival of the neuronal cell body. Thus far, this culture technique has been used primarily to study the effects of nerve growth factor on axon biology during development.
but it is possible to use the same technique to study the mechanism of action of various toxic insults to the neuron or the axon. Furthermore, using a coculture paradigm in the compartmentalized culture system, one can begin to examine the interplay between the axon and other cell types, such as glial cells and macrophages.

One recent example of such use of compartmentalized cultures is the exploration of the mechanism of axonal degeneration induced by human immunodeficiency virus envelope protein gp120.11 Colleagues and I12 have previously shown that gp120 can induce axonal degeneration and, at higher concentrations, neuronal cell death in dorsal root ganglion neurons through classic apoptotic pathways. This was mediated, in part, through an indirect mechanism that involved chemokine receptors on Schwann cells. What was unknown was whether gp120 had any direct local toxic effect on the axons. Using the compartmentalized culture system, we showed that gp120 has 2 types of action on sensory neurons that lead to axonal degeneration. In 1 case, gp120 caused neuronal death mediated through the Schwann cells near the neuronal body. Subsequent to this, there was axonal degeneration. But this was different from the distal axonal degeneration seen in the side chambers when gp120 was applied directly on the axons. In the axonal chamber, gp120 was able to bind to the chemokine receptors on the axons and induce degeneration through typical apoptotic machinery (ie, cytochrome c release and activation of caspase-3), but this was independent of the events occurring in the neuronal cell body. Furthermore, the Schwann cells associated with the axons were protective and did not participate in the induction of axonal degeneration. This is in contrast to the role of perineuronal Schwann cells in which they act as executioners of gp120-induced neuronal death. Further studies using in vivo models of human immunodeficiency virus–induced peripheral neuropathies will be needed to examine the full biological relevance of these in vitro observations.

In another example that demonstrates the role of terminal Schwann cells in disease pathogenesis, Halstead et al13 used a murine model to study the mechanism of paralysis in a type of autoimmune neuropathy. In Miller Fisher syndrome, antidisialoside antibodies against epitopes in ganglioside GQ1b are present in patient serum and have a pathogenic role.14,15 Administration of antidisialoside antibodies in mice causes distal axonal degeneration, but the mechanism and involvement of perisynaptic Schwann cells are unknown. In a recent study, when Halstead et al13 administered antidisialoside antibodies to mice, there was clear evidence of cell death of perisynaptic Schwann cells, with concomitant degeneration of distal terminal axons. While it is possible that Schwann cell death was secondary to axonal degeneration, this is unlikely because most peripheral neuropathies and denervating illnesses such as motoneuron diseases do not result in terminal or perisynaptic Schwann cell death. This study, however, does not clearly demonstrate that axonal degeneration was secondary to Schwann cell death; future studies with more carefully planned time course experiments or use of transgenic mice that are resistant to apoptotic cell death16 could shed light on this issue.

Distal axonal degeneration is not limited to peripheral neuropathies. An earlier electrophysiologic study in a mouse model of familial amyotrophic lateral sclerosis (ALS) caused by mutations in the superoxide dismutase-1 (SOD-1) gene suggested that the initial electrophysiologic abnormality was explained by axonal degeneration rather than neuronal loss.17 More recently, another study provided histopathologic evidence of distal axonal degeneration long before any observable changes could be detected in spinal motoneurons or in proximal ventral roots in the transgenic SOD-1 mice.18 In the same study, Fischer et al19 also provided similar pathologic data from a patient with ALS who died unexpectedly. These studies raise more questions than they answer. What is the mechanism of distal axonal degeneration in ALS? Are glial cells involved? We do not have the answers, but the role of nonneuronal cells in ALS has recently been explored in more detail because of the realization that neuronal death in the SOD-1 transgenic mouse model of familial ALS is non–cell autonomous.18,19 Using a unique method with chimeric mice, Clement et al20 demonstrated that astrocytes with wild-type SOD-1 can protect spinal motoneurons possessing the mutant SOD-1 gene and, in reverse, that astrocytes with the mutant SOD-1 gene can kill wild-type spinal motoneurons. This observation is certainly intriguing, but one has to wonder about the role of Schwann cells, especially terminal Schwann cells, when recent observations2 and the ratio of axon to Schwann cells (Figure 1) are considered. Further studies are needed to elucidate the potential role of Schwann cells in the pathogenesis of ALS.

**AXON-GLIA INTERACTIONS IN NEUROPROTECTION**

Schwann cells likely have an important role in neuroprotection and axon protection, a property shared by astrocytes in the central nervous system. In the stroke literature, there has been a long-standing observation that ischemic preconditioning protects neurons and limits damage from future ischemic attacks (reviewed in Dirnagl et al21). This endogenous neuroprotection is mediated, in part, through astrocytes, and astrocyte-secreted erythropoietin likely has an important role in this neuroprotection.21 In recent years, a growing body of literature has shown that erythropoietin and its receptor are expressed widely in the central22,23 and peripheral24,25 nervous systems. In a recent study, we showed that there was an endogenous neuroprotective pathway in the peripheral nervous system that protected sensory neurons against axonal degeneration.25 Schwann cell–derived erythropoietin is important in this endogenous protective pathway (Figure 2).

In a coculture system, when dorsal root ganglion sensory neurons were injured by axonal transection or by exposure to toxic drugs that cause axonal degeneration, we observed that periaxonal Schwann cells upregulated their erythropoietin expression.25 This did not occur in Schwann cells away from the axons, which suggests that the signaling required close contact between the axon and the Schwann cells. A similar observation was also made in rats when the sciatic nerve was tran-
CONCLUSION

The foregoing examples of research raise more questions about the mechanism of distal axonal degeneration seen in most peripheral neuropathies and the role of Schwann cells in this process. Axon-glia interactions have been studied in detail in development (reviewed in Bartsch), but insight gained from these studies needs to be applied to research on disease mechanisms. Furthermore, we need more focused studies on how axons degenerate, especially in distal axonal degeneration. The literature includes numerous peripheral nerve regeneration studies, but we still do not have a single effective therapy. We are good at assessing peripheral nerve regeneration in rodents, but often studies are carried out without good understanding of the underlying mecha-

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