Regenerative Nerve Fiber Growth in the Adult Central Nervous System

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Neurite growth and regeneration in the adult central nervous system (CNS) is extremely limited. An important factor contributing to these restrictions is specific growth inhibitory proteins associated with oligodendrocytes and CNS myelin. A major inhibitory factor is the antigen of a monoclonal antibody; the application of this neutralizing antibody to spinal cord- or brain-lesioned adult rats induces long-distance regeneration of lesioned axons, as well as a specific increase in sprouting and rewiring of the cortical output system to the brain stem and the spinal cord. These anatomic changes are paralleled by important functional recoveries of locomotion and precision movements.

Axonal and dendritic growth successively decreases as the central nervous system (CNS) matures. When the major tract systems are established and become connected, the fast-conducting axons get myelinated by oligodendrocytes. Time and pattern of myelination are specific for each fiber tract and CNS region. In the rat, myelin formation starts around birth in the brain stem and spinal cord and progresses for ~2 mo. In humans, some CNS regions start to myelinate before birth, but most myelin formation occurs postnatally.

Neurite growth leading to changes in the wiring of the adult CNS would be required under three different circumstances. 1) The first circumstance is when major changes in functional demands are required, a condition that probably includes, at least in part, long-term memory. 2) Another circumstance is when single nerve cells or small groups of neurons are damaged by microlesions or degenerate. In several parts of the CNS, neighboring neurons can adjust their connections to compensate for these losses. However, loss of larger cell populations often leads to permanent deficits. 3) The third circumstance is when large fiber tracts are lesioned by trauma or vascular events, requiring long-distance regeneration. This process does not take place in the adult CNS of higher vertebrates; many observations in experimental animals and humans show that axonal and dendritic growth is limited to short distances, usually not exceeding 1–2 mm, in the differentiated mammalian CNS.

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Investigations of cell biological mechanism involved in the restriction of the plastic and regenerative capabilities of the adult CNS have yielded a series of classical experiments demonstrating the outstanding importance of the local tissue environment of growing fibers. Peripheral as well as central adult axons can regenerate over long distances in peripheral nerves, e.g., after implantation into the spinal cord, brain, or optic nerve (1). In contrast, they stop growing if they contact adult CNS tissue, e.g., at the distal end of a peripheral nerve bridge. These experiments demonstrate that adult CNS neurons can be switched back into a “growth mode” and that adult axons are able to elongate over distances of several centimeters in a peripheral nerve environment.

There are many differences between adult peripheral nerve and CNS tissue: the main glial cell type of peripheral nerves is the Schwann cell, whereas oligodendrocytes, astrocytes, and microglial cells populate the CNS. Schwann cells are known to be highly neurite growth promoting in vitro and in vivo, an effect that is due at least in part to their synthesis of several neurotrophic factors as well as growth-promoting membrane and extracellular matrix proteins (2). When central glial cells were analyzed for their effects on neurite growth, growth-promoting as well as nonpermissive effects were found for astrocytes. Oligodendrocytes induced growth cone collapse and growth inhibition for various types of neurons.

Oligodendrocytes and CNS myelin contain potent neurite growth inhibitory proteins

Cocultures of oligodendrocytes with embryonic or postnatal dorsal root ganglion (DRG) cells, neuronal cell lines (PC-12, neuroblastoma), or retinal ganglion cells showed a fast collapse response of the neurite growth cones on contact with oligodendrocyte processes (see Refs. 3 and 5 for review). The morphological col-

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lapse was preceded by an intracellular calcium release, which is an obligatory step in the oligodendrocyte- and myelin-induced growth arrest responses. Growth cone collapse and growth inhibition are long-lasting and can also be induced by CNS myelin protein extracts or a purified 220- to 250-kDa protein. When dissociated neurons were plated on a substrate coated with myelin or myelin protein extracts from adult rat, mouse, bovine, or human spinal cord or brain white matter, neurite outgrowth was strongly inhibited and could not be overcome by high concentrations of growth-promoting neurotrophic factors such as nerve growth factor. Interestingly, cellular spreading and migration of fibroblasts or primary culture astrocytes were also inhibited by myelin.

With the use of inhibition of neurite outgrowth, neurite growth cone collapse, and inhibition of fibroblast spreading as routine assays, an active, high-molecular-mass membrane protein was found in CNS myelin of all analyzed species (3, 5). In the rat, an additional 35-kDa active constituent was present. The neurite growth inhibitor (NI) 220/250 was recently purified to homogeneity from bovine spinal cords. The corresponding cDNA codes for a novel transmembrane protein with a large extracellular domain. Homologies to human genomic sequences show very high conservation of the nucleotide and amino acid sequences. These proteins are normal constituents of CNS myelin throughout adult life.

Several antisera as well as monoclonal antibodies (MAbs) were raised against this NI-220/250 protein. The MAb, inhibitory neutralizing antibody 1 (IN-1), recognizes the native, active constituent and neutralizes its neurite growth inhibitory activity. After extensive studies in vitro, the antibody was applied to spinal cord and brain-lesioned animals.

In addition to NI-220/250, other components with neurite growth inhibitory activity are present in myelin protein preparations that cannot be neutralized by the IN-1 antibody. One constituent, myelin-associated glycoprotein (MAG), has recently been shown to inhibit growth of adult sensory neurons and neuroblastoma cells in vitro. Interestingly, MAG has a neurite growth-promoting effect on newborn cultured DRG neurons. CNS myelin isolated from MAG-gene-ablated mice retains its potent inhibitory activity. In agreement with these in vitro observations are in vivo studies on optic nerve and corticospinal tract regeneration, which were not or only to a very minor extent different from results with wild-type mice. These results show that MAG is only a minor inhibitory component in the CNS.

In contrast, regeneration of peripheral axons following a sciatic nerve crush was significantly enhanced in mice lacking the MAG gene.

A proteoglycan with neurite growth inhibitory effects was also recently purified from CNS myelin. Its molecular identity and its physiological roles are being analyzed.

Regeneration of lesioned axons in the adult spinal cord and brain by neutralization of myelin-associated neurite growth inhibitors

A direct way to investigate the role of oligodendrocyte- and myelin-associated neurite growth inhibitors is to delete oligodendrocytes in parts of or in the whole CNS and thereby prevent myelin formation. Two different approaches have been chosen to this end, and both resulted in a great increase in the regeneration capacity of spinal cord fiber tracts following lesion (see references in Ref. 5). In the chick embryo, the capacity of spinal fiber tracts to regenerate or grow through a complete spinal cord lesion to their normal target sites abruptly ends at embryonic days 12–13. This time point coincides with the appearance of oligodendrocytes in the chick embryo spinal cord. When complement and antibodies against a myelin-specific glycolipid, galactocerebroside, were injected, oligodendrocyte development was greatly impaired and myelin formation was prevented. Massive regeneration of ascending and descending spinal cord fiber tracts was observed at embryonic days 15–17 following complete spinal cord transection. This anatomic restitution was associated with reestablishment of functional connections between the brain stem and the lumbar spinal cord. Enhancement of regeneration could also be obtained with the same procedure in newly hatched chickens. When parts of the spinal cord of newborn rats were irradiated repeatedly, it was possible to almost completely deplete the dividing oligodendrocyte precursor population. Transection lesions in these myelin-free parts of the spinal cord at 2 wk of age were followed by regeneration of a population of corticospinal tract axons over long distances (4–18 mm, i.e., from middle thoracic to lumbar and sacral spinal levels) within 2–3 wk. Because of the lack of myelin in these spinal cords, no functional assessments could be obtained, however.

A major limitation of the experimental approaches cited above is the massive intervention that, in addition to preventing myelin formation, could lead to the upregulation of neurite growth-promoting molecules, e.g., by astrocytes, microglial cells, or neurons. The availability of an antibody that neutralized the myelin- and oligodendrocyte-associated neurite
growth inhibitors (MAb IN-1) allowed for a much more defined intervention.

In adult rats, the MAb IN-1 was applied in three different ways with very similar results: as small hybridoma tumors, usually in the cortex or hippocampus, of immune-suppressed rats (for 2 wk); as antibody-secreting hybridoma cells included in Millipore filter capsules implanted close to the lesion sites; or as recombinant IN-1 Fab' fragments infused as purified protein via subcutaneous Alzet pumps. Bilateral transection of the dorsal half of the spinal cord at midthoracic levels, including both corticospinal tracts, resulted in a limited, spontaneous degree of regenerative sprouting from the lesioned fibers rostral to the lesion in control animals (no antibody or control antibodies) (Fig. 1C). In MAb IN-1-infused animals, but not in controls, long-distance regeneration of corticospinal axons was frequently observed (Fig. 1A and B). Position and distance of regeneration varied from animal to animal, with the longest fibers reaching distances of 8–20 mm from the lesion site, which correspond to the lumbar and sacral spinal cord. Branching of regenerating axons into the gray matter and terminal arborizations could be observed and suggested the establishment of connections to local spinal neurons. Control animals in all these experiments showed regeneration of 0.5–1 mm, rarely up to 1.5–1.8 mm from the transection site. These distances correspond to less than one spinal cord segment. Very similar enhancements of regenerating axon growth by MAb IN-1 were observed in the septohippocampal system and the optic nerve.

The functional assessment of spinal cord-lesioned (overhemisected) and antibody-treated animals showed massive improvements in several aspects of locomotion and hindlimb reflexes. Interestingly, the stride length during locomotion on the treadmill returned to almost normal values in the MAb IN-1-treated animals, and the placing response, a reflex known to depend on a functional corticospinal tract, reappeared in a large number of these animals. In contrast, no improvement could be seen in the error rate in hindfoot placing when the rats crossed a grid, a task that requires detailed sensory-motor feedback. Thus the relatively small number of regenerating axons in the MAb IN-1-treated animals could be sufficient for reestablishing cerebral control for local spinal pattern generator circuits involved in stepping or certain reflexes. Additional effects of these antibodies on the rearrangement of local circuitry may also contribute to these functional recoveries.

Treatment of spinal cord-lesioned adult rats with neurotrophic factors, in particular neurotrophin 3, greatly enhanced the sprouting rostral to the lesion and, in combination with MAb IN-1, the regeneration of lesioned axons, showing that growth-enhancing factors can be used to promote regeneration of transected central fibers. In some CNS areas, in particular in the retina, axonal lesions also induce cell death, a phenomenon that can be counteracted by the appropriate combinations of neurotrophic molecules.

Histological analyses of the lesion site in all these experiments showed the difficulty encountered by the sprouting axons in crossing the lesion site. Caverns and compact scars are often avoided, possibly by the presence of additional inhibitory molecules. Chondroitin-sulfate proteoglycans in particular have been suggested to play a crucial role; however, this is so far only based on in vitro and correlative evidence. A complex cascade of inflammatory reactions, including the local synthesis and release of several cytokines, accompanies the tissue destruction and secondary cell death and probably crucially influences the reaction of astrocytes, microglia, oligodendrocytes, and neurons.

Enhancement of structural plasticity of intact circuits in the adult brain and spinal cord

Specific lesions in the neonatal CNS can induce compensatory rearrangements of intact fiber connections and circuits, sometimes leading to a high degree of recovery of function. Well-studied examples are lesions of the motor cortex or even one entire hemisphere in humans at perinatal ages or specific lesions of the corticospinal system in rats and hamsters. In contrast to this situation in the immature CNS, very little structural changes and functional recovery are observed following lesions of the adult CNS. In anatomic and behavioral experiments, adult rats were analyzed following a unilateral interruption of one pyramidal tract in the caudal medulla oblongata (4). In a classical paradigm (the food pellet-reaching test) to test skilled forelimb movements, which are known to depend on an intact corticospinal system, lesioned animals were strongly and permanently impaired. Whereas infusion of control antibodies did not change this situation, infusions of MAb IN-1 for 2 wk led to a permanent and drastic recovery of function in most of the movement components analyzed. Anatomic analyses of the unlesioned contralateral corticospinal tract showed that many collaterals sprouted from this tract across the midline in the spinal cord and reinnervated the denervated side. Similarly, in the brain stem, the tract that lost its access to the spinal cord because of lesion sprouted across the midline in the pons and the ruber nucleus, thus establishing a bilateral projec-
tion to these nuclei. These phenomena of structural plasticity, as well as similar fiber growth, which may occur in other tract systems in response to the neutralization of myelin-associated neurite growth inhibitory proteins, may be causally involved in the functional recovery described above. On the anatomic as well as functional level, the effects of MAb IN-1 in these lesioned adult animals seemed to restore conditions that are normally only seen at perinatal ages.

**FIGURE 1.** Parasagittal sections of the lesioned spinal cord of adult rats. Six-week-old rats had full bilateral transection of the corticospinal tract at T8, and sensory-motor cortex was injected with biotin dextran amine (BDA) anterograde. Rats were subsequently treated for 2 wk with monoclonal antibody IN-1, which neutralizes myelin-associated neurite growth inhibitors (NI)-35-250 (A and B) or were treated with control solution (C). A: in rats treated with the IN-1 antibody, corticospinal tract fibers labeled from the sensory-motor cortex by BDA approach the injury site from the left and sprout extensively. Some of these regenerative sprouts seem to be arrested by the scar. Many fibers bypass the lesion through the ventral gray matter (arrowheads). Dotted lines delineate the debris zone and future cavern. B: terminal arborization of a regenerated corticospinal tract fiber in the spinal gray matter 7 mm caudal to the lesion site. Side-branch formation and synapselike swelling suggest that the fiber contacts local spinal neurons. C: in rats that received a control solution, lesion-induced sprouts occur rostral to the lesion but fail to elongate over long distances. No regenerating fibers bypassing the lesion site are observed. [From Schwab and Brösamle (6).]
In summary, one way in which the adult CNS tissue is distinct from adult peripheral nerves as well as the developing CNS is the nonpermissive property for nerve fiber growth. Important components of this tissue property are the myelin- and oligodendrocyte-associated neurite growth inhibitory proteins, in particular the high-molecular-mass protein NI-220/250, the antigen of the neutralizing IN-1 antibody. A variety of in vivo experiments show long-distance regeneration of lesioned axons as well as enhancement of structural plasticity of unlesioned circuits in adult animals following spinal cord or brain lesions and treatment with IN-1 antibodies. These anatomic changes were accompanied by a high degree of functional restitution in several motor tasks.

Because large CNS lesions are probably detrimental to animals in the wild, the regeneration of lesioned CNS fiber tracts may not have been particularly important during evolution. Rather, the stabilization of the enormously complex CNS structure by inhibition of fiber growth in the tract areas (white matter) and restriction of anatomic plasticity in gray matter to small spatial dimensions may have been advantageous. One way to achieve this goal was the expression of specific neurite growth inhibitory molecules by oligodendrocytes, a cell type that differentiates when axon growth subsides.

The fact that the experimental interventions described above led to functionally meaningful results shows that signals and mechanisms that specify functionally correct connections are present or can be reactivated in the adult CNS. These results also point to the possibility of new therapeutic approaches to spinal cord lesions, traumatic brain lesions, or stroke. These new therapies may include combinations of interventions that suppress the growth inhibitory components of the adult CNS, stimulate neuronal survival and neurite growth, e.g., by neurotrophic factors, and improve the conditions at lesions sites by manipulation of the scar or implantation of growth-permissive bridges.

References