Histol Histopathol (1997) 12: 857-868
DOI: 10.14670/HH-12.857
http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

Invited Review

Determinants of axonal regeneration

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Summary. Axons often regrow to their targets and lost functions may be restored after an injury in the peripheral nervous system. In contrast, axonal regeneration is generally very limited after injuries in the central nervous system, and functional impairment is usually permanent. The regenerative capacity depends on intrinsic neuronal factors as well as the interaction of neurons with other cells. Glial cells may, in different situations, either support or inhibit axonal growth. This review discusses the molecular mechanisms that are involved in promoting and inhibiting axonal regeneration in the nervous system after injuries.

Key words: Extracellular matrix, Macrophage, Nerve regeneration, Neurotrophin

Introduction

Transection of a nerve fiber triggers a complex set of events in the nerve cell body, in the axon proximal and distal to the lesion, in supporting glial cells and other non-neuronal cells. The neuronal perikaryon swells, the nucleus is displaced against the cell membrane, and the Nissl substance disintegrates, a sign of increased protein synthesis. This neuronal reaction to axotomy occurs to a different extent in different types of neurons and also depends on factors such as the distance to the cell body. Many proteins normally synthesized by a neuron will no longer be produced, or will be produced at low levels after axotomy, while other proteins that are normally synthesized only at low levels or not at all, will be expressed at high levels. This shift in gene expressing in response to injury probably occurs, at least in part, in order to optimize the neuronal capacity for regeneration (reviewed in Aldskogius and Svensson, 1993). After transection of a nerve fiber the axonal membrane will rapidly seal and an end swelling, the growth cone, forms. Thin filopodia emerging from the axonal endbulb can sense molecular cues in the environment and transmit signals to the growth cone, presumably resulting in the decision of which path to choose (Davenport et al., 1993). When an outgrowing axon has reestablished contact with the target, neuronal protein synthesis will return to normal. The alteration in neuronal molecular phenotype after axotomy seems to be triggered by the disrupted retrograde transport of target derived factor(s), since the phenotype can be reversed toward the normal if the required trophic factor is supplied (see e.g. Fitzgerald et al., 1985). However, axons often fail to regrow and reinnervate the target, and moreover, neurons may even die in response to transection of the axon. The susceptibility of a neuron to death after transection of the axon is greater during development than in adulthood, probably as a result of decreasing dependence on trophic support for survival in the adult animal.

While regeneration in the peripheral nervous system (PNS) is often rapid and successful, regeneration in the adult mammalian central nervous system (CNS) is generally not seen. After an injury in the PNS, debris is rapidly cleared from the denervated distal stump, through a process known as Wallerian degeneration. The Schwann cells in the distal nerve stump will line up to form channels, known as the bands of Bügner, through which the severed axons can regrow to their targets (Fig. 1). In contrast, in the injured CNS, axon-growth is in most cases limited to the scar that will be formed by astrocytes at the site of the injury. The Wallerian degeneration distal to a lesion in the CNS is much slower than in the PNS, and a dense scar will form there with time (Fig. 1). There may be important intrinsic differences between PNS and CNS neurons in response to injury. However, non-neuronal factors seem to be at least as important. Here follows an overview of some of the suggested key players in promoting and inhibiting axonal regeneration. This review focuses on differences between the adult mammalian PNS and CNS in their response to traumatic injuries. Factors that are likely to be key determinants of the capacity of axonal regeneration are discussed, and a few examples are mentioned in each case.

Intrinsic neuronal regenerative capacity

Aguayo and collaborators demonstrated that axons of CNS neurons can grow long distances through PNS tissue by grafting segments of peripheral nerve to the

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injured brain, spinal cord, and optic nerve (Reviewed by Aguayo et al., 1991). This clearly demonstrated that the lack of regeneration within the CNS is not due entirely to a weak intrinsic neuronal regenerative capacity. Instead, this indicates that the CNS environment fails to stimulate axon regrowth or that the CNS contains axon-regrowth inhibiting molecules. The latter theory was also supported by the lack of PNS axon-growth in a CNS graft (Aguayo et al., 1978). However, intrinsic neuronal factors may indeed limit axonal regrowth in the CNS in some situations (reviewed by Fawcett, 1992). For example, the capacity of CNS neurons to innervate a peripheral nerve graft inserted in the injured spinal cord, brain or optic nerve decreases with increasing distance between the neuronal cell body and the injury (David and Aguayo, 1981; Benfey and Aguayo, 1982; Richardson et al., 1984; Vidal-Sanz et al., 1987). This is probably a result of a weaker triggering of regenerative programs in the neuron when the lesion is far from the cell body, as indicated by the fact that the growth-associated protein GAP-43 is induced in retinal ganglion cells only if the axotomy is within the first mm of the optic nerve (Doster et al., 1991). The distance between the cell body and the site of axotomy does not seem to be an important determinant of axonal regeneration in the PNS, since axons can regrow successfully even after the axon is cut close to the target. Other intrinsic neuronal differences that may render CNS neurons less likely than PNS neurons to regenerate successfully may be related to the facts that central neurons are more likely to die after axotomy than PNS nerve cells (Sunderland, 1978), and that the formation of ectopic terminals on nearby neurons may impede axonal regrowth in the CNS (Bernstein and Bernstein, 1971).

That axons from neurons in the brain and spinal cord can grow in peripheral nerve grafts indicates that the extracellular environment provided by glial and other cell types may be crucial for the regeneration process. In different situations non-neuronal cells may either stimulate axon-growth by e.g. secreting neurotrophic molecules or extracellular matrix molecules or actively inhibit axonal regeneration by repelling growth cones.

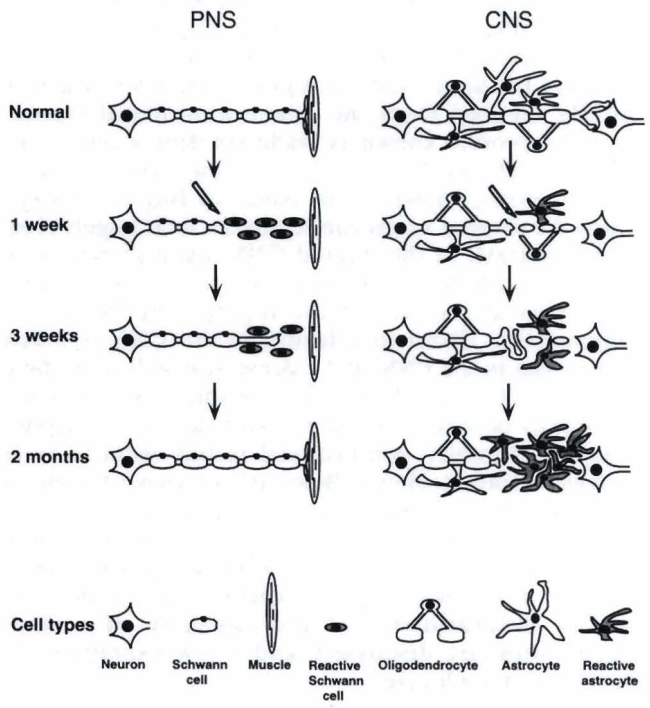

Fig. 1. Schematic drawing depicting the main cellular reactions after injuries in the PNS and CNS. In the PNS, the Schwann cells in the distal nerve stump line up to form channels through which the axons can regrow. In the CNS, clearance of axonal debris and myelin is slow distal to the injury, and astrocytes form a dense scar. The axonal growth in the injured CNS is limited to the site of the lesion.

Neurotrophic molecules

Much of the research on the role of neurotrophic factors in regeneration has focused on the neurotrophin family. This family consists of the prototypical neurotrophic factor, nerve growth factor (NGF) and the structurally and functionally related molecules brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and NT-4. The neurotrophins exert their functions by binding to specific cell surface receptors. The first identified neurotrophin-binding molecule is a widely expressed protein which can bind all neurotrophins with low affinity (designated p75 or low-affinity neurotrophin receptor). However, a group of tyrosine kinase receptors denoted trkA, trkB and trkC seem to be more important for mediating the effects of neurotrophins. NGF binds to trkA, BDNF and NT-4 selectively activate trkB, and the physiological receptor for NT-3 seems to be trkC (reviewed by Barbacid, 1995). The existence of alternative forms of the trkA, trkB and trkC tyrosine kinase receptors, as well as truncated forms of trkB and trkC which lack the tyrosine kinase domain add an extra level of complexity. The neuro-trophins, by activating their cognate receptors, stimulate differentiation and neurite outgrowth, and support the survival of different, but overlapping, groups of neurons (reviewed by Snider, 1994).

There is a wealth of circumstantial evidence implicating neurotrophins in PNS regeneration. When a peripheral nerve is transected, the normally low levels of NGF, BDNF and NT-4 in the nerve increase dramatically in the distal nerve stump (Heumann et al., 1987a; Meyer et al., 1992; Funakoshi et al., 1993). Moreover, some neurotrophins are expressed at increased levels in target tissues after denervation, i.e. BDNF mRNA expression is strongly elevated in muscle (Funakoshi et al., 1993; Koliatsos et al., 1993) and NGF mRNA levels increase in skin (Mearow et al., 1993).

Schwann cells normally express significant levels of truncated trkB and trkC receptors lacking the tyrosine kinase domain, but very low levels of p75. This expression pattern of neurotrophin receptors is reversed in denervated Schwann cells so that high levels of p75

are expressed and trkB and trkC expression is strongly decreased (Taniuchi et al., 1986, 1988; Heumann et al., 1987b; Frisén et al., 1993; Funakoshi et al., 1993). See Fig. 2 for a summary of changes in neurotrophin and neurotrophin receptor expression after injury.

It has been suggested that p75 can bind NGF on the surface of reactive Schwann cells and present NGF to regrowing axons (reviewed by Johnson et al., 1988). Indeed, NGF bound to distal segments of previously severed sciatic nerves can enhance sensory neurite growth in vitro (Sandrock and Matthew, 1987a). Since p75 can bind all neurotrophins with similar affinity, it is reasonable to extend this hypothesis to the other neurotrophins. The decrease in trkB and trkC mRNA in the distal nerve segment after transection limits competition for ligand, thus enabling binding of neurotrophins with low affinity to Schwann cell surfaces.

Most studies regarding the role of neurotrophins in peripheral nerve regeneration have hitherto focused on NGF sensitive nerve cells, such as sensory and sympathetic neurons. The weak sensory axon regeneration in C57BL/Ola mice (a mouse strain in which macrophage recruitment is virtually absent after injury and NGF-synthesis increases only marginally after injury) can be stimulated by exogenous NGF, supporting a role for NGF in sensory axon regrowth (Brown et al., 1991). Moreover, the rate of sensory axon regeneration through silastic tubes has been reported to increase when NGF is supplied (Derby et al., 1993). However, other reports suggest a lesser role for NGF in nerve regeneration. p75 and trkA expression as well as retrograde NGF-transport decrease in dorsal root ganglia neurons after axotomy (Raivich et al., 1991; Verge et al., 1992), and regeneration of sensory axons is not impeded when endogenous NGF is inactivated with anti-NGF antibodies (Rich et al., 1984; Diamond et al., 1987, 1992). Additionally, it does not appear that NGF is involved in survival of sensory neurons after injury (Rich et al., 1984) or regrowth of lesioned sympathetic axons (Gloster and Diamond, 1992). Thus, although NGF is synthesized at highly elevated levels after injury, it does not seem to be involved in sensory or sympathetic axon regeneration. However, data have been presented which indicate that NGF is involved in lesion-induced collateral sprouting of undamaged sensory neurons (which increase their p75 mRNA expression) and sympathetic neurons (Mearow et al., 1991; Diamond et al., 1992b; Gloster and Diamond, 1992). The decrease in p75 mRNA in sensory neurons after axotomy may indicate that neurotrophins are not involved in sensory axon regeneration. However, trkB and trkC mRNA are increased in dorsal root ganglia neurons after axotomy (Ernfors et al., 1993) suggesting that BDNF, NT-3, and/or NT-4 could be important for sensory neurons after injury.

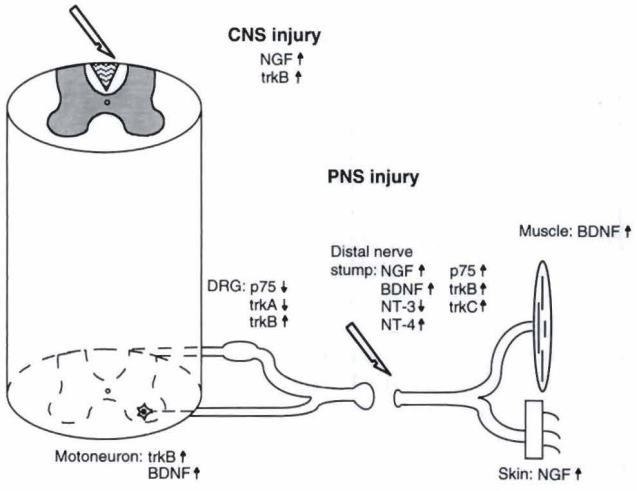

Fig 2. Schematic drawing illustrating alterations in immunoreactivity and/or mRNA for neurotrophins and neurotrophin receptors after central and peripheral nerve injury. See the text for references.

BDNF promotes the survival of axotomized motoneurons in neonatal rats (Sendtner et al., 1992a; Yan et al., 1992; Koliatsos et al., 1993). In contrast to trkA mRNA in sensory neurons, trkB expression by motoneurons increases after axotomy (Piehl et al., 1994; Kobayashi et al., 1996). The increase in trkB mRNA probably results in an accumulation of trkB receptors in the axonal growth cone, which could make motoneurons more sensitive to local and target-derived neurotrophin. A recent study in genetically engineered mice lacking trkB receptors, has indeed demonstrated that trkB is required for the survival of some facial motoneurons after axotomy (Alcántara et al., 1997).

The expression of neurotrophins and neurotrophin receptors is altered also after injuries in the central nervous system (see Fig. 2). NGF levels are transiently elevated at CNS injuries (Bakhit et al., 1991; Ishikawa et al., 1991; Lindholm, 1992). NGF synthesis in astrocytes can be induced in vitro by several cytokines and other molecules (Gadient, 1990; Lindholm et al., 1990; Spranger et al., 1990; Pechán et al., 1992, 1993; Zafra et al., 1992; Ladenheim, 1993), and the increase in NGF at a CNS injury may be due to increased levels of macrophage-derived cytokines. BDNF mRNA has been found to increase in neurons in the brain after ischemia and hypoglycemia (Lindvall et al., 1992) but not in glial cells after traumatic injuries (Lindholm et al., 1992). p75 and truncated trkB receptors are expressed at high levels by reactive astrocytes as well as by leptomeningeal cells at CNS injuries (Brunello et al., 1990; Frisén et al., 1992, 1993a,c; Risling et al., 1992; Beck et al., 1993).

At this point, only speculations can be made regarding the function of non-neuronal expression of p75 and truncated trkB receptors in the injured CNS. It is unlikely that truncated trkB receptors can transduce a signal to the cell since they lack the catalytic tyrosine kinase domain. It is also doubtful whether p75 on glial cells can mediate any biological effect of neurotrophins, although evidence has been presented for NGF signaling and internalization via p75 in glial cells (Kahle and Hertel, 1992; Carter et al., 1996). Both p75 and

truncated trkB receptors could perhaps serve a function by binding and accumulating neurotrophins at the site of the injury, thus making them available for axonal endbulbs. Neurotrophins bound to the surfaces of non-neuronal cells at a CNS lesion could possibly stimulate axonal growth. However, the immunoglobulin-like domains in the ectodomain of trk family receptors can inhibit neurite growth (Tannahill et al., 1995), and the high expression of truncated trkB receptors by reactive astrocytes may thus possibly inhibit axon-growth.

In addition to the neurotrophins, many other neurotrophic factors have been suggested to be of importance after neuronal injury. Moreover, several growth factors, best known for their effects on cells outside the nervous system, can stimulate axonal growth or promote neuronal survival and have been implicated in axonal regeneration, although the roles of these factors have not yet been studied as thoroughly as the effects of neurotrophins (see e.g. Raivich and Kreutzberg, 1993). The expression of ciliary neurotrophic factor (CNTF) decreases dramatically in the distal stump of a severed peripheral nerve (Sendtner et al., 1992b), but is expressed in reactive astrocytes after CNS injury (Ip et al., 1993). CNTF lacks a signal sequence and does not seem to be secreted from cells. However, CNTF may possibly be released from damaged cells, and the retrograde transport of this molecule is indeed increased after injury (Curtis et al., 1993). Glial cell-line derived neurotrophic factor (GDNF), a potent neurotrophic factor which is required for the survival of certain neurons during development (Moore et al., 1996; Sánchez et al., 1996), is another example of a neurotrophic factor that is produced at increased levels in injured peripheral nerves (Trupp et al., 1995; Hammarberg et al., 1996).

However, in spite of the wealth of circumstantial evidence implicating several neurotrophic factors in axonal regrowth after injury, there are in fact data suggesting that none of them may be required for regeneration. Axons can grow into acellular peripheral nerve segments, in which all cells have been killed and thus no neurotrophic factors can be synthesized (Ide et al., 1983; Sketelj et al., 1989).

Extracellular matrix

Acellular nerve segments predominantly consist of extracellular matrix tubes, and axon-growth in this environment suggests that extracellular matrix proteins may be of importance for regeneration. Much of the attention in this context has focused on laminin and related molecules, since this glycoprotein synthesized by Schwann cells is one of the strongest neurite outgrowth promoting matrix molecules known to date. Other matrix molecules which may affect neurite growth include for example collagen, fibronectin and tenascin (see Martini, 1994). Several different approaches have been used to study the role of laminin in axon-growth in mammals *in vivo*. Morphological studies have demonstrated that cut PNS axons regrow in close relation to laminin (Bignami et al., 1984; Liesi, 1985; Salonen et al., 1987; Kuecherer-Ehret et al., 1990). Other studies have shown the effect of blocking the interaction between axons and laminin. These experiments have demonstrated impeded axonal growth in acellular nerve segments pretreated with anti-laminin (or anti-fibronectin antisera) (Wang et al., 1992a,b), as well as weak axon-ingrowth into extracellular matrix-coated tubes in the presence of antibodies to the laminin/collagen receptor α1ß1-integrin (Toyota et al., 1990). Furthermore, the re-innervation of the denervated iris is slower in the presence of antibodies to a laminin-proteoglycan complex (Sandrock and Matthew, 1987b). Taken together, these data suggest that laminin is important in axonal regeneration in the PNS.

In the CNS, astrocytes express laminin during development and reexpress laminin after injuries in the adult. However, laminin is only synthesized locally at injuries and not in degenerating tracts undergoing Wallerian degeneration (Liesi et al., 1984). Axonal sprouts which grow into the scar tissue that forms at the lesion after a spinal cord injury are closely related to laminin expressing astrocytes (Risling et al., 1993; Frisén et al., 1995a). Furthermore, when neuronal cells are cultured on spinal cord cryostat sections, the neurons attach to laminin-rich regions of the sections and orient their neurites after laminin in the tissue (Frisén et al., 1995a). These data suggest that laminin may be involved in lesion-induced sprouting in the injured spinal cord. However, no relation was found between axonal sprouting and laminin in the injured optic nerve (Giftochristos and David, 1988). Possible explanations for this discrepancy may be differences in axonal and/or glial properties in the spinal cord and optic nerve. Differences in laminin-sensitivity in the different neuronal cell types could possibly be of importance; indeed, whereas sensory neurons (which project to the spinal cord) remain laminin-sensitive in adult animals (Unsicker et al., 1985), retinal ganglion cell laminin-receptors decrease after innervation of the target tissue during development, and the neurite-outgrowth promoting effect of laminin is lost (Cohen et al., 1986, 1989). Moreover, different populations of astrocytes in various parts of the CNS may respond differently to injuries, and the ability of astrocytes to support neurite growth in vitro depends on which part of the CNS the glial cells were taken from (reviewed by Wilkin et al., 1990; Hatten et al., 1991).

Other matrix molecules which are upregulated after CNS injuries include, among others tenascin, collagen, and fibronectin (Egan and Vijayan, 1991; McKeon et al., 1991; Bartsch et al., 1992; Laywell et al., 1992; Risling et al., 1993) all of which may be of importance for axon growth. The expression and role of cell adhesion molecules belonging to e.g. the immunoglobulin and cadherin families, which could be important for neuron-glial interactions (Neugebauer et al., 1988; Tomaselli et al., 1988), remains to be studied in the injured nervous

system. Several cell adhesion molecules have been implicated in developmental and regenerative axon-glial interactions (Reichardt et al., 1990).

Axon-growth inhibiting molecules

Through the years theories regarding cell extension and migration have mostly focused on stimulating cues in the environment, but more recently growth control by repulsion has gained increasing interest. Schwab and collaborators have provided a large body of evidence for the presence of neurite growth-inhibiting molecules within the brain and spinal cord (reviewed in Schwab et al., 1993). One of the first strong indications for the presence of axon-growth inhibiting activity was the finding that cultured neurons can attach to and send out neurites on slices of peripheral nerves and CNS gray matter, but not on CNS white matter even in the presence of neurotrophic factors (Schwab and Thoenen, 1985; Carbonetto, 1987). Cocultures of neurons and glial cells demonstrated that neurites readily grew over astrocytes but when the growth cones came in contact with oligodendrocytes they not only ceased to grow, but collapsed and were retracted (Schwab and Caroni, 1988). That oligodendrocytes are important inhibitors *in vivo* is supported by studies demonstrating that regeneration in the embryonic spinal cord is enhanced when the onset of myelination is delayed (Keirstead et al., 1992) or oligodendrocyte precursor cells are eliminated by x-irradiation (Savio and Schwab, 1990). Recently, some functional recovery was made possible in spinal cord transected paraplegic rats when axons were directed from the white matter to the gray matter with PNS grafts bridging the lesion (Cheng et al., 1996), thus probably circumventing the inhibitory oligodendro-cytes in the white matter.

Two oligodendrocyte-derived molecules that are at least in part responsible for this dramatic effect on axon-growth are myelin-associated proteins designated NI-35 and NI-250 (Caroni and Schwab, 1988). They seem to cause growth cone collapse and retraction by binding to neuronal receptors which, via G-proteins, induce rapid influx of extracellular calcium (Bandtlow et al., 1993; Igarashi et al., 1993). Inhibition of axonal growth *in vivo* by these oligodendrocyte proteins is indicated by the fact that transected cortico-spinal and septo-hippocampal axons show extensive axon-growth in the presence of neutralizing antibodies to NI-35 and NI-250 (Schnell and Schwab, 1990, 1993; Cadelli and Schwab, 1991). Myelin-associated glycoprotein (MAG) is another oligodendrocyte-derived molecule that inhibits axonal growth *in vitro* (McKerracher et al., 1994; Mukhopadhyay et al., 1994), but it may not be a major inhibitor *in vivo* (Bartsch et al., 1995). Several other molecules produced by oligodendrocytes that can inhibit axon-growth have been described, but their significance remains to be established (Schwab et al., 1993).

Myelin-related axonal-growth inhibiting molecules thus seem to be very important inhibitors of axonal regeneration in the CNS. However, myelin is removed from degenerating tracts with time, but the axons still do not regrow, suggesting that other factors may be important. The finding that the initially strong re-generative response of retinal ganglion cells ceases within a few weeks after injury (Thanos and Vanselow, 1989), led to the suggestion that the slow myelin removal in Wallerian degeneration in the CNS (where myelin is cleared away only after the regenerative attempts by retinal neurons have stopped) is a major reason for the lack of regeneration in the CNS (David et al., 1990). However, neurons fail to attach *in vitro* to sections of tracts undergoing Wallerian degeneration in the spinal cord even long after the injury, at a time when little myelin can be detected (Frisén et al., 1994). Furthermore, lack of axon ingrowth in the adult spinal cord dorsal horn after dorsal root injury, where few oligodendrocytes are found (Carlstedt, 1985), indicates that factors other than myelin-related molecules may counteract CNS regeneration. Indeed, other axon-growth inhibiting factors (e.g. glial hyaluronate-binding protein, GHAP) are expressed by astrocytes in areas of Wallerian degeneration, but not, or at substantially lower levels, in astrocytic scars formed at traumatic injuries (Mansour et al., 1990; Bovolenta et al., 1993).

In the last few years, a plethora of novel axon-growth inhibiting molecules have been identified as a result of searches for molecules involved in axonal guidance during the development of the nervous system. Emerging evidence strongly implicate several of these proteins, for example semaphorins, netrins and ligands for Eph-related tyrosine kinase receptors, in axonal pathfinding (Goodman, 1996), but very little is yet known regarding their possible role in axonal re-generation.

Cooperation with non-nervous system cells

Transection of a peripheral nerve results in the rapid disintegration of the barrier between the blood and the nerve tissue, i.e. the blood-nerve barrier, distal to the injury (Olsson, 1966; Mellick and Cavanagh, 1968). This enables the fast recruitment of mononuclear leukocytes which phagocytize degrading axons and myelin (see Beuche and Fride, 1984) (Fig. 3). The importance of macrophages in the repair after injuries in the nervous system is indicated by, for example, the finding that regeneration of sensory axons is very slow in the mouse strain C57BL/Ola, in which macrophage recruitment to an injured nerve is almost absent (Lunn et al., 1989). Macrophages which invade an injured nerve seem, via interleukin-1, to induce NGF-synthesis by non-neuronal cells (Lindholm et al., 1987; Heumann et al., 1987b), and the weak sensory axon regrowth in C57BL/Ola mice can be supported by exogenous NGF (Brown et al., 1991).

After an injury in the CNS, macrophages rapidly invade the lesion area. However, macrophage infiltration

distal to a CNS injury is slow (Fig. 3), resulting in the delayed clearance of debris in tracts undergoing Wallerian degeneration (Frisén et al., 1994). Macrophages may be detrimental or beneficial following CNS injury. Suppression of macrophage invasion after transient spinal cord ischemia in rabbits improves functional recovery and promotes motoneuron survival (Giulian and Robertson, 1990). A possible role for macrophages could be to phagocytize debris from axotomized neurons undergoing retrograde cell death. Furthermore, it is well known that a large number of synapses on motoneurons disappear after axotomy. In addition to phagocytosis of necrotic neuronal somata, macrophages could therefore be involved in phagocytosis of terminals on moto-neurons (Blinzinger and Kreutzberg, 1968). Morphological indications of macrophage phagocytosis of neuronal elements have been demonstrated on seemingly intact neurites during development (Innocenti et al., 1983) as well as on axons in sciatic nerve-end neuromata (Frisén et al., 1993b). An alternative way in which macrophages may negatively affect neurons unaffected by the initial trauma is by secreting neurotoxic molecules (Théry et al., 1991). In contrast, macrophages can counteract the inhibitory action of optic nerve white matter on neurite growth *in vitro*, and could therefore be important in axonal sprouting in the injured CNS (David et al., 1990). Macrophages are thus implicated in injury reactions in both the PNS and CNS, in part by acting as regulators of some of the other determinants of axonal regeneration mentioned above.

The glial scar - a physical barrier for axonal

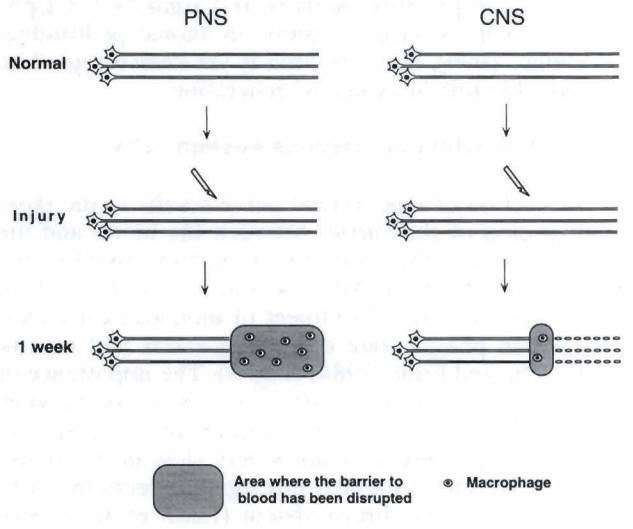

Fig. 3. Disruption of the barrier to blood and recruitment of macrophages is different in the PNS and in the CNS. In the PNS, the blood-nerve barrier is disrupted throughout the distal segment of a severed nerve. This enables rapid invasion of macrophages which engulf myelin and axonal degradation products. In the CNS, the disruption of the blood-brain barrier is limited to the lesion are. Macrophages will rapidly enter the CNS at the lesion and clear this area from debris, but macrophage recruitment and removal of debris distal to the lesion is very slow.

The glial scar - a physical barrier for axonal regrowth?

After lesions in the CNS, astrocytes both at the injury and in denervated tracts undergo hypertrophy and increase the synthesis of the intermediate filament proteins glial fibrillary acidic protein, nestin and vimentin (Eddleston and Mucke, 1993; Frisén et al., 1995b). Within a few weeks the astrocytes form a dense network of processes, referred to as the glial scar. The fact that this tissue appears compact histologically led to the suggestion that it may constitute a physical barrier for axon regrowth (reviewed in Reier et al., 1989). An axon-growth inhibitory effect of astrocytes has been supported for example by studies on regeneration of the central branch from primary sensory neurons: after a dorsal root crush in the adult rat, the sensory axons regrow to the dorsal root transitional zone, where most axons then stop growing and form terminals on astrocytes (Carlstedt, 1985). When the axons make contact with astrocytes, the neurofilament synthesis in the neuronal cell body ceases. Astrocytes have therefore been suggested to activate an inherent neuronal physiological stop mechanism (Liuzzi and Lasek, 1987; Liuzi and Tedeschi, 1992).

Despite the wealth of data suggesting axon-growth inhibition by astrocytes, these glial cells are believed to be important in axonal growth promotion and guidance during embryogenesis. Furthermore, astrocytes in culture form a suitable substrate for cocultured neurons, and neurites readily grow over the astrocytes (reviewed in Hatten et al., 1991). Moreover, already by the turn of the century scientists such as Santiago Ramón y Cajal noticed that after injuries in the CNS, axons sent sprouts into the scar formed at the lesion, although they failed to grow beyond this scar. The axonal sprouts in the scar tissue are closely associated with astrocytes. In many situations scar tissue sprouts seem to be aborted within one month after an injury (Ramón y Cajal, 1928). However, several studies have demonstrated that axonal sprouts in the spinal cord can persist for more than a year in the glial scar formed after an incision (Risling et al., 1983, 1992; Frisén et al., 1993a). After a ventral funiculus incision or after ventral root avulsion and replantation, many motoneuron axons manage to traverse the glial scar and reinnervate the ventral root, and may even reinnervate the target tissue in a functional manner (Cullheim et al., 1989; Carlstedt et al., 1993, 1995).

Ramón y Cajal hypothesized from the fact that axonal sprouts grow into the scar tissue formed at lesions that stimulatory substances for axon growth may be present in the scar (Ramón y Cajal, 1928). Many decades later this hypothesis proved to be true (Nieto-Sampedro et al., 1982). Among the neurotrophic factors that have been described to be expressed at increased levels in glial scars are NGF (Ishikawa et al., 1991; Lindholm et al., 1992), basic fibroblast growth factor (Frautschy et al., 1991; Ishikawa et al., 1991; Logan et al., 1992; Koshinaga et al., 1993), insulin-like growth

factor 1 (IGF-1; García-Estrada et al., 1992), transforming growth factor-ß (Lindholm, 1992), and CNTF (Ip et al., 1993). Furthermore, expression of neurite-growth promoting extracellular matrix molecules such as laminin, collagen, fibronectin, and tenascin may be of importance (discussed above). The majority of these axon-growth promoting molecules are synthesized by astrocytes. In addition to these axon growth-promoting molecules, axon growth-inhibiting activity is also found at CNS lesions (Rudge and Silver, 1990; McKeon et al., 1991).

One explanation to the contradictory reports on the role of astrocytes and glial scars on axonal elongation is that astrocytes react differently to different types of injuries. Glial scars are formed both at the site of an injury, such as an incision, and distal to this point where the distal segments of the transected axons degenerate, i.e. Wallerian degeneration. These two types of scars, the type that is formed at the site of a penetrating injury and the type that forms in a tract undergoing Wallerian degeneration, differ in many respects, not least in their capacity to promote axonal growth. The increased synthesis of axon-growth promoting molecules is limited to the type of glial scar that forms at the site of the injury, and does not take place in the scar that forms in the zone undergoing Wallerian degeneration. The differential capacity to promote axon-growth in these two types of glial scars has been demonstrated in *in vitro* experiments where neuronal cells have been cultured on tissue sections from the injured CNS. Neurons can attach and extend neurites on astrocytes in sections from a glial scar formed at a penetrating injury, but fail to attach to sections of a denervated tract undergoing Wallerian degeneration (David et al., 1990; Frisén et al., 1994).

The regulation of the astrocytic phenotype with regard to neurite-growth promotion is poorly understood. Possible factors leading to a change in astrocyte properties after injury may for example be the direct trauma to the cell, a change in the extracellular environment caused by the blood-brain barrier disruption with concomitant influx of blood cells and plasma, or signals from severed neurons or glial cells. *In vitro* studies have identified molecules able to induce synthesis of NGF, BDNF, bFGF, and laminin by astrocytes (Gadient et al., 1990; Lindholm et al., 1990; Spranger et al., 1990; Pechán et al., 1992, 1993; Toru-Delbauffe et al., 1992; Zafra et al., 1992; Baghdassarian et al., 1993; Ladenheim et al., 1993). Interestingly, some data suggest that macrophages may be involved in the regulation of astrocytic properties after injury. Local high levels of TGF-ß at the lesion site, probably at least in part derived from macrophages invading the site after CNS injury, may inhibit glial proliferation and induce laminin- and NGF-synthesis by astrocytes (Lindholm et al., 1990, 1992; Toru-Delbauffe et al., 1992; Baghdassarian et al., 1993).

The ingrowth of axons into CNS scar tissue demonstrates that the adult mammalian CNS has a potential to support axon-growth. This might perhaps involve reactivation of developmental programs used to support and guide growing axons during embryogenesis. An understanding of the molecular basis for the axon growth promotion by reactive glial cells, and the elucidation of the signals which induce this permissive glial phenotype may in the future lead to strategies to manipulate glial features and promote axonal regeneration.

Is the blood-brain barrier a barrier for axonal regeneration?

In the intact brain and spinal cord, as well as in peripheral nerves, the nervous tissue is protected from the outer environment by a barrier to the blood. The barrier between the blood and the CNS, the blood-brain barrier (BBB), resides in the endothelium of blood vessels in the CNS: these endothelial cells do not take up molecules by endocytosis to the same extent as do those outside the CNS and they are interconnected by tight junctions which hinder passive diffusion of molecules through the vessel wall. These features are believed to be induced in the endothelial cells by soluble factors from neighboring astrocytes (Janzer and Raff, 1987; Lobrinius et al., 1992).

The role of the barrier between blood and nervous tissue in the context of nerve regeneration is intriguing. In most, if not all, regenerating systems no barrier is present during axon regrowth. In lower animals such as amphibians, in which CNS regeneration is possible, the BBB is disrupted in the denervated part of an injured tract (Kiernan and Contestabile, 1980). In mammals the blood-nerve barrier is similarly disrupted distal to a peripheral nerve lesion (Olson, 1966; Mellick and Cavanagh, 1968). Moreover, olfactory receptor neurons residing in the nasal mucosa are exchanged throughout life in mammals, and axons from new receptor cells grow to the glomeruli in the olfactory bulb (Graziadei and Monti Graziadei, 1979). The BBB is incomplete in the superficial olfactory bulb where the axon ingrowth to the CNS occurs (Balin et al., 1986). After injuries in the CNS, the BBB is disrupted locally at the site of the injury, and not, as in the PNS, throughout the denervated area (Olsson, 1966; Mellick and Cavanagh, 1968) (Fig. 3). This defect in the BBB is usually repaired within a month (Kiernan, 1985), which correlates with the retraction of lesion-induced sprouts in the CNS (Ramón y Cajal, 1928). However, in the situation of spinal cord injuries, where axonal sprouts may persist for much longer times, the BBB defect is much more persistent (Risling et al., 1989, 1990; Frisén et al., 1993a).

Is the correlation between axon-growth and absence of a barrier to the blood merely a coincidence or can the contact between the blood and nervous tissue be causally related to axonal growth? The absence of a complete barrier could enable molecules as well as cells to pass from the blood to the nervous tissue. In a peripheral nerve the rapid disruption of the blood-nerve barrier makes it possible for macrophages to enter the nerve

distal to an injury (Fig. 3). These blood-derived cells phagocytize myelin and axonal debris, and may secrete potent cytokines and growth factors. Macrophages rapidly enter the lesion area after brain or spinal cord trauma, but fail initially to enter the denervated tract (see above and Fig. 3). Macrophages may also be of importance in the injured CNS not least because of their ability to neutralize inhibitory properties in the mammalian CNS (David et al., 1990). Macrophage-derived cytokines may also be intimately involved in the glial reactions seen at the injury. In addition, molecules that normally circulate in plasma, such as growth factors and cytokines, which may enter the injured spinal cord at a site of failing blood-brain barrier, could be important for axon growth.

Future perspectives

Very much has been learned about axonal regeneration during the past few decades, and the industrious activity in this field is likely to increase our understanding substantially during the coming years. The first step on the road to enhancing axonal regeneration in the CNS must be to promote neuronal survival and axonal growth. Elucidating the mechanisms behind injury-induced nerve cell death and determinants of intrinsic neuronal regenerative capacity will be of fundamental importance. Other pivotal studies for the near future will be further characterization of axon growth-inhibiting molecules in the CNS. This will facilitate the search for neuronal receptors transmitting signals which cause axons to stop growing. Identification of neuronal receptors for CNS axon growth-inhibiting molecules, and the development of antagonist drugs to such receptors may prove instrumental in the treatment of brain and spinal cord injuries. Increased knowledge regarding the regulation of astrocyte features, enabling the targeted induction of an axon growth permissive phenotype after injury may also prove valuable. However, even if axon growth can be induced in the adult CNS, will these axons be able to navigate to their appropriate target? Misdirected growth of axons and innervation of inappropriate targets may very well constitute a worse situation than no axon growth. Axonal pathfinding during embryogenesis is directed by distinct molecular cues in the path of the growing axons and on the target cells. The molecular nature of some of these axon guidance cues have recently been unveiled. Are these guidance cues only expressed during embryogenesis, or are they present also in the adult tissues (or are they reexpressed after injury) so that they could direct regrowing axons to the appropriate target? Some experiments give reason to hope that this is the case (Wizenmann et al., 1993; Bähr and Wizenmann, 1996). The identification of molecules that support or inhibit axonal growth have resulted in a major leap forward in our understanding of axonal regeneration, and it is likely that as more molecular key players in axonal growth are being unveiled that it soon may be possible to restore severed neural circuits in the injured mammalian CNS.

Acknowledgments. I am indebted to Drs. D. Clarke, M. Jaramillo and I. Silos-Santiago for thoughtful discussions and suggestions on the manuscript. The experiments from the authors lab described here were supported by the Swedish Medical Research Council, Stiftelsen Elsa och Sigurd Goljes minne, Hans och Loo Ostermans fond, Åke Wibergs stifelse, Jeanssons stifelse, Tore Nilssons fond and the Swedish Medical Society.

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