REVIEW ARTICLE

CANADIAN ASSOCIATION OF NEUROSCIENCE REVIEW: Axonal Regeneration in the Peripheral and Central Nervous Systems – Current Issues and Advances

Keith Fenrich, Tessa Gordon

ABSTRACT: Injured nerves regenerate their axons in the peripheral (PNS) but not the central nervous system (CNS). The contrasting capacities have been attributed to the growth permissive Schwann cells in the PNS and the growth inhibitory environment of the oligodendrocytes in the CNS. In the current review, we first contrast the robust regenerative response of injured PNS neurons with the weak response of the CNS neurons, and the capacity of Schwann cells and not the oligodendrocytes to support axonal regeneration. We then consider the factors that limit axonal regeneration in both the PNS and CNS. Limiting factors in the PNS include slow regeneration of axons across the injury site, progressive decline in the regenerative capacity of axotomized neurons (chronic axotomy) and progressive failure of denervated Schwann cells to support axonal regeneration (chronic denervation). In the CNS on the other hand, it is the poor regenerative response of neurons, the inhibitory proteins that are expressed by oligodendrocytes and act via a common receptor on CNS neurons, and the formation of the glial scar that prevent axonal regeneration in the CNS. Strategies to overcome these limitations in the PNS are considered in detail and contrasted with strategies in the CNS.

RÉSUMÉ: Régénérescence axonale dans le système nerveux périphérique et dans le système nerveux central – Questions actuelles et progrès. Revue émanant de l'Association canadienne des neurosciences: Les nerfs du système nerveux périphérique (SNP) qui ont subi une lésion régénèrent leurs axones alors que ceux du système nerveux central (SNC) ne le font pas. Cette différence a été attribuée à un environnement permissif conféré par les cellules de Schwann dans le SNP et à un environnement inhibiteur de la croissance conféré par les oligodendrocytes dans le SNC. Dans cette revue, nous opposons la réponse régénératrice robuste des neurones du SNP lésés à la réponse faible des neurones du SNC et la capacité des cellules de Schwann de supporter la régénérescence axonale à l'incapacité des oligodendrocytes de le faire. Nous considérons ensuite les facteurs qui limitent la régénérescence axonale dans le SNP et dans le SNC. Dans le SNP, ces facteurs sont la régénérescence lente des axones pour franchir la lésion, et l'axotomie et la dénervation chronique des cellules de Schwann qui diminuent progressivement la capacité régénératrice des neurones lésés. D'autre part, dans le SNC, la réponse régénératrice faible, les protéines inhibitrices qui sont exprimées par les oligodendrocytes et qui agissent via un récepteur commun situé sur les neurones du SNC et la formation de tissu glial cicatriciel empêchent la régénérescence axonale. Nous examinons en détail et nous comparons les stratégies pour surmonter ces limites dans le SNP et dans le SNC.

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The degenerative processes following peripheral nerve and central nerve injuries are similar in many respects but there are several intrinsic and environmental differences that distinguish the two processes. Neurons whose axons travel in the peripheral nerves regenerate their axons within the permissive growth environment of the Schwann cells. Nonetheless, neurons and the Schwann cells of the peripheral nervous system (PNS) progressively fail to sustain the regenerative response with time after injury. In the central nervous system (CNS), the intrinsic growth potential of injured neurons contrasts with that of injured PNS neurons in being insufficient even immediately after axotomy, to overcome the inhibitory growth environment of the oligodendrocytes and the astrocytes in the CNS.

Several reviews that consider either axonal regeneration after injuries in the PNS or CNS, but rarely both, have appeared in

From the Centre for Neuroscience, Division of Physical Medicine and Rehabilitation, University of Alberta, Edmonton, AB Canada.

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Reprint requests to: Tessa Gordon, Centre for Neuroscience, Division of Physical Medicine and Rehabilitation, University of Alberta, Edmonton, AB Canada T6G 2S2

recent years (PNS;[1-5] and CNS[6-15]). In the current review, we consider both PNS and CNS axonal regeneration in an attempt to provide insights into the problems of poor axonal regeneration in the PNS and the lack of regeneration in the CNS as a basis on which to consider experimental approaches to promote axonal regeneration in both systems.

NEURONAL AND NONNEURONAL RESPONSES TO INJURY IN THE PNS AND CNS

PNS nerve injury

Wallerian degeneration

Axons that are physically separated from the neuronal cell body after nerve injury degenerate. The axonal degeneration after axotomy was first observed and described by Augustus Waller[16] in 1850, and more fully elucidated in the nineteenth and twentieth centuries by Ramon Y Cajal and others.[17] Cajal's detailed histological work identified the axonal degeneration, the infiltration of leukocytes into the distal nerve stumps, the formation of ovoids as the Schwann cells fragment the myelin sheaths, and the dedifferentiation of the Schwann cells from myelinating to nonmyelinating. The degenerative process is now referred to as Wallerian degeneration.[1,18,19] The axonal degeneration is mediated by calcium influx via ion specific channels which, in turn, activates axonal proteases; disintegration and degeneration of the axolemma and axoplasm occurs within 24 hours in small and 48 hours in large nerve fibers.[18,20-22] Within two days of injury, Schwann cell gene regulation is altered as the cells begin to down-regulate genes that transcribe myelin proteins and begin to express regeneration associated genes (RAGs). The RAGs include genes that transcribe the growth associated protein-43 (GAP-43), neurotrophic factors and their truncated receptors, the Schwann cell proliferative factor, neuregulin, and its erb receptors.[1,2,23-25] The dedifferentiated Schwann cells scavenge myelin debris, form ovoids from their own myelin debris, proliferate, and form the bands of Bungner. The bands guide and support the axons that regenerate from the proximal nerve stump into and through the endoneurial tubes of the distal nerve stumps.[20,26,27] Release of the prototypical neurotrophic factor, nerve growth factor (NGF) from fibroblasts and the Schwann cells in the distal nerve stump, may play an important role in the proliferation and migration of the Schwann cells across the injury site, thereby assisting in the guidance of growing neurites into the distal nerve stump.[28]

Hematogeneous macrophages play an essential role both in the phagocytosis of myelin following nerve injury as well as in the change in the functional state of the Schwann cells.[27,29,30] The macrophages are recruited into the distal nerve stump in large numbers by the third day after injury.[30-32] They infiltrate into the nerve stump in response to chemoattractive factors, including cytokines such as interleukin-1β leukemia inhibitory factor, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1, which are released by the Schwann cells.[33] The critical involvement of the proinflammatory cytokine, TNF-α, in macrophage recruitment is evident from the reduced invasion of macrophages seen in the distal nerve stump in TNF-α knockout mice.[34] Macrophages permeate the entire distal nerve stump where they remain over at least a one month period and are responsible for removing the majority of the myelin debris.[29,30,35] The debris includes myelin associated proteins such as myelin-associated glycoprotein (MAG)[36] which have been demonstrated to have strong inhibitory effects on axonal growth[37-39] (Section *Oligodendrocyte derived myelin-associated inhibitors*).

There is a highly ordered pattern of release of pro- and anti-inflammatory cytokines from resident Schwann cells, fibroblasts and recruited macrophages during Wallerian degeneration in the PNS.[40,41] An example of the pro-inflammatory cytokines is TNF-α which is expressed in macrophages and Schwann cells as well as in fibroblasts and endothelial cells in the injured peripheral nerve.[42] The anti-inflammatory cytokines include IL-10.[41] The pattern of cytokine release in the injured peripheral nerve closely follows the pattern of release of the same cytokines in the injury-induced inflammatory responses of nonneural tissues, the orchestrated production of cytokines serving to provoke a time-limited inflammatory response.[40,43] The time-limited inflammatory response is effective in removing myelin debris in the injured PNS, an effect that contrasts with the tardy removal of myelin debris in the injured CNS by the resident CNS macrophage population of microglia (see below in: CNS nerve injury: *Wallerian degeneration*).

Neuronal response

While the axons distal to the injury, are undergoing Wallerian degeneration, axons of the proximal nerve stump undergo "die back" to the first node of Ranvier.[1,19,42] The cell body of axotomized PNS neurons undergoes characteristic morphological changes that are collectively referred to as "chromatolysis". These include the breakup of the ordered arrays of rough endoplasmic reticulum and the movement of the nucleus from the center of the cell body; the changes are believed to be the basis for the marked alterations in mRNA synthesis and change in gene expression in the axotomized neurons, concurrent with the conversion of the neurons from the normally "transmitting" to the "growth" mode that supports axonal regeneration.[1,44-46] The altered gene expression includes the upregulation of RAGs that are responsible for growth cone stability and elongation, as well as axonal guidance and sprouting.[47] The upregulated RAGs include the genes that transcribe the cytoskeletal proteins, tubulin and actin, and the growth associated proteins, GAP-43 and cytoskeleton-associated protein-23 (CAP-23), which have been shown to be very important mediators of growth cone elongation.[48-50] Concurrently, other genes are downregulated, including the genes for the neurofilament cytoskeletal proteins.[50-52] Reduced transport of neurofilament proteins accounts for the reduced diameter of the axotomized nerves.[53] The upregulation of tubulin and actin, possibly in association with the reduced neurofilament-tubulin ratio and in turn, reduced neurofilament microtubule interactions, allows axons to regenerate at 1-3mm/day, a rate which corresponds with the rate of the slow component b axonal transport of these cytoskeletal proteins.[50,51,54]

Axotomized PNS neurons also express proteins that are essential for the interaction between the growth cones and the Schwann cells in the permissive growth environment of the distal nerve stumps. These include receptors for neurotrophic factors that are expressed in the denervated Schwann cells as

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described below (Section ***Response of the* nonneuronal *cells***), as well as proteins such as neuregulin that binds to erb receptors on the Schwann cells to mediate, at least in part, the interaction of the growing axons and the Schwann cells in the growth pathway.[55] Neuregulin derived both from the growth cones and the Schwann cells contributes to the mitogenic signal for Schwann cell proliferation on contact of the growth cones with the Schwann cells.[56] Growth cones that emerge from the axons in the proximal nerve stump extend along the surface of Schwann cells and/or the inner surface of the basal lamina of the Schwann cell column in the distal nerve stump.[57,58] The neurons express several adhesion molecules in the growth cone membranes, including neural adhesion molecule, in addition to the integrins that bind to extracellular matrix proteins such as laminin.[1,5,58]

Response of the nonneuronal cells

The expression of cytokines and the resulting inflammatory response during Wallerian degeneration play an important role in regulating the degradation of myelin and the conversion of the denervated Schwann cells from their myelinating to their growth supportive nonmyelinating phenotype. The latter phenotype is similar to that of nonmyelinating Schwann cells which normally surround several unmyelinated axons and which do not form myelin.[58-62] The switch in phenotype of the denervated Schwann cells in the distal nerve stump involves downregulation of myelin-associated genes and upregulation of several RAGs.[5,25] Genes that are upregulated include those for several neurotrophic factors, truncated trk receptors and the p75 neurotrophic factor receptor.[2] The neurotrophic factors belong to three families, the neurotrophins, glial cell derived neurotrophic factor (GDNF) and the neuropoeitic cytokine families.[2] The neurotrophin family consists of NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5. Glial cell derived neurotrophic factor neurotrophic factors include GDNF, neurturin, persephin, and artemin.[55,58-61,63] Of these neurotrophic factors, NGF, BDNF, GDNF and the cytokines interleukin-6 and leukemia inhibitory factor are factors that are upregulated in denervated Schwann cells.[64-69] Several cytokines, including transforming growth factor-β (TGF-β) that are secreted by both macrophages and the denervated Schwann cells are released in the distal nerve stump after nerve injury and have been implicated in expression of neurotrophic factors in the denervated Schwann cells.[5,41,70,71] Presently techniques that include gene arrays and differential screening of genes are now being used to identify novel injury-induced genes and their time course of expression during the transition from the myelinating to the nonmyelinating Schwann cell phenotype.[72]

CNS nerve injury

Wallerian degeneration

While CNS axons undergo Wallerian degeneration at approximately the same rate as injured PNS axons, the removal of the degenerating myelin of the oligodendrocytes is tardy requiring very prolonged periods of time.[73,74] Following injury, microglia become phagocytic at the restricted site of the injury. However, because their phagocytic capacity is limited, the microglia fail to clear the myelin debris of the denervated oligodendrocytes[73,75] and, most importantly, they do not effectively remove the myelin and its associated growth inhibitors, which include Nogo[73,76,77] and MAG.[38,78,79] The microglia also release cytokines. These further activate the immune response to the region, but because of the poor regional blood flow at the injury site, the immune response is slowed significantly and, in turn, inflammation is prolonged.[31,73,80]

Hematogeneous macrophages accumulate in high densities only at the immediate site of injury in both the CNS and PNS.[81] However, the macrophages fail to accumulate at more distant sites in the CNS,[31,73,80] further reducing the removal of myelin debris in the CNS in contrast to the PNS. This means that the removal of myelin debris is limited to the point of injury in the CNS, and the overall immune response is delayed.[82] Consequently, myelin debris remains within the white matter tracts for long periods of time and, in the absence of effective phagocytosis by the microglia, the injured neurons are exposed to the inhibitors of axonal regeneration that are directly associated with the myelin (See section *Oligodendrocyte derived myelin-associated inhibitors*).

Neuronal responses

Prior to injury, most CNS neurons, like the neurons of the PNS, do not express high levels of RAGs.[83-92] However, in contrast to the PNS, the injured CNS neurons normally fail to upregulate and/or to express RAGs to support axonal regeneration.[93-96] Co-expression of certain RAGs may be required to elicit CNS axonal regeneration: GAP-43 or CAP-23 expression was not sufficient to induce axonal regeneration while the co-expression of these two RAGs in transgenic mice was very effective in promoting CNS regeneration.[48] The failure of injury to induce a robust change in RAG expression may arise because axotomized CNS neurons have multiple collateral axons that remain connected to targets, especially for the long axon tracts in the spinal cord. Hence, the transition of injured neurons from the “transmitting” to the “growth” mode that occurs in PNS neurons may not occur in CNS neurons. Findings of minimal upregulation of RAGs in axotomized CNS neurons, unless axotomy was performed very close to the cell bodies[89,93,97] concur with this explanation.

The capacity for axonal growth in injured CNS neurons was clearly demonstrated in the classical experiments of Aguayo and his colleagues.[98-102] These workers showed that CNS neurons regenerated axons through peripheral nerve grafts that were inserted into the CNS. Central nervous system neurons also regenerate axons through purified Schwann cell implants and myelin free spinal cord in accordance with the earlier findings of CNS neuronal regeneration through Schwann cell-containing peripheral nerve grafts. Even so, the number of axons that regenerate and the distance over which they traverse is small at best.[103,104] At least a component of this poor regenerative capacity may be attributed to the low levels of expression of neurotrophic factors in the CNS neurons because endogenous delivery of NGF, BDNF and neurotrophin-3 to injured neuronal populations that express the appropriate trk receptors has been demonstrated to elicit more robust axonal outgrowth through permissive cell grafts[93,105-108] in concert with their effectiveness in promoting neuronal survival.[13,109,110] The ability of neurotrophic factors to elicit axonal outgrowth may depend on where their receptors are located: the contrasting ability of

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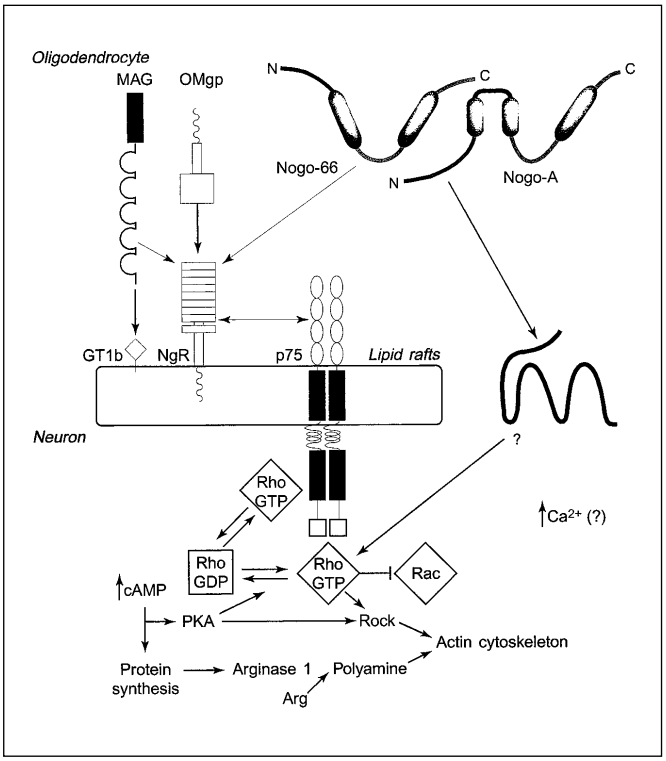

Figure 1: *Myelin-associated proteins within the oligodendrocytes membranes that include myelin associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp) and Nogo-66 and Nogo-A are currently believed to act as growth-inhibitory molecules by acting via a common receptor subunit, NgR on the neuronal membranes in the CNS. The NgR subunit is now recognised to be complexed with p75, probably acting as a signal-transduction unit that activates the small GTPase, RhoA, simultaneously with inhibition of Rac and elevation of intracellular Ca^{2+} ions. Experimental elevation of intracellular cAMP levels leads to activation of protein-kinase A (PKA), arginase-I upregulation and the synthesis of neurite growth promoting polamines.[39] Thereby, measures that increase intracellular cAMP may bypass the interaction of the inhibitory ligands in oligodendrocyte membranes and the receptor complex of Ng/p75 on the neurons, and thereby, promote axonal regeneration. Adapted from Oertloe and Schwab (2003).[130]*

neurotrophin-secreting cell grafts to promote axon outgrowth in lesioned coerulospinal neurons and not in lesioned corticospinal neurons has been suggested to be due to localization of trk receptors on the axons, in addition to the dendrites and soma, of the former but not the latter neurons.[111]

Non-neuronal cells

The oligodendrocytes and microglia in the CNS fail to sequester the myelin debris, in contrast to the effectiveness in this regard of Schwann cells and macrophages after injury in the PNS. The denervated oligodendrocytes also do not dedifferentiate in the same manner as the denervated Schwann cells in the PNS. They fail to form the bands of Bungner that guide regenerating axons in the PNS. Growth-inhibitory molecules of the CNS myelin that are not effectively removed by the nonneuronal cells, also promote the proliferation of astrocytes.[112] Astrocytes normally serve to transfer nutrients to the axons and perikarya of the CNS; they protect neurons by contributing to blood-brain barrier functions and by channeling metabolic wastes from the parenchyma and excess neurotransmitters from synapses.[113] After injury, the proliferating astrocytes are a major limiting factor in the dedifferentiation of the denervated oligodendrocytes. Ultimately the proliferation of the astrocytes creates a glial scar that not only forms a physical barrier to growth cones but also produces additional inhibitory compounds. These compounds include tenascin and proteoglycans which further inhibit axonal regeneration.[114,115] However, it is unclear whether it is the physical barrier or the inhibitory molecules released by the glial scar tissue, which have a greater inhibitory effect on advancing growth cones. Traditionally the dense physical barrier created by the glial scarring has been believed to be the major inhibitor of axonal regeneration.[116-119] Recent evidence now indicates that the physical barrier of the glial scar may play a relatively minor role compared to the inhibitory molecules released in the scar, especially chondrotin sulfate proteoglycans (CSPGs) such as NG2, versican, neurocan, and phosphocan, by glial cells including astrocytes, the oligodendrocyte precursors, meningeal cells and the microglia.[6,114,119-122]

Oligodendrocyte derived myelin-associated inhibitors

Nogo – Neurite growth inhibitory fractions from CNS tissue were used to prepare a monoclonal antibody IN-1 that was able to reduce the inhibitory activity of CNS myelin *in vitro*[123] and *in vivo*[124] and to promote neurite outgrowth and regeneration of injured nerve fibers respectively. *In vivo* local administration of IN-1 antibody at the site of a corticospinal lesion in adult rats led to extensive sprouting and significant regeneration together with improved functional reflex and sensory ability.[125] Thereafter, the inhibitory fractions were fully purified and sequenced. The corresponding cDNA, now called Nogo-A, was cloned[126-128] and thought to be inhibitory to CNS regeneration in line with the evidence that 1) blockade of the Nogo-A by IN-1 antibody promoted CNS regeneration, 2) only Nogo-A, of three splice variants, -A, -B, and -C, is expressed primarily in the CNS,[12] and 3) transgenic expression of Nogo-A in the Schwann cells of peripheral nerve, delayed axonal regeneration after nerve crush in the PNS.[129] Since the identification of the Nogo gene family, several different possible functions of the Nogo proteins have been acknowledged; the subcellular localization to membranes of the endoplasmic reticulum and other cellular structures suggests a possible role in structural stability of the endoplasmic reticulum network for example.[130]

Nogo-A is a membrane bound protein with two trans-membrane domains and a 66-amino-acid extracellular loop[131] (Figure 1), that is localized to both CNS myelin in the white matter as well as neurons in the grey matter[126,127,132] that have strong regenerative capacity (Section PNS axonal regeneration – counteracting chronic axotomy, Schwann cell denervation and staggered axonal regeneration: *Neuronal phenotype*). A receptor that binds to Nogo-66 has recently been cloned and referred to as NgR.[133] It is attached to neuronal growth cone membranes by a glycosylphosphatidylinositol anchor.[133,134] NgR associates with p75 to activate transduction pathways that involve Rho[135] (Figure 1). In addition to Nogo-A, NgR binds to other myelin associated inhibitors that include MAG and oligodendrocyte-

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myelin glycoprotein (OMgp) with high affinity.[134,135] Amino-Nogo, another growth inhibitory sequence of Nogo-A protein has also been identified[126] for which no receptor has yet been found.

Even while present research is focusing on a role of Nogo-A as one of the inhibitors associated with CNS myelin, the distribution of mRNA expression for Nogo-A is much more widespread than simply in the oligodendrocytes as anticipated for a growth-inhibitor: Nogo-A is present in neurons in the grey matter including the motoneurons[132,136] that have strong regenerative capacity. The *in vivo* evidence for regeneration of axons of sensory ganglion neurons within the CNS even within pathways in which the axons are exposed to slowly fragmenting CNS myelin,[121,137,138] also argue against the role of Nogo-A being inhibitory to CNS regeneration. The evidence of delayed peripheral nerve regeneration in transgenic mice that expressed Nogo-A in the PNS, supports a role of Nogo-A as inhibitory to CNS regeneration.[139] However, the failure or just minimal CNS regeneration observed in three different Nogo-A knockout mice[140-142] indicates that the inability of CNS neurons to regenerate after injury cannot be attributed only to the inhibitory effects of myelin-associated Nogo-A binding to the NgR on axons.[78,143] In fact, the amounts of NgR that were detected on axons was surprisingly low, even after axotomy in the CNS and NgR mRNA appeared to be more strongly expressed in neurons in areas of the brain than in spinal cords of adult rats.[144,145] These findings taken together indicate that the basis for the strong negative effects of CNS glial cells on the capacity for injured neurons to regenerate remains subject to debate[132] and the CSPGs that are released by the glial scar tissue in damaged CNS, remain strong contenders to explain the failure of CNS regeneration (see Section *Non-neuronal cells*.)

Myelin-associated glycoprotein (MAG) – Like Nogo, MAG is also expressed in the inner loop of myelin and on the surface of oligodendrocytes[78,146,147] (Figure 1). Myelin-associated glycoprotein is a potent inhibitor of axonal growth of a wide variety of neurons in the adult.[78,79,146-149] Myelin-associated glycoprotein induces the collapse of the growth cone.[150,151] Following CNS injury, membrane-bound MAG can undergo proteolysis to form a soluble form of MAG called dMAG, which also contributes to axon growth inhibition.[37] Myelin-associated glycoprotein does not inhibit neurite outgrowth during early postnatal development when intracellular levels of neuronal cAMP are high; MAG becomes inhibitory in association with the dramatic fall in cAMP levels in maturing neurons.[79] Although the mechanisms of MAG-induced growth cone inhibition have yet to be fully elucidated, the recent finding that MAG has high affinity binding kinetics to the Nogo receptor supports the convergence of the inhibitory effects of different myelin proteins on neurons via the same receptors.[152,153] It has also been shown that the inhibitory signal of MAG is mediated through the actions of p75,[154] Rho,[155] and more specifically through a NgR/p75 complex.[135,156] Evidence is now beginning to emerge that the p75 receptor may be ubiquitously involved in inhibiting axon growth, not only in the peripheral neuron populations such as sympathetic and motoneurons,[2] but as a key initiator of the inhibitory signals in central myelin that limit axon regeneration after CNS injury.[157]

OMgp – Oligodendrocyte-myelin glycoprotein is similar to Nogo in its distribution and effects: it is highly expressed in oligodendrocytes, it is found on the cell surface and in myelin[158,159] it binds to NgR with similar affinity, and it is equally as potent at collapsing growth cones of rat cerebellar and chick DRG neurons.[134,135] The NgR/p75 complex is required for OMgp signal transduction consistent with NgR being the high affinity receptor for all the known myelin-associated growth inhibitors.[130,135]

Contrasting responses of the PNS and CNS to nerve injuries

The key differences between the described cellular responses in the PNS and CNS (Sections on PNS Nerve Injury and CNS Nerve Injury) that are important in their contrasting capacities for axonal regeneration are summarized as follows: first, despite Wallerian degeneration of axons that are separated from the neuronal soma in both the PNS and the CNS, it is the rapid phagocytosis of myelin debris by macrophages and Schwann cells of the PNS that prevents the growth cones of the injured neurons from exposure to myelin-associated inhibitory proteins, many of which are expressed by Schwann cells in addition to CNS oligodendrocytes. Even though removal of PNS myelin debris is surprisingly slow, occurring over a month's duration, it is considerably more rapid than the tardy removal of myelin debris in the CNS. Most importantly, the interacting macrophages and Schwann cells in the distal stumps of injured peripheral nerves are effective in removing the inhibitory proteins associated with the myelin, in contrast to the microglia and oligodendrocytes in the CNS.

A second related difference is the slow release of cytokines from microglia, which is slowed even further by the poor regional blood flow to sites of CNS injury, in contrast to the cytokine release from macrophages and their interaction with Schwann cells in the distal stumps of injured peripheral nerves. The inflammatory response in the CNS, in contrast to the PNS, does not appear to support neural repair.

A third difference is that, at least in the case of axotomized motoneurons in the PNS, a section of the peripheral nerve removes all the nerve-muscle connections and thereby converts the motoneurons from a "transmitting" to a "growth" mode in concert with the loss of the target connections. Axotomy of the motor axons removes all nerve-muscle connections because each motor axon branches only within the intramuscular nerve branches to innervate the many muscle fibers within the target muscle.[161] Central nervous system long tract axons have many collaterals compared to PNS axons and, therefore, even after the axotomy of large spinal tracts such as the corticospinal tract, these axotomized CNS neurons may retain a significant number of functional connections with target neurons. This might explain the comparatively low expression of RAGs in injured CNS axons compared to the pronounced expression of these genes in axotomized PNS neurons.

A fourth difference is in the responses of the nonneuronal cells in the PNS and CNS to the neuronal damage. The Schwann cells proliferate and undergo dedifferentiation into a growth supportive nonmyelinating phenotype that effectively supports axonal regeneration in concert with the extracellular matrix of the distal nerve stumps. In contrast, the oligodendrocytes fail to dedifferentiate into growth supportive cells. This occurs in concert with the proliferation of astrocytes, which contribute additional inhibitory molecules that prevent axonal regeneration.

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Finally, as a fifth difference that has not been considered in any detail above, concerns the connective tissue structures of the peripheral nerve, including the endo-, peri- and epi-neural sheaths which, in contrast to the CNS where these structures are absent, contain the regenerating axons in the PNS and provide guidance.[162,163]

Approaches to improve PNS axonal regeneration and to promote CNS regeneration

The capacity for axon sprouts that emerge from the proximal stump of the injured peripheral nerve to regenerate axons within the Schwann cell environment of the denervated distal nerve stumps contrasts with the failure of axon sprouts in the injured CNS neurons to grow and regenerate[14,164] The contrasting failure of the injured CNS neurons to regenerate their lost axons as opposed to the ability of injured PNS nerves to regenerate theirs, has often led to the misconception that axonal regeneration in the injured PNS is always successful. In fact, axonal regeneration in the PNS may fail to restore any functional recovery, especially for injuries that severe nerve trunks close to the spinal cord and far from the target organs of the axotomized neurons.[3] Rates of regeneration of 1-3 mm/day require periods of years rather than months for axons to regenerate over the long distances of more than a meter to reinnervate denervated targets. Animal experiments to explore the basis for the poor functional recovery despite regenerative capacity in the PNS, have shown that the capacity of injured PNS neurons to regenerate their axons and the capacity of denervated Schwann cells to support axonal regeneration declines with time and distance.[1,5,57,165,166] On the other hand, the capacity of injured CNS neurons to regenerate is counteracted by the limited neuronal growth response and the inhibitory growth environment that the axons encounter.[15,167,168] In the injured PNS, approaches to improve the capacity for axonal regeneration and, in turn, functional recovery depend on sustaining the growth response of the neurons and the growth supportive properties of the Schwann cells in the growth pathway, both of which are time-dependent, declining with time to a nonsupportive state.[1,2] In the injured CNS, the approaches to promote regeneration are similar with respect to promoting the growth response of the injured neurons; however, the nonpermissive growth environment of damaged oligodendrocytes and the astroglial scarring that occur in the injured CNS disallow axonal growth.

PNS axonal regeneration – counteracting chronic axotomy, Schwann cell denervation and staggered axonal regeneration

Neuronal phenotype

As previously discussed, the transition of a neuron from a "transmitting" to a "regenerating" state follows the loss of synaptic transmission by that neuron.[1,44] Upregulation of RAGs including GAP-43, and the cytoskeletal proteins, tubulin and actin, although immediate, is not sustained in chronically axotomized motoneurons: the expression of the genes declines with time in parallel with a progressive decline in the capacity of the motoneurons to regenerate their axons and reinnervate muscles.[57,169,170] The number of chronically axotomized motoneurons that regenerate their axons declines to a third of the number after immediate nerve repair. However, their capacity to reinnervate as many as five times the number of denervated muscle fibers and thereby to expand motor unit size by five-fold, allows for reinnervation of all the denervated muscle fibers and their recovery from denervation atrophy. Thereby, the full recovery of muscle size and force-generating ability effectively conceals the reduced numbers of motoneurons that regenerated their axons.[170] Application of exogenous neurotrophic factors, BDNF and GDNF to chronically axotomized motoneurons has potential for sustaining the regenerative capacity[2,171] (Figure 2) and there have been advances in sustaining the release of such factors by retroviral expression of these growth factors.[172,173] In addition, we have also recently demonstrated that the immunophilin ligand, FK506, that enhances neurite outgrowth both *in vivo* and *in vitro* independent of its potent immunosuppressive affects,[174] is very effective in counteracting the negative effects of chronic axotomy on axonal regeneration.[175]

Schwann cells

A long-standing view has been that a progressive and irreversible atrophy of denervated target organs accounts for the poor functional recovery that is noted particularly for the most proximal nerve injuries.[176] However, it is the combination of the regression of the growth state of the chronically axotomized neurons and the progressive decline in the number and capacity of the nonmyelinating, growth permissive Schwann cells in the denervated distal nerve pathways to support axonal regeneration that accounts fully for the progressive and very marked decline in the capacity of injured nerves to regenerate back to their denervated targets.[2,57,170,177] The Schwann cells atrophy, their growth supportive phenotype regresses, and many die with time.[30,177-179] Yet, the few axons that succeed in regenerating through the chronically denervated Schwann cells, are well-myelinated by the remaining Schwann cells.[155]

An important link made between the deterioration of the growth environment of the distal nerve stumps within the second month of chronic Schwann cell denervation, with the decline in numbers of macrophages in the distal nerve stumps[30] suggested the possibility that the atrophic Schwann cells may be reactivated by inflammatory cytokines which are normally released during macrophage invasion. Indeed, chronically denervating Schwann cells could be reactivated by exposure to the cytokine TGF-β that is normally secreted by macrophages and dividing Schwann cells; the reactivated Schwann cells were shown to be very effective in supporting axonal regeneration[177] (Figure 3).

Axonal outgrowth

The slow rate of regeneration that corresponds with the rate of slow transport, 1-3 mm per day, is a key rate limiting step in axonal regeneration.[3,5,180] In addition, a very sluggish outgrowth of axons from the proximal nerve stump has only recently been recognized as the major contributer to a major time delay in axonal regeneration: a period as long as a month may pass before all axons regenerate across a surgical site of reunion of cut nerves in an animal model of nerve injury[181,182] (Figure 4.1 and 4.2). This period of "staggered motor axonal regeneration" may be shortened substantially by a brief period of low frequency electrical stimulation at the time of surgical reunion,

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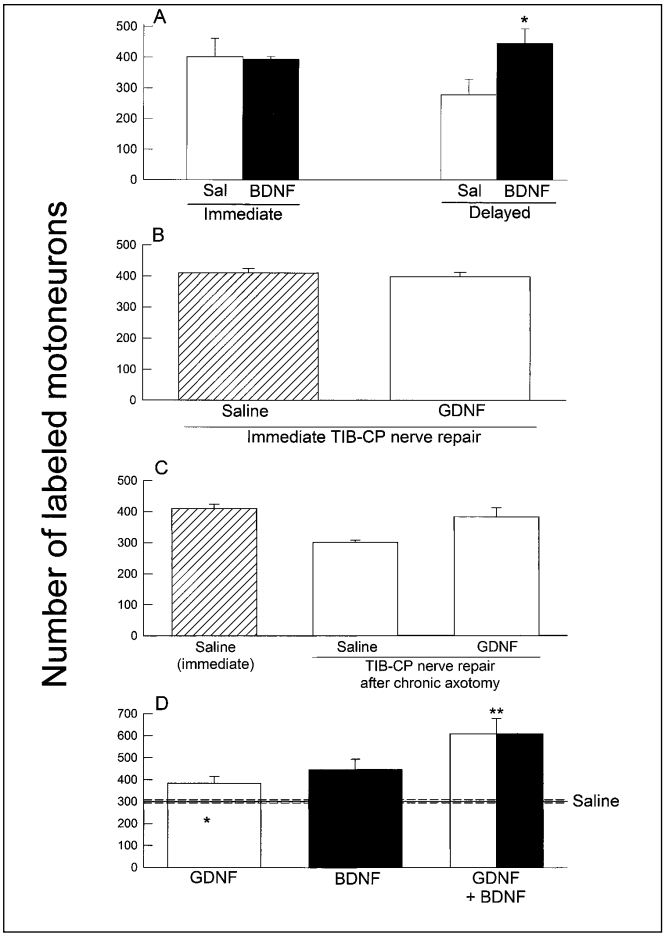

Figure 2: *Application of exogenous neurotrophic factors, BDNF and GDNF to chronically axotomized motoneurons reverses the negative effects of prolonged axotomy on regenerative capacity. Rat tibial (TIB) nerve was sectioned and the proximal nerve stump either sutured immediately to the distal nerve stump of the cut common peroneal (CP) nerve (immediate nerve suture) or two months later (delayed nerve suture). Either saline or exogenous neurotrophic factors, BDNF, GDNF or both BDNF and GDNF were delivered to the cross-suture for a period of one month after which the regenerated tibial axons were exposed to rubyred dye to backlabel the TIB motoneurons which had regenerated their axons over a distance of 20mm.* ***A)*** *Exogenous BDNF in low dose did not change the number of TIB motoneurons that regenerated their axons immediately after axotomy in contrast to the effectiveness of the same dose of BDNF to significantly increase the number of chronically axotomized motoneurons that regenerated their axons over the same distance.* ***B)*** *Exogenous GDNF also did not change the number of motoneurons that regenerate their axons after immediate TIB-CP nerve repair in contrast to* ***C)*** *where exogenous GDNF like BDNF significantly increased the number of motoneurons that regenerated their axons.* ***D)*** *Exogenous delivery of both GDNF and BDNF significantly increased the number of chronically axotomized tibial motoneurons above the number that regenerated axons after application of either neurotrophic factor.*

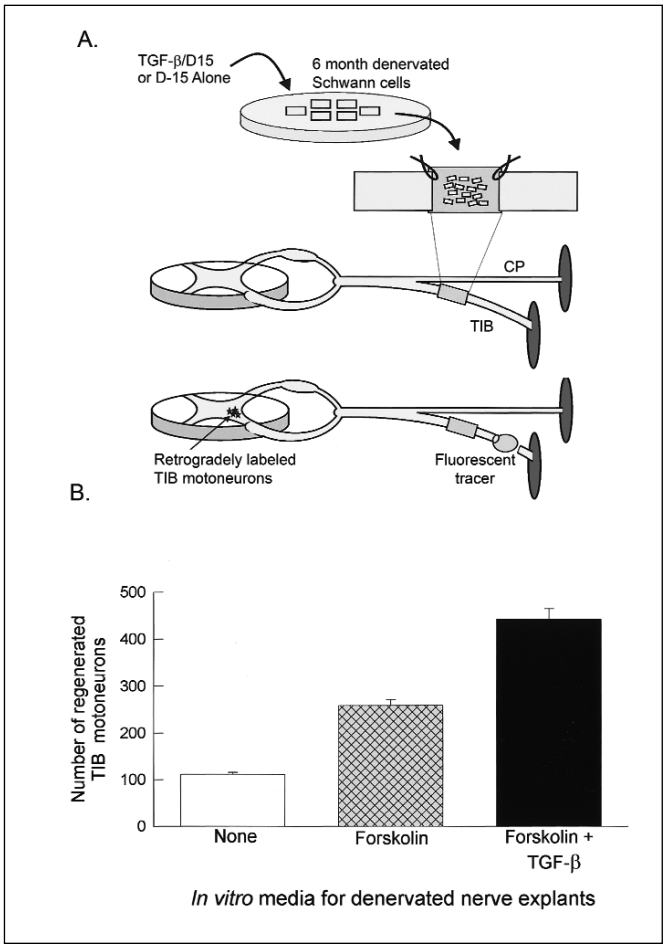

Figure 3: A) *Rat sciatic explants of six month chronically denervated Schwann cells were incubated in vitro for two days with either Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum (D-15 medium) or with D-15 medium containing 1ng/ml transforming growth factor-β (TGF-β) and 0.5 μM forskolin. The explants were placed in a silastic tube that bridged between cut tibial proximal and distal nerve stumps and the number of TIB motoneurons that regenerated their axons through the bridge was determined three months later by counting the motoneurons that had regenerated their axons and were backlabelled with fluorogold.* ***B)*** *Significantly higher numbers of motoneurons regenerated their axons through a silastic tube containing the TGF-β+forskolin-treated Schwann cell explants than in D-15 medium alone, or D-15 + forskolin. (Adapted from Sulaiman OA, Gordon T. Transforming growth factor-beta and forskolin attenuate the adverse effects of long-term Schwann cell denervation on peripheral nerve regeneration in vivo. Glia 2002; 37:206-218.)*

demonstrating that electrical stimulation may be used effectively to synchronize the outgrowth of axons from the proximal nerve stumps of cut peripheral nerves into the distal nerve stumps[182] (Figure 4.3). The complex growth of axons from proximal nerve stumps results in axons growing in many directions, including back into the proximal nerve stump, as first described by Ramon Y Cajal[164] (Figure 5). The electrical stimulation accelerates motor axonal outgrowth from the proximal nerve stumps in direct association with accelerated upregulation of the neurotrophic factor, BDNF and their receptors, trkB and p75 in the motoneurons,[183] consistent with increasing recognition of an activity-dependent regulation of gene expression in neurons.[184-187] It is likely that the altered expression of neurotrophic factors and their receptors is causally linked to the axonal outgrowth in view of evidence for a sequential upregulation of RAGs that include

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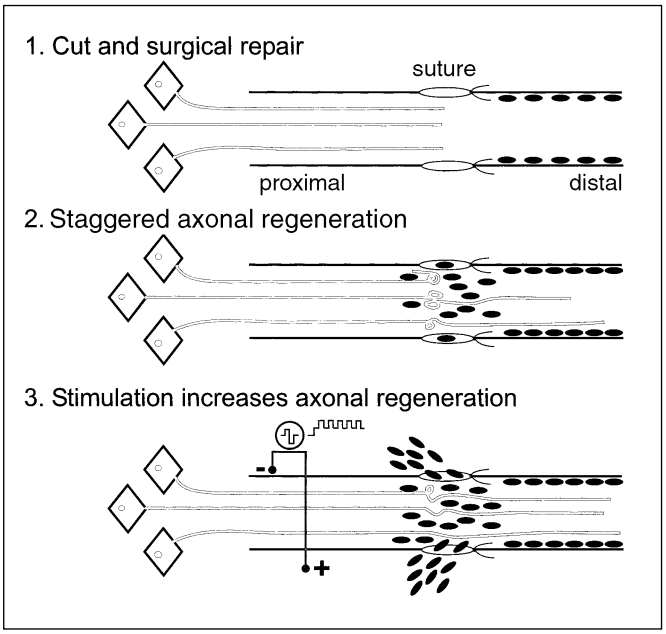

Figure 4: *1) After section of a peripheral nerve and surgical suture of proximal and distal nerve stumps. 2) Motoneurons (♦) regenerate their axons across the suture site in a "staggered" fashion, individual axons growing in complex patterns prior to entering the distal nerve stumps where they regenerate their axons within the Schwann cell (⬬) lined endoneurial tubes of the distal nerve stumps. 3) Low frequency electrical stimulation of the axons proximal to the nerve cut and suture, accelerates regeneration of axons across the suture site and into the distal nerve stump.*

tubulin, actin and GAP-43 within two days of the high levels of expression of BDNF and trkB mRNA in stimulated motoneurons that are regenerating their axons.[188]

CNS axonal regeneration – counteracting the poor regenerative response, the inhibitory environment and the glial scar

Neuronal phenotype

The low and transient expression of RAGs in injured central neurons that normally sustain many of their target connections may be counteracted by exogenous application of neurotrophins. While this application has been shown to be effective in promoting axonal regeneration in the CNS, the application is often damaging to surrounding neural tissue and attempts are being made to improve the application.[93-96,189] Altering RAG expression to promote CNS regeneration may one day be a viable therapeutic option but, because gene therapy is still in its infancy, it may be a long time before it is considered a viable alternative to existing therapies. Meanwhile engineered cells that secrete the neurotrophic factors directly into the lesion site or gene therapy with viral constructs that produce the required factors where they are needed, are being developed and used experimentally.[189]

Co-expression of GAP-43 and CAP-23 in transgenic mice promotes axonal regeneration in the CNS[48] so that pharmacologically targeting the expression of these genes may prove to be highly beneficial as a regenerative therapy. There have been no attempts, thus far, at attempting GAP-43 and CAP-43 gene therapy following spinal cord injury (SCI). Nearly all gene therapy research for SCI has focused on manipulating neurotrophic factor and metabolic enzyme expression.[27] There has also been minimal progress in deducing the precise expression pathway for the GAP-43 gene[190] or the CAP-23 gene.

Non-neuronal cells

The immune response

Following axonal injury in either the PNS or the CNS, the primary role of the immune system is to remove toxic debris from the injury site, especially the myelin debris of the damaged Schwann cells and oligodendrocytes, as discussed earlier (Sections CNS Nerve Injury and *Wallerian degeneration*.) Prolonged inflammation develops in concert with the release of cytokines from microglia, following SCIs, and has been thought to be a major contributor to secondary tissue damage to the injured spinal cord. The effectiveness of a high dose of methylprednisolone, administered early after SCI to counteract the inflammatory response, led to a standard drug treatment in North America of intravenous administration of the drug at the time of injury on the recommendation of the National Acute Spinal Cord Injury Studies.[191] However, there is now much controversy regarding the efficacy of the drug treatment and its use as a primary therapy is fast decreasing.[192,193] There are features of the immune response that are both beneficial in helping to initiate axonal regeneration, as well as detrimental by contributing to tissue loss. The neuroprotective and regenerative qualities of the immune response that are outlined in the review of Hauben and Schwartz[194] are, however, still controversial in light of confounding factors which may aggravate the injury, following the selective activation of the desirable qualities.[195]

Macrophages secrete neural and glial toxins that contribute to the prohibitive environment of the injured spinal cord.[196] Not surprisingly, inhibiting monocyte and/or macrophage activity has been shown to reduce secondary injury and improve neurological recovery.[197-200] Conversely, by inducing macrophage cytokine production, using *in vivo* lipopolysaccharide injections, a slight increase in neurite sprouting after SCI was observed in rats[201] as well as increasing the rate of myelin debris clearance in mice.[202,203] A combination therapy of lipopolysaccharide, anti-inflammatory steroids, and a cyclo-oxygenase inhibitor,[201] resulted in significant improvement in spinal cord tissue repair and functional recovery in rats.

It is evident that the side effect-to-benefit ratio is currently a matter of some debate when manipulating the immune response after SCI, in order to induce spinal cord regeneration. Using this approach, as either a primary or secondary means of regenerating spinal cord tissue, is best summarized by the words of Popovich and Jones:[195] "Until we better appreciate the ligand-receptor pathways and molecular signaling cascades that are used by macrophages after SCI and whether they can be controlled, we argue against the intentional activation [of the immune response] and/or introduction of these [immune] cells into the injury site, which could provoke tissue injury beyond a level sustained by trauma."

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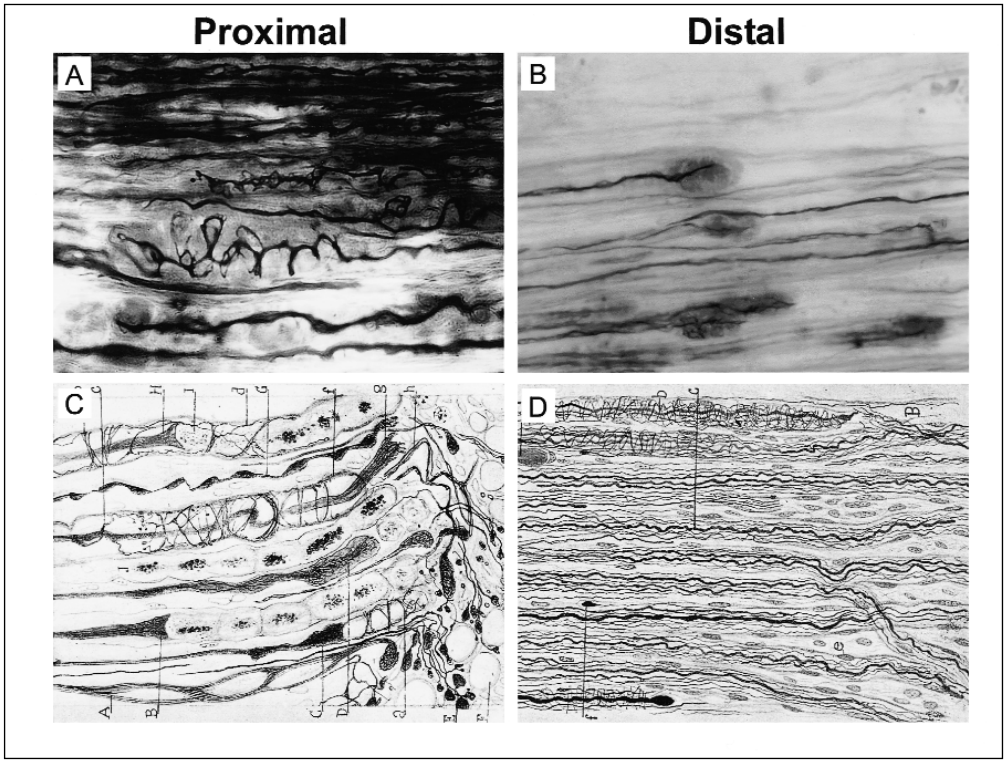

Figure 5: *Silver staining of axons in* ***A)*** *the proximal and* ***B)*** *distal nerve stumps after cross-suture of the proximal tibial nerve stump to the distal common peroneal nerve stump confirms the findings of Ramon Y Cajal*[17] *that growth cones that emerge from the proximal nerve stump regenerate in many different directions, including the regeneration of axons back into the proximal nerve stump. This contrast with the regeneration of axons in straight lines within the endoneurial tubes of the distal nerve stumps* ***(B,D)****. The meandering of regenerating axons seen in* ***C)*** *in our silver stained regenerating axons in the distal nerve stump, are similar to the "meandering" of axons seen in* ***D)*** *Cajal's drawings of silver impregnated axons in a distal nerve stump.*

Counteracting the inhibitory effects of the myelin outgrowth inhibitors

Receptor blockade – In addition to attempting to use immunological methods to remove the growth inhibition attributed to exposed Nogo and other oligodendrocytic myelin inhibitors, important experimental approaches are being investigated to block the inhibitory ligands and their common NgR receptor, and in turn, to promote axonal growth (Figure 1). These approaches extend from the use of the IN-1 antibody that effectively inhibits the actions of both Nogo-66 and Amino-Nogo in collapsing the growth cone. Potential therapeutic approaches may include creating a more selective IN-1 antibody,[204] that will more effectively bind to and inhibit Nogo,[205] or disrupting the NgR transduction pathway.[156] Because all three of the myelin-associated growth inhibitor molecules have been found to act primarily through the NgR, the possibility of promoting axonal regeneration by blocking the receptor are even more promising than attempting to block the inhibitory ligands themselves. It has recently been shown that the competitive block of the NgR with a synthetic Nogo-66 extracellular peptide can significantly increase axonal sprouting and growth caudal to the lesion site of corticospinal and serotonergic-containing raphespinal nerve fibers in rat spinal cords.[206]

Cyclic AMP – Since cAMP is a very common intracellular signaling secondary messenger, and guidance molecules such as semaphorin III, netrin I and BDNF can be made attractive or repulsive based on intracellular cAMP concentrations,[151,207] it has been hypothesized that the intracellular cAMP concentration is a common growth cone mediator, whose levels are controlled by the summated actions of many different signaling pathways. CNS axons are able to regenerate despite myelin inhibition when their cAMP levels are elevated; a peripheral lesion of dorsal root ganglion one day before central axotomy of the same neurons, elevated cAMP levels of the pre-lesioned neurons three-fold and thereby allowed the centrally lesioned axons to overcome myelin inhibition in the CNS and to regenerate.[208] A dramatic demonstration of axonal regeneration of mouse dorsal root ganglion neurons over long distances in degenerating spinal cord white matter of injured adult rat CNS[121] may also be linked to elevations in cAMP in the transplanted neurons. The dissection of the mouse neurons up to five hours prior to their implantation in the rat spinal cords may constitute a sufficient conditioning stimulus to raise cAMP in the neurons and, in turn, promote axonal growth as demonstrated previously for a conditioning lesion made at the same time as a central lesion of the dorsal root ganglion neurons *in vivo.*[137,138]

This phenomenon of integration of signals via cAMP can be

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therapeutically advantageous because it means that instead of attempting to control the individual mediators of regeneration such as the neurotrophins or myelin-derived inhibitors, a single variable can be targeted with equally favorable results. Filbin and her colleagues[130,209] demonstrated that elevated cAMP levels result in polyamine synthesis through the increased production of Arginase I in the injured CNS (Figure 1). By blocking polyamine synthesis, the regenerative qualities of cAMP are inhibited, thus the regenerative qualities of cAMP are mediated primarily through its actions on polyamine synthesis.[209] Analogues of cAMP have been shown to induce axonal regeneration in primary sensory neurons *in vivo*,[138] and retinal ganglion cells *in vivo*.[210]

Tissue transplantation – Transplantation of either neuronal or neuronal supportive tissue from the PNS or progenitor tissue into the CNS appears to be able to significantly facilitate regeneration and recovery. These transplants, often called bridges, can either shield CNS neurons, allowing the neurons to regenerate into and through the bridges, or replace CNS neurons.[7,12] Tissue used for these bridges includes peripheral nerves, Schwann cells, olfactory ensheathing glia, fetal tissue, stem cells, and neuronal precursor cells.[7,12] Transplantation of olfactory ensheathing glia appears to be one of the most promising transplantation strategies. The glia guide regenerating axons through the injury site and they synthesize cell adhesion molecules and neurotrophic factors; the glia can now be prepared so that they are not able to spread or migrate beyond the site of administration.[12,211,212] Perhaps the most convincing evidence of successful functional restoration using olfactory ensheathing cell transplantation, is the increased breathing and climbing ability of rats that was recently demonstrated after cervical transections.[211]

Suppressing glial inhibition

The glial cells of the damaged spinal cord release a wide variety of inhibitory CSPGs within days after a lesion and their expression persists in the glial scar for several weeks.[122] Intrathecal injection into a lesioned spinal cord, of chondroitinase, an enzyme that inactivates CSPGs, promoted axonal sprouting and elongation in dorsal columns and corticospinal tracts and there was significant improvement in functional locomotor and proprioceptive behaviour.[213] However, this treatment was not as successful as IN-1 antibody treatment.[124,214] Based on the results of these studies, it has been suggested that a combination therapy of suppressing myelin inhibitors and scar inhibitors simultaneously would be highly beneficial.[12]

Conclusions

Since the time of Waller,[164] our understanding of the degenerative and regenerative processes of injured neurons has increased substantially. Our current knowledge of PNS degeneration and regeneration, as well as the factors that inhibit such regeneration in the CNS, is sufficient to begin rationally designing therapies to overcome the limitations in PNS axonal regeneration and the intrinsic and environmental barriers of injured CNS axons, thus increasing the possibility of functional recovery. Indeed, new and promising therapies are emerging which may be able to induce axonal sprouting and elongation, with the possibility of creating functional connections. For instance, the application of certain growth factors or growth inhibitor antibodies has been shown to induce both PNS and CNS axonal growth. As well, the novel approach of using cAMP analogues and inducers to mimic the actions of neurotrophic factors, and induce intrinsic regenerative pathways within CNS neurons appears to be very promising. Despite these advances, there are still many pathological pathways of SCI that have yet to be elucidated and integrated into the etiology of SCI. Also, there are other more practical problems that must be overcome. These include having to locally administer most of the currently researched drugs in order to achieve appropriate target specificity, guidance of regenerating axons to create functional and appropriate target connections and remyelination of the regenerated axons. Although there have been many significant advances in the study of axonal regeneration in the PNS and the CNS, the development of clinically viable treatment strategies that will allow significant functional regeneration is probably still many years away.

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