Original Article

The role of complement in immunological demyelination of the mammalian spinal cord

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Study design: Specificity of serum complement component to elicit immunological demyelination.

Objectives: To assess the role of complement components and pathways in experimental immunological demyelination of the adult rat spinal cord.

Setting: ICORD, University of British Columbia, Vancouver, Canada.

Subjects: We used 32 adult male Sprague–Dawley rats, of approximately 220 g weight.

Methods: Rats received intraspinal infusions of demyelinating reagents, delivered by osmotic minipump, for a 7-day infusion at 0.5 μ l/h. Reagents consisted of a polyclonal antibody to galactocerebroside and human serum complement. Complement sera deficient for a single component were used to assess the role of the alternative pathway, the classical pathway, and the membrane attack complex. Demyelination was assessed, at 7 days, ultrastructurally.

Results: Removal of C3 protein, common to classical and alternative complement pathways, or C4 protein, a classical pathway protein, resulted in no demyelination. However, complement deficient in Factor B, an alternative pathway protein, produced effective demyelination. Upon removal of C5 or C6, membrane attack complex proteins, demyelination was also observed.

Conclusion: This suggests that the classical pathway is sufficient for the protocol to demyelinate the adult rat spinal cord, and that the membrane attack complex is also not required.

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Keywords: galactocerebroside; antibody; myelin; macrophage; membrane attack complex; demyelination

Introduction

After an adult central nervous system (CNS) injury, it has been suggested that a barrier to axonal regeneration is the presence of intrinsic 'inhibitory growth molecules' associated with glial cells. For example, axonal growth inhibitory properties have been attributed to CNS myelin-associated molecules, including Nogo (or NI35/250),^{1–4} NI220,⁵ myelin-associated glycoprotein (MAG),^{6,7} and oligodendrocyte-myelin glycoprotein (OMgp),^{8,9} all of which are expressed by oligodendrocytes. Recent studies have shown that either the selective disruption of myelin or the targeted functional inhibition of the aforementioned myelin proteins within either the developing and adult avian or mammalian spinal cord can facilitate functional axonal sprouting and/or regeneration.^{10–16}

To reduce transiently the axonal growth inhibition activity associated with CNS myelin, we have developed an immunological intraspinal infusion protocol.¹⁷ The immunological process is activated by infusing a myelin-specific IgG antibody to an outer cell surface myelin-specific epitope (eg anti-galactocerebroside or GalC) that has a high affinity for activating the accompanying infusion of serum complement. Only when combined will these two reagents selectively target CNS myelin to focally demyelinate an injured spinal cord region in a concentration-dependent, diffusion-limited manner.^{11,12,14,17,18}

When applied to the embryonic chick spinal cord, prior to transection of the cord,^{12,17} this treatment suppressed CNS myelin development and extended the normal permissive period for axonal regeneration and functional recovery to later stages of development.^{12,17} In the mature spinal cord of posthatching birds, the same immunological protocol transiently demyelinates

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Figure 1 Cartoon of complement cascade that may be involved in immunological demyelination, indicating experimental complement serum-deficiency points. Two pathways of activity are possible in the complement cascade: the classical pathway (molecules C1, C4, and C2 at bottom of diagram), which is antibody mediated, and the alternative pathway (molecules B and D at top of diagram), which is mediated by the spontaneous conversion of C3 to C3b. Both pathways converge to form the membrane attack complex (MAC). This results in the permeabilization of the cell membrane and cell lysis. Complement proteins (C4, Factor B, C3, C5) removed from the human serum complement used in these experiments are highlighted with a circle at their respective positions in the cascade. The infusion of serum deficient in C4 with GalC antibody halts the classical pathway of the complement cascade after the binding of C1 to the GalC antibody, thereby rendering this pathway inactive. The alternative pathway (including opsonizing deposition fragment C3b), and the MAC (including anaphylatoxin C5a), still has the ability to activate through to completion. If immunological demyelination is dependent on antibody mediated complement activation, this will result in the preservation of myelin. Infusing serum deficient in Factor B with GalC antibody halts the alternative pathway of the complement cascade after initial C3 deposition, thereby rendering this pathway inactive. The classical pathway (including opsonizing deposition fragment C3b and anaphylatoxins C4a and C3a), and the MAC (including anaphylatoxin C5a), retains the ability to activate through to completion, inducing demyelination. Infusing serum deficient in C3 with GalC antibody halts the classical pathway of the complement cascade after C4b2a deposition and the alternative pathway before initiation, thereby rendering both pathways inactive. Only C4b2a and anaphylatoxin C4a should be active, and no C3 opsonization will take place. Infusing serum deficient in C5 with GalC antibody stops the complement cascade after deposition of the C5 convertases (C4b2a3b and C3b,Bb,3b) from both the classical and alternative pathways, thereby preventing MAC assembly. The classical pathway (including opsonizing deposition fragment C3b and anaphylatoxins C4a and C3a), and the alternative pathway (including C3b), still has the ability to activate through to completion. However, the MAC (including anaphylatoxin C5a) is unable to form

and disrupts compact myelin, thereby facilitating some functional axonal regeneration of axotomized avian brainstem-spinal neurons, albeit at a reduced level of repair when compared to the injured developing spinal cord.¹⁴ The compacted form of myelin subsequently reappears after the immunological protocol is discontinued.^{14,19}

More recent work in the adult rat spinal cord indicates that a similar immunological treatment after a thoracic cord lesion also facilitates anatomical regrowth of axonal projections from specific brainstem-spinal nuclei,¹¹ as well as ascending dorsal column neurons.¹⁸ These studies indicated that this immunological protocol is effective in

transiently removing the inhibitory effects of CNS myelin against axonal repair in both the developing and adult spinal cord of different species and will facilitate functional axonal regeneration.¹⁰

The complement system is a major effector pathway of the humoral immune system for the incorporation of targeted cellular components by phagocytotic cells.²⁰ It is involved in the control of inflammation, the activation of phagocytes, and the lytic attack on cell membranes. Complement consists of a complex collection of approximately¹⁷ plasma-soluble proteins (Figure 1), many of which are zymogens (ie inactive proenzymes that require cleavage to become active).

Activation of complement can occur along two possible pathways, the classical and alternative pathways reviewed by Devine²⁰ both of which result in the formation of the membrane attack complex (MAC).

In the present study, we have investigated the classical and alternative pathways of the complement cascade, and also of particular complement enzymes themselves (C3, C4, C5, C6, and Factor B), for their contributions to the focal CNS demyelination observed, as outlined above. By infusing serum, specifically deficient for each of these complement proteins, we have rendered either the classical, alternative, or MAC pathways inactive. Through the subsequent examination of axonal-myelin ultrastructure, we were able to determine whether the complete complement cascade was essential for the observed myelin changes. Furthermore, we were able to assess the roles of the classical, alternative, or MAC pathways of complement activation.

Materials and methods

Choice of depleted sera

All human complement sera were obtained from Sigma Inc. (St Louis, MO, USA). Deficient sera were prepared and verified by Sigma Inc. using affinity chromatography, a highly sensitive hemolytic assay, and Ouchterlony microimmunodiffusion.²¹ All intact or deficient human complement sera were infused into the intact spinal cord along with a complement-fixing IgG antibody to GalC (see below). Sera deficient for a specific protein were selected on the basis of their potential interruption of specific pathways within the complement cascade. C4-deficient serum (Sigma # C-0913) ($n=4$ animals) was used to test the role of the classical pathway, Factor B-deficient serum (Sigma # C-0535) ($n=4$) to test the role of the alternative pathway, C3-deficient serum (Sigma # C-8788) ($n=4$) to test the importance of this zymogen that is common to both the classical and alternative pathways, as well as the possible role of macrophage chemotaxis, and finally, C5-deficient serum (Sigma # C-1163) ($n=2$) and C6-deficient serum (Sigma # C-1288) ($n=4$) to examine the necessity of the MAC to demyelination. Normal (ie intact) human sera (Sigma # C-9473) with anti-GalC antibody ($n=4$) were used as a positive control. Other control animals received either an infusion of the polyclonal IgG anti-GalC alone ($n=4$), human serum complement alone ($n=4$), or received no treatment ($n=2$). Polyclonal anti-GalC antibody was raised in rabbits, and partially purified to a predominance of the IgG subclass as described previously.¹¹

Transient immunological myelin disruption

Adult male rats (Sprague–Dawley, 10–12 weeks old), approximately 220 g in weight, were anesthetized with ketamine/xylazine (60, 7.5 mg/kg, respectively). A dorsal incision was performed and lidocaine was injected into the musculature surrounding the dorsal aspect of the tenth thoracic spinal column (T10). After a limited

dorsolateral laminectomy, an intraspinal cannula was implanted at T10 ($n=30$ total) and connected to an Alzet osmotic mini-pump (Model 1007D; Alza Corp., Palo Alto, CA, USA) to subsequently deliver a continuous intraspinal infusion (at 0.5 μ l/h, for 7 days) of one of the various complement sera (33% v/v, see above) along with a complement-fixing IgG antibody to GalC (0.25 mg/ml). Each cannula was held in place by means of dental acrylic applied to the vertebral bone and to a stainless-steel screw inserted at T11. The osmotic pump was then placed in a subcutaneous cavity. Muscle layers were then sutured over the dental acrylic, and the superficial tissue and skin closed. All surgical procedures and subsequent animal care protocols were in accordance with the Canadian and UBC Animal Care Committee Guidelines.

Electron microscopy

Tissue for ultrastructural analysis was obtained from 10–12-week-old adult male Sprague–Dawley rats killed at 7 days postimplantation of the osmotic pumps. Animals were lethally anesthetized with ketamine/xylazine (120, 15 mg/kg, respectively), and then perfused intracardially with 200 ml of 0.1 M PBS (pH 7.4) followed by 100 ml of 4% glutaraldehyde in 0.1 M PB (pH 7.3) and subsequently postfixed overnight in the same fixative. The infusion site and surrounding cord was cut into 1 mm transverse blocks and processed to preserve rostral–caudal sequence. Blocks were washed in 0.1 M sodium cacodylate buffer (24 h), postfixed in 2% OsO₄, dehydrated through increasing concentrations of ethanol (70, 85, 95, 100, 100%) and propylene oxide, and embedded in Spurr's resin according to standard protocols. Tissue blocks from experimental and untreated control animals were processed in parallel. Semithin sections (1 μ m) were cut from each block, mounted on Superfrost slides, stained with 2% alkaline Toluidine Blue, and examined under a light microscope. For electron microscopic examination, blocks were trimmed and sections cut at 80–100 nm, mounted on copper grids, stained with 2% uranyl acetate, and lead citrate, and viewed under a Zeiss EM 10C (at 80 kV).

Results

Results are based on the ultrastructural analysis of the adult rat thoracic spinal cord, following the direct intraspinal infusion of myelin-specific IgG antibody along with whole complete serum (as a positive control) or serum depleted, through immunoadsorption, for a specific complement enzyme. Since the implantation of an osmotic pump cannula into the dorsolateral cord can cause some limited nonspecific focal damage at the insertion point, all ultrastructural assessments of spinal myelin integrity were made from the adjacent dorsal column region (1 mm rostral or caudal) to the cannula insertion site.

As reported previously,¹¹ direct intraspinal (T10) infusion over 7 days (0.5 μ l/h) with normal 33% human

Figure 2 Ventral demyelination during dorso-medial infusion. Infusion (for 7 days) of complete human complement sera and anti-GalC IgG antibody results in widespread demyelination. Ventral aspects of the rat spinal cord are heavily, but not completely, demyelinated. The outermost rim of the cord still has intact myelin (dark band); however, there is an absence of myelin in the medial half of the ventral white matter tracts. The dotted white line delineates gray–white matter boundary (white matter below, gray matter above line). The demyelinated zone is also indicated

Figure 3 The effects of complete human complement and anti-GalC IgG antibody infusion on myelin ultrastructure compared to control infusions of antibody alone or complement alone. Electron photomicrographs of transverse sections through dorsal columns of adult rat thoracic spinal cord. (a) Control untreated animals showing normal appearance of myelin. (b) After 7 days of continuous intraspinal infusion of full human complement serum and anti-GalC antibody, large regions of demyelinated axons were visible within 1 mm of the infusion site. Oligodendrocytes (O), appearing normal, are observed. Macrophages (M) are observed among regions of healthy naked axons. (c, d) Myelin structure following 7-day infusion of GalC IgG antibody alone (c) or human complement alone (d) within 1 mm of infusion site, showing compact nature of myelin with minimal nonspecific damage

serum complement along with an IgG polyclonal antibody to GalC (0.25 mg/ml) in PBS resulted in profound demyelination within the dorsal column and

ventral horns (Figure 2) of all animals extending at least 1 mm, in a rostral and caudal direction, on either side of the cannula insertion site (Figure 3b) after 7 days of immunological infusion. Oligodendrocytes were observed to survive within this demyelination zone (data not shown, but have been characterized in previous studies of immunological demyelination¹⁸). Some cell death was observed in the central region of demyelination, and presumably some of this can be attributed to oligodendrocytes cell death, as previously reported. It is, however, unclear as to the mechanism of this cell death, be it by apoptotic, necrotic, or phagocyte-mediated mechanisms. In addition, large numbers of macrophages or activated CNS microglia containing myelin debris were present within the demyelinated spinal tissue. These macrophages/microglia contained myelin ovoids and were closely associated with the demyelinated (naked) axons. In addition, a few blood-borne cells such as neutrophils or other polymorphonucleocytes were observed to infiltrate the infusion region. At rostral–caudal distances further removed from the infusion site (2–8 mm), myelin disruption, as characterized by a delaminating appearance, was observed. This was characterized by an unraveling on the compact nature of myelin,¹⁴ distinguishable from processing artifacts.

Control infusions of human serum complement alone (Figure 3d) or antibodies to GalC alone (Figure 3c) showed normal myelination comparable to that of untreated animals (Figure 3a). Minimal myelin disruption was observed, and the compact nature of the myelin was preserved.

Upon direct intraspinal infusion of C4-deficient human serum complement (blocking complete activation of the classical pathway), along with the IgG anti-GalC in PBS, normal myelination (ie no demyelination) was observed within and around the infusion site (Figure 4a), and a normal distribution of spinal neural cells and processes was found throughout all the cords examined.

In animals infused with the IgG anti-GalC and human complement deficient in C3 (thereby blocking both the classical and alternative complement pathways), no apparent damage within the dorsal column of all animals examined resulted (Figure 5a). Axons, CNS myelin, and oligodendrocytes all appeared normal, as did all of the neural cell types. Spinal myelin retained its compact nature, with little, if any, disruption or delamination.

Infusion of human serum complement deficient in Factor B (blocking complete activation of the alternate complement pathway), along with the IgG anti-GalC in PBS, resulted in a demyelinated CNS environment, similar to that observed after infusion of normal (intact) human complement along with the IgG anti-GalC (as shown in Figure 3b). Myelination was suppressed for at least 1 mm on either side of the infusion site, and large regions of naked axons were observed (Figure 4b), once again associated with activated macrophages/microglia (Figure 5b). These demyelinated axons maintained an

Figure 4 Classical and alternative pathway contributions to demyelination. (a) Electron photomicrograph of a transverse section through the dorsal column of adult rat thoracic spinal cord following 7-day infusion of GalC antibody with human complement deficient in C4, within 1 mm of the infusion site. The compact nature of myelin remains, with minimal disruption and no demyelination observed, similar to that seen for control animals. (b) Electron photomicrograph of a transverse section through the dorsal column of adult rat thoracic spinal cord following 7-day infusion of GalC antibody with human complement deficient in Factor B, within 1 mm of infusion site. Extensive demyelination is observed, with axons appearing otherwise healthy, similar to that seen for treatment with complete human complement and anti-GalC IgG antibody. Occasional axons are seen with disrupted thin myelin sheaths

altered (eg reduced spacing of cytoskeletal elements) but healthy (ie no evidence of axonal loss) appearance at the ultrastructural level.

Figure 5 C3 and macrophage contributions to demyelination. (a) Electron photomicrograph of a transverse section through the dorsal column of adult rat thoracic spinal cord following 7-day infusion of GalC antibody with human complement deficient in C3, within 1 mm of infusion site. Normal myelin is observed, with minimal disruption and no demyelination, similar to that seen for control animals. Oligodendrocytes (O) and axons again appear healthy, and there is no evidence of macrophage activity. (b) Electron photomicrograph of a transverse section through the dorsal column of adult rat thoracic spinal cord following 7-day infusion of GalC antibody with human complement deficient in Factor B, within 1 mm of the infusion site, as in Figure 3b. Naked demyelinated axons are associated with macrophages (M), which contain lysosomes containing myelin breakdown products (arrowheads) or myelin debris (arrow)

Direct intraspinal infusion of IgG anti-GalC and human complement deficient in C5 (Figure 6) or C6 (not shown), which are enzymes of the MAC (Figure 1) proteins, resulted in focal demyelination. This zone of induced demyelination was accompanied by the presence of macrophages containing myelin debris. As in

Figure 6 The effects of C5-deficient serum and anti-GalC IgG infusion on myelin. Electron photomicrograph of a transverse section through the dorsal column of adult rat thoracic spinal cord, following 7-day infusion of GalC antibody with human complement deficient in C5, within 1 mm of the infusion site. Extensive demyelination is observed, with axons appearing otherwise healthy, similar to those seen for treatment with full human complement and GalC antibody. Again, naked demyelinated axons are associated with macrophages (M), which contain myelin ovoids or myelin debris (arrows)

the above, there was no evidence to suggest that axonal loss was occurring.

In cases where focal demyelination was observed, a region of myelin delamination was also observed. This extended 6–8 mm on either side of the infusion site. Beyond this zone neural tissue composition and myelin compaction appeared normal (see reference Keirstead *et al*¹⁴).

Discussion

We have examined some of the potential mechanisms of an immunological demyelination protocol by analyzing the effects of deleting individual complement proteins, thereby disrupting distinct aspects of the pathways involved in the complement cascade. By infusing serum deficient for one of a group of specific proteins in the complement cascade (C3, C4, Factor B, C5, or C6) along with an IgG anti-GalC antibody, we rendered either the classical, alternative, or MAC pathways inactive. Through examination of axonal-myelin ultrastructure, we determined that the complete complement cascade is not required for serum complement-mediated, GalC antibody-dependent demyelination of the adult rat spinal cord.

By functionally blocking activation of the alternative pathway (through the use of Factor B deficient serum), or blocking activation of the MAC (through the use of C5 or C6 deficient serum), substantial demyelination was still observed. This suggested that activation of the classical pathway alone is sufficient to trigger transient demyelination when infused, directly into the spinal cord, along with anti-GalC antibody.

In previous studies, we utilized guinea-pig complement and an IgG anti-GalC antibody^{11,14,17} to induce transient myelin suppression/demyelination in the developing and mature avian and adult rat spinal cords. Control infusions (anti-GalC antibody alone, complement alone, vehicle alone) did not cause any demyelination of the spinal cord in these cases, indicating that both serum complement and a myelin-specific, complement-fixing antibody are essential for this immunological CNS demyelination to occur. While it is clear that neural cells such as microglia and astrocytes are capable of producing complement protein, the levels produced are not sufficient to compensate for individual components absent in our infusate. Our demyelination protocol requires high concentration of complement – much higher than endogenous levels. Our control experiments clearly indicate that exogenous complement is required, and without it, the antibodies alone are not capable of eliciting demyelination.

In the study presented here, we have utilized human complement since it is readily available as deficient sera; human serum complement is more applicable to the development of a potential clinical therapeutic for the treatment of neurotrauma.

Observations of the requirement for the early stages of the complement cascade (C1–C4), and the nonroles for the alternative pathway and the membrane attack complex, in mediating immunological demyelination, suggest that other mechanisms other than membrane lysis contribute to the demyelinating process. There are features that are common to all the experiments where demyelination was observed (ie with complete serum complement treatment, Factor B-deficient complement, and C5- or C6-deficient serum).

First, without exception, macrophages containing myelin debris were observed in close association with naked axons (Figures 3b and 4a). Macrophage recruitment could occur in a number of ways. These include their chemoattraction by complement-derived inflammatory mediators, C4a for example, and/or nonspecifically through the injury caused by the cannula insertion. Activated microglia/macrophages can be either phagocytic, as in the case of EAE²² and early MS lesions,²³ or they can release potentially cytotoxic substances, such as proteolytic or lipolytic enzymes,²⁴ free oxygen radicals,²⁵ and inflammatory cytokines such as IL-1 and TNF.^{26–28} Cytokines such as TNF α and IL-1 are then thought to elicit a complex inflammatory response and have been shown to facilitate myelin destruction.²⁹ In addition, TNF α is believed to act in other demyelinating lesions such as in models EAE^{26,30} and in MS,^{31–33} but its exact contribution is not clear. It is possible that the

initial contact between microglia and oligodendrocytes requires complement-receptor binding, but the ultimate fate of the oligodendrocyte/myelin may then be governed by the local release of cytokines.³⁴

Although macrophages appeared to be the only cells that elicited the removal of myelin, we cannot discount the possible, minor, contribution of other phagocytes. Indeed, we occasionally observed the infiltration of polymorphonucleocytes (PMN) (eg neutrophils), in animals where demyelination was observed (data not shown). It is possible that these cells may have been attracted to the demyelination site due to the penetrating injury of the cannula insertion during surgery, and at the time the spinal tissue was collected, have left the tissue. Macrophage recruitment is slower than PMN recruitment, usually taking 2–3 days until significant numbers are present throughout the area that will ultimately be demyelinated, and peaking in numbers 7 days after spinal cord injury (SCI).

Second, in all cases, amplified C3b deposition could be occurring on the myelin surface via C3 convertase activity. C3 is the most abundant complement component in plasma, and the complement cascade becomes dramatically amplified through increasing C3b deposition.³⁵ The C3 protein is common to both the classical and alternative complement pathways and becomes activated at the point where the pathways converge. The lack of demyelination (ie preservation of normal myelin) when C3-deficient sera were used indicates that C3 is an essential complement protein to the immunological demyelinating procedure. The opsonization (ie coating of cell surface with complement or antibody) of the target membrane by C3b is thought to be necessary in the phagocytosis of myelin by microglia/macrophages.^{36–38} These results, together with the observations that macrophages play an active phagocytic role, support the suggestion that microglia/macrophages are involved in the transient demyelination procedure.

Third, anaphylatoxins C3a and C4a are released as early cleavage products of the classical pathway of the complement cascade. C3a, C4a, and C5a increase vascular permeability, have a chemottractive effect on macrophages and other phagocytic cells, enhance the phagocytic ability of these cells, and, as mentioned, increase the expression of complement receptors CR1 and CR3 on macrophage surfaces.³⁹ In addition, C3a, C4a, and C5a can induce macrophages to synthesize or secrete other inflammatory mediators such as interleukin-1 (IL-1)⁴⁰ and interleukin-6 (IL-6).⁴¹ These common features suggest that demyelination could be occurring through the C3b opsonization of target myelin via the classical pathway, followed by subsequent macrophage infiltration and phagocytosis of myelin, all being promoted by local inflammation induced by complement cleavage products C3a and C4a.

As the alternative pathway and the MAC do not appear to be crucial for demyelination to occur, refinements of the composition omitting the alternative pathway and MAC proteins could be used to induce demyelination, and yet limit any possible side effects (eg

on astrocytes) caused by nonspecific damage resulting through the activation of these pathways.⁴² Future experiments might utilize a refined version of the original infusion protocol, modified to include only classical pathway proteins C1, C4, C2, and C3, along with IgG anti-GalC, to achieve substantial focal demyelination of the adult spinal cord, thereby transiently removing the inhibitory effects of CNS myelin on axonal regrowth. Focal demyelination (ie near the SCI) is desirable over extensive demyelination of regions distant from the site of infusion. Large demyelinated zones would be detrimental to the animal in the short term, and possibly in the long term. We also believe that long distance demyelination is not required for axonal regrowth. Studies have indicated that axons only need to 'escape' the inhibitory environment where they are injured and are then capable of growing between myelin sheaths in an uninhibited manner. Such short distance growth is sufficient for axonal sprouting and with the intrinsic plasticity of the spinal pathways can lead to reorganization through use-dependent activation.⁴³

It has been suggested that macrophages may also promote remyelination. Surviving oligodendrocytes have been shown to have limited capacity for remyelination, while oligodendrocyte precursor cells, which mature in demyelinated lesions, are the predominant cell type that will initiate central remyelination.⁴⁴ Macrophages have been observed to stimulate myelin formation *in vitro*,^{45,46} and inflammation has been shown to be important for stimulating remyelination.⁴⁷ Macrophages have also been shown to express a variety of growth factors and cytokines, including platelet-derived growth factor, epidermal growth factor, transforming growth factor beta, insulin-like growth factor, and nerve growth factor, that have been implicated in such processes as regulation of oligodendrocyte proliferation and survival, myelination, and remyelination; for a review see Diemel *et al.*⁴⁸ Therefore, the presence of macrophages within demyelinated regions of the spinal cord might even facilitate the subsequent remyelination of demyelinated axons.

Finally, in addition to possible contributions to demyelination and remyelination, the presence of macrophages at lesion sites may be 'directly' beneficial for axonal regeneration, perhaps through the secretion of cytokines or growth factors. In addition, studies in lower vertebrates, where some CNS axons retain a natural regenerative ability throughout adult life, have shown vigorous phagocytic responses to nerve injury.^{49,50} Macrophage invasion of damaged white-matter sites is slower and less extensive in the CNS than in the growth-permissive peripheral nervous system (PNS).⁵¹ In addition, the macrophages are normally less efficient at clearing myelin debris in the CNS than in the PNS,⁵² allowing potential inhibitory debris to remain for a longer period of time within the CNS. *In vitro*, the nonpermissive nature of the CNS white matter can be modified by medium conditioned with activated macrophages.⁵³ *In vivo*, the transplantation of activated monocytes into the injured rat CNS stimulates

axonal regeneration of both the injured optic nerve⁵⁴ and transected spinal cord.⁵⁵

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