Impaired remyelination and depletion of oligodendrocyte progenitors does not occur following repeated episodes of focal demyelination in the rat central nervous system

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Summary
It has been hypothesized that the progressive failure of remyelination in chronic multiple sclerosis is, in part, the consequence of repeated episodes of demyelination at the same site, eventually depleting oligodendrocyte progenitor cells (OPCs) and exhausting the remyelinating capacity. We investigated the effect of previous focal, ethidium bromide-induced demyelination of brain stem white matter (with intervening recovery) on the efficiency of the remyelination process during second and third subsequent episodes of demyelination, and the OPC response during a second episode of demyelination. Previous focal demyelinating lesions followed by recovery did not result in any retardation of the remyelination process, nor did they alter the proportion of Schwann cell versus oligodendrocyte remyelination. The OPC response during remyelination was quantified by in situ hybridization using a probe to platelet-derived growth factor-α receptor (PDGFαR), an OPC-expressed mRNA. Following recovery from focal, toxin-induced CNS demyelination, the OPC density returned to levels equivalent to those in normal white matter. Furthermore, there was no depletion of OPCs following repeated episodes of focal, toxin-induced CNS demyelination at the same site. These results indicate that repeated CNS demyelination, which has the opportunity to repair in the intervening period, is not characterized by impaired remyelination or depletion of OPCs.

Keywords: demyelination, multiple sclerosis, oligodendrocyte progenitor, platelet-derived growth factor-α receptor, remyelination

Abbreviations: CCP = caudal cerebellar peduncle; EAE = experimental autoimmune encephalomyelitis; EB = ethidium bromide; FGF = fibroblast growth factor; MBP = myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; OPC = oligodendrocyte progenitor cell; PLP = proteolipid protein; PDGF = platelet-derived growth factor; PDGFαR = platelet-derived growth factor-α receptor

Introduction
Although remyelination occurs in many acute plaques of multiple sclerosis (Prineas and Connell, 1979; Prineas et al., 1993a; Lassmann et al., 1997), remyelination failure becomes an increasingly prominent feature of the pathology of chronic multiple sclerosis (Prineas et al., 2002). The progressive failure of remyelination during the later stages of the disease results in impaired axonal conduction (Smith and McDonald, 1999), and may contribute to the axonal atrophy that is an important component of the secondary progressive phase of the disease (Kornek et al., 2000). The reasons for remyelination failure in multiple sclerosis are not fully understood, and understanding why a relatively robust regenerative process should lose momentum is an important prerequisite for developing an effective therapeutic solution (Franklin, 2002). One widely entertained hypothesis is that repeated episodes of demyelination at the same site eventually exhaust the capacity of the tissue to mount a remyelinating response (Ludwin, 1980), possibly due to the gradual depletion of oligodendrocyte progenitor cells (OPCs) in and around the lesion (Johnson and Ludwin, 1981; Carroll et al.,...
1998; Keirstead et al., 1998; Franklin, 1999). We call this hypothesis the repeat demyelination-OPC depletion hypothesis. Some supportive evidence has been obtained from the examination of multiple sclerosis lesions (Prineas et al., 1993b), and from experimental models such as repeated or sustained exposure to the dietary demyelinating agent cuprizone, which lead to impaired remyelination (Ludwin, 1980; Johnson and Ludwin, 1981). A similar impairment of remyelination occurs in a chronic relapsing experimental autoimmune encephalomyelitis (EAE) model induced by repeated adoptive transfer of encephalitogenic T-cells and anti-MOG (myelin oligodendrocyte glycoprotein) antibodies (Linnington et al., 1992). However, in most of these experimental models, cuprizone being an exception, it is often difficult to establish whether the same area of CNS white matter has undergone successive bouts of demyelination, remyelination and further demyelination. This makes it difficult to address the repeat demyelination-OPC depletion hypothesis of remyelination failure directly.

Experimental models of demyelination/remyelination that involve direct injection of demyelinating agents such as the gliotoxins ethidium bromide (EB) and lysolecithin should, in principle, be amenable to repeat demyelination by re-injection at the same site. For the most part, these models have involved injection into spinal cord white matter, where the relatively small size of the tracts makes the tissue vulnerable to axonal injury following repeat injections. However, these limitations are substantially resolved in a model involving stereotaxic injections of gliotoxins into the caudal cerebellar peduncles (CCPs; equivalent to the inferior cerebellar peduncle in humans) of adult rats (Woodruff and Franklin, 1999). Using this model we have been able to test three predictions arising from the repeat demyelination-OPC depletion hypothesis of remyelination failure: (i) that following an episode of focal demyelination and remyelination, there will be an impairment in the extent of remyelination after subsequent episodes of demyelination affecting the same area; (ii) that the local OPC population will be depleted following successful remyelination of a demyelinating lesion; and (iii) that this depletion of the local OPC population results in an impaired OPC response to subsequent episodes of demyelination.

Materials and methods

Induction of focal demyelination

Female Sprague–Dawley rats (young adults, 8–10 weeks of age, ~ 200 g) were used in all experiments. Experiments were performed in compliance with UK Home Office regulations and institutional guidelines. Anaesthesia was induced using 4% isoflurane in oxygen. Immediately following loss of consciousness the rats were removed from the 4% isoflurane–oxygen mixture and general anaesthesia was maintained by a continuous intravenous infusion of propofol (Rapinovet; Mallinckrodt Veterinary Ltd, Uxbridge, UK) via the lateral tail vein. Diazepam (C.P. Pharmaceuticals Ltd, Wrexham, UK) was administered at 1.7 mg/kg i.p. to facilitate anaesthesia, and buprenorphine hydrochloride (Vetergesic; Animalcare Ltd, Hull, UK) was administered at 0.03 mg/kg i.m. to provide analgesia. Three millilitres of warmed sterile saline was injected (i.p.) to maintain normal hydration during the surgical procedure and recovery. Demyelination was induced unilaterally or bilaterally by stereotaxic injection of 4 ml of 0.01% ethidium bromide (EB) into the CCP as described previously in detail (Woodruff and Franklin, 1999). In the experiments involving remyelination analysis following a second injection of EB, we confirmed that stereotaxic coordinates were correct for both the first and second injection by injecting Evans Blue dye into the peduncles of an age-matched control rat and identifying the location of the dye following sectioning of the brain on a sledge microtome.

Histological analysis of remyelination

Groups of four to six animals were used for histological comparison of the character and extent of remyelination following focal demyelination in the presence of no, one or two previous demyelinating lesions at the same site, with complete recovery between, as detailed in Fig. 1. Animals were perfused with 4% glutaraldehyde in phosphate buffer and the brains were post-fixed overnight. Tissue blocks...
encompassing the CCP were cut as detailed previously (Woodruff and Franklin, 1999). While maintaining their correct orientation and sequence, blocks were processed through osmium tetroxide, dehydrated and embedded in TAAB (TAAB Laboratories, Aldermaston, UK) resin. Sections (1 μm) were cut and stained with alkaline toluidine blue. In these sections, remyelinated axons can be readily distinguished from normally myelinated axons outside the lesion by the decreased thickness of the myelin sheath. Within the lesion, remyelinated axons can be distinguished from demyelinated axons because the former possess myelin sheaths recognizable as a dark staining rim around the axon. The myelin of Schwann cell remyelination has a slightly darker staining hue than that of central myelin, and the proximity of the Schwann cell nucleus frequently gives the myelinating Schwann cell unit in transverse section a characteristic ‘signet ring’ appearance, a combination of features that enable Schwann cell remyelination to be distinguished from oligodendrocyte remyelination. Using these morphological criteria, the estimated proportions of axons that had been remyelinated by oligodendrocytes, by Schwann cells, or had remained demyelinated were recorded on two separate occasions, blinded to status and survival time. The mean scores and SEM were calculated for each group of animals, and statistical analysis was performed using the Mann–Whitney test.

**PDGFαR in situ hybridization**

The platelet-derived growth factor-α receptor (PDGFαR) probe was transcribed from a 1637 bp EcoRI cDNA fragment, encoding most of the extracellular domain of mouse PDGFαR cloned into pBluescript KS+ (a gift from Dr N. P. Pringle and Professor W. D. Richardson, University College London, UK). Groups of four animals were used for the comparison of PDGFαR-positive (+) cells in the CCP. As detailed in Fig. 2, comparisons were performed between PDGFαR+ cells in normal white matter and following complete remyelination (10 weeks post lesioning), as well as 5, 10 and 21 days following demyelination in the presence or absence of a previous demyelinating lesion at the same site. Animals were perfused with 4% paraformaldehyde in PBS (phosphate-buffered saline) and tissue was prepared for in situ hybridization as described previously (Fruttiger et al., 1999), except that both the RNA polymerases and the RNA transcription reactions were run as recommended by Boehringer Mannheim Biochemica (Mannheim, Germany). After in situ hybridization, RNA hybrids were visualized in situ by a standard technique as described previously (Fruttiger et al., 1999). The density of the PDGFαR+ cells within the lesion was assessed by image analysis (MCID model M4; Imaging Research Inc., Toronto, Canada). EB-injected or normal regions of the CCP, identified by solochrome cyanine staining, were captured under the 4× objective using a red filter to accentuate the blue-stained PDGFαR nuclei. The automatic target detection feature of the MCID system was used to select positive nuclei, and the threshold level was set according to the lesion background level such that all positive nuclei were selected. The valid criteria for counting a single cell were set as an area of >20 μm². The average area of PDGFαR+ cells was found to be ~60 μm². Therefore, to allow estimation of PDGFαR+ cells that appeared in ‘clumps’, a target with an area of >80 μm² was counted as two or more cells. However, to exclude areas of above-threshold non-specific staining such as section tears and folds, targets with an area of >200 μm² were excluded. The MCID image analysis system then calculated the cell density of positive cells. Representative sections were analysed from each lesion side of each animal to derive a mean score (a mean of 10 sections per lesion side were analysed per mean score). The individual mean scores were then used to calculate the mean score and SEM for each group of animals, and statistical analysis was performed using the Mann–Whitney test.

**Results**

*Previous focal demyelination–remyelination does not impair subsequent remyelination following a repeated episode of demyelination at the same site*

The repeat demyelination-OPC depletion hypothesis predicts that the extent and rate of the remyelinating response would become progressively impaired at that site following repeated cycles of superimposed focal demyelination and remyelination. In order to test this, we used a model that involves stereotaxic injection of EB into the CCP, a relatively large cross-sectional tract of large-diameter myelinated proprioceptive fibres en-route from the spinal cord to the cerebellar...
Toxin-induced CNS demyelination model. The solochrome cyanine-stained section demonstrates focal demyelination of the CCP induced by stereotaxic injection of EB into the CCP, with the adjacent normally myelinated spinal tract of the trigeminal nerve (sp5) indicated for comparison. Following successful repair, the region of remyelination in the CCP appears paler than the normally myelinated sp5 (insert: toluidine blue-stained section).

Scale bars: 500 μm.

We have demonstrated previously that this model results in rapid demyelination (Woodruff and Franklin, 1999; Sim et al., 2000, 2002) associated with loss of mRNAs of myelin basic protein (MBP), proteolipid protein (PLP) and Gtx (markers of myelinating oligodendrocytes), PDGFαR (a marker of OPCs) and Olig-1 (a marker of all oligodendrocyte lineage cells) (Sim et al., 2000, 2002b). To confirm that remyelination had occurred prior to induction of further serial lesions at the same site, the extent of remyelination was assessed at 9 weeks following EB injection. As indicated in Fig. 4, the process of remyelination was complete by this time, predominantly through oligodendrocyte remyelination, but also with some Schwann cell peripheral-type myelin and with occasional axons that had not yet remyelinated (Shields et al., 1999; Sim et al., 2002a).

To assess the rate and extent of remyelination in a demyelinating lesion created in an area of remyelination, EB was injected a second time (using modified stereotaxic coordinates to ensure correct positioning) in the CCP of rats that had been injected with EB 10 weeks previously. The EB was also injected into the normal white matter of the contralateral CCP. The extent of remyelination was assessed at 4 and 12 weeks following the second EB injection and compared with the remyelination in the contralateral CCP (Fig. 5). The data demonstrated that there was no difference in the remyelination at 4 weeks following the second serial (double) EB injection as compared with a single EB injection. At 12 weeks, although the extent of remyelination had increased, there was similarly no difference between the double and single lesions. At neither survival times was there a significant difference in the proportion of oligodendrocyte versus Schwann cell remyelinated axon between the double and single lesions.

Since a single episode of repeat demyelination may not be regarded as a rigorous test of the hypothesis, we next assessed the extent and character of remyelination 10 weeks after a third serial EB injection at the same site, as compared with 10 weeks after a single contralateral EB injection (Fig. 6). In this experiment all the lesions were mainly in the dorsal part of the trigeminal tract, which lies immediately beneath the CCP (see Fig. 3). The distribution of the lesion on the side that had received three injections was the same as that on the side receiving a single injection, indicating that these three injections had all been into the same white matter area. We found that there was no difference in remyelination following three episodes of demyelination at the same site where complete recovery is allowed in the intervening period, as compared with a single episode of demyelination.

The introduction of a syringe needle and the injection of EB inevitably cause some degree of axonal injury. It might be argued that second and third injections into the same area will result in enough axonal damage to cause a significant decrease in the lesion size. For a lesion that remyelinates from its edge to its centre (Sim et al., 2000, 2002b), this would decrease the time required for complete remyelination in the multiple injection lesions, thereby potentially masking a decrease in remyelination efficiency. However, when we compared the area of the trigeminal tract containing both the remyelinated lesion and the surrounding intact white matter in single- (n = 4) and triple- (n = 4) injected animals with the trigeminal tract in unlesioned animals (n = 8) we could find no evidence of a significant decrease in lesion size [control area = 0.86 mm² ± 0.01 (SEM); single injection = 0.85 mm² ± 0.02; triple injection = 0.89 mm² ± 0.01]. Thus, while there is likely to be cumulative axonal damage it is unlikely to be sufficiently large to alter the interpretation of the results significantly.

**PDGFαR+ oligodendrocyte progenitor cell numbers return to levels equivalent to those in normal white matter following recovery from EB-induced focal demyelination**

The OPC depletion hypothesis predicts that reduced numbers of OPCs occur within and around an area of remyelination when compared with normal white matter. This was addressed by quantifying the numbers of PDGFαR mRNA-expressing OPCs at 10 weeks following an EB demyelinating lesion in a group of four animals, as compared with levels in the unlesioned CCP. At 10 weeks following demyelinating injury we could detect no difference between the numbers of PDGFαR mRNA-expressing OPCs within the area of
remyelination and in the equivalent area of the contralateral intact CCP (Fig. 7). Both the area of remyelination and the intact CCP were identified on adjacent solochrome cyanine-stained sections. These findings indicate that following successful remyelination, PDGFαR mRNA-expressing OPCs return to levels equivalent to those in normal white matter, with no demonstrable sustained depletion of OPCs. Moreover, since there was a similar distribution of OPCs throughout the CCP containing an area of remyelination, we could find no evidence that remyelinated areas of white matter contain different densities of OPCs to normally myelinated white matter.

**The PDGFαR+ oligodendrocyte progenitor cell response following focal demyelination is not impaired by a previous episode of remyelination at the same site**

The OPC depletion hypothesis also predicts that the OPC response will be impaired during the repair response to subsequent episodes of superimposed demyelination. The EB model of demyelination is characterized by depletion of PDGFαR and Olig-1 mRNA-expressing OPCs within the lesion, and recruitment of new OPCs occurs from the surrounding intact white matter (Sim *et al.*, 2002b). We could therefore use this model to establish whether a previous episode of successful remyelination altered the number of PDGFαR mRNA-expressing OPCs generated following a subsequent episode of EB-induced demyelination at the same site. We determined the PDGFαR mRNA+ cell density within the area of demyelination in groups of four animals 5, 10 and 21 days after EB injection in the presence or absence of a previous demyelinating lesion at the same site 10 weeks previously. The area of demyelination for determining PDGFαR mRNA+ cell density was delineated on adjacent solochrome cyanine-stained sections. We found that there was no depletion of the PDGFαR mRNA+ OPC response in the presence of a previous demyelinating lesion (Fig. 8) and that the absolute densities were comparable to levels that we have previously been demonstrated in rats of a similar age and strain (Sim *et al.*, 2002b). This observation suggests that the OPC response is the same regardless of the previous history of demyelination–remyelination at the same site.

**Discussion**

The variable nature of the reparative response following primary demyelination of the CNS, where some lesions...
should undergo extensive and even complete remyelination while others remain largely demyelinated, constitutes one of the most frustrating aspects of multiple sclerosis. If each new lesion were to remyelinate fully then in all likelihood the disease would carry a more favourable prognosis. Understanding why remyelination is incomplete or fails entirely is critically important in devising therapeutic approaches through which it might be improved.

A number of hypotheses have been proposed to explain remyelination failure (reviewed in Franklin, 2002), one of which has as its basis the predisposition in multiple sclerosis for demyelinating episodes to repeatedly affect the same area of white matter (Prineas et al., 1993b). The repair of focal demyelination involves the local recruitment of OPCs, which engage demyelinated axons and differentiate into remyelinating oligodendrocytes. It has been proposed that should the same area of white matter be required to mount a repair response repeatedly following cycles of demyelination and remyelination then the number of OPCs available for recruitment declines, resulting in an impairment of remyelination. Indeed, one study reported a persistent decrease in OPC number around a demyelinating lesion following a single episode of demyelination (Keirstead et al., 1998), although this observation is at variance with other studies indicating the converse (Cenci di Bello et al., 1999; Levine and Reynolds, 1999; Sim et al., 2002b). We have called this

![Fig. 5](image1.png)

**Fig. 5** Comparison of the character and extent of remyelination (± SEM) 4 and 12 weeks following EB injection into a remyelinated area (filled bars) and normal white matter (shaded bars). No retardation of either the rate or extent of remyelination is evident at 4-weeks and 12-weeks post lesion induction, with remyelination primarily oligodendrocyte-derived.

![Fig. 6](image2.png)

**Fig. 6** No evidence of impaired remyelination is present following three episodes of demyelination at the same site (black bars) where complete recovery is allowed in the intervening period, as compared with a single episode of demyelination (grey bars) (± SEM).
Fig. 7 (A) The CCP at 70 days post-lesion (70 dpl) was identified histologically by solochrome cyanine (SC) staining on adjacent sections, and this was then used to define the region for oligodendrocyte progenitor determination following in situ hybridization for PDGFαR mRNA. (B) No depletion of oligodendrocyte progenitors is evident when comparing the density of PDGFαR mRNA-positive cells in the CCP 10 weeks following a demyelinating lesion (grey bar) with normal CCP (white bar) (±SEM). Scale bars: 500 μm.

Fig. 8 (A) Density of PDGFαR mRNA-positive oligodendrocyte progenitors 10 days post (dp) a single EB lesion, with the extent of the demyelinating lesion defined by solochrome cyanine (SC) staining of the adjacent serial section, and (B) 21 days after an EB lesion superimposed on a remyelinated region. (C) Quantification of the PDGFαR mRNA-positive cell density 5, 10 and 21 days following an EB lesion demonstrated no significant alteration in the presence (black bars) or absence (grey bars) of a demyelinating lesion at the same site 10 weeks previously (±SEM). Scale bars: 500 μm.
hypothesis the repeat demyelination-OPC depletion hypothesis, and have tested it using a model in which the same area of white matter can be repeatedly targeted for demyelination by the stereotaxic injection of a toxin that causes acute demyelination. We have found that the same region of white matter can undergo complete remyelination after each of three sequential episodes of demyelination, implying that an area of remyelination will remyelinate as efficiently as an area of normally myelinated tissue should they become demyelinated. While it is possible that one or more of the injections failed to induce a lesion, we regard this as unlikely given that failure to induce a lesion by stereotaxic injection of EB into brain stem white matter occurs infrequently. Moreover, we have also shown that when remyelination is complete the numbers of OPCs have returned to levels equivalent to those that existed before demyelination and remyelination occurred, and that a repeat wave of remyelination does not cause a progressive depletion of OPCs.

These results are consistent with recent studies indicating efficient repopulation by OPCs of areas of normal white matter from which they have been depleted by X-irradiation (Hinks et al., 2001; Chari and Blakemore, 2002), and provide further evidence of the robust capacity of OPCs to repopulate areas from which they are depleted even though the rate at which this occurs may decline with age (Sim et al., 2002b). The toxin model we have used, however, differs from multiple sclerosis in many ways and is not intended to provide an experimental ‘facsimile’ of multiple sclerosis, which is in any case a disease of considerable diversity. It does, however, provide a powerful model for studying the biology of the repair process in a manner that is not confused by the persistence of a demyelinating stimulus. One can argue that results obtained with these models should be extrapolated to immune-mediated inflammatory disease such as multiple sclerosis with some caution, and should be supported by data from a model more similar to multiple sclerosis in its aetiology and pathogenesis. On the other hand, one can also argue that it provides insights into the fundamental principles of the myelin repair process, broadly applicable across the spectrum of demyelinating disease, that do not emerge with similar clarity in more complex models.

If the process of remyelination per se does not inhibit subsequent remyelination, why then do some models of repeat demyelination, such as the EAE model based on repeated co-transfer of MBP-specific T cells in combination with antibodies specific to MOG (Linnington et al., 1992), lead to persistent demyelination? A key feature of the experimental design in the present study was the period of time between successive episodes of demyelination. These times were chosen to allow ample opportunity for the regenerative response to occur within an environment free of a demyelinating stimulus. The results indicate that in such circumstances, efficient remyelination occurs regardless of whether the region has undergone remyelination previously. A more severe challenge to the remyelinating process would occur if the region were exposed to demyelinating stimuli while remyelination was still in progress, where its repeated interruption may indeed then lead to remyelination failure. Such a situation is likely to occur in the chronic relapsing form of MBP-induced EAE augmented by MOG antibodies, where demyelinating stimuli may persist while remyelination takes place and are supplemented at ~3-week intervals (Linnington et al., 1992). More deleterious still would be a situation where there are attempts at remyelination in the face of a constant exposure to a demyelinating stimulus. As soon as new remyelinating oligodendrocytes are generated they would succumb to the demyelinating stimulus and thus lead to a relentless demand for the provision of new OPCs. Such a situation has been modelled in mice fed the demyelinating agent cuprizone over a prolonged period (6–7 months), a protocol that leads to sustained demyelination with very few oligodendrocytes and hence very little remyelination (Ludwin, 1980). However, whether the poor remyelination in chronic cuprizone is due to a reduction in OPCs has not been demonstrated and, despite the apparent ultrastructural normality of both the axons and astrocytes in the chronic cuprizone model (Ludwin, 1980), it may still be the case that the expression of remyelinating signals from both these cell types is altered. For example, the expression profile of secreted growth factors involved in remyelination by astrocytes, such as PFG or PDGF, may change without there being any histologically obvious change in their morphology (Redwine and Armstrong, 1998; Hinks and Franklin, 1999; Hinks and Franklin, 2000; Armstrong et al., 2002).

A confounding feature of models in which demyelinating agents are repeatedly or chronically administered is the effect on remyelination attributable to ageing. As animals age there is a profound decrease in the rate at which remyelination occurs (Gilson and Blakemore, 1993; Shields et al., 1999; Sim et al., 2000). This phenomenon is associated with an impairment of both OPC recruitment and differentiation, and not with an age-associated decline in the number of OPCs in normal white matter available for recruitment (Sim et al., 2002b). Experiments conducted over several months in rodents are of sufficient duration for there to be a significant slowing of remyelination rate as the animals age, and this alone may contribute to an apparent failure of remyelination, independent of the pattern or manner of demyelination.

The results of the present study challenge the concept that remyelination fails because of OPC depletion. Further evidence challenging this notion has come from studies on multiple sclerosis tissue, which indicate that areas of chronic demyelination can exist in which OPCs and immature oligodendrocytes can be detected (Scolding et al., 1998; Wolswijk, 1998; Chang et al., 2000, 2002). In these situations it would appear that remyelination may fail not as a result of a shortage of OPCs, but rather due to their inability to differentiate fully into myelin-forming cells. Whether this is due to the absence of differentiation inducing signals, the presence of differentiation inhibitors such as the notch-jagged signalling pathway (Wang et al., 1998; John et al., 2002) and PSA-NCAM (polysialylated neural cell adhesion molecule)
(Charles et al., 2002), or due to changes in the properties of demyelinated axons that render them incapable of being remyelinated (Chang et al., 2002) is not fully resolved.

A case can thus be made that the onus of blame for the eventual failure of remyelination lies more with the environmental signals to which the OPCs respond during remyelination than with the OPCs themselves. The environment that leads to remyelination is likely to have many diverse components, the timing and extent of their expression being critical for the success of the repair process. If these signals are inappropriately expressed—the dysregulation hypothesis of remyelination failure (Franklin, 2002)—then remyelination may fail. Recent years have seen some significant advances in the identity of growth factors critical for remyelination (reviewed in Franklin et al., 2001), although whether there is a therapeutic role for individual growth factors is not clear, contrasting results being obtained with different models and different routes of delivery (Yao et al., 1995; Cannella et al., 1998; Cannella et al., 2000; O'Leary et al., 2002). A significant development has been the identification of the pro-remyelinating role of the inflammatory response to demyelination (Ludwin, 1980; Graca and Blakemore, 1986; Warrington et al., 2001; Franklin et al., 2002). Depletion of macrophages or of key inflammatory cytokines such as interleukin-1β and tumour necrosis factor-α results in an impairment of remyelination following toxin-induced demyelination (Arnett et al., 2001; Kotter et al., 2001; Mason et al., 2001), while an impaired macrophage response in old animals compared with young following lysolecithin-induced demyelination is associated with less efficient remyelination (Hinks and Franklin, 2000). An argument has been made that in large demyelinated plaques in multiple sclerosis, which are many fold greater in volume than those that can be obtained in rodent models, then the abatement of the pro-remyelinating environment is more likely to occur before remyelination is complete, especially in those lesions in which OPCs are deficient (Blakemore et al., 2002). Identifying the key environmental signals for remyelination and those whose absence accounts for remyelination failure is thus important for developing therapeutic strategies by which remyelination can be enhanced.

Acknowledgements
The authors wish to thank Anil Kalupahana for technical assistance and Dr Chao Zhao for his help. This work was funded by the Wellcome Trust and Cambridge Neuroscience Inc., MA, USA.

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