*Commentary*

# Surprising Pleiotropy of Nerve Growth Factor in the Treatment of Experimental Autoimmune Encephalomyelitis

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The use of neurotrophic factors to treat multiple sclerosis (MS), a spontaneous inflammatory demyelinating disease of young adults, seems intuitively appealing: the process entails death of cellular constituents of the central nervous system (CNS), including oligodendroglia and neurons as well as damage to myelin (the hallmark of MS). It appears that demise of these neural cells, some of which are considered irreplaceable, results in part from the consequences of chronic inflammatory destruction of the myelin membrane. This conclusion comes from several lines of evidence: most persuasive is the axonal pathology observed in mutant mice deficient for the proteolipid protein of CNS myelin (1). By analogy, the ruin of demyelinated axons in the CNS of patients with MS is attributed in part to loss of the protective and trophic influences of myelin itself (2, 3). It also appears likely that oligodendroglia, challenged to remyelinate repeatedly within the inflammatory microenvironment of the MS lesion, undergo extensive cell death (4). Therefore, remyelination is viewed as a critical neuroprotective event, protecting axons from lysis and restoring normal oligodendrocyte physiology.

Furthermore, remyelination, different from other forms of CNS regeneration in mammals, seems tantalizingly feasible: it has long been known that remyelination of denuded axons can be vigorous and effective in the early phases of MS (5). Remyelination gives rise to structures termed "shadow plaques" in which axons are ensheathed by shortened, thinned myelin internodes that are nonetheless highly functional. The failure of remyelination during chronic MS has been attributed to insufficient numbers or impaired differentiation of resident oligodendrocyte precursors.

Work in vitro and in vivo has produced a substantial body of knowledge of the growth factors required for proliferation and differentiation of oligodendroglia. Not surprisingly, many of the trophic factors (including nerve growth factor [NGF], ciliary neurotrophic factor, platelet-derived growth factor, basic fibroblast growth factor, neurotrophin-3, and insulin-like growth factor [IGF]-1]) that support oligodendrocyte survival or proliferation also act towards subpopulations of neurons. It could be hoped that provision of neural growth factors to the CNS under inflammatory demyelinative siege could aid both oligodendroglia and neurons to survive.

In this context, preclinical treatment trials of various neurotrophins have been undertaken. In most cases, the hypothesis to be tested was that trophic support for oligodendroglia or their progenitors might promote remyelination. In the main, these studies were conducted in rodent models of MS, including experimental autoimmune encephalomyelitis (EAE) and virus-induced demyelination, caused by Theiler's murine encephalomyelitis virus (TMEV). It has been a formidable technical challenge to demonstrate that individual factors could promote remyelination in these systems. In part, the reason for this difficulty lies in the sharing of growth factors and/or cytokines between the immune and nervous systems. Therefore, agents that are predicted to act solely on neurons or oligodendroglia frequently exhibit immunomodulatory activities as well. This attribute of the protein factors studied in parallel by immunologists and neurobiologists has been intriguing and perplexing experimentalists for more than a decade.

There is another level of complexity imposed by the multistage disease process of EAE, during which demyelination (destruction) is succeeded by remyelination (tissue repair). Remyelination, as quantitated at the endpoint of an EAE experiment, reflects the aggregate of destruction and repair. Therefore, it can be impossible to disentangle the restraint of inflammation from the promotion of remyelination or other forms of tissue repair. In a head-to-head comparison, two agents, one of which produces a purely antiinflammatory effect while the other exhibits solely remyelinative properties, may pari passu generate identical net increases in remyelination.

An appreciation of the challenges that complicate experimental use of neurotrophins in EAE can be obtained by reviewing the results of studies using IGF-1 to treat this model disease. IGF-1 has many attributes of a promising remyelinative agent: it is expressed vigorously and early in detergent-mediated models of demyelination, before the onset of remyelination (6). Further, IGF-1 promotes remyelination in organotypic neural cultures in vitro after myelin

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J. Exp. Med. © The Rockefeller University Press • 0022-1007/2000/05/1625/06 $5.00
Volume 191, Number 10, May 15, 2000 1625–1629
http://www.jem.org/cgi/current/full/191/10/1625

lysis, mediated by antibodies and complement (7). Upon administration to Lewis rats with acute passive-transfer EAE, IGF-1 produced beneficial histological and clinical outcomes that were initially proposed to result from enhanced remyelination (8). However, further analysis indicated that effects on the inflammatory component of acute EAE, rather than myelin repair, determined the therapeutic benefit of IGF-1 administration (9). Subsequent experiments in chronic murine EAE also demonstrated benefits that appeared to result from reduced inflammatory tissue injury (10). Somewhat disconcertingly, it was recently shown that minor variations in timing or dose of IGF-1 could switch results of treating mice with chronic EAE from beneficial to deleterious outcomes (11).

Villoslada et al. in this issue provide evidence that continuous intracerebroventricular (ICV) infusions of recombinant human NGF reduced the severity of EAE in nonhuman primates (12). Use of the marmoset model for these studies imposes both advantages and disadvantages for generalizing the results and is worthy of comment. The use of the New World primate species *Callithrix jacchus* in EAE was developed by Hauser and Genain during the past decade, and the model has been continuously refined as its attributes emerged (13). Evident benefits of the model included salient similarities to MS: chronicity with relapse, primary inflammatory demyelination, and changes on magnetic resonance imaging (MRI) brain scans. The naturally occurring bone marrow chimerism between littermate

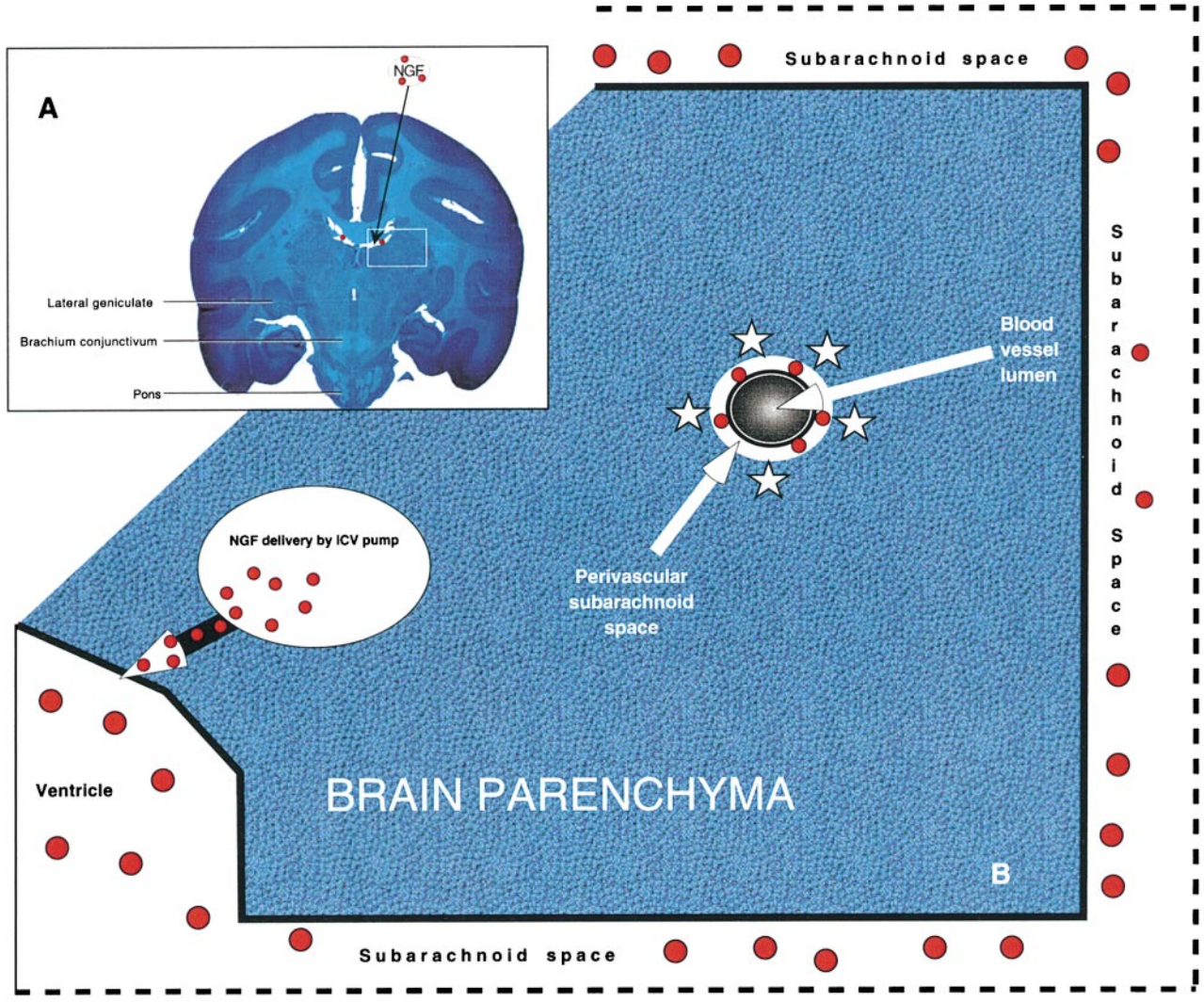


**Figure 1.** Distribution of NGF after ICV administration. (A) A coronal section of marmoset brain at the level of the thalamus, stained with methylene blue. Indicated for localization are the lateral geniculate nucleus, the brachium conjunctivum, and the pons. The arrow shows the trajectory of the ICV cannula, terminating in the lateral ventricle. NGF (filled red circles) is delivered from the pump into the ventricle. The box indicates the region shown in the cartoon in B. (B) The brain parenchyma is shown in blue, with NGF shown as filled red circles. Astrocytes are denoted by stars. After ICV administration, NGF distributes in the subarachnoid space: the ventricle, the subarachnoid space over the cerebral convexities, and the perivascular subarachnoid space, each of which is labeled. The pial surface (between brain parenchyma and subarachnoid space) is shown as a heavy solid line; the arachnoid membrane is shown as a dashed line.

marmosets (fraternal twins that share placental circulatory support in utero) provided the opportunity for adoptive transfer studies in an outbred species (14). With persistent study, it became clear that myelin oligodendroglial glycoprotein (MOG), a minor myelin constituent, was a major T cell and B cell target of the encephalitogenic process in this species, as is currently suspected in human MS (15). There are also several problems with this model: because of their expense and scarcity, marmosets are not used for EAE experiments in the numbers with which rodent EAE experimentalists are familiar. Thus, statistical power may be lost, and in-depth follow-up mechanistic studies are not feasible. Another less obvious obstacle is that, perhaps contrary to expectations, adult marmosets weigh between 250 and 500 g. For that reason, limited analysis of blood and cerebrospinal fluid (CSF) from these animals can be performed. Obviously, immunological reagents that are prepared for analyzing human material often, but not always, function well in marmoset studies (*C. jacchus* are New World monkeys, unlike *Homo sapiens sapiens*). However, if anti–human antibodies don't detect marmoset determinants, limited alternatives are available. Genetic manipulations, so convenient and frequently informative in mice, are not practical in marmoset EAE experiments. Perhaps the most significant benefit of EAE studies in nonhuman primates concerns the direct application of reagents that are targeted against human receptors (16).

The current study (12) was founded on the hypothesis that neurotrophin treatment could improve remyelination, and thus clinical recovery. The early onset of the treatment effect and its antiinflammatory mechanism were both unexpected. These unforeseen results could open new vistas on the analysis and treatment of immunopathological diseases of the CNS, including most prominently MS.

Certainly, the results reported by Villoslada et al. (12) demonstrated impressive and convincing benefit from NGF administration. Receiving agent in a preventive fashion (before the onset of EAE), NGF-treated animals experienced delay in EAE onset and milder disease; one marmoset was completely protected from clinical disease while showing modest histological inflammation. Five of the six treated animals fared better than any of the controls. The immunological effects of NGF in this model were fascinating: there was no effect on priming of antigen-specific, encephalitogenic T cells in vitro or in vivo and no change in production of MOG-specific antibodies. However, NGF-treated marmosets demonstrated a marked decrease in histological inflammatory scores and much less demyelination.

Importantly, inflammation and demyelination in both control and NGF-treated marmosets were concordant, arguing against an effect at the level of myelin repair. Taken together, these results suggested a change in the ability of primed T cells to orchestrate the CNS inflammatory response. To address the mechanism of this effect, tissue sections were analyzed for cells immunoreactive for IFN-γ or IL-10. There was a significant decrease in IFN-γ–immunoreactive cells and an increase in IL-10–producing cells. Perhaps most interesting, the cells expressing IL-10 were astrocytes, resident neuroepithelial elements. Accordingly, it appears likely that the beneficial effect of NGF treatment was mediated by eliciting an immunoregulatory response from neural cells. Also of note, astrocytes were previously shown to be the principal source of IL-10 in MS lesions, consistent with the possibility that astrocyte IL-10 represents a CNS-intrinsic mechanism for suppressing inflammation (17).

The site of action of NGF, which was given directly ICV, may also be addressed by these results. During continuous ICV administration, high concentrations of NGF would be present in the extracellular fluid of the perivascular subarachnoid space, which is in equilibrium with the ventricular CSF (see Fig. 1). Therefore, potent effects on perivascular T cells (such as the observed downregulation of IFN-γ) might be anticipated, given the expression of the high-affinity NGF receptor, trkA, by T cells (reference 18; see Fig. 2). It is also important to consider whether NGF, delivered ICV, might diffuse or be transported across the blood–brain barrier into the circulation, and achieve sufficient concentration to act on circulating cells.

Less secure would be the potential of NGF to reach the cells of the CNS parenchyma. It appears possible, although far from certain, that the high expression of IL-10 by parenchymal astrocytes was driven directly by NGF, suggesting an effect on cells distant from the site of delivery. The ability of NGF to stimulate astroglial production of IL-10 in vitro has not been addressed. However, it has been reported that IL-10 treatment enhances NGF production by astrocytes in culture, opening the possibility of an autoregulatory feed-forward circuit (19).

If NGF did not directly elicit IL-10 production by astrocytes, how else could one explain this observation? One alternative possibility is that NGF acted on mast cells that are bathed by CSF within the subarachnoid space, thereby eliciting immunosuppressive mediators such as prostanoids (reference 20; see Fig. 2). Mast cells are an established target for NGF in the CNS, and in some reports, the response of these enigmatic cells is to produce immunoregulatory secreted factors, which could stimulate nearby parenchymal cells (21–23). Regardless of the detailed mechanism, it is highly intriguing to consider that NGF administration rendered the CNS nonpermissive for development of immune-mediated demyelinative lesions, by inducing local mechanisms of restraining inflammatory reactions.

The implications of this study (12) clearly extend our knowledge of the biology of NGF in CNS inflammation, while posing new questions. Previous demonstrations that NGF was present in the tissues of humans with MS and rodents with EAE led to divergent conclusions, ranging from speculation that NGF was a contributor to the inflammatory pathology to conjecture that recovery from demyelination was attributable to NGF upregulation (24–27). In the event, it seems that none of these formulations would have predicted the results of treatment with NGF, sounding a precautionary note for MS researchers.

Given these caveats, what are the practical lessons for possible clinical application of NGF to be drawn from the

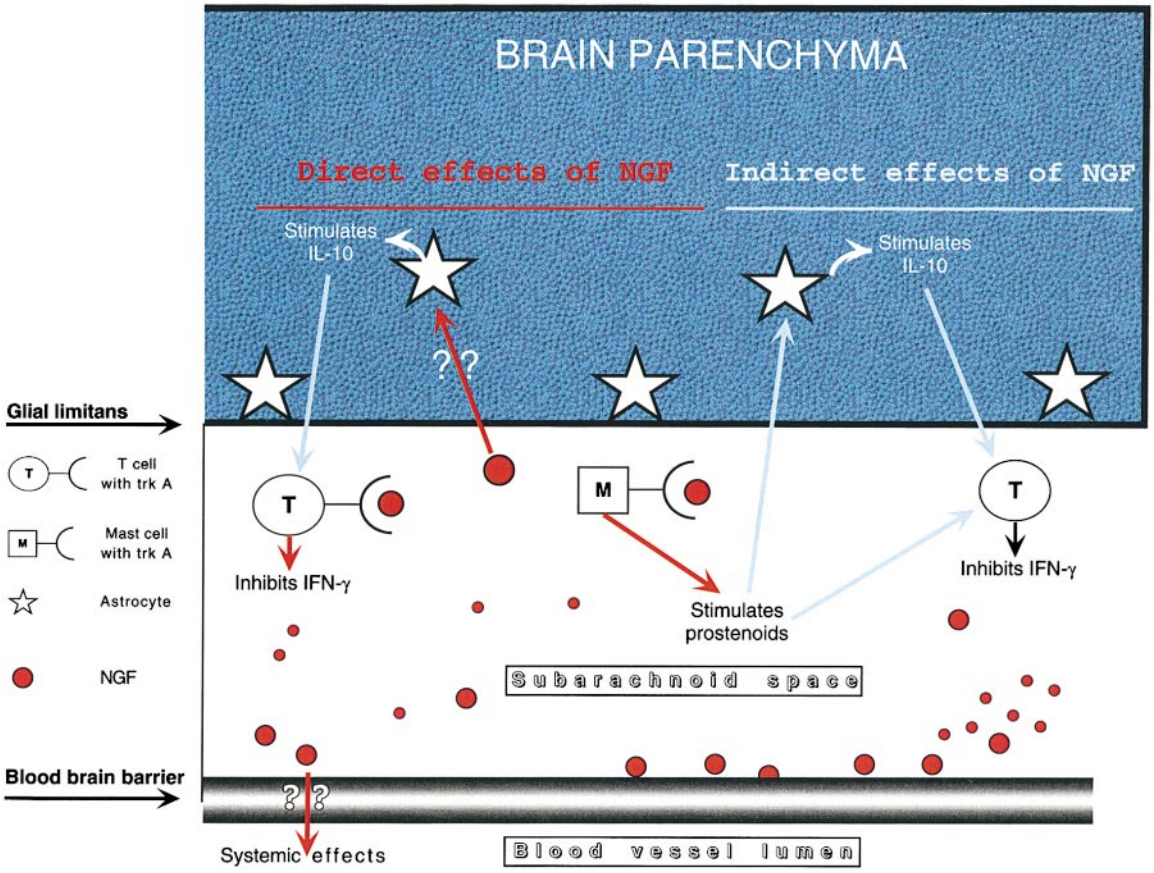


**Figure 2.** ICV NGF: potential sites of action in EAE. Shown are the brain parenchyma (blue), the perivascular subarachnoid space, and the glial limitans (a network of astrocyte processes), which separates the two compartments. The subarachnoid space is delimited from the blood vessel lumen by the blood–brain barrier. At left are the symbols for T cells (open circles), mast cells (open squares), and astrocytes (stars). Direct and indirect actions of NGF (filled red circles) are shown on the left and right, respectively. NGF may potentially act directly (red arrows) on all receptor-bearing cells within the subarachnoid compartment, including T cells and mast cells, with consequences as shown. Possibly (red arrows surrounded by question marks), NGF could act on parenchymal CNS cells or on circulating leukocytes. Thus, increased production of IL-10 by astrocytes (open stars) within the brain parenchyma could be a direct effect of the NGF, or secondary to stimulation (light blue filled arrow) by products elicited from mast cells. Suppression of T cell IFN-γ expression may be secondary to astrocyte-derived IL-10, mast cell products, or due to direct action of NGF on T cells (red arrow).

report by Villoslada et al. (12)? First, it should be determined if systemic administration of NGF (already used in a short trial for diabetic neuropathy [28]) can generate beneficial outcomes of this magnitude in the marmoset model. This will be an important distinction: MS typically becomes symptomatic at about age 30, and many experts currently favor early and continuous treatment thereafter for life. In that context, ICV infusions are unlikely to be feasible for the majority of MS patients. It bears recalling that IFN-β was initially shown to be effective in MS by the intrathecal route and subsequently demonstrated to have equal efficacy when delivered by peripheral injections. However, if systemic injections of NGF prove to be ineffective in modifying the course of EAE, other "blue sky" alternatives may become more attractive. Thus, this report may provide additional impetus to use gene therapy to deliver factors such as NGF in CNS demyelinating diseases, via genetically modified T cells that recognize CNS determinants and produce NGF or other factors upon antigen encounter (29, 30). Second, and concurrently, it will probably be determined if the proposed cellular mechanisms of the NGF-mediated treatment effect can be demonstrated in vitro (inducing IL-10 expression by astrocytes and blocking IFN-γ production by primed antigen-stimulated T cells). Certainly, concerns that engagement of the p75 low-affinity NGF receptor could deliver death signals to oligodendrocytes, raised by results of in vitro studies, will need to be resolved (31, 32). If questions about long-term safety, efficacy, and tolerability of NGF treatment of humans are resolved satisfactorily, then the report by Villoslada et al. (12) may represent a landmark in defining new therapeutic strategies for MS.

*Received: 15 March 2000*
*Accepted: 3 April 2000*

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