Growth factor treatment of demyelinating disease: at last, a leap into the light

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Researchers seeking treatments for multiple sclerosis (MS) have long dreamed of using neurotrophic factors to enhance remyelination. Previous attempts to apply trophic support for oligodendrocytes in experimental demyelination uniformly produced complicated outcomes that reflected unexpected effects on immune or inflammatory responses and could be interpreted only with caution. Now, two recent publications have demonstrated convincingly that cytokines of the interleukin (IL)-6 superfamily can ameliorate experimental autoimmune encephalomyelitis and promote oligodendrocyte survival, without demonstrable effect on inflammation or immune responses.

'All growth (factor treatment for demyelinating disease) is a leap in the dark, a spontaneous unpremeditated act without benefit of experience.' pace Henry Miller, The Wisdom of the Heart (1947)

Inflammatory demyelinating diseases of the human central nervous system (CNS) such as multiple sclerosis (MS) harm delicate neural elements including myelin, oligodendrocytes, axons and neurons [1]. Neurotrophins, including the nerve growth factors and neuropoietic cytokines, comprise a heterogeneous group of protein factors that support proliferation or survival of CNS cells. MS researchers have long dreamed of using these powerful agents to enhance remyelination, which is often vigorous early in the disease but ultimately fails for unknown reasons. The major workhorse of MS preclinical therapeutics has been the model disorder experimental autoimmune encephalomyelitis (EAE). All previous attempts at trophic support for oligodendrocytes in EAE produced complicated outcomes that reflected unexpected effects on the inflammatory response and could be interpreted only with caution. Two recent publications have demonstrated convincingly that cytokine members of the interleukin (IL)-6 superfamily can ameliorate EAE and promote oligodendrocyte survival, without demonstrable effect on inflammation or immune responses [2,3]. Preliminary studies oo a small cohort of MS patients lacking functional genes encoding ciliary neurotrophic factor (CNTF), one protein studied in these experiments, supported the relevance of these results for human MS [4]. These findings, for the first time, indicate persuasively that provision of growth factor-mediated trophic support for oligodendrocytes can modulate the outcome of inflammatory demyelination.

Axonal degeneration in MS lesions

MS, a disease of unknown cause, is characterized by a unique tissue pathology featuring inflammatory mononuclear infiltrates, primary demyelination, glial reaction and axonal degeneration (for review, see [5]). Although it has been clear for more than a century that axons are destroyed during the MS disease process, contemporary quantitative microscopy revealed the extent of axon loss in both acute and chronic lesions. As CNS axons are non-regenerating, it was a loud tocsin when recent reports showed continuous destruction of axons in persistently demyelinated MS lesions, and it is now considered highly likely that this slow, unremitting axonal degeneration is a crucial factor in the relentless symptoms of progressive MS, which is the most disabling feature of the disease.

The flip-side to this rather bleak picture of axonal degeneration in MS lesions was that remyelination appeared to be completely protective. Classical neuropathological studies of MS patients who died shortly after onset revealed abundant remyelination [6], indicating that this type of tissue repair is clearly feasible in the human CNS; the great conundrum in this field is why remyelination fails as the disease continues. A complete account of the reasons for failure of spontaneous remyelination in MS remains elusive. Potential causes include limiting numbers of oligodendrocytes and their progenitors; dense astroglial scar; nonpermissive axonal substrate [7]; or adverse environmental factors.

Oligodendrocyte cell death

It is evident that without oligodendrocytes, the CNS myelinating cells, remyelination cannot occur. The extent and mechanisms of oligodendrocyte cell death in MS lesions is controversial [8]. Such disagreement is perhaps unavoidable, given the extensive heterogeneity of lesion character, differing investigative strategies and wide biological variability of the disease. One scheme recently proposed for categorizing MS lesions focuses on forms of the disease that either do or do not exhibit extensive oligodendrocyte apoptosis. The pathological hallmark of one major lesion type that features oligodendrocyte apoptosis is the selective destruction of the periaxonal myelin-associated glycoprotein (MAG)-containing processes of oligodendrocytes [9,10]. A plausible cause for this distinctive pathological pattern is that oligodendrocyte cell injury, producing a 'dying-back oligodendrogliopathy', lies at the core of this form of MS, which constituted about 25% of all cases in one carefully characterized autopsy and biopsy series [11]. In this series, and other detailed investigations, the remainder of cases (>60%) exhibited pathological changes consistent with immune-mediated inflammatory demyelination [12]. Taking all relevant observations into account, it seems most likely that mature oligodendrocytes are tremendously reduced in chronic, fully demyelinated lesions; that oligodendrocyte progenitors persist in many of these chronic lesions; and that some degree of apoptotic

oligodendrocyte cell death occurs during the active phase of formation of new lesions. The relevance of these reports for the new publications is twofold. First, it seems clear that persistently demyelinated CNS axons exhibit reduced viability. Therefore, remyelination is perhaps the single most potent neuroprotective therapy that could be applied to ameliorate disability in MS. Second, if there are different pathological subtypes of MS, a diversity of remyelination strategies might be required.

Oligodendrocyte growth factors

What is known about oligodendrocyte growth factors? It is possible to generate cell cultures that recapitulate the development of the oligodendrocyte lineage [13] and these simplified systems have been used to uncover a substantial number of factors, which, alone or in combination, promote proliferation, survival or differentiation of oligodendroglial progenitors *in vitro*. Extensively characterized elements include: developmental factors such as sonic hedgehog; homeostatic endocrine factors such as the thyroid hormones; pleiotropic growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF)-2, and insulin like growth factor (IGF)-1; retinoic acid; members of the neurotrophin (NT) family including nerve growth factor (NGF), NT-3 and -4/5; chemokines such as growth-regulated oncogene (GRO)-α/CXCL1; neuregulins such as glial cell growth factor (GGF)-2; and neuropoietin cytokines such as IL-6, ciliary neurotrophic factor (CNTF) and leukemia-inhibitory factor (LIF) ([14–16] and references therein). In many cases, transgenic or knockout mice exhibited phenotypes supporting the relevance of these findings for the developmental origin of the oligodendrocyte lineage.

Animated by such observations, and by the hope that tissue repair could recapitulate development, researchers attempted to modify outcomes of various models of CNS demyelination by administering such factors. The most frequently used model has been EAE, provoked by immunization with myelin proteins or derivative peptides, emulsified in adjuvants. Formidable technical problems confront investigators who attempt to modify remyelination during the evolution of EAE. First, it is difficult to quantitate myelin rescue that results from limiting destruction from that which arises from promoting repair. This problem is amplified by the unexpected fact that agents that might be predicted to act selectively on oligodendroglial progenitors also affect the function of the inflammatory/immune apparatus. The complex, pleiotropic effects of molecules such as NGF, IGF and GGF-2 in EAE exemplified these challenges, despite being deployed by experienced, expert and committed investigators [17–23]. Similarly, we noted some years ago that IL-6 administration provided clinical benefit associated with complex beneficial effects on antiviral responses, inflammation and tissue injury in a virus-induced model of demyelination [24].

Butzkueven and colleagues studied LIF in EAE [3]. They demonstrated moderate activity of LIF injections in reducing disease severity, in two different models of disease. Oligodendrocyte populations in both gray and white matter were reduced by about one-third in mice receiving control protein injections but maintained in mice that received LIF. The treatment worked equally well when given at the time of immunization or after onset of disease, arguing that the benefit acted by enhancing oligodendrocyte survival rather than reducing inflammation. Consistent with this proposal, inflammatory infiltrates were not reduced by LIF treatment and *in vitro* recall responses of T cells were unaffected in magnitude or quality by exposure to high concentrations of LIF. The investigators provided several lines of evidence that LIF acted directly on oligodendrocytes. Most importantly, the binding/signaling receptor subunit LIFRβ was expressed and activated in affected tissues and LIFRβ was expressed on oligodendrocytes. Of course, it remains plausible that factors which support remyelination and oligodendroglial survival exert their most important functions through effects on adult glial progenitors [25].

Linker *et al.* examined the role of CNTF [2]. Mice that lacked CNTF following targeted gene deletion developed EAE slightly later than their wild-type littermates but recovered much more slowly. The neuropathological characteristics of EAE in CNTF-deficient mice were delineated in detail: significantly more apoptotic oligodendrocytes were observed and myelin vacuolation was more severe in mutant mice with EAE, especially at late time points. Ultrastructural features of this pathology resembled those described in lesions of MS associated with the postulated 'dying-back oligodendrogliopathy'. The investigators noted similarities between the pathological changes in CNTF-null mice with EAE and transgenic mice that over-expressed tumor necrosis factor (TNF)-α in the CNS and also recalled previous *in vitro* studies demonstrating that CNTF could protect oligodendroglia from TNF-mediated apoptosis [26,27]. Therefore, anti-TNF-α antiserum was administered to $CNTF^{-/-}$ mice with established EAE, and this treatment reduced oligodendroglial pathology without suppressing ongoing inflammation. Interestingly, anti-TNF antibodies produced marked benefit for CNTF-null mice whereas wild-type mice exhibited slightly worse outcomes, as evidenced by increased oligodendrocyte cell death and aggravation of myelin vacuolation. Presumably, the anti-TNF antibodies impaired the process of apoptosis in autoaggressive T cells, an effect that outweighed any benefit for oligodendroglial survival in wild-type (but not CNTFR knockout) mice. Of course, selective alterations of type 2 TNF receptor expression on the oligodendrocytes [28] of CNTF-mutant mice could also produce this discrepant result and must be considered as this work is extended. This result is reminiscent of the deleterious effect of TNF sequestrant therapy in humans with MS and points to the urgent need for deeper insights into the pathogenetic variants of this disease [29].

Comparisons between the two studies are not straightforward, given the differing technical approaches employed. Importantly, both experiments unequivocally showed that members of the IL-6 family neuropoietic cytokines produce benefit in EAE. Furthermore, no apparent effect on either inflammation or immunity was noted, focusing attention on the neurobiological functions of these agents. Presence of either cytokine (LIF injections or $CNTF^{+/+}$ genetic background) significantly but incompletely promoted oligodendrocyte survival and clinical benefit. Evidence supporting LIF as a local oligodendroglial survival factor in mice with EAE was forthcoming from these studies. Although CNTF lacks a signal peptide and its mode of secretion is uncertain, the presence of the gene was required for timely recovery from EAE, implying this peptide also has an endogenous function. The function of the two factors in normal physiology has not been easy to unravel. Mice lacking either LIF or CNTF exhibited unimpressive phenotypes for motor neuron survival and development [30]. More remarkable still, approximately 2.5% of the Japanese population is homozygous for a null CNTF allele, without any obvious consequences for healthy development or susceptibility to disease [31]. Furthermore, it seems highly likely that the LIFRβ subunit is relevant to the effects of LIF and CNTF in EAE, as it is required for binding and signaling in response to both cytokines (Fig. 1) and is expressed by oligodendroglia in the inflamed CNS. It remains a matter of speculation whether LIF and CNTF exert redundant functions in EAE, and whether other members of this cytokine family could provide similar benefit.

The major, irreplaceable target of the MS disease process is the axon and the neuron that gives rise to it. In this regard, there is a substantial body of experimental data showing neuroprotective effects of IL-6-family members toward various populations of neurons, subjected to challenge in a diverse array of model diseases of the nervous system (Table 1). Furthermore, resident glia cells are the major source of IL-6-family cytokines in the inflamed CNS, a pattern that is also mimicked in the lesioned peripheral nervous system as well [32]. Very little is known of the regulation of these crucial factors in the CNS *in vivo;* in axotomized peripheral nerve it seems likely that IL-6 induces LIF [32].

Concluding remarks

The application of these new findings to human MS will now be a matter of great interest. Ideally, one would favor therapies that evade the complexity of injecting proteins; it would thus be very appealing to have detailed information about the post-receptor signaling events that support oligodendroglia survival in LIF-treated mice with EAE, for example. In simpler model systems, evidence has accumulated implicating the phosphoinositol 3-kinase/Akt or p42/44 mitogen-activated protein (MAP) kinase pathways as mediators of neurotrophic effects of the neuropoietins ([33]and references therein). It is possible that selective pharmacological manipulation of post-receptor signaling pathways in patients with MS could mediate protective effects towards oligodendrocytes. Favoring consideration of direct administration of these cytokines, it should be noted that both CNTF or closely related analogues (Regeneron Pharmaceuticals; http://www.regeneron.com/products/product_candidate.asp?v_c_id=6) and LIF (Amrad Corporation Ltd; http://www.amrad.com.au/AMRAD/News/News.asp?NID=90) have been administered to humans in well-executed clinical trials and that the side-effects of such treatments were tolerable. It is thus not impossible to envision clinical trials in MS patients arising from the current studies. Positive results would truly provide a leap into the light.

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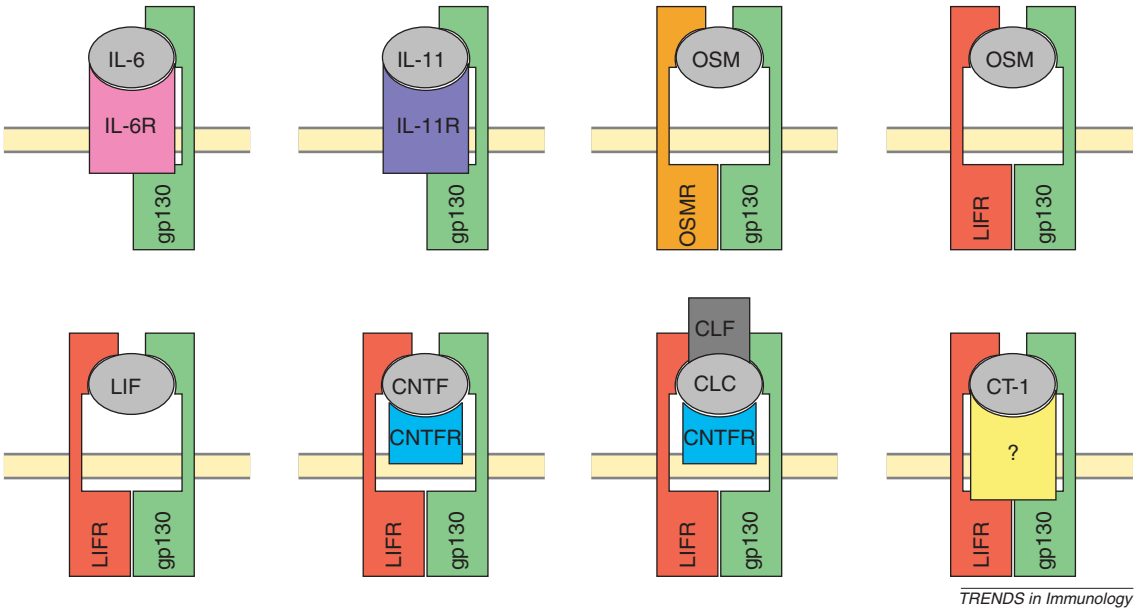

Fig. 1. The functional redundancy of the IL-6 cytokine family is explained by the modular nature of the receptors involved in binding the various family members. All members of the family bind to multimeric receptor complexes that share the signal-transducing gp130 receptor as a common subunit. Interleukin (IL)-6 and IL-11 bind to gp130 in complex with specific non-signal-transducing α chain receptors (Il-6R and Il-11R, respectively). By contrast, leukemia-inhibitory factor (LIF) binds to gp130 complexed with the LIF receptor β or the oncostatin M (OSM) receptor signal-transducing subunits. Ciliary neurotrophic factor (CNTF) binds to both gp130 and LIFRb, as well as to a non-signal-transducing α chain (CNTFR). Likewsie, cardiotrophin 1 (CT-1) binds a multimeric complex comprising gp130, LIFRb and an unidentified a chain subunit. Finally, the cardiotrophin-like cytokine (CLC), also known as novel neurotrophin 1, binds to the same multimeric receptor complex as CNTF, with the additional requirement of the soluble cytokine-like factor 1 (CLF) receptor.

Table 1. Neuroprotective effects of cytokines of the IL-6 family[a]

Protected neuron population	Cytokine	Refs
Motor neurons	IL-6 CNTF, LIF, CT-1, CLC-CLF	[34,35]
Cortical neurons	IL-6, CNTF	[34,35]
Cerebellar neurons	IL-6	[34]
Dorsal root ganglia neurons	IL-6, OSM, CT-1, IL-11	[34]
Nodose and trigeminal ganglia neurons	CNTF, OSM, LIF, CT-1	[35]
Ciliary ganglia neurons	CNTF, CT-1	[35]
Sympathetic neurons	CLC-CLF	[36]

[a]Abbreviations: CLC-CLF, cardiotrophin-like cytokine and cytokine-like factor 1 soluble receptor; CNTF, ciliary neurotrophic factor; CT, cardiotrophin; IL, interleukin;. LIF, leukemia-inhibitory factor; OSM, oncostatin M.