PERSPECTIVE

Interferon beta (IFN-β) treatment exerts potential neuroprotective effects through neurotrophic factors and novel neurotensin/neurotensin high affinity receptor 1 pathway

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by coexisting processes of inflammation, demyelination, axonal neurodegeneration, and gliosis. It is the most common disabling neurological disease in young adulthood. Although autoimmune inflammation contributes to axonal pathology and demyelination, more recent evidence suggests that inflammation may also be beneficial. Protective autoimmunity is partly mediated *via* neurotrophic factors and neurotrophin regulation. Elevated neurotrophin levels have been observed during inflammatory processes, in MS lesions, whole peripheral blood mononuclear cells (PBMCs), serum, and cerebrospinal fluid (CSF). Furthermore, we recently demonstrated that T cells of MS patients produce neurotrophins (Soltys et al., 2014).

Neurotrophins and neurotrophic cytokines (*e.g.*, interleukin 6 [IL-6], ciliary neurotrophic factor [CNTF], nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], neurotrophin-3 [NT-3]) exert their biological effects through interaction with membrane receptors to activate intracellular signal cascades resulting in a spectrum of effects. Neurotrophins and neurotrophic cytokines that regulate neuronal and oligodendroctye development are deficient in MS. Neurotrophins include prototype NGF family, glia cell line-derived neurotrophic factor (GDNF) family and other neurophins, as shown in **Table 1**. The immune system may be modulated by neurotrophins and neurotrophic factors. Most members of the neurotrophin family (BDNF, NGF, GDNF, neurotrophin NT3, neurotrophin NT4) can be produced and secreted by lymphocytes and monocytes. Antigen activation significantly increases neurotrophin secretion by lymphocytes, suggesting a possible role in protective autoimmunity. In turn, immune cells seem to be the target of neurotrophin autocrine and paracrine actions. Neurotrophins also act as neuroprotective mediators in CNS injury, indicating interactions between the immune cells and nervous systems. Neurotrophins are able to promote neuronal survival and differentiation and prevent neural death favoring the recovery process including neural regeneration and remyelination.

Interferon beta (IFN-β) is a widely used disease-modifying therapy in relapsing remitting MS (RRMS). The mechanism(s) of action of IFN-β are not completely clear, though it has been shown to significantly reduce relapse rate and the appearance of new lesions. The peripheral immune modulating effect of IFN-β is better understood; whether it plays a role in neuroprotection is more debatable. Caggiula et al. (2006) found no differences in NGF, GDNF, or neurotrophin NT3/NT4 production among RRMS or SPMS patients compared to the healthy controls in their PBMCs. However, they found a significant increase in BDNF production 6 months after starting IFN-β therapy in RRMS patients who did not present clinical exacerbation of disease up to the end of the study.

Since MS disease progression is associated with neurodegeneration, axonal loss, promoting NPC survival and/or differentiation may be helpful in neuroregeneration and slowing MS disease progression. NPCs can differentiate into all neural lineage cells, which could contribute to the remyelination, neuroregeneration, and repair of MS lesions. IFN-β is known as an immunomodulator, but its effects on NPCs in the CNS are not clear. Therefore we first investigated whether IFN-β has direct neuroprotective effects on NPCs. We found that treatment of mouse NPCs *in vitro* with IFN-β increased their survival and decreased apoptosis. However, there was no effect on differentiation or proliferation. The null effect on differentiation or proliferation could be due to the fact that IFN-β is a cytokine but not a mitogen or growth factor. Furthermore, we demonstrated that IFN-β treatment upregulated the signal transducer and activator of transcription 1/2 (STAT 1/2) signaling pathway, GDNF family receptor alpha-2 (GFRα2), nucleotide-binding oligomerization domain-containing protein 1 (NOD1), caspases 1/12, tumor necrosis factor (ligand) superfamily, member 10 (TNFSF10), and GDNF. These results provided a novel mechanism by which IFN-β can directly affect NPC survival, possibly playing a neuroprotective role in the CNS by modulating neurotrophic factors in addition to the peripheral immunomodulatory effect (Hirsch et al., 2009). We further tested the effects of IFN-β on human NPCs (hNPCs) *in vitro* and demonstrated for the first time a direct effect of IFN-β-1b treatment on modulating hNPCs proliferation and differentiation, in contrast to our result in the murine NPCs system. This highlights the differences between murine and human NPCs and the need for further characterization of the effects of drug treatment on various NPCs physiology. Giving the finding that IFN-β-1b sustains hNPCs proliferation, protects cells from apoptosis, and promotes differentiation in a concentration-dependent fashion, our findings support the hypothesis that therapeutic strategies that enhanced IFN-β-1b delivery directly to the CNS would be beneficial (Arscott et al., 2011). Using "Neurotrophins and Receptor Pathway" RT^2 Profiler^{TM} PCR array in hNPCs, we also showed that IFN-β induced the expression of a novel neurotensin high affinity receptor 1 (NTSR1) (Arscott et al., 2011).

As discussed earlier, inflammation can provide neuroprotective effects *via* altered cytokine/neurotrophin homeostasis in RRMS, as increased neurotrophin production in inflammatory infiltrates during EAE and in MS patients were reported. A significant number of immune cells containing BDNF were detected in actively demyelinating areas of MS lesions, and increases of NGF and CNTF levels in cerebrospinal fluid (CSF) were demonstrated during relapse and recovery phases, respectively. BDNF production by PBMCs in MS patients is higher during relapse and in the recovery phase, as compared with values detected in the stable phase of the disease. Caggiula et al. (2005) have measured the production of neurotrophins (BDNF, NGF, GDNF, NT3 and NT4), proinflammatory cytokine tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) by PBMCs in RRMS patients at different phases of disease (stable, relapse and postrelapse), and correlated the clinical recovery with different neurotrophic factor levels. They found that during the acute relapsing phase of the disease, considerable increases of BDNF, TNF-α, and IFN-γ production were detected, while significantly higher levels of GDNF, NGF, neurotrophin NT3 and NT4 were found during the post-relapse phase in subjects with complete remission only. The neuroprotective potential of immune cells seems to be inversely correlated with disease duration and with the age of patients. These suggest that the distinct neurotrophin production from specific cell populations could perhaps provide better understanding of the complex regulation in MS pathogenesis.

Previous reports from Caggiula et al. (2005, 2006) used MS patient PBMCs. Taking a step further, we utilized broader unbiased SuperArray gene screen technology to examine the neurotrophin gene expression of purified T cells from MS patients. Since T cells are the main drivers for MS disease and a main mechanism of action of IFN-β is to reduce the level of T cell activation thereby decreasing inflammation. Investigating how IFN-β modulates T cell neurotrophin expression therefore represents a novel approach to exploit key factors that are functioning to reduce inflammation as well as promote neuroprotection during RRMS inflammatory attacks. In T cells isolated from RRMS patients with or without IFN-β treatment, we found that IFN-β induced anti-inflammatory cytokine expression and upregulated the expression of neurotensin high affinity receptor 1 (NTSR1). NTSR1 is expressed in active demyelinating lesions (Soltys et al., 2014). NTSR1 belongs to the large superfamily of G-protein (Gq) coupled receptors, and its agonist neurotensin (NT) is a 13-amino acid endogenous neuropeptide involved in regulation of immunity and inflammation. Neurotensin and its receptors are expressed by T lymphocytes, macrophages and dendritic cells. The peptide is involved in regulation of cytokine production, neutrophil chemotaxis, nitric oxide (NO) generation and mast cell activation (Saada et al., 2012). Furthermore, we demonstrated that neurotensin (NT) was a potent inducer of human neural stem/progenitor cell survival, indicating its therapeutic potential for neuroregeneration (Soltys et al., 2014). Expanding these findings here, we also demonstrated that neurotensin, but not IL-10 (as an anti-inflammatory cytokine), promoted hNPC survival. Other report in literature showed that IL-10 and BDNF levels in monocytes were high during RRMS, but low in progressive MS and T cells produced less IL-10 under IFN-β therapy (Hamanicioglu and Reder, 2007). In contrast, we did not observe BDNF nor IL-10 gene expression in T cells of MS patient. Collectively, our critical finding is the neurotrophic capabilities of the NT/NTSR1 pathway which may play novel roles in T cell and NPC interactions. Understanding how the NT/NTSR1 system is involved in this feedback loop may reveal additional

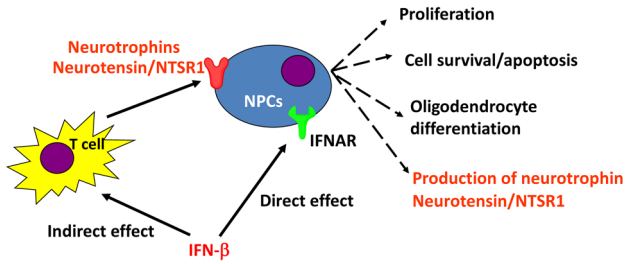

Figure 1 Model of neuroprotective effects of IFN-β on NPCs: neurotrophic capabilities of the neurotensin/NTSR1 pathway.
IFN-β treatment exerts both direct and indirect effects on NPCs. The direct effect is through the IFNAR receptor and the indirect effect is through T cells for specific neurotrophins and neurotensin/NTSR1-mediated cross-talk between immune (T cells) and CNS (NPCs) cells. IFN-β plays important roles in balancing immunomodulation and neuroprotection through NT/NTSR1 signaling as well as other neurotrophin receptors in RRMS. IFNAR: Interferon-α/β receptor; IFN-β: interferon beta; NPCs: neural progenitor cells; NT: neurotensin; NTSR1: neurotensin high affinity receptor 1.

insights into MS pathogenesis.

IFN-β appears to directly increase expression and concentration of anti-inflammatory cytokines while downregulate the expression of proinflammatory cytokines in MS. It may also reduce the trafficking of inflammatory cells across the blood-brain barrier (BBB) (Kochanowski et al., 2015). Most recently, additional mechanisms by which type I-interferons ameliorate MS have been reported. Interleukin 27 is an important regulator of inflammation through the induction of regulatory Tr1 cells, as well as a suppressor of Th17-cell development (El-Behi et al., 2014). IFN-β, IL-27, and IL-10 exert a range of similar immunoregulatory effects in murine and human experimental systems (Fitzgerald et al., 2013). IFN-β drives IL-27 production in activated monocytes and dendritic cells (DCs). IFN-β and IL-27 both induce human IL-10 and suppress human Th17 responses. In addition, dendritic cells (DCs) control the balance between effector T cells and regulatory T cells *in vivo*. IL-27 signaling in mouse DCs limited the generation of effector cells of the Th1 and Th17 subsets of helper T cells indicating that IL-27 signaling in DCs limited pathogenic T cell responses and the development of autoimmunity (Mascanfroni et al., 2013).

Similar to IFN-β, glatiramer acetate (GA, copaxone) is another first line disease modifying drug available to treat MS. Accumulated evidence from EAE model and MS patients indicates that GA affects various levels of the innate and the adaptive immune response, generating deviation from the pro-inflammatory to the anti-inflammatory pathway. GA has *in situ* immunomodulatory effect and its ability to generate neuroprotective repair in the CNS (Aharoni, 2013). GA-specific T cells did express BDNF in the brain and GA can restore impaired expressions of BDNF, NT-3, NT-4 and IGF-2 in MS patients supporting the importance of neurotrophic factors in MS treatment.

Our studies support that increased production of neurotrophic factors from T cells in MS patients and human NPCs could be a potential mechanism in neuronal survival and repair. Interestingly, the most prominent neurotrophin changes in our study were generally observed on receptors, rather than an soluble ligand. We hypothesize that the critical neurotrophic receptors that promote immune cell regulation or neural protection in RRMS may always have some lower level of expression/activity. When this activity on CNS and immune cells eventually exhausts, the delicate balance between inflammation and neuroprotection is lost and a more progressive disease course ensues. Thus, IFN-β may upregulate these factors to prolong their time for depletion. In our model (**Figure 1**), IFN-β treatment exhibits the direct neuroprotective effect through the interferon-α/β receptor (IFNAR). The indirect effect could be mediated through T cells through specific neurotrophins and neurotensin/NTSR1-faciliated cross-talk between immune (T cells) and CNS (NPCs) cells. Our findings support that neurotensin/NTSR1 signaling pathway as well as other neurotrophin receptors are important in immunomodulation and neuroprotection in RRMS. Ongoing and future studies on neurotrophins and neurotensin/NTSR1 pathways will further increase our understanding of the actions of IFN-β on the immune system as well as the CNS, which will in turn aid advances in the treatment of MS.

Table 1 Classic neurotrophin families and their receptors

	Family I	Family II	Family III
Family name	Nerve growth factors (NGFs)	Glial cell line-derived neurotrophic factor family ligands (GFLs)	Neuropoietic cytokines
Ligand	NGF, BDNF, NT3, NT4/5	GDNG, GDNF, neurturin, artemin, persephin	CNTF, LIF
Receptor	TrkA/B/C, $p75^{NTR}$	Ret receptor tyrosine kinase	CNTFR-α, gp130/LIFR-β

BDNF: Brain-derived neurotrophic factor; CNTF: ciliary neurotrophic factor; LIF: leukemia inhibitory factor; NT3: neurotrophin 3; $p75^{NTR}$: p75 neurotrophin receptor; TrkA/B/C: tropomyosin receptor kinase A/B/C.

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