Neuroprotective Effects of Interferon-β in Multiple Sclerosis

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1. IMMUNE DYSREGULATION IN MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is considered an immune-mediated, demyelinating, and degenerative disease of the central nervous system (CNS). The immune-mediated pathogenesis is supported by the significant infiltration of leukocytes into the CNS parenchyma and by the finding of a substantial similarity between active MS plaques and lesions in the CNS of animals with experimental autoimmune encephalomyelitis (EAE); EAE is produced by an autoreactive T-cell response. The favorable response of many MS patients to immunomodulatory drugs, including use of β interferons (IFNs) and glatiramer acetate to decrease the number of relapses and reduce magnetic resonance imaging (MRI) disease activity (1–4), also corroborates the hypothesis of immune-mediated injury.

The pathology of MS lesions provides clues to the important role of T cells in the disease. Active MS lesions are characterized by perivascular lymphocyte infiltration that spreads into the parenchyma. A prevailing hypothesis of MS pathogenesis is that T cells are activated in the periphery by unknown antigens (5,6) and both myelin and nonmyelin antigens may be involved (7–9). Although auto-antigen specific T cells are present in the immune system of MS patients, they are also found in healthy people, leading to the concept that these cells need to be activated to become mediators of disease. Different mechanisms have been suggested for the activation of autoreactive T cells, such as molecular mimicry, epitope spreading, mistaken self- and bystander activation (Fig. 1).

Molecular mimicry describes conformational similarities between epitopes of myelin proteins and epitopes of foreign antigens (e.g., viral or bacterial origin). For this reason, T cells would recognize self-antigen as non-self. More recently, it has been reported that structural similarities between different proteins of the major histocompatibility complex (MHC) human leukocyte antigen class II, one recognizing the foreign antigen and the other a self-antigen, can induce an autoimmune response (10). Another proposed mechanism of T-cell activation is epitope spreading; inflammation and tissue destruction would induce antigen presentation of previously hidden epitopes and cause the activation of T cells of different specificities. In this way, the immune response spreads from the first self-antigen to others that induce their own immune responses. Although epitope spreading is documented in EAE, there is still little evidence that this process occurs in MS (11).

In addition to epitope spreading, the same inflammatory environment, because of the high concentration of cytokines, can induce activation of immune cells per se by a mechanism called...
bystander activation. Others have suggested the possibility of a unique MS response called “mistaken self,” in which microbial infection of immune cells induces a T-cell response not only against the infective agent but also against a stress protein called B-crystallin, which is expressed in infected immune cells (12); however, this model has not been fully proven in animal studies, and, because of the wide distribution of B-crystallin in human tissues, does not fully explain the organ-specific response in MS.

When activated, T cells cross the blood–brain barrier (BBB) into the CNS parenchyma, where they accumulate and proliferate in response to antigen restimulation (13–15). These activated T cells secrete proinflammatory cytokines that activate resident microglia, infiltrating macrophages and B cells. This inflammatory response is thought to damage axons and myelin through antigen-specific or nonantigen-specific mechanisms.

2. NEURODEGENERATION IN MULTIPLE SCLEROSIS

Although MS is described as a demyelinating disease of the CNS, Charcot (16) recognized a neurodegenerative component more than a century ago. Nonetheless, because of the significant and obvious demyelination in MS, the disease was referred to for many years as one with loss of myelin and relative sparing of axons. The pendulum has swung, however, and the concept of significant neurodegeneration in MS has taken hold. Several studies have confirmed axonal loss or injury in MS. For example, immunohistochemical studies of amyloid precursor protein (APP) have demonstrated APP-positive profiles in active MS lesions axons and at the border of chronic active MS lesions. APP is a normal constituent of neurons transported down axons by fast axoplasmic flow; it accumulates in injured neurons to a level detectable by immunohistochemistry and is thus a useful marker of axonal injury. Some of the APP-positive structures in MS lesions resemble the terminal ends of axons and likely represent transected axons (17). Significantly, the number of APP-positive axons correlates with the degree of inflammation (18).
More evidence of neuronal and/or axonal damage in MS arose from clinical outcomes (19). Neurological disability has been correlated with atrophy of the spinal cord, cerebellum, and cerebral cortex (18,20,21). MRI spectroscopy for N-acetyl-aspartate (NAA), a marker of axonal integrity, has found reduced levels in brain tissue of MS patients. The decrease of NAA per lesion volume was significantly higher in secondary progressive (SP) MS patients with irreversible neurological disability than in relapsing–remitting (RR) MS patients with little fixed disability (21). More recently, a technique quantifying whole-brain NAA showed that widespread axonal pathology was independent of MRI-enhancement and was present from the earliest clinical stage of the disease (22).

A correlation also exists between the persistence of black holes (described as hypointense lesions on T1-weighted images with low signal intensity compared with the surrounding white matter) and the duration of the inflammatory phase of lesions (measured as length of persistence of the enhancement) (23). Black holes have been interpreted as areas of axonal loss (24,25). Neuronal loss in the thalamus of patients with SPMS (26) and RRMS (27) has been reported and confirmed by a substantial neocortical volume loss in RR and progressive MS as measured by MRI (28). Neuronal death in cortical MS lesions has also been observed (29).

3. MULTIPLE SCLEROSIS: NEURODEGENERATION INDUCED BY INFLAMMATION?

Because of the correlation between inflammation and signs of axonal injury or loss, it is likely that inflammatory molecules may aggravate and/or cause the degenerative process. If so, it is unknown whether the axonal injury seen in MS lesions results from an immune response against a particular neural antigen, a nonspecific injury related to secretion of soluble factors, or a cell–cell-contact-mediated mechanism. In addition, modification in the CNS microenvironment induced by inflammatory processes, such as axon hyperexcitability following chronic demyelination, may facilitate the neurodegenerative processes (30). Classical proinflammatory cytokines (tumor necrosis factor [TNF]-α and interleukin [IL]-1) can be neurotoxic, whereas anti-inflammatory cytokines (IL-10) may be neuroprotective; however, this is controversial. In one study, TNF-α killed human fetal neurons in culture (31), but in another study, the cytokine had no effect (32). Another study reported that TNF-α killed neurons by preventing the action of a survival factor, insulin-like growth factor-1 (33). IFN-γ, also a proinflammatory cytokine, was shown to induce neurons to express MHC class I on their surface and thus facilitate neuronal recognition and killing by cytotoxic T cells (34).

A number of studies indicate that endogenous IL-1 contributes to experimentally induced neurodegeneration. Administration of recombinant IL-1 receptor antagonist (IL-1ra) into the brain or periphery of rodents markedly inhibits brain damage caused by cerebral ischemia, brain injury, and excitotoxins (35–37). Conversely, injections of IL-1β antibody is neuroprotective (38). We have shown that IL-1β toxicity for oligodendrocytes is mediated by glutamate (39). Glutamate imbalance has been associated with oligodendrocyte and axonal damage in MS lesions (40). Indirect evidence comes from a recent study in which a small number of MS patients were treated with riluzole, an inhibitor of glutamate transmission that is used for treatment of amyotrophic lateral sclerosis, and this reduced the cervical cord atrophy and development of hypointense T1 lesions (41). Although this result is interesting, the number of patients was too small and the study was not randomized.

In addition to cytokines, other molecules elaborated by inflammatory cells can injure axons. This includes free radicals such as nitric oxide, which damages small axons in vitro (42,43), particularly when they are demyelinated. Interestingly, levels of nitric oxide synthase are elevated in MS lesions (44–46).

Matrix metalloproteinases (MMPs), enzymes that can destroy the extracellular matrix (ECM), are associated with the pathogenesis of MS. There is good evidence that T cells and other leukocytes use MMPs to cross the BBB (47). MMP-1 (48) and MMP-2 (49) are toxic to neurons in
vitro, although MMP-2 also has a role in promoting regeneration of denervated nerves in rat peripheral nerve explants (50). These observations confirm the duality of molecules, such as MMPs and cytokines: they can have opposing roles in different environments.

Much attention has recently focused on the possible role of antibodies in MS. Their function in demyelination seems to be important and supported by findings in EAE (51), but there is little data on the role of antibodies in axonal and neuronal injury. Antibodies can cause injury through an antibody-dependent cellular cytotoxicity mechanism or by inducing complement activation. When activated, the complement cascade induces a membrane attack complex (MAC) that breaks the cell membrane. In MS, complement components and activation products have been isolated from cerebrospinal fluid (CSF) (52) and brain lesions (53,54). In addition, a good correlation was demonstrated between C9, a component of the MAC, and areas of axonal degeneration (55).

Information on whether and how T cells kill neurons is limited. The first evidence of neuronal susceptibility to T-cell cytotoxicity came from a study using mouse peripheral nervous system neurons in vitro (56); only allogeneic T cells were cytotoxic to neurons. Since then, antigen-specific T cells were found to be toxic to syngeneic mouse neurons but required the neurons to express MHC-I, which was achieved by prior treatment with tetrodotoxin and IFN-γ (57,58).

Neurons in the CNS express minimal or negligible levels of MHC-I (34,59), and given that many infiltrating T cells in MS are probably CNS antigen nonspecific (60), we investigated other means of producing neuronal cytotoxicity. We demonstrated that polyclonally activated T cells have a potent cytotoxic effect on human neurons. This occurred in an allogeneic and syngeneic system in the absence of added antigen, required T-cell activation, and was mediated through contact-dependent non-MHC-I mechanisms. These results show the high and selective vulnerability of human neurons to T cells, and suggest that when enough activated T cells accumulate in the CNS, neuronal cytotoxicity can result (32).

After the initial involvement of an inflammatory insult, neurodegenerative processes of MS can progress for other reasons such as synaptic disconnection and lack of trophic support; possibly, these mechanisms are prevalent in SP and primary progressive (PP)MS. This hypothesis can also explain the absence of any improvement of these forms of MS to the currently available immunomodulatory treatments.

4. INTERFERON-β: A BRIEF REVIEW OF MECHANISMS OF ACTION

IFNs are a family of cytokines that were first described by Isaacs and Lindenmann in 1957 as naturally occurring proteins that interfere with viral replication (61). Subsequently two types of IFNs were identified: type I and type II IFNs. Type I IFN include IFN-α, -β, -ω and -τ, and some IFNs (IFN-α, and -β) are currently used in many therapeutic protocols. On the contrary, IFN-γ, considered a proinflammatory cytokine, is the only type II IFN. Type I and II IFNs engage different receptors expressed on their target cells.

IFN-β has been in clinical use as an immunomodulatory drug for the treatment of MS for more than 10 yr. There are three commercial forms of IFN-β: Betaseron® (IFN-β1b; Berlex Laboratories), Avonex® (Biogen IDEC), and Rebif® (Serono) (both IFN-β1a). All three preparations are referred to in this chapter interchangeably as IFN-β unless otherwise indicated. In MS, the mechanism of the immunomodulatory effect of IFN-β likely occurs at several levels. In the periphery, IFNβ can affect antigen presentation and the cytokine milieu. The activity on antigen presentation is exerted through a mechanism of downregulation of surface molecules involved in this process. IFN-β is effective in preventing the IFN-γ-induced upregulation of MHC-II on antigen-presenting cells (62).

IFN-β can also downregulate the expression of costimulatory molecules and inhibit the activation and proliferation of T cells (Fig. 1). In this regard, IFN-β treatment decreases the expression of CD54 (intercellular adhesion molecule [ICAM-1]) and CD80 (B7.1) on CD14-positive peripheral blood mononuclear cells of MS patients (63). In addition, IFN-β inhibits the expression of FLIP, an antiapoptotic protein, leading to an increased incidence of death of T cells (64). Corresponding with
these mechanisms, the frequency of myelin basic protein-reactive T cells in MS patients was found to be reduced following treatment with IFN-β, compared to pretreatment levels (65).

Perhaps predominantly through its effect on antigen presentation, an outcome of IFN-β treatment is its alterations of cytokine levels. Several studies have described an elevation of IL-10 in the mononuclear cells, serum, and CSF of IFN-β-treated MS patients. In addition, the majority of studies have shown that IFN-β decreases IFN-γ, IL-12 and TNF-α (T helper [Th1]1 cytokines) (62), although others found no difference or an increase (66,67). A decrease in both Th1 and Th2 cytokine-producing T cells has also been reported following IFN-β treatment, suggesting a general suppression of T cells. Although the majority of data indicate a diminution of Th1 responses, a clear effect on Th2 cytokines is equivocal.

A major effect of IFN-β therapy seems to involve the BBB. During the migration of leukocytes across the BBB, different adhesion molecules are expressed at the interface between T cells and endothelial cells. An increased expression of some of these adhesion molecules, such as VLA-4, leukocyte function antigen (LFA)-1, vascular cell adhesion molecule (VCAM)-1, and ICAM-1, has been described in MS. Many adhesion molecules are present in two forms: membrane bound and soluble. The soluble form can interact with receptors on the T-cell membrane and prevent the interaction with endothelial cells. The conversion of cell-associated VCAM-1 to its soluble form is facilitated by IFN-β (68). In addition, IFN-β increases soluble ICAM-1 levels in the serum of MS patients (69). Furthermore, IFN-β decreases the expression of several chemokines (70,71), reducing the chemokine gradient that facilitates leukocyte access to the CNS.

When leukocytes traverse the endothelial barrier, they encounter the ECM proteins of the basement membrane. To pass through the basement membrane, digestion of the ECM proteins appears necessary; here, leukocytes employ the action of proteolytic enzymes, such as MMPs. IFN-β reduces the production of MMP-9 by activated T cells (72,73). This decrease impairs the ability of T cells to cross the BBB. Furthermore, MMP-9 serum levels are decreased in RRMS patients treated with IFN-β. (69) On the other hand, in PPMS patients treated with IFN-β, serum levels of MMP-9 were not different from those of the placebo-treated group (74). The lack of difference can be related to the reduced inflammatory activity in progressive patients compared to RR ones, and this seems to be confirmed by another observation showing reduced MMP-9 and MMP-7 transcript levels in mononuclear cells of patients with RRMS but not SPMS (75). Overall, IFN-β decreases the number of inflammatory cells infiltrating the CNS parenchyma (Fig. 1) and this appears to be a major mechanism for its clinical effect.

5. IS IFN-β NEUROPROTECTIVE IN MS? ASSESSING CLINICAL OUTCOMES

As the neurodegenerative aspects of MS are strongly correlated with clinical disability, neuroprotection is a primary goal of treatment. A neuroprotective treatment can be defined as one that slows or stops disease progression by protecting, rescuing, or restoring the degenerated neurons and/or axons. In clinical trials, the available treatments for MS have shown some efficacy in reducing the relapse rate and number of gadolinium-enhancing lesions; however evidence for whether these treatments have any neuroprotective effect is weaker.

The progression of disability in MS may be a reflection of the neurodegenerative aspect of MS. IFN-β has a robust effect on inflammation (gadolinium enhancement) on brain MRI (1) and a less robust one on the frequency of clinical relapses (2). To assess the effect of these drugs on progression of disability is a challenge, as trials have not been long enough to make such conclusions. Furthermore, MS is a chronic disease, and it takes about 15 yr from onset for a patient with RRMS to reach an Expanded Disability Status Scale (EDSS) of 6 (use of a cane) (76).

The first randomized controlled trial (RCT) of IFN-β used recombinant IFN-β1b at two different doses (1.6 MIU and 8 MIU) subcutaneously every other day and enrolled 372 patients with an EDSS of up to 5.5 inclusive. Patients were followed for 5 yr. Changes in the neurological rating
and EDSS were used as secondary outcomes. This trial did not show a change in the neurological rating at 3 yr. The EDSS slightly increased, with a trend for statistical significance only at 3 yr ($p = 0.043$) (2).

IFN-β1a (Avonex) once weekly by intramuscular route was tested in another phase III trial involving 301 patients with a mild to moderate EDSS (1–3.5) who were followed for 2 yr. Because the drop-off rate was lower than expected, the trial was terminated earlier than 2 yr, after 172 patients had completed 2 yr of treatment. The primary outcome in this study, contrary to other trials, was time to onset of sustained worsening of disability instead of relapse rate. There was a 37% reduction in the probability of progression by one EDSS point at 2 yr, analyzing all patients in the study ($p = 0.02$) (77); however, for the patients who completed 2 yr of treatment, the difference in the proportion of patients with progression of disability was 21.1% for the IFN-β1a group, compared with 33.3% in the placebo group ($p = 0.07$) (78).

The third RCT is known as Prevention of Relapses and Disability by Interferon β Subcutaneously in Multiple Sclerosis (PRISMS); this compared 22 and 44 µg IFN-β1a (Rebif) three times a week vs placebo (79). The study (PRISMS-2) enrolled 560 patients with an EDSS of 0 to 5.5 inclusive and followed them for 2 yr. Progression in disability again was a secondary outcome and defined as at least a one-point EDSS increase that was sustained at least for 3 mo. Time to progression was significantly longer in both treatment groups compared to the placebo arm ($p < 0.05$). The Integrated Disability Status Scale (IDSS), defined as the area under the time/EDSS plot, was not increased at 2 yr (median IDSS at 0) in both treated groups and increased by 0.4 in the placebo group. Patients in the placebo group were then rolled over to 22 or 44 µg Rebif and followed for another 2 yr (PRISMS-4). Again, time to progression of disability was significantly longer in the high-dose vs the crossover group (42.1 vs 24.2 mo), and no statistically significant difference was found between the low-dose vs the crossover groups or between the low- vs high-dose groups. IDSS at 4 yr was significantly lower in the high-dose vs the crossover group ($p = 0.034$), and no difference was observed for the low-dose vs the crossover group or low- vs high-dose group. In PRISMS-2, 61.7, 70.3, and 73.2% of patients in the placebo, low-, and high-dose groups, respectively were progression-free, and these were not statistically significant. In PRISMS-4 (80), the percentages of progression-free patients in the aforementioned three groups were 46, 51, and 56%, respectively, and these were also not statistically significant from one another, except for a trend found when comparing the placebo to the high-dose group ($p = 0.07$).

IFN-β was also studied in SPMS. This group might better reveal a neuroprotective effect of IFN-β, as disability in SPMS might be more clearly correlated with axonal loss. In the European SPMS trial, 718 patients were randomized to receive 8 MIU IFN-β1b or placebo. In this trial, 70% of the randomized patients had relapses in addition to progression. The time to confirmed neurological deterioration was statistically significant in the treated group ($p = 0.0008$). About 50% and 39% of the patients progressed in the placebo and treatment group, respectively, representing a 22% decrease in the proportion of treated patients who progressed compared to placebo subjects (81).

Although the above trial did show some therapeutic effect of IFN-β in SPMS, unfortunately, a North American trial failed to replicate these findings. In the North American trial, 939 patients were randomized to receive every other day either placebo, 8 MIU IFN-β1b, or an IFN-β1b dose adjusted to body surface area. This trial was ended early because of the lack of efficacy in reducing disease progression (82).

The above two studies in SPMS were followed by a third one, the Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-β-1a in MS trial, which randomized patients in North America and Europe to 22 or 44 µg IFN-β1a or placebo. Again, this trial did not show a difference in time to sustained progression in disability. Interestingly, women (62% of the whole population) in the high-dose ($p = 0.006$) and low-dose groups ($p = 0.038$) and men in the placebo group showed a statistically significant delay in progression compared to controls (19).
As noted, the above studies described progression of disability using the EDSS, which relies heavily on ambulation, especially in the higher numerical range; the scale overlooks hand functions and does not include cognitive performance. Therefore, any beneficial effect of immunomodulatory drugs could be an underestimation of their real values. For instance, the last phase III trial in SPMS, the International Multiple Sclerosis Secondary Progressive Avonex Clinical Trial (IMPACT) study, used Avonex at the standard approved dose for 2 yr. The primary objective of IMPACT again was time to disease progression but used the Multiple Sclerosis Functional Composite score (MSFC) based on timing of a patient’s ability to walk 25 ft and the nine-hole peg test, which evaluates arm and hand function and ability to perform calculations. The results of the IMPACT study showed a significantly positive effect on the overall MSFC score but not on ambulation (83).

Clinical trials increasingly are relying on MRI of the brain and, very recently, of the spinal cord as a biomarker of the disease. MRI measures used for assessment of neurodegeneration include brain atrophy measurements and T1-hypointense lesions or black T1 (black holes). T2-hyperintense lesions are very sensitive but lack specificity as they reflect edema, demyelination, axonal loss, or gliosis and are therefore not useful as markers for neurodegeneration.

In a longitudinal study of brain atrophy using Avonex, 237 patients had a baseline scan, 124 had a follow-up at yr 1, and 85 at yr 2. This study showed a weak positive correlation between EDSS and third ventricle width \( r = 0.26, p = 0.0001 \), EDSS and lateral ventricle width \( r = 0.18, p = 0.0007 \), and EDSS and corpus callosum atrophy \( r = -0.15, p = 0.016 \) (84). Patients were then randomized to IFN-\(\beta\)-1a once weekly vs placebo and followed for at least 2 yr. Brain atrophy was measured using brain parenchymal fraction (BPF), defined as the ratio of brain parenchyma tissue volume over total volume contained within the brain. At 1 yr, the rate of brain atrophy was similar between the two groups but this was significantly different at yr 2 \( p < 0.01 \), with less atrophy in the treatment group. In the whole group, BPF decreased as the number of relapses increased. This decrease was statistically significant even in patients with no or a single relapse. Therefore, brain atrophy occurs even without relapses. This could be caused by subclinical inflammation but could also be caused by neurodegenerative aspect of MS, in which axonal loss continues independent of inflammation. This dichotomy could be caused by Wallerian degeneration or lack of trophic support (85). Alternately, the discordance between axonal loss and inflammation could represent a temporal separation, in which axons continue to degenerate slowly some years after the causative inflammatory insult. If so, this could account for the observation that agents, such as CAMPATH-1 (86,87) and Linomide (88), reduced gadolinium enhancements but did not affect clinical outcomes.

More recently, an open-label, baseline-vs-treatment crossover study of 30 RRMS patients showed that IFN-\(\beta\)-1b slows the progression of brain atrophy during the second and third year of treatment, although 43% of patients had an increase in EDSS of at least 1 point and 70% of at least 0.5 points, consistent with the rate of disease progression observed in other studies. In the same cohort of patients, the presence or absence of neutralizing antibodies had no significant effect on brain atrophy (89).

T1-hypointense lesions or black T1 are markers of destructive pathology, principally of axonal loss. Black holes, however, are not synonymous with destruction in general, as new lesions could present as such, although these new black holes are less hypointense than the persistent lesions, might be reversible, and do enhance with gadolinium. Henceforth, we refer to black T1 as the lesions associated with irreversible damage.

The same group that studied the effect of IFN-\(\beta\)-1a on brain atrophy also evaluated the effect of this drug on black holes. Black holes increased by 8% \( p < 0.05 \) and 34% \( p < 0.05 \) in the treated and placebo group, respectively. The presence of enhancing lesions appeared to be the most important factor determining the future development of T1-hypointense lesions \( r = 0.45, p < 0.001 \). These enhancing lesions also predicted third ventricle atrophy (90).
T1-hypointense lesions were also studied in a subgroup of SPMS patients (85) treated with IFN-β1b in the European trial. Forty-one patients were randomized to placebo and 44 to treatment, and patients were followed-up every 6 mo for 36 mo. At baseline, T1-hypointense lesion volume was 5.1 cc and 4.9 cc in placebo and treatment group and showed a linear increase in volume by 2.4 cc and 0.76 cc, respectively. This increase from baseline was statistically significant for both arms (p = 0.0002 and p = 0.006, respectively). The average increase of black-hole volume per year was 14% in the placebo group and 7.7% in the treatment group, and this difference was statistically significant (p = 0.0003) (91).

As shown above, the correlation between cerebral atrophy and EDSS, although statistically significant, remains weak. It is thought that locomotor disability is more correlated with spinal cord atrophy than with cerebral atrophy (92,93). The assessment of spinal cord atrophy continues to be challenging, as image resolution remains problematic; however, a recent study computed the upper cervical cord area (UCCA) in a series of 38 patients with RRMS (20) and SPMS (18) randomized to IFN-β1a (22 vs 44 µg) matched to nontreated patients. The UCCA decreased by 5.7% and 4.5% at 48 mo in the placebo and IFN-treated group, respectively (p = 0.35) (94).

We still have no standardized way to measure the impact of neurodegenerative processes in MS. Characteristics measured by MRI, such as brain atrophy, have not been correlated with disability in MS, and measures of axonal loss, such as the magnetization transfer ratio (MTR) and spectroscopy, have not been used in large-scale clinical trials. The effects of IFN-β on MTR measures in RRMS remain controversial. A recent study of SPMS has shown no effect of IFN-β1b on MTR measures. No data are available for other treatments. In addition, small studies have shown conflicting results regarding the effect of IFN-β on N-acetyl-aspartate peak. In one study, 10 patients with RRMS who followed by MRI/MRS for 1 yr pretreatment and 1 yr posttreatment with IFN-β1b were compared to 6 untreated patients. An increase of N-acetyl-aspartate levels in brains of patients with RRMS following IFN-β treatment was noted (p = 0.03) (95,96). Another study failed to show an increase in N-acetyl aspartate peak following treatment with IFN-β1a, however, the study lasted only 6 mo (97). A third study involving 11 patients taking IFN-β (1 Avonex, 1 Betaseron, and 9 Rebif) for 12 mo showed a decrease in the number of T2 lesions and, contrary to expectations, a decrease in N-acetyl-aspartate peak. This study suggested that reduction of new inflammatory activity with IFN-β does not invariably halt progression of axonal injury, although it appeared that an inverse relationship existed between the rate of progression of axonal injury and relapse rate for the preceding 2 yr (96).

In conclusion, what can we learn from these studies? First, IFN-β seems to have a minimal effect on EDSS, as it is mainly used for short-term purposes in clinical trials. Second, if IFN-β has an effect on EDSS, it may reduce disability secondary to inflammation. Third, although their effect on EDSS is less obvious, the IFN-β preparations have a statistically significant effect on brain atrophy and T1-lesion volume, underlining again the weak correlation between EDSS and measures of atrophy. This weak correlation could potentially be improved using measurement of spinal cord atrophy. Fourth, brain atrophy increased as the number of relapses increased and patients with SPMS and superimposed relapses do better on IFN-β than do patients with SPMS without relapses. In these patients without relapses, neurodegeneration might be occurring independently (or as a delayed consequence) of inflammation, and immunomodulators may not provide neuroprotection at this irreversible stage.

6. PROPOSED MECHANISMS OF NEUROPROTECTION BY IFN-β

The clinical studies above suggest a neuroprotective effect for IFN-β in MS, even if it is not robust. What might be the mechanism of neuroprotection? We think it is likely that the neuroprotective mechanism is linked to the neurodegeneration that inflammatory molecules and T cells can effect, as discussed earlier. Thus, by reducing the influx of pathogenic T cells into the CNS, IFN-β would in essence be preventing further CNS insults from these cells. The outcome would be
neuroprotection, even if it were exerted indirectly by preventing detrimental leucocytes from reaching their sites of action within the CNS parenchyma (Fig. 1).

Is there a potential for IFN-β to exert direct neuroprotective effects within the CNS? This seems unlikely, as IFN-β is not thought to cross into the CNS. Although, if it does and the BBB in MS patients is not intact, IFN-β possibly may act within the CNS. In this regard, we have described that IFN-β treatment of astrocytes in culture resulted in the significant upregulation of an important survival factor, nerve growth factor (98); however, it remains to be demonstrated that this occurs in vivo within the CNS.

7. PERSPECTIVES

To design new neuroprotective treatments or confirm the neuroprotection conferred by existing therapeutics, we need to understand the mechanisms responsible for axonal and neuronal loss and find measurable clinical outcomes more related to the neurodegenerative aspects of MS. Furthermore, we need to modify trial designs, which do not adequately reveal the potential beneficial effect of existing immunomodulatory drugs. There is a prospect for drug-related neuroprotection in MS, and medications (e.g., glatiramer acetate) may have this efficacy (99). By decreasing pathogenic inflammation, it appears that some degree of neuroprotection is also afforded by IFN-β, and it is up to the research community to demonstrate this convincingly and to design appropriate strategies to enhance this capacity.

REFERENCES

Effects of IFN-β in MS


CME QUESTIONS

1. All of the following can be direct targets of interferon (IFN)-β except:
   A. T-cell activation
   B. Blood–brain barrier
   C. Major histocompatibilty complex class II expression
   D. Microglia activation
   E. Matrix metalloproteinases production

2. Which of the following is not a marker of neuronal injury?
   A. Amyloid precursor protein
   B. Interferon γ
   C. Black holes
   D. N-acetyl-aspartate decrease

3. All of the following have been shown to induce neuronal injury except:
   A. Tumor necrosis factor-α
   B. activated T cells
   C. Matrix metalloproteinase-1
   D. Complement C9
   E. N-acetyl-aspartate

For questions 4 and 5, choose the correct answer:
A. If A, B, and C are correct
B. If A and C are correct
C. If B and D are correct
D. If D is correct
E. If all are correct

4. The reason(s) why IFN-β is not robust on disability/neurodegeneration is:
   A. The lack of sensitivity of the Expanded Disability Status Scale (EDSS)
   B. The lack of methods to assess spinal cord atrophy
   C. IFN-β’s lack of a direct neuroprotective effect in the brain
   D. IFN-β might be used too late in the disease degenerative process

5. IFN-β’s effect on disability in multiple sclerosis (MS):
   A. Has been shown in all randomized controlled trials
   B. Is strong on the EDSS
   C. Seems to be more obvious in secondary progressive MS
   D. Cannot be assessed reliably because of the relatively short duration of clinical trials