How Type I Interferons Work in Multiple Sclerosis and Other Diseases: Some Unexpected Mechanisms

Anthony T. Reder and Xuan Feng

Type I interferons (IFNs) are important in innate and adaptive immunity. They are used to treat virus infections, cancer, and multiple sclerosis (MS). There are 5 type I IFN families in humans—IFN- α with 13 subtypes, plus IFN- β , ϵ , κ , and ω . Because their receptor binding affinities vary, these IFNs have different gene induction profiles and quite variable therapeutic effects. IFN- α subtypes may each be specific for certain viruses, but can be neurotoxic. IFN- β induces IFN- α , plus has additional direct effects on target cells. IFN- β was the first therapy approved that could change the course of MS. It has broader specificity than IFN- α , enhances cognition in MS, and may be neuroprotective and can potentially enhance fertility in women. Priming the IFN signaling system with an injection of IFN- β can enhance subnormal type I IFN signals in MS. Many other commonly used drugs and vitamins may potentiate clinical benefits of IFN- β .

Type I Interferons and Multiple Sclerosis

TYPE I INTERFERONS (IFNs; IFN- α , β , ϵ , κ , and ω in humans, plus IFN-tau [IFN- τ] in ungulates) are produced by many cells. Typically, fibroblasts produce IFN- β ; plasmacytoid dendritic cells (DC) produce IFN- α . Type I IFNs have tissue-specific and gene-specific roles that are dependent on a balance of the different IFN subtypes, the timing of exposure, and interactions with drugs and environmental factors. The $\sim 1,000$ genes that are controlled by these IFNs are critical for antiviral immunity and also impact cell proliferation, immune regulation, cytoprotection, and possibly fertility.

In multiple sclerosis (MS), cells from the innate and adaptive arms of the immune system cause central nervous system (CNS) inflammation. Adaptive immunity is prominent in the earlier, relapsing/remitting phase of MS (RRMS). Innate immune responses appear to underlie the later secondary progressive (SPMS) phase but are likely to contribute to brain damage at all times. IFN- β therapy for MS prevents and shortens relapses and also reduces new magnetic resonance imaging (MRI) brain lesions, MRI T1 black hole formation, progression, and cognitive loss (Lacy and others 2013). Long-term therapy induces neuroprotectant proteins such as BDNF, NGF, Nrf2, and NCOA7-AS (Croze and others 2013). Five years of IFN- β -1b therapy, begun 8 years after the first symptoms, reduces the death rate by 47% (Goodin and others 2012). The survival benefit is based on less all-cause mortality, but is predominantly

from less MS-related death. Its method of action is complex, with dose-dependent changes in different cells and tissues, and variation at different phases of MS.

Type I IFNs Differ from Each Other: Implications for Therapy of MS and Other Diseases

Different effects of IFN- α and IFN- β on MS, cancer, and virus infections

Multiple sclerosis. IFN- β is used to treat MS, whereas IFN- α is an approved therapy to treat virus infections and cancer. The rationale for these applications is a mix of scientific evidence and tradition. In MS, early small studies showed that moderate to high doses of IFN- β -1b (4–16 M units every other day) reduced relapses (Knobler and others 1993).

With human leukocyte IFN- α , however, there were no or minimal benefits (Frith and the AUSTIMS Research Group 1989). Magnetic resonance imaging was still in its infancy and was not performed in this early study. Other small studies of human leukocyte IFN- α , which is composed of multiple subtypes of IFN- α , showed trends for benefit (Knobler and others 1984; Squillacote and others 1996). At lower doses of IFN- α (2 M units 3 times per week) than current MS IFN- β treatments, there was no benefit on MS exacerbations (Camenga and others 1986). There were significant side effects such as flu-like symptoms, neutropenia, transient neurological worsening, and slower visual-evoked potentials. High-dose IFN- α -2a, 9 MU intramuscular, every other day for 6 months, reduced MRI lesions and attacks in a

placebo-controlled trial of 20 patients (Durelli and others 1994). It is likely, but not certain, that IFN- β is more potent than IFN- α in MS. The utility of IFN- α versus IFN- β in treating other diseases is instructive.

Viruses. IFN- α is approved for treatment of hepatitis B and C. Different IFN- α can reverse virus-induced disorders of cell proliferation, including condylomata acuminata (genital warts from HPV) and laryngeal papillomatosis. IFN- α -2a, -2b, -n1, -n3, and peginterferon α -2a and α -2b vary in their specific antiviral indications. In HIV-infected patients with progressive multifocal leukoencephalopathy (PML), IFN- α triples survival times (Cabrera-Gomez and Lopez-Saura 1999). IFN- α is as effective as acyclovir in speeding recovery from herpes zoster (shingles) and in reducing postherpetic neuralgia (Duschet and others 1988; Cabrera-Gomez and Lopez-Saura 1999), although with more side effects. IFN- α subtypes are not equal. For instance, IFN- α 2 enhances CD4 and CD8 motility and induces interferon regulatory factor-7 (IRF-7) and 2',5'-oligoadenylate synthetase (OAS), but IFN- α 8 does not (Foster and others 2004). IFN- β could also reduce the incidence, duration, and consequences of shingles, and there are no reports of an increased incidence during therapy, but this has not been studied in detail. Of note, MS patients with active virus infections, based on high serum antibody titers against herpes viruses and endogenous retroviruses, have less response to IFN- β therapy (Petersen and others 2012).

Side effects of type I IFNs. Side effects of IFN- α and IFN- β include fatigue, weight loss, myalgia, and arthralgia (flu-like symptoms). IFN- α , however, is more or solely able to provoke depression and immune-mediated complications such as autoimmune hemolytic anemia/thrombocytopenia, collagen vascular disorders, hypothyroidism, immune-mediated nephritis, and, rarely, cardiac dysfunction. Chronic fatigue and neurotoxicity, such as depression and cognitive impairment, are common dose- and time-limiting side effects of IFN- α (Talpoz and others 2013).

Similar doses of IFN- α and IFN- β sometimes have different effects. At equivalent antiviral doses, IFN- α 2 and IFN- α 8 induce the mRNA coding for MxA, an antiviral protein, at 0.5 ng/mL (ie, $\sim$ 200 U/mL). However, a high dose of either IFN- α , 5 ng/mL ($\sim$ 2,000 U/mL), is less effective (Foster and others 2004). Such dose-dependent reversal in gene activation is not seen with IFN- β and IFN- τ , consistent with the clinical toxicity of IFN- α > IFN- β . IFN- β also induces a larger number of genes than IFN- α (Der and others 1998), and presumably some of them obviate IFN- α -induced side effects (Reder and Feng 2013).

Equivalent doses of IFN- β and IFN- α have different effects on viruses, cancer, and inflammatory diseases. Since all type I IFNs bind to the IFNAR, how can type I IFNs differ in their effects? There are at least 3 ways.

Receptor binding by IFN- β is longer than with IFN- α

All type I IFNs bind to the same receptor (IFNAR). However, time on receptor is longer with IFN- β than with IFN- α .

Fluorescent IFN- β binds to the IFNAR1 30 times longer (30 s with 100 nM affinity, K_D) than IFN- α (1 s, 5 μ M affinity) (Jaitin and others 2006). Cells with few IFNAR are most responsive in this differential sensitivity to IFN- β , whereas those with abundant IFNAR respond better to IFN- α 2 (Moraga and others 2009). IFN- β -1a and IFN- β -1b have

not been compared in affinity for the IFNAR. However, a difference in binding affinity is the likely explanation for the greater gene induction by IFN- β versus IFN- α . In an early study using medium-scale microarrays, 268 genes were induced by IFN- β , but only 94 were induced with IFN- α in the human HT1080 fibrosarcoma line (Der and others 1998).

Longer time on the receptor will lead to more and longer gene induction. However, this is partially counterbalanced by more endocytosis of the IFN-receptor complex and faster termination of signaling (Thomas and others 2011). Could time on receptor modify the effect of IFNs on cancer cells?

In the United States and Europe, forms of IFN- α are used to treat chronic myeloid leukemia (CML; Philadelphia chromosome abnormality), B (hairy) cell leukemia, follicular lymphoma, non-Hodgkin's lymphoma, mycosis fungoides, multiple myeloma, malignant melanoma, AIDS-related Kaposi's sarcoma, carcinoid, and also bladder, renal, epithelial ovarian, and skin cancer. IFN- α also benefits CML patients who have failed tyrosine kinase inhibition, likely by targeting imatinib-resistant CML stem cells, and activating B and T lymphocytes, natural killer cells, and antigen-presenting DC that destroy tumor cells (Talpoz and others 2013).

Greater receptor-binding affinity of IFN- β compared with IFN- α may account for the more potent and different antiproliferative and perhaps immunoregulatory actions of IFN- β , despite their equivalent antiviral effects. Antiviral potency is already maximal at intermediate IFN/IFNAR binding affinity. Higher affinities, in contrast, are needed to attain maximal antiproliferative strength (Thomas and others 2011). Because of differential energetics of shared anchor points between IFN and the IFNAR, IFN- β would be ascendant over IFN- α in preventing growth of cancers. With equivalent IFN doses (based on antiviral activity), *in vitro* IFN- β is more potent than IFN- α at inhibiting gliomas (Rosenblum and others 1990), adrenocortical, pancreatic (Vitale and others 2007), and carcinoid cancers (Vitale and others 2006).

IFN- α is an interferon- β -stimulated gene

Virus nucleic acids bind to pattern recognition receptors, including TLR7 (toll-like receptor7; activated by virus RNA), TLR9 (by virus DNA), retinoic acid-inducible gene-I (RIG-I), melanoma differentiation-associated protein 5) (MDA-5), CD11b/CD18 (activated by Mac1), stimulator of interferon genes (STING; activated by DNase II), and the Trex system, all of which activate IRF3 to induce transcription of IFN (Reder and Feng 2013). The first IFNs produced are IFN- β and IFN- α 1 (IFN- α 4 is the homolog in mice). Secreted IFN- β in turn induces transcription factor IRF7 through the JAK-STAT pathways in surrounding cells, which in turn induces multiple subtypes of IFN- α , amplifying the initial antiviral reaction (Marie and others 1998).

MS therapy with IFN- β has a similar effect. Injections of IFN- β induce a peak of serum type I IFN (the injected IFN- β) at 1 h, but then there is a second slower rise in serum type I IFNs (induced IFN- α and IFN- β) over 8 h (Fig. 1) (Feng and others 2012a). This prolongs and amplifies the effect of an injection of IFN- β .

Immune regulation differs between type I IFNs

IFN- α and IFN- β equivalently induce naïve CD4 T cells to differentiate into Th1 cells. However, IFN- α promotes

FIG. 1. IFN- β induces 2 peaks of type I IFN. An otherwise healthy 32-year-old man with MS injected IFN- β -1a subcutaneously after a 3-day washout. Serum type I IFN activity was quantitated with a sensitive assay that measures mRNA induction of 3 IFN-stimulated genes. This assay is 50 times more sensitive than conventional type I IFN ELISAs. The initial rise and fall is classic for the injected IFN- β . The second prolonged peak of type I IFN is likely from a combination of induced IFN- β and subtypes of IFN- α (Feng and others 2012b; Reder and Feng 2013), based on blocking experiments with specific anti-IFN- α and anti-IFN- β antibodies. Predicted curves are dashed. Note nonlinear x-axis. ELISA, enzyme-linked immunosorbent assay; IFN, interferon; MS, multiple sclerosis.

differentiation of DC that activate different genes and secrete different cytokines than IFN- β -induced DC. For instance, IFN- α regulates tissue inhibitor of metalloproteinase (TIMP3), CD52, and the receptor for Fc fragments of immunoglobulin (FcR); IFN- β regulates regulator of G-protein signaling (RGS1) and indoleamine 2,3-dioxygenase (IDO) (Garcin and others 2013). IFN- α and IFN- β generate different phagocytic processing of nucleic acids (more so with IFN- α -induced DC) in systemic lupus erythematosus (SLE) (Garcin and others 2013) and perhaps in the mechanistically related demyelinating disease, neuromyelitis optica (NMO) (Javed and others 2008; Feng and others 2012b).

Interferon levels are normally maintained at ~1 unit/mL in serum for optimum signaling, but IFN levels are low in MS

IFN- α/β binds to the IFNAR and activates JAK1 and TYK2. These kinases in turn tyrosyl phosphorylate transcription factors STAT1 and STAT2 on specific tyrosine residues, which in association with a non-STAT protein, IRF9 (p48), bind to DNA and activate or repress ~1,000 genes. A second phosphorylation modifies gene expression. Five to ten minutes after tyrosine is phosphorylated, STAT1 is sometimes also phosphorylated on serine 727 to boost the signal (Sadzak and others 2008).

A modicum of serum IFN is needed to maintain type I IFN signaling. The physiologic level of IFN- α/β is likely around the average of 1 U/mL seen in healthy controls (Fig. 2) (Feng and others 2012a). IFN starvation in culture scales down the intracellular levels of these STAT transcription factors that are central to the IFN signaling cascade. When intracellular levels of unphosphorylated STAT1 and acti-

FIG. 2. Serum type I IFN activity is reduced in progressive MS compared with stable RRMS and healthy controls. Values below the level of detection are plotted at 0.01 U/mL; assay as in Fig. 1. The number below detection is 5/19 (26%) for controls, 34/59 (58%) for RRMS, and 13/21 (62%) for secondary and primary progressive MS. These serum levels parallel separate *in vitro* functional assays and *in vivo* clinical responses to IFN- β (Feng and others 2012b). Significance based on Mann-Whitney *U*-test; medians and standard errors of mean are given for orientation. Note log scale on y-axis. RRMS, relapsing/remitting phase of MS; SP, secondary progressive MS; PP, primary progressive MS; HC, healthy controls.

vated phospho (p)-STAT1 are low, cells have reduced or absent responses to IFN- β . Most MS patients have low levels of serum IFN (Feng and others 2012b), and this seems to parallel their low responses to IFN- β *in vitro* and *in vivo* (Fig. 2). Serum type I IFN levels are higher than normal in SLE and NMO, but are low in MS. SPMS patients have even lower levels than RRMS patients.

During MS exacerbations (Reder and others 2011) and active clinical progression (Feng and others 2002a), cultured mononuclear immune cells (MNC) are resistant to IFN- β and need high *in vitro* IFN doses to trigger a response. These findings of an endogenous, state-dependent defect in IFN signaling in MS may translate to the clinic. Low type I IFN levels in MS could affect immune regulation, determine the threshold of response to IFN therapy, and could be corrected or “primed” by IFN therapy.

Circulating MNCs from clinically stable relapsing/remitting (RRMS) patients have lower levels of the IFN-induced MxA protein. Moreover, MxA levels fall further during exacerbations and progression (Feng and others 2002a). This IFN resistance *in vitro* is actually reflected *in vivo*. IFN- β therapy is most effective in early RRMS, but has little benefit on progression in the later stages of MS (Panitch 2004). Similarly, low- and high-dose subcutaneous IFN- β -1a have equivalent clinical benefit in mild MS, but the high dose of IFN is more effective in MS with greater disability scores (EDSS of >3.5, on a 10-point scale) (Ebers and PRISMS Study Group 1998). Patients spontaneously report that doubling the IFN- β dose during an exacerbation, to daily high-dose injections, is as effective as high-dose glucocorticoid therapy in speeding recovery from an exacerbation ($N=40$, uncontrolled; A.T.R. personal observation, 2013).

FIG. 3. Priming of MNC in culture overnight in IFN- β -1b (160 U/mL) enhances cellular responses to a second *in vitro* dose of IFN- β . The effect is seen in healthy controls and in patients with stable and active RRMS. MxA protein is measured in MNC lysates with Western blots after 24 h restimulation with IFN- β (Reder and others 2013). MNC, mononuclear immune cells.

Priming the Pump: IFN Signaling Depends on Endogenous IFN Levels

Can subnormal responses to IFN therapy be corrected? A “priming” dose of IFN- α , β , or IFN- γ induces synthesis of STAT1 protein, and allows more vigorous activation after a second pulse of IFN. Overnight priming with 160 U/mL of IFN- β -1b, a dose optimal for cytokine induction *in vitro* without toxicity, triples production of the MxA protein after a second pulse of IFN- β (Fig. 3) (Feng and others 2013). Perhaps long-term IFN- β therapy is able to prime the subnormal IFN setpoint in MS, elevate STAT1 levels, and rev up an endogenous defect in IFN signaling, bringing IFN responses back up to normal levels.

A small percentage of MS patients has normal or excessive reaction to IFN- β . This 15%–20% is more often non-responsive to IFN- β therapy (Comabella and others 2009; Axtell and others 2010; Rudick and others 2011). SLE and NMO patients also have excessive responses to type I IFN *in vitro* and poor therapeutic results with IFN- α (“too much of a good thing”) (Feng and others 2012b). The majority of MS patients, however, has low levels of serum IFN- α/β and diminished responses to IFN- β (Feng and others 2002a, 2012b). We argue that IFN- β therapy primes the pump and brings IFN signaling back up to normal levels. This would affect a large number of genes and could affect many biological systems, in some unexpected ways.

Type I IFNs Enhance Fertility and Modify Estrogen-Regulated Genes

In sheep and other ungulates, IFN-tau is produced by the trophoblast to aid implantation of the fertilized ovum into the uterine endometrium (Roberts 2007). It is possible that type I IFNs could modify fertility and egg implantation in humans. Some cases may be relevant.

1. Luna was a 30-year-old woman with 2 children. Her MS symptoms began soon after delivering her first baby. She felt more energy and fewer MS symptoms during her second pregnancy, but after the second child was born, she began birth control with depot medroxyprogesterone acetate implants. Five years later, she started thrice-weekly IFN- β -1a, and soon became pregnant again, despite the implant.

2. Five women with infertility received multiple *in vitro* fertilization (IVF) treatments. The hormones were ineffective. While IVF continued, they started or restarted IFN- β therapy because of concerns about impending MS exacerbations. They soon became pregnant.

These 6 pregnancies in our clinic population may not have been a coincidence.

IFN- τ silences expression of estrogen receptor alpha and then downregulates the oxytocin receptor in order to prevent destruction of the corpus luteum and subsequent menstruation and cycling. In women, the role of IFN- τ is filled by human chorionic gonadotrophin, which maintains the corpus luteum. IFN- τ also increases expression of many genes critical for uterine receptivity to implantation and conceptus development through nonclassical IFN pathways (Bazer 2013). IFN- α and IFN- τ , infused into the uterine lumen of nonpregnant ewes, are both able to extend the estrous cycle length (Roberts 2007).

IFN- τ is not present in human cells (Reder and Byskosh, 1995, unpublished; Chon and Bixler 2010), despite an early report of IFN- τ in human immune cells. It is not induced by viruses, suggesting that it has a role remote from that of other type I IFNs. IFN- τ blocks experimental allergic encephalomyelitis (EAE), however, and effects of IFN- τ in MS are under study.

Could type I IFNs such as IFN- β affect uterine cells or blastocyst implantation in women? Transcription profiling shows that as the trophoblast implants into the endometrium and forms the deciduum, there is limited, but definite, induction of human IFN-stimulated genes (Roberts 2007). The anecdotal reports above suggest that IFN- β could facilitate fertility.

Estrogens affect the course of MS. Women are 3 times more likely than men to have MS, exacerbations rebound postpartum, and MRI lesions increase before menstruation (Gold and Voskuhl 2009). IVF with gonadotrophin releasing hormone (GnRH) agonists can trigger MS exacerbations (Correale and others 2012; Michel and others 2012). Estriol rises throughout pregnancy, and has effects on immunity. Oral estriol prevents enhancing MRI lesions (Gold and Voskuhl 2009). Estriol’s ability to reduce MS exacerbations is being studied.

Pregnancy prevents MS exacerbations. Pregnant patients often say that many of their symptoms have dissipated or disappeared. With each succeeding month of pregnancy, MS

relapses plummet and a blissful aura waxes in the mother-to-be. Estriol levels increase during the gravid period, and are being tested as a therapy for MS (Voskuhl and others, 2014). Pregnancy in NMO, although not studied, would be expected to reflect the profile in SLE. NMO is similar to SLE in linkage to autoimmune diseases, high serum IFN- α/β and overblown reactions to IFN- β , and inefficacy of IFN- β therapy (Javed and others 2008). Active SLE during pregnancy is high risk for the woman and triples pregnancy loss the first 2 trimesters (Buyon 2008). SLE flares appear in 50% of pregnant women (Ostensen and others 2011).

Could type I IFNs affect estrogens? IFNs occasionally modify menstrual cycles, indicating that there are multiple, largely unexplored interactions between the 2 signaling pathways. IFN- β therapy modifies the composition of at least 1 estrogen-related protein that is present in lymphocytes and neurons. Nuclear receptor coactivator protein 7 (NCOA7) couples with the estrogen receptor, allowing it to turn on estrogen-responsive genes. IFN- β -1b therapy instigates alternate transcription of the gene, forming NCOA7-AS. This new protein has antioxidant and neuroprotective function (Croze and others 2009).

Cognition in MS Improves with IFN Therapy

IFN- β enhances cognition in MS (Pliskin and others 1996; Ebers and PRISMS Study Group 1998; Fischer and others 2000) and may preserve neuropsychological function over decades (Lacy and others 2013). There may be a direct effect of type I IFN on CNS cells. It could also be argued that improved cognition is simply from less brain inflammation. However, chemotherapy largely abolishes inflammation but also diminishes cognitive function and damages neurons (Petzold and others 2010). Other approved disease-modifying drugs have variable effects on cognition.

Type I IFN production and IFN therapy can modify gene expression in the brain and spinal cord (Goelz and others 2007). Although little serum IFN crosses the blood–brain barrier, some IFN is able to cross through the circumventricular areas that have weaker barrier functions. Its effects may be region-specific. In the CNS, IFN- α can excite neurons in the cortex, hypothalamus, and hippocampus, but has no effect in the thalamic gray (Reyes-Vazquez and others 2012). IFN- β is a growth factor for neural progenitor stem cells and enhances differentiation into oligodendroglia at high doses (Arscott and others 2011). Local type I IFN could act alone, and could also enhance the benefits of exercise and antidepressants to promote stem cell migration into the adult hippocampus.

IFN levels are altered within CNS lesions caused by direct trauma or inflammation. Axonal transection of entorhinal afferents in the hippocampus induces IFN-stimulated genes and inhibits MMP9 in the hippocampal macrophages and microglia. The local IFN prevents macrophage infiltration into these damaged areas (Khoroshii and Owens 2010).

CNS inflammation enhances the ability of type I IFN to penetrate the blood–brain barrier. EAE induces 3513 CNS-specific genes. Intraperitoneal murine IFN- β induces 57 genes in the CNS that are not induced in the periphery and spleen. IFN- β given during EAE, however, induces 725 unique genes in the brain and spinal cord (Goelz and others 2007). Thus, systemic IFN crosses the blood–brain barrier, and CNS inflammation increases the ability of IFN- β therapy to regulate CNS genes.

Drug Combinations with IFN in MS

Interferons influence other signaling systems, sometimes in surprising ways. The external and internal environment and therapies for MS and its symptoms modify effects of IFNs.

Vitamin D and type I IFNs: clinical studies in MS

Vitamin D potentiates the reduction of MRI lesions and clinical activity by IFN- β -1b therapy. By itself, IFN- β prevents 85%–90% of new Gd⁺ MRI lesions. When 20,000 U of weekly oral vitamin D3 is added for a year in patients on chronic IFN- β -1b therapy, the remaining Gd⁺ lesions fall from 0.6/year to 0.1/year in a comparison of 30 patients on IFN alone versus 32 on IFN plus vitamin D3 (Soilu-Hanninen and others 2012). Accumulated T2 burden of disease trended lower (287 versus 83 mm³; $P < 0.11$), as did clinical measures of walking speed and disability ($P < 0.07$). These effects tend to be more potent in patients who had more active MS before IFN- β therapy began (Äivo and others 2012). In a second study, when patients were on IFN- β -1b, high serum vitamin D levels (>49 nM) cause a 3-fold drop in the relapse rate. IFN- β was much less effective when vitamin D levels were <50 nM (Stewart and others 2012). (For conversion between studies, 20 ng/mL of 25-OH vitamin D = 50 nM.)

In a clinical trial of IFN- β -1b in clinically isolated syndromes (first demyelinating attack), low serum vitamin D levels (<50 nM) (Ascherio and others 2014) predicted more Gd⁺ MRI lesions, more brain atrophy, more relapses, and more conversion to clinically definite MS compared with the cohort with high serum levels. With low serum vitamin D, there was a trend for more progression in this cohort of patients with early MS that was not expected to show progression. The vitamin D effect was statistically independent of IFN therapy, but epidemiologic factors such as sun exposure, perhaps vitamin D supplementation, and other lifestyle factors could confound the analysis, as sunlight seems to have a vitamin D-independent benefit (Lucas and others 2011). IFN- β seems to directly elevate serum vitamin D levels through an unknown mechanism (Stewart and others 2012). One small study found no additive effect of vitamin D3 in 23 patients: 4 were therapy-naïve, 5 on long-term glatiramer, and 14 on short-term IFN- β -1b. However, the groups were significantly mismatched at baseline, including ominous prognostic demographics in the high-dose vitamin D group, vitamin D supplements were changed in mid-study, and the placebo group was actually treated with moderate levels of vitamin D (Stein and others 2011).

Sunlight, and by implication vitamin D, has potent epidemiologic effects on preventing onset of MS. The incidence of MS is 50% lower in sun-exposed Tasmanian children than in those who avoided sunlight (van der Mei and others 2003). Low serum vitamin D levels in U.S. army recruits predisposed to onset of MS (Munger and others 2006). Sunlight has additional benefits beyond increasing serum vitamin D levels. It elevates IL-10 and α -MSH, both of which can suppress function of Th1 cells and macrophages (Arnason and others 2012).

Vitamin D is the exemplar of a second agent in MS therapy—it is inexpensive and safe, and seems to enhance responses to at least one of the approved MS therapies, IFN- β -1b. There are low endogenous levels of type I IFN in MS before any therapy, and low levels of vitamin D predispose

to MS onset and exacerbations. In both cases, supplementation benefits MS. Combination studies of vitamin D plus IFN- β , glatiramer, and other agents are in progress to determine possible synergies.

Multiple drugs interact with IFNs to govern immunity

In MS, Th1 and perhaps Th17 cell function is increased and regulatory/suppressor cell function is decreased. In some studies, proinflammatory Th17 cell function is excessive in MS, but nonetheless there is no rise in polymorphonuclear neutrophils in MS, as would be expected from high IL-17 levels (Durelli and others 2009). In contrast, regulatory T cells (Treg) control Th1/inflammatory immunity. CD4 Treg have subnormal function in MS (Dalla Libera and others 2011), perhaps allowing unopposed inflammation. CD8 regulatory cell dysfunction is even more pronounced than CD4 dysregulation in MS (Antel and others 1979; Antel and others 1989; Karandikar and others 2002; Correale and Villa 2008). These cells are targeted by IFN- β therapy.

IFN- β modifies immunity. Many assume that the benefit in MS is forged through an IFN-induced Th1 to Th2 shift. Type I IFNs, however, actually enhance Th1 development and function in mice and in MS (Byskosh and Reder 1996). IFN- β corrects the cardinal defect in MS CD8 suppressor function to normal levels (Noronha and others 1992) and restores CD4, CD25 Treg numbers and function back up to normal levels (Venken and others 2008; Vandenbark and others 2009; Chen and others 2012). IFN- β inhibits function and causes apoptosis of Th17 cells through the IFNAR1 without affecting Th1 cells (Durelli and others 2009) and also causes apoptosis of activated T cells (Ahn and others 2004).

Many drugs alter immunity. Could a drug with a mechanism of action that potentially benefits MS enhance IFN therapy?

1. cAMP agonists generally inhibit activation and function of CD4 Th1 effector cells, Th17 cells, and macrophages, and can ameliorate EAE (Mosenden and Tasken 2011; Arnason and others 2012). They increase CD4 and CD8 Treg function. CD4 Treg secrete prostaglandin E2 (PGE2) to induce cAMP in responder T cells and thus inhibit T cell function (Mahic and others 2006) and reduce inflammation. However, counterbalancing the inhibitory effects on Th1 cytokine secretion, cAMP agonists promote differentiation of Th1 and Th17 cells (Li and others 2012). cAMP reduces secretion of IL-17F, but increases proinflammatory IL17A in SLE (Hedrich and others 2012).
2. Histamine 2 receptor agonists activate cAMP and increase CD8 suppressor cell function (Sansonini and others 1985). In macrophages and DC, H2 receptor agonists inhibit IL-12 and increase IL-10 (Idzko and others 2002). They inhibit EAE (Passani and Ballerini 2012) and SSPE, a chronic CNS virus infection (Anlar and others 1993). Antihistamines inhibit these regulatory T cells and activate the immune system to provoke DTH and possibly prevent cancer (Sahasrabudhe and others 1987). Histamine blockers are potentially injurious in MS. Histamine effects on the course of MS, the combination of IFN- β plus histamine agonists, and combinations with modafinil, which indirectly activates histaminergic neurons (Ishizuka and others 2012), have not yet been studied.
3. PGE2 binds to EP2R and increases cAMP. PGE is secreted by macrophages, inducible CD4 Treg, microglia, and astrocytes. PG effects are brief systemically and largely local, because 95% of blood PGE is cleared in one pass through the pulmonary circulation. Misoprostol, a long-acting PGE1 agonist ($t_{1/2}$ = 30 min), ameliorates the pain of trigeminal neuralgia in MS, even when multiple other agents have failed (Reder and Arnason 1995). The mechanism is possibly through cAMP effects on local immune cells in the trigeminal pathway or on PGE receptors in the trigeminal neurons themselves, which have a peculiar propensity to enhance in MS (Nakashima and others 2001). Misoprostol also inhibits EAE (Reder and others 1994). Could PGE synergize with IFN- β ? The answer is likely to depend on the local cell composition. Activated monocytes secrete 10 times more IL-10 than T cells. The monocyte to T cell ratio increases over time in CNS plaques as lesions become less Gd⁺ and more chronic and quiescent. The ratio of monocytes to T cells could control how drugs regulate inflammation. PGE decreases IL-10 in T cells, but enhances IL-10 secretion in monocytes (Feng and others 2002b). IFN- β exerts the opposite effect, increasing IL-10 in T cells, but reducing IL-10 production by monocytes. In progressive MS, perhaps PGE and other cAMP agonists would increase IL-10 within high-macrophage chronic CNS lesions.
4. Phosphodiesterase inhibitors elevate cAMP. Rolipram and ibudilast, 2 PGE4 inhibitors, block EAE, reduce inflammatory cytokines, and increase production of IL-10 and neurotrophic factors (Barkhof and others 2010). Ibudilast did not modify relapses in MS, but it did slow clinical progression and brain volume loss and prevented T1 black hole formation (Barkhof and others 2010). This suggests that ibudilast had little effect on T cells, but that it may have activated monocytes or other brain cells that could produce IL-10 (Feng and others 2002b) and neurotrophic factors.
5. The β_2 -adrenergic agonist, terbutaline, increases CD8 suppressor function and reduces fatigue in MS (Reder and Arnason, 1988, unpublished) and inhibits EAE (Wiegmann and others 1995). CD8⁺, CD28⁻ regulatory/suppressor cells have the highest levels of cAMP and high-affinity β -adrenergic receptors. Immune cell subsets have different levels of expression of β_2 -AR (CD8⁺, CD28⁻ suppressor cells > B cells > CD4 cells > monocytes (Karaszewski and others 1993). Isoproterenol induces more cAMP in CD8 cells from chronic progressive MS than in CD8 cells from healthy controls. This suggests that cAMP agonists potentially have a significant effect on the specific regulatory immune subset that is most dysfunctional in MS. Extending these studies, oral albuterol enhances the benefit of glatiramer in RRMS on relapses and reduces IL-13 and IFN- γ , but not IL-5 (Khoury and others 2010); IL-10 was not measured.
6. Adrenocorticotropin (ACTH) binds to melanocortin receptors (MCR1-5), inducing cAMP (Arnason and others 2012). ACTH is FDA-approved to shorten the duration of relapses in MS, based on an early MS trial (Rose and others 1970). In 3 groups of 20 RRMS patients in each study arm, 80% of patients treated with ACTH plus cyclophosphamide were stable or

improved for a year, compared with 20% with ACTH alone, and 50% with ACTH, oral cyclophosphamide, and plasmapheresis (Hauser and others 1983). Cyclophosphamide decreases effector T cell function but also CD8 suppressor cell function (Sahasrabudhe and others 1987). It is hard to determine relative mechanisms of ACTH and chemotherapy on immune cell function in this study. The FDA-approved form of ACTH is purified from pituitaries and contains different fragments of the pro-opiomelanocortin (POMC) molecule. The first 13 amino acids of ACTH are actually α -MSH, which binds to MCR1 and MCR3. α -melanocyte stimulating hormone (α -MSH) inhibits EAE, a model of MS (Taylor and Kitaichi 2008). Trials of ACTH in conjunction with IFN- β are tantalizing. Monthly ACTH added to ongoing IFN- β -1b reduced relapses, FLAIR MRI lesions, and neuropsychiatric side effects compared with added monthly intravenous methylprednisolone (Amezcuca and others 2013).

7. Statins are antiinflammatory (Ehrenstein and others 2005). They lower cholesterol and LDL, but also deactivate macrophages in atherosclerotic plaques. Their antiinflammatory effect is more closely correlated with prevention of strokes and heart attacks than is their depression of serum cholesterol. Because they are antiinflammatory, it is logical to combine statins with IFN- β to treat MS. Surprisingly, adding high-dose atorvastatin (40–80 mg/day) to ongoing subcutaneous IFN- β -1a therapy caused clinical or MRI exacerbations in 10/15 patients, compared with only 1/10 in the IFN- β -only group (Birnbau and others 2008). Why would 2 agents with potential therapeutic benefit in MS not synergize? Statins directly inhibit IFN signaling (Feng and others 2012a). Ordinarily, type I IFNs bind to the IFNAR and activate JAK1 and TYK2, which phosphorylate STAT2 and STAT1. p-STAT1, p-STAT2, and IRF9 form a complex that binds to DNA promoters and activates genes. Statins, *in vitro* and *in vivo*, prevent phosphorylation of STAT1 on tyrosine and hamper synthesis of downstream antiviral proteins (MxA and viperin) and IFN-regulated immune proteins (IRF1 and type I IFN itself). There was no blockade of STAT2 phosphorylation. In this case, combination of 2 drugs that individually benefit MS is detrimental. This disruption of IFN signaling demonstrates the need to understand the mechanism of action and potential interactions between therapies, especially in MS, a disease where the undelaying cause is still a mystery.

Neutralizing Antibodies and Type I IFNs

How many neutralizing antibodies (NAb) are needed to neutralize the IFN response? NAb to IFN- β can block IFN signaling *in vitro*. *In vivo*, mid to high titers of NAb allow more MRI lesions, but clinical effects are less clear and perhaps even counterintuitive. In a retrospective study of over 2,000 patients, NAb to IFN- β -1b actually correlated with fewer clinical attacks despite an adverse effect on MRI (Goodin and others 2007). IFN- β cuts down Gd⁺ lesions by 85%–90% within a week of starting therapy, but attack rate does not fall for 3 months. This may indicate that effects on endothelial cells are immediate, but that reversal of immune dysregulation in the brain parenchyma may take longer.

Possible mechanisms for the dissociation of measured NAb potency from their ability to block the clinical benefit of IFN- β are detailed by Reder (2007): (1) *in vitro* measures of NAb may not reflect *in vivo* function; (2) Ig-Fc-IFN complexes suppress immunity; (3) Ab-IFN aggregates prolong IFN action; (4) contemporaneous binding Ab (BAB) enhance IFN signaling; (5) there are no excess virus infections in NAb-positive patients; (6) patients with high levels of clinical activity are less likely to be NAb-positive.

In addition, there may be different effects on endothelial cells (Gd⁺ lesions) compared with effects on each step of the sequence of immune cell penetration through endothelial cells (Åström and others 1968), and then the basement membrane, and then into the brain parenchyma (MS exacerbations). IFN- β therapy reduces MMP-9, and this likely prevents the second step required for immune cells to enter the CNS parenchyma. This is a putative mechanism of preventing the ensuing exacerbations and permanent CNS damage, reflected in less MRI T1 black hole formation (Filippi and others 2011).

A well-controlled study seemed to show that NAb to type I IFN actually enhanced IFN signaling (Moll and others 2008). Unfortunately, the antibodies to IFN were prepared on the same columns that earlier had been used to purify IFN (Moll and others 2011). Residual IFN from the columns was bound by the antibodies, IFN-Ab complexes formed, and then IFN dissociated from the Abs during *in vitro* experiments. This led to apparent enhancement of IFN signaling by blocking antibodies. At least 2 suppliers have provided IFN-contaminated NAb. However, this case is not closed: NAb-IFN complexes could also dissociate *in vivo* and release bioactive IFN, depending on the half-life of the complex, the Ab versus receptor affinity, and local conditions. Further implications are as follows: *in vitro* blocking experiments that use antibodies must be viewed with caution because NAb titers, affinity, and *in vitro* half-life may vary. Moreover, therapy with intravenous immunoglobulin (IVIG)—which can contain complexes of cytokines and anticytokine antibodies—could deliver multiple cytokines *in vivo*.

Viruses, Bacteria, and Type I IFNs

Virus infections cause MS exacerbations (Sibley and others 1985). IFN therapy does not reduce the frequency of virus infections, but it does prevent the sequelae of virus infections (Panitch 1994). In addition, endogenous viruses could play a role in MS (Perron and others 2012). Ancient human endogenous retrovirus (HERV) sequences are 8% of the genome. Although HERV DNA does not code for intact virions, HERV nucleic acids are transcribed. These virus traces include nucleic acids, proteins, and protein fragments, and can induce IFNs. Activated macrophages, microglia, and astrocytes within active MS lesions contain significant amounts of HERV proteins (Perron, personal communication, 2013).

Viral nucleic acids are policed in multiple ways. Trex (DNA polymerase III) repairs DNA double-strand breaks and also destroys virus DNA and endogenous DNA fragments. In Aicardi-Goutières syndrome, mutations in Trex allow an excess virus load and high systemic and CNS IFN- α . IFN- β -1b markedly reduces the retrovirus signature in MNC in this syndrome, perhaps by blocking inflammation

in the peripheral blood and in CNS lesions with high HERV burden. Possibly related, HIV patients on antiretroviral agents may have a lower than expected incidence of MS (Nexo and others 2013). It could be argued that HIV infection suppresses the immune system and prevents MS, but a pre-HAART study showed rapid worsening of MS in HIV-infected MS patients (Berger and others 1989).

The 2 kg gut microbiome contains 1,000× more bacteria than the cells in the rest of the human body. This consortium of 100 trillion microbes provides a diverse and changing constellation of proteins that shape the repertoire of the immune system. Current thinking holds that a highly diverse gut microbiome prevents autoimmune diseases and MS. IFN is likely to have a complex influence on gut bacteria, as well as viruses and phages that infect bacteria and their owner, and the consequential control by the immune system. IFNs, as well as perhaps other disease-modifying therapies, are likely to alter the microbiota in MS, which provides a potentially fertile direction for improving MS therapy.

IFN- β is not approved for any diseases other than MS. However, type I IFNs do curtail infections and cancer. When evaluating the benefit versus side-effect profile of MS therapy, the safety and potential benefit of IFN- β on cervical HPV infections, chronic virus infections, herpes zoster, and cancer should be included in the calculus, along with effects on cognition, fertility, neuroprotection, and mortality.

Acknowledgments

This study was supported by a grant (RG#4509A) from the U.S. National MS Society. The authors thank Suad Causevic for technical help.

Author Disclosure Statement

A.T.R. has received funding for unrestricted research grants, blinded trials, and consulting from the following groups attempting to find a cure for MS: Bayer, Biogen, Novartis, Questcor, and Serono, as well as the National MS Society.

X.F. has received unrestricted research grants from Bayer, Biogen, Novartis, and the National MS Society.

References

- Ahn J, Feng X, Patel N, Dhawan N, Reder AT. 2004. Abnormal levels of interferon-gamma receptors in active multiple sclerosis are normalized by IFN- β therapy: implications for control of apoptosis. *Front Biosci* 9:1547–1555.
- Äivo J, Lindsröm BM, Soilu-Hänninen M. 2012. A Randomised, double-blind, placebo-controlled trial with vitamin D3 in MS: subgroup analysis of patients with baseline disease activity despite interferon treatment. *Mult Scler Int* 2012:802796.
- Amezcuca L, Axtell RC, Cen S, Tauhid S, Neema M, Subhani D, Bakshi R, Steinman L, Berkovich R. 2013. Pilot study of monthly pulse adrenocorticotrophic hormone or methylprednisolone as an add-on therapy to β -interferons for long-term treatment of multiple sclerosis. *ECTRIMS* (online), P1097.
- Anlar B, Gücüyener K, Imir T, Yalaz K, Renda Y. 1993. Cimetidine as an immunomodulator in subacute sclerosing panencephalitis: a double blind, placebo-controlled study. *Pediatr Infect Dis J* 12:578–581.
- Antel JP, Arnason BGW, Medof ME. 1979. Suppressor cell function in multiple sclerosis: correlation with clinical disease activity. *Ann Neurol* 5:338–342.
- Antel JP, Freedman MS, Brodovsky S, Francis GS, Duquette P. 1989. Activated suppressor cell function in severely disabled patients with multiple sclerosis. *Ann Neurol* 25:204–207.
- Arnason BG, Berkovich R, Catania A, Lisak RP, Zaidi M. 2012. Mechanisms of action of adrenocorticotrophic hormone and other melanocortins relevant to the clinical management of patients with multiple sclerosis. *Mult Scler* 19:130–136.
- Arscott WT, Soltys J, Knight J, Mao-Draayer Y. 2011. Interferon β -1b directly modulates human neural stem/progenitor cell fate. *Brain Res* 1413:1–8.
- Ascherio A, Munger KL, White R, Kochert K, Simon KC, Polman CH, Freedman MS, Hartung HP, Miller DH, Montalban X, Edan G, Barkhof F, Pleimes D, Radu EW, Sandbrink R, Kapos L, Pohl C. 2014. Vitamin D as an early predictor of multiple sclerosis activity and progression. *JAMA Neurology* 71:306–314.
- Åström KE, Webster HDF, Arnason BGW. 1968. The initial lesion in experimental allergic neuritis: a phase and electron microscopic study. *J Exp Med* 128:469–495.
- Axtell RC, de Jong BA, Boniface K, van der Voort LF, Bhat R, De Sarno P, Naves R, Han M, Zhong F, Castellanos JG, Mair R, Christakos A, Kolkowitz I, Katz L, Killestein J, Polman CH, de Waal Malefyt R, Steinman L, Raman C. 2010. T helper type 1 and 17 cells determine efficacy of interferon- β in multiple sclerosis and experimental encephalomyelitis. *Nat Med* 16:406–413.
- Barkhof F, Hulst HE, Drulovic J, Uitdehaag BM, Matsuda K, Landin R. 2010. Ibudilast in relapsing-remitting multiple sclerosis: a neuroprotectant? *Neurology* 74:1033–1040.
- Bazer FW. 2013. Pregnancy recognition signaling mechanisms in ruminants and pigs. *J Anim Sci Biotechnol* 4:23.
- Berger JR, Sheremata WA, Resnick L, Atherton S, Fletcher MA, Norenberg M. 1989. Multiple sclerosis-like illness occurring with human immunodeficiency virus infection. *Neurology* 39:324–329.
- Birnbaum G, Cree B, Altafullah I, Zinser M, Reder A. 2008. Combining beta interferon and atorvastatin increases disease activity in multiple sclerosis. *Neurology* 71:1390–1395.
- Buyon JP. 2008. Systemic lupus erythematosus. In: Klippel JH, editor. *Primer on Rheumatic Diseases* (Chapter 15). New York, NY: Springer Science + Business Media, pp 303–337.
- Byskosh PV, Reder AT. 1996. Interferon- β effects on cytokine mRNA in peripheral mononuclear cells in multiple sclerosis. *Mult Scler* 1:262–269.
- Cabrera-Gomez JA, Lopez-Saura P. 1999. [Recent advances in the treatment the nervous system disorders with interferon-alpha]. *Rev Neurol* 29:1225–1235.
- Camenga DL, Johnson KP, Alter M, Engelhardt CD, Fishman PS, Greenstein JI, Haley AS, Hirsch RL, Kleiner JE, Kofie VY, Koski CL, Margulies SL, Panitch HS, Valero R. 1986. Systemic recombinant α -2 interferon therapy in relapsing multiple sclerosis. *Arch Neurol* 43:1239–1246.
- Chen M, Chen G, Deng S, Liu X, Hutton GJ, Hong J. 2012. IFN- β induces the proliferation of CD4+CD25+Foxp3+ regulatory T cells through upregulation of GITRL on dendritic cells in the treatment of multiple sclerosis. *J Neuroimmunol* 242:39–46.
- Chon TW, Bixler S. 2010. Interferon- τ : current applications and potential in antiviral therapy. *J Interferon Cytokine Res* 30:477–485.
- Comabella M, Lunemann JD, Rio J, Sanchez A, Lopez C, Julia E, Fernandez M, Nonell L, Camina-Tato M, Deisenhammer F, Caballero E, Tortola MT, Prinz M, Montalban X, Martin R. 2009. A type I interferon signature in monocytes is

- associated with poor response to interferon- β in multiple sclerosis. *Brain* 132(Pt 12):3353–3365.
- Correale J, Farez MF, Ysrraelit MC. 2012. Increase in multiple sclerosis activity after assisted reproduction technology. *Ann Neurol* 72:682–694.
- Correale J, Villa A. 2008. Isolation and characterization of CD8+ regulatory T cells in multiple sclerosis. *J Neuroimmunol* 195:121–134.
- Croze E, Velichko S, Tran T, Yamaguchi K, Salamon H, Reder A, Knappertz V, Paz P. 2009. Betaferon[®] induces a novel alternate start transcript in cells obtained from relapsing-remitting multiple sclerosis patients and human brain that is associated with control of oxidative resistance. *Mult Scler* 15(Suppl 2):P475, S138.
- Croze E, Yamaguchi KD, Knappertz V, Reder AT, Salamon H. 2013. Interferon-beta-1b-induced short- and long-term signatures of treatment activity in multiple sclerosis. *Pharmacogenomics J* 13:443–451.
- Dalla Libera D, Di Mitri D, Bergami A, Centonze D, Gasperini C, Grasso MG, Galgani S, Martinelli V, Comi G, Avolio C, Martino G, Borsellino G, Sallusto F, Battistini L, Furlan R. 2011. T regulatory cells are markers of disease activity in multiple sclerosis patients. *PLoS One* 6:e21386.
- Der SD, Zhou A, Williams BR, Silverman RH. 1998. Identification of genes differentially regulated by interferon α , β , or γ using oligonucleotide arrays. *Proc Natl Acad Sci USA* 95:15623–15628.
- Durelli L, Bongioanni MR, Cavallo R, Ferrero B, Ferri R, Ferrio MF, Bradac GB, Riva A, Vai S, Geuna M, Bergamini L, Bergamasco B. 1994. Chronic systemic high-dose recombinant interferon alfa-2a reduces exacerbation rate, MRI signs of disease activity, and lymphocyte interferon gamma production in relapsing-remitting multiple sclerosis. *Neurology* 44:406–413.
- Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, Ferrero B, Eid P, Novelli F. 2009. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon- β . *Ann Neurol* 65:499–509.
- Duschet P, Schwarz T, Soyer P, Henk A, Hausmaninger H, Gschnait F. 1988. Treatment of herpes zoster. Recombinant alpha interferon versus acyclovir. *Int J Dermatol* 27:193–197.
- Ebers GC, PRISMS (Prevention of Relapses and Disability by Interferon b-1a Subcutaneously in Multiple Sclerosis) Study Group. 1998. Randomised double-blind placebo-controlled study of interferon β -1a in relapsing/remitting multiple sclerosis. *Lancet* 352:1498–1504.
- Feng X, Anzai L, Abdallah M, Kim H-I, Li L, Reder AT. 2013. Serum cytokines, neuroprotective proteins, and interferon signaling in IFN therapy responders (R) and non-responders (NR) in MS. *Neurology* 80 (Meeting Abstracts 1), P05.182. Available at www.neurology.org online
- Feng X, Han D, Kilaru BK, Franek BS, Niewold TB, Reder AT. 2012a. Inhibition of interferon-beta responses in multiple sclerosis immune cells associated with high-dose statins. *Arch Neurol* 69:1303–1309.
- Feng X, Petraglia AL, Chen M, Byskosh PV, Boos MD, Reder AT. 2002a. Low expression of interferon-stimulated genes in active multiple sclerosis is linked to subnormal phosphorylation of STAT1. *J Neuroimmunol* 129:105–115.
- Feng X, Reder NP, Yanamandala M, Hill A, Franek BS, Niewold TB, Reder AT, Javed A. 2012b. Type I interferon signature is high in lupus and neuromyelitis optica but low in multiple sclerosis. *J Neurol Sci* 313:48–53.
- Feng X, Yau D, Holbrook C, Reder AT. 2002b. Type I interferons inhibit Interleukin-10 production in activated human monocytes and stimulate IL-10 in T cells: implications for Th1-mediated diseases. *J Interferon Cytokine Res* 22:311–319.
- Filippi M, Rocca MA, Camesasca F, Cook S, O'Connor P, Arnason BG, Kappos L, Goodin D, Jeffery D, Hartung HP, Comi G, Wolinsky JS, Bogumil T, Pohl C, Beckmann K, Sandbrink R, Croze E, Brown C, Desimone TM, Arnold DL, Cutter G, Knappertz V. 2011. Interferon β -1b and glatiramer acetate effects on permanent black hole evolution. *Neurology* 76:1222–1228.
- Fischer JS, Priore RL, Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, Goodkin DE, et al. 2000. Neuropsychological effects of interferon β -1a in relapsing multiple sclerosis. *Ann Neurol* 48:885–892.
- Foster GR, Masri SH, David R, Jones M, Datta A, Lombardi G, Runkell L, de Dios C, Sizing I, James MJ, Marelli-Berg FM. 2004. IFN- α subtypes differentially affect human T cell motility. *J Immunol* 173:1663–1670.
- Frith J, the AUSTIMS Research Group. 1989. Interferon- α and transfer factor in the treatment of multiple sclerosis: a double-blind, placebo-controlled trial. *J Neurol Neurosurg Psychiatry* 52:566–574.
- Garcin G, Bordat Y, Chuchana P, Monneron D, Law HK, Piehler J, Uze G. 2013. Differential activity of type I interferon subtypes for dendritic cell differentiation. *PLoS One* 8:e58465.
- Goelz SE, Hahm B, Luo Y, Engber T, Gorelik L. 2007. Positive regulation of neuroprotective genes in the CNS of mice with experimental autoimmune encephalitis (EAE) by interferon beta (IFN- β) treatment. *Neurology* 68(Suppl 1):P03.031A.
- Gold SM, Voskuhl RR. 2009. Estrogen and testosterone therapies in multiple sclerosis. *Prog Brain Res* 175:239–251.
- Goodin DS, Hurwitz B, Noronha A. 2007. Neutralizing antibodies to interferon β -1b are not associated with disease worsening in multiple sclerosis. *J Int Med Res* 35:173–187.
- Goodin DS, Reder AT, Ebers GC, Cutter G, Kremenchtzky M, Oger J, Langdon D, Rametta M, Beckmann K, DeSimone TM, Knappertz V. 2012. Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFN β -1b trial. *Neurology* 78:1315–1322.
- Hauser SL, Dawson DM, Leirich JR, Beal MF, Kevy SV, Propper RD, Mills JA, Weiner HL. 1983. Intensive immunosuppression in progressive multiple sclerosis. A randomized, three-arm study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH. *N Engl J Med* 308:178–180.
- Hedrich CM, Rauen T, Kis-Toth K, Kyttaris VC, Tsokos GC. 2012. cAMP-responsive element modulator α (CREM α) suppresses IL-17F protein expression in T lymphocytes from patients with systemic lupus erythematosus (SLE). *J Biol Chem* 287:4715–4725.
- Idzko M, la Sala A, Ferrari D, Panther E, Herouy Y, Dichmann S, Mockenhaupt M, Di Virgilio F, Girolomoni G, Norgauer J. 2002. Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 109:839–846.
- Ishizuka T, Murotani T, Yamatodani A. 2012. Action of modafinil through histaminergic and orexinergic neurons. *Vitam Horm* 89:259–278.
- Jaitin DA, Roisman LC, Jaks E, Gavutis M, Piehler J, Van der Heyden J, Uze G, Schreiber G. 2006. Inquiring into the differential action of interferons (IFNs): an IFN- α 2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN- β . *Mol Cell Biol* 26:1888–1897.
- Javed A, Balabanov R, Arnason BGW, Kelly TJ, Sweiss NJ, Pytel P, Walsh R, Blair EA, Stemer A, Lazzaro M, Reder AT.

2008. Minor salivary gland inflammation in Devic's disease and longitudinally extensive myelitis. *Mult Scler* 14:809–814.
- Karandikar NJ, Crawford MP, Yan X, Ratts RB, Brenchley JM, Ambrozak DR, Lovett-Racke AE, Frohman EM, Stastny P, Douek DC, Koup RA, Racke MK. 2002. Glatiramer acetate (Copaxone) therapy induces CD8⁺ T cell responses in patients with multiple sclerosis. *J Clin Invest* 109:641–649.
- Karaszewski JW, Reder AT, Anlar B, Arnason BGW. 1993. Increased high affinity beta-adrenergic receptor densities and cyclic AMP responses of CD8 cells in multiple sclerosis. *J Neuroimmunol* 43:1–7.
- Khoroshki R, Owens T. 2010. Injury-induced type I IFN signaling regulates inflammatory responses in the central nervous system. *J Immunol* 185:1258–1264.
- Khoury SJ, Healy BC, Kivisakk P, Viglietta V, Egorova S, Guttman CR, Wedgwood JF, Hafler DA, Weiner HL, Buckle G, Cook S, Reddy S. 2010. A randomized controlled double-masked trial of albuterol add-on therapy in patients with multiple sclerosis. *Arch Neurol* 67:1055–1061.
- Knobler RL, Greenstein JI, Johnson KP, Lublin FD, Panitch HS, Conway K, Grant-Gorsen SV, Muldoon J, Marcus SG, Wallenberg JC. 1993. Systemic recombinant human interferon- β treatment of relapsing-remitting multiple sclerosis: pilot study analysis and six-year follow-up. *J Interferon Res* 13:333–340.
- Knobler RL, Panitch HS, Braheny SL, Sipe JC, Rice GPA, Huddleston JR, Francis GS, Hooper CJ, Kamin-Lewis RM, Johnson KP, Oldstone MBA, Merigan TC. 1984. Systemic alpha-interferon therapy of multiple sclerosis. *Neurology* 34:1273–1279.
- Lacy M, Hauser M, Pliskin N, Assuras S, Valentine MO, Reder A. 2013. The effects of long-term interferon- β -1b treatment on cognitive functioning in multiple sclerosis: a 16-year longitudinal study. *Mult Scler* 19:1765–1772.
- Li X, Murray F, Koide N, Goldstone J, Dann SM, Chen J, Bertin S, Fu G, Weinstein LS, Chen M, Corr M, Eckmann L, Insel PA, Raz E. 2012. Divergent requirement for G α s and cAMP in the differentiation and inflammatory profile of distinct mouse Th subsets. *J Clin Invest* 122:963–973.
- Lucas RM, Ponsonby AL, Dear K, Valery PC, Pender MP, Taylor BV, Kilpatrick TJ, Dwyer T, Coulthard A, Chapman C, van der Mei I, Williams D, McMichael AJ. 2011. Sun exposure and vitamin D are independent risk factors for CNS demyelination. *Neurology* 76:540–548.
- Mahic M, Yaqub S, Johansson CC, Tasken K, Aandahl EM. 2006. FOXP3⁺CD4⁺CD25⁺ adaptive regulatory T cells express cyclooxygenase-2 and suppress effector T cells by a prostaglandin E₂-dependent mechanism. *J Immunol* 177:246–254.
- Marie I, Durbin JE, Levy DE. 1998. Differential viral induction of distinct interferon- α genes by positive feedback through interferon regulatory factor-7. *EMBO J* 17:6660–6669.
- Michel L, Foucher Y, Vukusic S, Confavreux C, de Seze J, Brassat D, Clanet M, Clavelou P, Ouallet JC, Brochet B, Pelletier J, Labauge P, Lebrun C, Lepage E, Le Frere F, Jacq-Foucher M, Barriere P, Wiertlewski S, Laplaud DA. 2012. Increased risk of multiple sclerosis relapse after *in vitro* fertilisation. *J Neurol Neurosurg Psychiatry* 83:796–802.
- Moll HP, Freudenthaler H, Zommer A, Buchberger E, Brostjan C. 2008. Neutralizing type I IFN antibodies trigger an IFN-like response in endothelial cells. *J Immunol* 180:5250–5256.
- Moll HP, Freudenthaler H, Zommer A, Buchberger E, Lin XH, Crisafulli S, Pandya Y, Pestka S, Lavoie T, Brostjan C. 2011. Contamination with recombinant IFN accounts for the unexpected stimulatory properties of commonly used IFN-blocking antibodies. *Eur J Immunol* 41:252–254.
- Moraga I, Harari D, Schreiber G, Uzé G, Pellegrini S. 2009. Receptor density is key to the alpha2/beta interferon differential activities. *Mol Cell Biol* 29:4778–4787.
- Mosenden R, Tasken K. 2011. Cyclic AMP-mediated immune regulation—overview of mechanisms of action in T cells. *Cell Signal* 23:1009–1016.
- Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 296:2832–2838.
- Nakashima I, Fujihara K, Kimpura T, Okita N, Takase S, Itoyama Y. 2001. Linear pontine trigeminal root lesions in multiple sclerosis: clinical and magnetic resonance imaging studies in 5 cases. *Arch Neurol* 58:101–104.
- Nexo BA, Pedersen L, Sorensen HT, Koch-Henriksen N. 2013. Treatment of HIV and risk of multiple sclerosis. *Epidemiology* 24:331–332.
- Noronha A, Toscas A, Jensen M. 1992. Contrasting effects of alpha, beta, and gamma interferons on nonspecific suppressor function in multiple sclerosis. *Ann Neurol* 31:103–106.
- Ostensen M, Brucato A, Carp H, Chambers C, Dolhain RJ, Doria A, Forger F, Gordon C, Hahn S, Khamashta M, Lockshin MD, Matucci-Cerinic M, Meroni P, Nelson JL, Parke A, Petri M, Raio L, Ruiz-Irastorza G, Silva CA, Tin-can A, Villiger PM, Wunder D, Cutolo M. 2011. Pregnancy and reproduction in autoimmune rheumatic diseases. *Rheumatology (Oxford)* 50:657–664.
- Panitch HS. 1994. Influence of infection on exacerbations of multiple sclerosis. *Ann Neurol* 36(Suppl):S25–S28.
- Panitch HS. 2004. Interferon beta-1b in secondary progressive MS: results from a 3-year controlled study. *Neurology* 63:1788–1795.
- Passani MB, Ballerini C. 2012. Histamine and neuroinflammation: insights from murine experimental autoimmune encephalomyelitis. *Front Syst Neurosci* 6:32.
- Perron H, Germi R, Bernard C, Garcia-Montojo M, Deluen C, Farinelli L, Faucard R, Veas F, Stefas I, Fabrick BO, Van-Horssen J, Van-der-Valk P, Gerdil C, Mancuso R, Saresella M, Clerici M, Marcel S, Creange A, Cavaretta R, Caputo D, Arru G, Morand P, Lang AB, Sotgiu S, Ruprecht K, Rieckmann P, Villoslada P, Chofflon M, Boucraut J, Pelletier J, Hartung HP. 2012. Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. *Mult Scler* 18:1721–1736.
- Petersen T, Møller-Larsen A, Ellermann-Eriksen S, Thiel S, Christensen T. 2012. Effects of interferon-beta therapy on elements in the antiviral immune response towards the human herpesviruses EBV, HSV, and VZV, and to the human endogenous retroviruses HERV-H and HERV-W in multiple sclerosis. *J Neuroimmunol* 249:105–108.
- Petzold A, Mondria T, Kuhle J, Rocca MA, Cornelissen J, te Boekhorst P, Lowenberg B, Giovannoni G, Filippi M, Kappos L, Hintzen R. 2010. Evidence for acute neurotoxicity after chemotherapy. *Ann Neurol* 68:806–815.
- Pliskin NH, Hamer DP, Goldstein DS, Towle VL, Reder AT, Noronha A, Arnason BGW. 1996. Improved delayed visual reproduction test performance in multiple sclerosis patients receiving interferon β -1b. *Neurology* 47:1463–1468.
- Reder AT. 2007. Neutralizing antibodies in multiple sclerosis: a complex impact on interferon responses, magnetic resonance imaging findings, and clinical outcomes. *Mult Scler* 13(Suppl):553–562.
- Reder AT, Abdallah N, Kim H-I, Anzai L, Li L, Feng X. 2013. Correction of non-response to interferon-beta in multiple sclerosis. *Neurology* 80 (Meeting Abstracts 1), P01.203. Available at www.neurology.org

- Reder AT, Arnason BGW. 1995. Trigeminal neuralgia in MS relieved by a prostaglandin E analogue. *Neurology* 45:1097–1100.
- Reder AT, Feng X. 2013. Aberrant type I interferon regulation in autoimmunity: opposite directions in MS and SLE, shaped by evolution and body ecology. *Front Immunol* 4:1–13.
- Reder AT, Li L, Feng X. 2011. Disease activity affects responses to IFN- β -1b therapy in multiple sclerosis. *Neurology* 76(Suppl. 3):P01.215.
- Reder AT, Thapar M, Sapugay AM, Jensen MA. 1994. Prostaglandins and inhibitors of arachidonate metabolism suppress experimental allergic encephalomyelitis. *J Neuroimmunol* 54:117–127.
- Reyes-Vazquez C, Prieto-Gomez B, Dafny N. 2012. Interferon modulates central nervous system function. *Brain Res* 1442: 76–89.
- Roberts RM. 2007. Interferon-tau, a type I interferon involved in maternal recognition of pregnancy. *Cytokine Growth Factor Rev* 18:403–408.
- Rose AS, Kuzma JW, Kurtzke JF, Namerow NS, Sibley WA, Tourtellotte WW, Dixon WJ, Foley JM, Geschwind N, MacKay RP, McDowell FH, Scheinberg LC, Schumacher GA, White PT, Brown AJ, Fusillo MG, Johnson W, Kane CA, Lee JE, de Oliveira MM, Rumberg J, Simpson JF. 1970. Cooperative study in the evaluation of therapy in multiple sclerosis: ACTH vs. placebo: final report. *Neurology* 20:1–70.
- Rudick RA, Rani MR, Xu Y, Lee JC, Na J, Shrock J, Josyula A, Fisher E, Ransohoff RM. 2011. Excessive biologic response to IFN β is associated with poor treatment response in patients with multiple sclerosis. *PLoS One* 6:e19262.
- Sadzak I, Schiff M, Gattermeier I, Glinitzer R, Sauer I, Saalmuller A, Yang E, Schaljo B. 2008. Recruitment of Stat1 to chromatin is required for interferon-induced serine phosphorylation of Stat1 transactivation domain. *Proc Natl Acad Sci U S A* 105:8944–8949.
- Sahasrabudhe DM, McCune CS, O'Donnell RW, Henshaw EC. 1987. Inhibition of suppressor T lymphocytes (Ts) by cimetidine. *J Immunol* 138:2760–2763.
- Sansoni P, Silverman ED, Khan MM, Melmon KL, Engleman EG. 1985. Immunoregulatory T cells in man. Histamine-induced suppressor T cells are derived from a Leu 2+ (T8+) subpopulation distinct from that which gives rise to cytotoxic T cells. *J Clin Invest* 75:650–656.
- Sibley WA, Bamford CR, Clark K. 1985. Clinical viral infections and multiple sclerosis. *Lancet* I:1313–1315.
- Soilu-Hanninen M, Aivo J, Lindstrom BM, Elovaara I, Sumelahti ML, Farkkila M, Tienari P, Atula S, Sarasoja T, Herrala L, Keskinarkaus I, Kruger J, Kallio T, Rocca MA, Filippi M. 2012. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 83:565–571.
- Squillacote D, Martinez M, Sheremata W. 1996. Natural alpha interferon in multiple sclerosis: results of three preliminary series. *J Int Med Res* 24:246–257.
- Stein MS, Liu Y, Gray OM, Baker JE, Kolbe SC, Ditchfield MR, Egan GF, Mitchell PJ, Harrison LC, Butzkueven H, Kilpatrick TJ. 2011. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. *Neurology* 77: 1611–1618.
- Stewart N, Simpson S Jr., van der Mei I, Ponsonby AL, Blizzard L, Dwyer T, Pittas F, Eyles D, Ko P, Taylor BV. 2012. Interferon- β and serum 25-hydroxyvitamin D interact to modulate relapse risk in MS. *Neurology* 79:254–260.
- Talpaz M, Hehlmann R, Quintas-Cardama A, Mercier J, Cortes J. 2013. Re-emergence of interferon- α in the treatment of chronic myeloid leukemia. *Leukemia* 27:803–812.
- Taylor AW, Kitaichi N. 2008. The diminishment of experimental autoimmune encephalomyelitis (EAE) by neuropeptide alpha-melanocyte stimulating hormone (α -MSH) therapy. *Brain Behav Immun* 22:639–646.
- Thomas C, Moraga I, Levin D, Krutzik PO, Podoplelova Y, Trejo A, Lee C, Yarden G, Vleck SE, Glenn JS, Nolan GP, Piehler J, Schreiber G, Garcia KC. 2011. Structural linkage between ligand discrimination and receptor activation by type I interferons. *Cell* 146:621–632.
- van der Mei IA, Ponsonby AL, Dwyer T, Blizzard L, Simmons R, Taylor BV, Butzkueven H, Kilpatrick T. 2003. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. *BMJ* 327:316.
- Vandenbark AA, Huan J, Agotsch M, La Tocha D, Goelz S, Offner H, Lanker S, Bourdette D. 2009. Interferon-beta-1a treatment increases CD56^{bright} natural killer cells and CD4+CD25+ Foxp3 expression in subjects with multiple sclerosis. *J Neuroimmunol* 215:125–128.
- Venken K, Hellings N, Thewissen M, Somers V, Hensen K, Rummens JL, Medaer R, Hupperts R, Stinissen P. 2008. Compromised CD4⁺ CD25^{high} regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. *Immunology* 123:79–89.
- Vitale G, de Herder WW, van Koetsveld PM, Waaijers M, Schoordijk W, Croze E, Colao A, Lamberts SW, Hofland LJ. 2006. IFN- β is a highly potent inhibitor of gastroenteropancreatic neuroendocrine tumor cell growth *in vitro*. *Cancer Res* 66:554–562.
- Vitale G, van Eijck CH, van Koetsveld Ing PM, Erdmann JJ, Speel EJ, van der Wansem Ing K, Mooij DM, Colao A, Lombardi G, Croze E, Lamberts SW, Hofland LJ. 2007. Type I interferons in the treatment of pancreatic cancer: mechanisms of action and role of related receptors. *Ann Surg* 246:259–268.
- Voskuhl R, Wang HJ, Wu TCJ, Sicotte N, Bates A, Beaver G, Corboy J, Cross A, Dhib-Jalbut S, Ford C, Giesser B, Jacobs D, Lynch S, Pachner A, Racke M, Ratchford J, Reder AT, Rose J, Wingerchuk D, Burnett M, Tseng CH, Elashoff R. 2014. A combination trial of estriol plus glatimer acetate in relapsing-remitting multiple sclerosis. *Neurology* (Meeting Abstracts 1), S23.003. Available at www.neurology.org online
- Wiegmann K, Muthyala S, Kim DH, Arnason BGW, Chelmickas-Schorr E. 1995. β -Adrenergic agonists suppress chronic/relapsing experimental allergic encephalomyelitis (CREAE) in Lewis rats. *J Neuroimmunol* 56:201–206.

Address correspondence to:

Dr. Anthony T. Reder
Department of Neurology, MC-2030
University of Chicago
5841 S Maryland Avenue
Chicago, IL 60637

E-mail: areder@neurology.bsd.uchicago.edu

Received 27 December 2013/Accepted 12 February 2014