The Journal of Immunology

BRIEF REPORT | SEPTEMBER 01 2002

Cutting Edge: Oral Type I IFN-τ Promotes a Th2 Bias and Enhances Suppression of Autoimmune Encephalomyelitis by Oral Glatiramer Acetate[1]

Jeanne M. Soos; ... et. al

J Immunol (2002) 169 (5): 2231–2235.
https://doi.org/10.4049/jimmunol.169.5.2231

CUTTING EDGE

Cutting Edge: Oral Type I IFN-τ Promotes a Th2 Bias and Enhances Suppression of Autoimmune Encephalomyelitis by Oral Glatiramer Acetate[1]

Jeanne M. Soos,[2]* Olaf Stüve,[†] Sawsan Youssef,[‡] Manuel Bravo,[†] Howard M. Johnson,[§] Howard L. Weiner,* and Scott S. Zamvil[3†]

IFN-τ, a novel type I IFN that possesses immunomodulatory properties, lacks toxicity normally associated with other type I IFNs. We examined the effects of oral IFN-τ alone and in combination with oral glatiramer acetate in experimental allergic encephalomyelitis (EAE). By comparison of oral administration of IFN-α, -β, and -τ to myelin basic protein-specific TCR-transgenic mice, we demonstrate these type I IFNs promote secretion of the Th2 cytokine IL-10 with similar efficiency. Whereas IFN-α and -β induced IFN-γ secretion, a Th1 cytokine, IFN-τ did not. Oral IFN-τ alone suppressed EAE. When suboptimal doses were administered orally in combination to wild-type mice, IFN-τ and glatiramer acetate had a synergistic beneficial effect in suppression of EAE. This combination was associated with TGF-β secretion and enhanced IL-10 production. Thus, IFN-τ is a potential candidate for use as a single agent or in combination therapy for multiple sclerosis. ***The Journal of Immunology*****, 2002, 169: 2231–2235.**

Interferon-β (1a and 1b) and glatiramer acetate (GA[4]; Copaxone, Cop1) are currently the only approved medications for treatment of relapsing-remitting multiple sclerosis (MS) (1, 2). These two classes of medications have distinct immunoregulatory characteristics. IFN-β exerts several effects in an Ag-nonspecific manner (1). Among its activities, IFN-β induces IL-10 secretion (3) and suppresses IFN-γ-inducible MHC class II up-regulation on APC (4). In contrast, GA, a synthetic basic random copolymer composed of tyrosine (Y), glutamate (E), alanine (A), and lysine (K), appears to preferentially affect T cells specific for CNS autoantigens (5), altering their Ag/MHC recognition not unlike altered peptide ligands (6, 7). GA also induces populations of GA-reactive Th2 regulatory cells that may provide bystander suppression in the CNS (8). Despite approval, IFN-β and GA are only partially effective MS treatments, and IFN-β, in particular, can be associated with significant side effects and potential toxicity, underscoring the importance for developing treatments that are more potent but also possess fewer potential side effects. Because currently available MS treatments alone are not entirely satisfactory, there is enthusiasm for testing medications in combination for enhanced efficacy (9, 10). In this regard, IFN-β and GA are currently being tested in combination in relapsing-remitting MS (10).

IFN-τ, a type I IFN first identified as a pregnancy recognition hormone in ruminants (11), possesses antiviral and immunoregulatory properties (11). Like IFN-β, IFN-τ induces T cell secretion of IL-10 and suppresses IFN-γ-inducible class II up-regulation on APC. Similar to other type I IFNs, IFN-τ is acid stable (11). However, in contrast to other type I IFNs, the biological activities of IFN-τ have not been associated with either significant side effects or toxicities (12, 13). IFN-τ was effective in the prevention of both acute and relapsing (11, 12) experimental allergic encephalomyelitis (EAE), a model for MS (14). IFN-τ also reversed ongoing relapsing EAE (15). In addition, IFN-τ was equally effective in EAE when given orally as well as parenterally (12, 13). Based upon these observations oral IFN-τ was tested in a phase I MS clinical trial (16). No toxicity was observed. Thus, because IFN-τ lacks toxicity and can be given orally, it is considered an attractive candidate for further evaluation in MS therapy.

In the present study, we evaluated the combination of oral IFN-τ and GA in EAE. First, we examined how oral IFN-τ alone influences T cell cytokine secretion in myelin basic protein (MBP)-specific TCR-transgenic mice. These mice, which contain a homogeneous population of naive MBP Ac1-11-specific CD4$^+$ (Th0) cells, serve as a valuable resource to test how immunomodulatory agents influence T cell activation and differentiation of CNS Ag-specific T cells. IFN-τ administration induced lymphocyte secretion of IL-4, IL-5, and IL-10 but, in contrast to IFN-α and -β, IFN-τ did not induce IFN-γ. Thus, IFN-τ supported a Th2 pattern of T cell differentiation. When administered in combination to wild-type mice at suboptimal doses, IFN-τ and GA had a synergistic beneficial effect in suppression of EAE. The combination of

*Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; †Department of Neurology, University of California, San Francisco, CA 94143; ‡Department of Neurology, Stanford Medical School, Stanford, CA 94305; and §Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611

Received for publication December 31, 2001. Accepted for publication June 28, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Support for this study was provided to S.S.Z. by Maisin Foundation Grant 98-416, National Institutes of Health Grant KO2 NS02207, and National Multiple Sclerosis Society Grant RG 3206-A-3, to H.L.W. by National Institutes of Health Grants AI 43458 and NS 38037, to S.S.Z. and H.L.W. by the Nancy Davis Foundation, and to H.M.J. by National Institutes of Health Grant R01-CA69959. J.M.S. and O.S. were supported by fellowships from the National Multiple Sclerosis Society and S.Y. was supported by a fellowship from the Katherine McCormick Foundation.

[2] Current address: Immunologic Toxicology, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA 19406.

[3] Address correspondence and reprint requests to Dr. Scott S. Zamvil, Department of Neurology, University of California, 521 Parnassus Avenue, C-440, San Francisco, CA 94143. E-mail address: zamvil@itsa.ucsf.edu

[4] Abbreviations used in this paper: GA, glatiramer acetate; MS, multiple sclerosis; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein.

0022-1767/02/$02.00

GA and IFN-τ promoted lymphocyte secretion of TGF-β and enhanced IL-10.

Materials and Methods

Mice

PL/J MBP Ac1-11-specific TCR-transgenic female mice (17) were provided by Dr. C. A. Janeway, Jr. (New Haven, CT). PL/J female mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Experimentation was conducted at Brigham and Women's Hospital (Boston, MA) and the University of California (San Francisco, CA) with institutional approval according to the U.S. Public Health Service's *Policy on Human Care and Use of Laboratory Animals*.

Antigens

MBP was prepared from mouse brains and purity was confirmed by gel electrophoresis and amino acid analysis. MBP Ac1-11 (Ac-ASQKRPSQRHG) was synthesized and HPLC purified.

EAE induction

EAE was induced in 10- to 12-wk-old female PL/J mice using 300 μg MBP in CFA containing 8 mg/ml H37Ra (Difco, Detroit, MI). Mice were injected on the flanks and base of the tail. A total of 400 ng of *Bordetella pertussis* toxin (List Biologicals, Campbell, CA) was administered i.v. on days 0 and 2.

Production and purification of IFN-τ

Ovine IFN-τ gene was expressed in *Pichia pastoris* using a synthetic gene construct and purified by sequential DEAE-cellulose and hydroxyapatite chromatography (12). Homogeneity was determined by SDS-PAGE and silver staining. Purified IFN-τ had a specific activity of $0.29-0.44 \times 10^8$ U/mg as measured by antiviral activity on Madin Darby bovine kidney cells. Murine IFN-α and IFN-β were obtained from Lee Biomolecular (San Diego, CA).

Administration of IFN-τ and GA

IFN-τ and GA were administered (100 μl each; 200 μl total volume/day) using 18-gauge feeding needles from Fisher Scientific (Norcross, GA). A total of 100 μl PBS was administered to mice treated with IFN-τ or GA alone. Mice were treated for 30 days in experiments testing EAE suppression.

T cells, proliferation, and cytokine measurements

Spleen and lymph node cells were cultured in 96-well plates at 5×10^5 cells/well in X-vivo 20 (BioWhittaker, Walkersville, MD) and appropriate Ag concentrations. Separate $CD4^+$ and $CD8^+$ T cells from MBP Ac1-11-

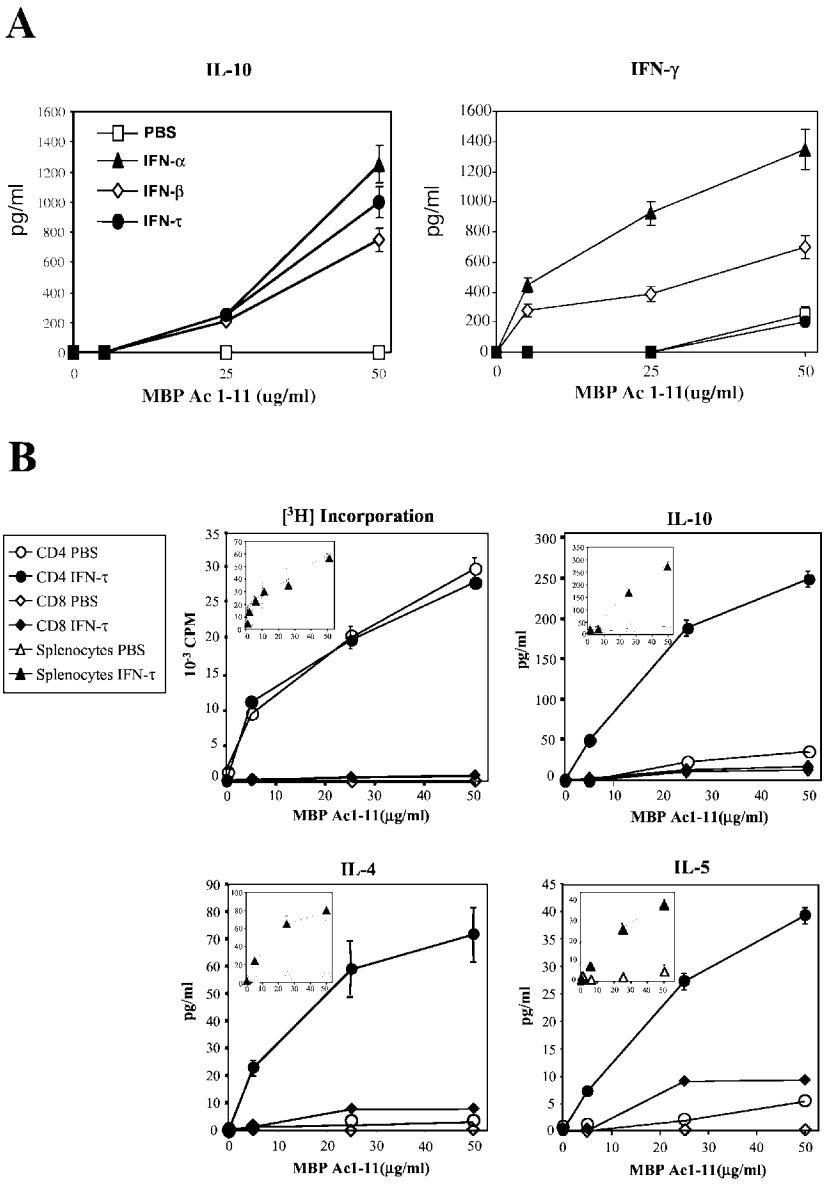

FIGURE 1. Cytokine production by T cells from MBP Ac1-11-specific TCR-transgenic mice treated with oral IFN. *A*, IFN-α, -β, and -τ induce IL-10 secretion while IFN-α and -β, but not IFN-τ, promote IFN-γ secretion. *B*, $CD4^+$ T cells from MBP Ac1-11-specific TCR-transgenic mice secrete IL-4, IL-5, and IL-10. Insets show cytokine secretion and proliferation by unseparated splenocytes from mice fed either IFN-τ or vehicle (PBS). Mice were fed daily with 1×10^5 U of IFN-α, IFN-β, or IFN-τ for 3 days. Splenocytes (5×10^5) were harvested 1 day after the last feeding. $CD4^+$ and $CD8^+$ T cells were separated as described in *Materials and Methods*. A total of 1×10^4 separated $CD4^+$ or $CD8^+$ T cells were cultured with 5×10^5 irradiated PL/J splenocytes and MBP Ac1-11. Cytokine secretion was measured by ELISA and proliferation by [^{3}H]thymidine incorporation as described in *Materials and Methods*.

specific TCR-transgenic mice were prepared by high-affinity negative selection using columns containing Ab-coated glass beads to remove B cells, monocytes, and either $CD4^+$ or $CD8^+$ cells (R&D Systems, Minneapolis, MN). $CD4^+$ and $CD8^+$ T cells were 95 and 85–90% pure, respectively, as measured by flow cytometry. For proliferation, 1×10^4 $CD4^+$ or $CD8^+$ T cells were cultured in the presence of 5×10^5 irradiated PL/J splenocytes, pulsed with 1 μCi/well [^{3}H]thymidine at 72 h, and harvested 16 h later. For cytokine measurements, culture supernatants were collected at 24 h for IL-2 measurement, 48 h for IFN-γ, IL-10, and TNF-α, 72 h for TGF-β, and 120 h for IL-4 and IL-5. Cytokines were measured by ELISA using kits from BioSource International (Camarillo, CA) as described previously (18). SE measurements for proliferation and cytokine measurements were within 10% of the mean.

Results and Discussion

The type I IFNs can induce lymphocyte secretion of the Th2 cytokine IL-10 (11). Therefore, we initially compared IFN-τ with IFN-α and -β for in vivo induction of IL-10. Unimmunized MBP Ac1-11-specific TCR-transgenic mice, used as a source of naive MBP-specific T (Th0) cells, were fed three times with 1×10^5 U of IFN-α, IFN-β, or IFN-τ. Splenocytes, isolated 1 day after the last feeding, were stimulated with MBP Ac1-11 in vitro. As shown in Fig. 1*A*, each of the three type I IFNs induced substantial IL-10 secretion. It can also be seen that IFN-τ was at least as potent as, if not more potent than, IFN-β at inducing IL-10 secretion.

It is also recognized that the type I IFNs can induce T cell secretion of the Th1 cytokine IFN-γ (11, 19). In fact, it has been observed that the frequency of IFN-γ-secreting cells increases during the first 2 mo of IFN-β 1b treatment, possibly contributing to the prominent "flu-like" symptoms that MS patients commonly experience during initial treatment (19). Thus, we compared IFN-α, -β, and -τ for in vivo induction of IFN-γ. IFN-α was approximately twice as potent as IFN-β (Fig. 1*A*). In contrast, IFN-τ did not stimulate IFN-γ secretion above the level seen in control (vehicle (PBS)-fed) mice.

While the experiments described above demonstrated that IFN-τ promoted IL-10 secretion and did not induce IFN-γ, they did not establish whether IFN-τ treatment promoted secretion of other Th2 cytokines. Thus, we examined for secretion of IL-4 and IL-5 (Fig. 1*B*). Splenocytes from IFN-τ-fed MBP Ac1-11 TCR-transgenic mice secreted IL-4, IL-5, and IL-10 when stimulated with MBP Ac1-11 (Fig. 1*B*, insets), but reduced levels of IL-2 and IFN-γ (data not shown). Similarly, lymphocytes from IFN-τ-fed wild-type PL/J mice immunized with MBP Ac1-11 or SJL/J mice immunized with encephalitogenic proteolipoprotein peptide p139–151 also secreted Th2 cytokines IL-4, IL-5, and IL-10 and reduced levels of IL-2 and IFN-γ (data not shown). To examine whether $CD4^+$ or $CD8^+$ cells were responsible for Th2 cytokine secretion, $CD4^+$ and $CD8^+$ cells were purified from IFN-τ-fed MBP Ac1-11-specific TCR-transgenic mice and restimulated in the presence of fresh APC and MBP Ac1-11. As shown in Fig. 1*B*, these Th2 cytokines were produced in cultures containing $CD4^+$ T cells. In contrast, a lower amount of IL-5 and only minimal levels of IL-4 or IL-10 were detected in cultures containing $CD8^+$ T cells. Similarly, Th2 cytokine secretion was observed in cultures containing $CD4^+$, but not $CD8^+$, T cells from IFN-τ-fed proteolipoprotein p139–151-immunized SJL/J mice (data not shown). Monocytes from IFN-β-treated MS patients can produce IL-10 (3, 20). Interestingly, the level of IL-10 production in cultures containing total spleen cells from IFN-τ-treated mice was similar to that observed when purified $CD4^+$ T cells from IFN-τ-treated mice were restimulated with fresh APC (Fig. 1*B*). While our results did not negate the possibility that monocytes may have contributed to the secretion of IL-10, they indicate that IFN-τ-induced IL-10 secretion was driven by $CD4^+$ T cells.

In a previous study, it was observed that when IFN-τ was applied in vitro at 100 or 1000 U/ml it inhibited Ag-induced proliferation by 40 or 61%, respectively (12). In contrast, while Th2 deviation was observed when IFN-τ was administered orally at 1×10^5 U and examined in vitro without additional IFN-τ, no significant inhibition of proliferation was observed in total spleen cells or in cultures containing separated $CD4^+$ or $CD8^+$ cells (Fig. 1*B*). In addition, we did not observe inhibition of proliferation in other experiments using 1×10^5 U IFN-τ or less. However, when mice were administered 1×10^6 U or higher doses in separate experiments >50% inhibition of proliferation was observed (O. Stuve, J. M. Soos, and S. S. Zamvil, unpublished observations).

To examine how IFN-τ modulates Th1 and Th2 cytokine responses in EAE, mice were immunized for EAE induction with MBP and treated with either IFN-τ or vehicle (PBS). While MBP-specific TCR-transgenic mice are quite useful for examining regulation of Th cell differentiation (17), it can be preferable to test treatment effects on EAE induced in wild-type mice, which contain a normal T cell repertoire. Cytokine production by MBP-reactive lymphocytes was examined 20 days after immunization of PL/J

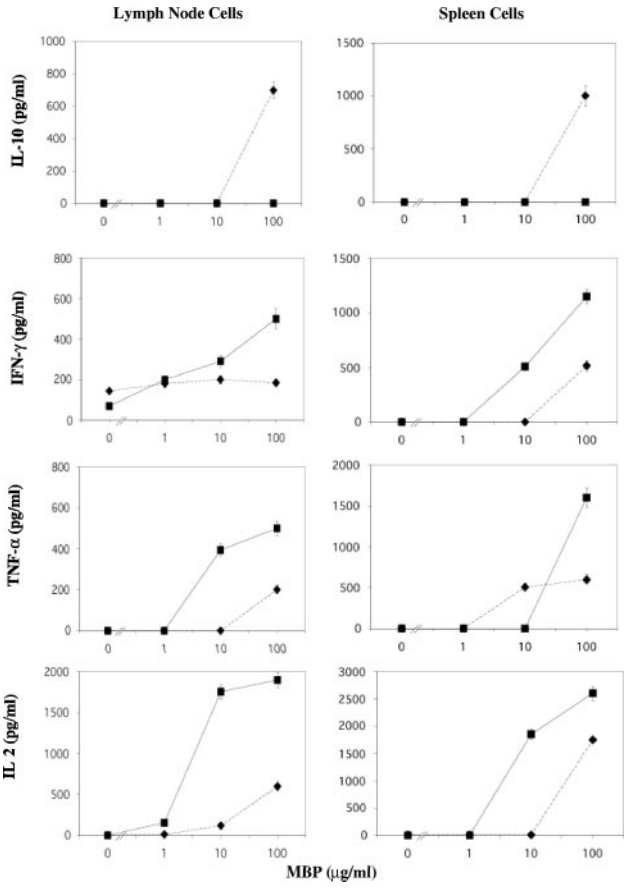

FIGURE 2. Cytokine profile of mice protected from EAE by oral IFN-τ administration. Five PL/J mice immunized with native MBP for EAE induction were fed daily for 20 days with either 1×10^5 U/feeding of oral IFN-τ (◆) or PBS (■) used as a control. Mice treated with IFN-τ did not show signs of clinical EAE (EAE score of 0 for the entire duration), whereas mice fed PBS developed EAE with mean severity of 2.5. Lymph node cells and spleen cells from two mice in each group were harvested on day 21, then pooled and cultured with MBP at the concentrations indicated. One of the two mice in the PBS-fed group that was used for this analysis had a clinical EAE score of 3, while the other mouse had a score of 2. Cytokine secretion was analyzed by ELISA as described in *Materials and Methods*.

Table I. *EAE protection by combination of oral IFN-τ and GA*[a]

Treatment	Incidence	Day of Onset	Mean Severity[b]
PBS	10/10	15.7	3.3
IFN-τ (1×10^5)	1/5	16	0.6
IFN-τ (5×10^4)	9/10	17.8	1.6
IFN-τ (10^4)	8/10	16.7	2.2
GA (100 μg)	7/10	18.5	1.6
IFN-τ (5×10^4) + GA (100 μg)	2/10[c]	18.0	0.5
IFN-τ (10^4) + GA (100 μg)	5/10	16.2	0.9

[a] PL/J mice were immunized s.c. with 300 μg MBP in CFA on day 0. A total of 400 ng pertussis toxin was administered on days 0 and 2. IFN-τ and GA were administered separately (100 μl each; 200 μl total) daily. A total of 100 μl PBS was administered to mice treated with IFN-τ or GA alone. Severity was graded as follows: 0, normal; 1, loss of tail tone; 2, mild hind limb monoparesis or paraparesis; 3, moderate paraparesis or paraplegia; 4, quadraparesis; 5, moribund/death.

[b] Mean maximal severity for each group.

[c] A value of $p < 0.001$ ($Z = 4.4$) in comparison with IFN-τ (5×10^4 U) alone; $p < 0.005$ ($Z = 2.6$) in comparison with GA alone.

mice. At this time PBS-treated mice reached an average paralysis grade of 2.5 while mice treated with IFN-τ mice did not develop EAE (see Fig. 2). Control (PBS-treated) mice that developed EAE exhibited a classic Th1 response with production of IFN-γ and TNF-α (Fig. 2). These mice also produced robust levels of IL-2 but did not produce any detectable IL-10. In contrast, MBP-stimulated lymph node cells or splenocytes from IFN-τ-treated mice secreted substantial IL-10. Lymph node cells and splenocytes from these same mice secreted less IFN-γ, TNF-α, and IL-2. Thus, IFN-τ prevention of EAE correlated with induction of IL-10 and a concomitant reduction of the Th1 cytokines, IFN-γ, TNF-α, and IL-2.

Because IFN-τ and GA have distinct modes of action (2, 8, 11) and oral administration of either one can ameliorate EAE (13, 21), we investigated whether they could complement each other when administered in combination. In preliminary experiments various doses (1×10^5, 5×10^4, and 1×10^4 U) of IFN-τ were tested alone. Whereas 1×10^5 U IFN-τ protected mice from EAE, below this amount there was a dose-dependent loss in efficacy. One hundred micrograms of GA alone was also suboptimal. However, as shown in Table I, when IFN-τ and GA were administered in combination at suboptimal doses, mice were protected. In comparison with IFN-τ (5×10^4 U) treatment alone, there was a significant reduction ($Z = 4.4$; $p < 0.001$) in EAE incidence when mice were given combination therapy. In comparison with GA alone, combination therapy was also associated with a significant reduction ($Z = 2.6$; $p < 0.005$) in EAE incidence. These in vivo results indicated that these two immunomodulatory agents could function in an additive or synergistic manner.

GA can induce TGF-β secretion by CNS autoantigen-specific T cells (2, 21). Because previous studies attributed the beneficial affects of IFN-τ to production of IL-10 (11, 12, 15) and we observed that IFN-τ induced substantial quantities of IL-10, we evaluated whether the combination of suboptimal doses of oral IFN-τ and oral GA, which was effective in EAE protection, facilitated MBP-specific T cells to secrete TGF-β and IL-10. As shown in Fig. 3*A*, lymphocytes from MBP Ac1-11 TCR-transgenic mice treated with oral GA alone produced relatively small amounts of IL-10 when stimulated with MBP. Lymphocytes isolated from TCR-transgenic mice fed IFN-τ alone produced a moderate level of IL-10. However, mice treated with suboptimal doses of oral IFN-τ and oral GA produced approximately two times the sum of the IL-10 produced by lymphocytes from mice treated with either agent alone. In contrast to oral GA, oral IFN-τ did not induce TGF-β, and the amount of TGF-β produced by MBP-specific T cells from TCR-transgenic mice treated with the combination of oral GA and oral IFN-τ was similar to that of mice treated with GA alone (see Fig. 3*B*). Thus, the clinically beneficial effects observed when suboptimal doses of oral IFN-τ and oral GA were administered in combination (Table I) could have reflected enhanced IL-10 secretion as well as a potential additive or synergistic effect of both IL-10 and TGF-β secretion.

It was reported that IFN-τ could either prevent or reverse relapsing EAE (12, 15). IFN-τ also prevented superantigen-induced EAE relapses (12). Furthermore, we have observed that oral IFN-τ could suppress murine collagen-induced arthritis (J. M. Soos, H. L. Weiner, and S. S. Zamvil, unpublished observation). Thus, the beneficial anti-inflammatory properties of IFN-τ are not restricted to immune responses to CNS autoantigens but may be applicable to other organ-specific autoimmune conditions. Like other type I IFNs, IFN-τ induces IL-10 when given as monotherapy (11, 12, 15). The results from previous studies (12, 15) and this investigation demonstrated that the beneficial clinical effects of IFN-τ in EAE were dose dependent. Larger in vivo doses of IFN-τ induced

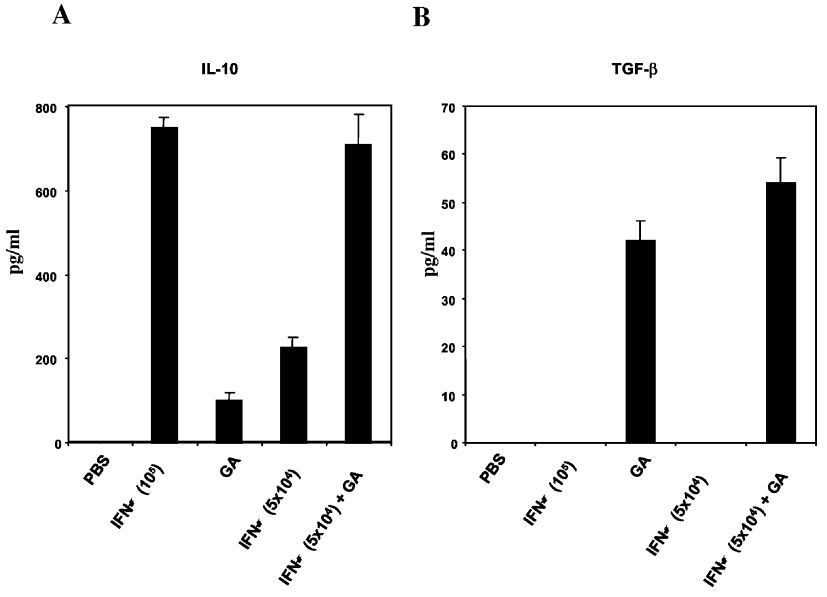

FIGURE 3. Secretion of IL-10 and TGF-β by MBP-specific TCR-transgenic mice treated with oral IFN-τ, oral GA, or a combination of oral IFN-τ and oral GA. Mice were fed daily for 3 days with PBS, IFN-τ (5×10^4 or 1×10^5 U), GA (100 μg), or the combination of IFN-τ and GA. Splenocytes and lymph node cells, harvested 1 day after the last feeding, were cultured with MBP Ac1-11 (50 μg/ml). Cytokine secretion was analyzed by ELISA as described in *Materials and Methods.*

higher levels of IL-10 by Ag-reactive T cells examined in vitro. However, IL-10 may not be solely responsible for the clinical efficacy of IFN-τ. For example, it is also known that IFN-τ suppresses IFN-γ-inducible MHC class II up-regulation (11) and, as we have shown, IFN-τ also promotes secretion of IL-4 and IL-5. Thus, it is possible that these other immunoregulatory characteristics of IFN-τ may also contribute to its beneficial effects.

Previously, it was reported that administration of oral or parenteral IFN-α in combination with GA did not improve clinical EAE (22), raising concern for the safety of using GA in combination with a type I IFN. The mechanism(s) responsible for their observation was not clearly elucidated. Interestingly, an initial clinical MS trial designed to test the safety of IFN-β 1a and GA concluded that combination was safe (10). In our study we have clearly shown that the combination of oral IFN-τ and GA is beneficial in EAE. Among other differences between the earlier EAE study and this investigation is that GA was administered parenterally in their study and not orally. Most importantly, we have also demonstrated that IFN-τ, in contrast to IFN-α or IFN-β, does not promote secretion of IFN-γ, an attractive feature of this type I IFN, which could also contribute to the different clinical observations made in these two studies. This key pharmacodynamic difference between IFN-τ and IFN-α should be considered in view of the results from a recent pilot MS trial using oral IFN-α that suggested that oral IFN-α may not be effective in treatment of relapsing-remitting MS (23). In addition, patients in that trial received either 10,000 or 30,000 U of IFN-α. In contrast, in the phase I oral IFN-τ trial (16), patients were given from 4×10^7 to 3.6×10^8 antiviral units daily, without significant toxicity.

The goal of combination therapy in MS is to improve efficacy without increasing side effects (9). Thus, medications chosen for combination therapy should not have overlapping toxicities. Theoretically, these medications should produce an additive or synergistic effect. Thus, MS medications that have different modes of action, possibly acting on different parts of the pathogenic cascade, may be preferred. In this regard, IFN-τ, like IFN-β, exerts effects in an Ag-independent manner (1), whereas GA appears to affect primarily T cells specific for CNS autoantigens (6, 8). In this report, we have demonstrated for the first time that combination of a type I IFN and GA can suppress EAE. Because oral or parenteral administration of IFN-τ is effective in EAE and is not associated with significant side effects or toxicities, IFN-τ is an excellent candidate for use as a single agent or in combination in MS.

Acknowledgments

We thank Drs. C. P. Genain, S. L. Hauser, A. J. Slavin, P. A. Nelson, E. Waubant, and D. S. Goodin for helpful discussions.

References

1. Yong, V. W., S. Chabot, O. Stuve, and G. Williams. 1998. Interferon β in the treatment of multiple sclerosis: mechanisms of action. *Neurology 51:682.*
2. Neuhaus, O., C. Farina, H. Wekerle, and R. Hohlfeld. 2001. Mechanisms of action of glatiramer acetate in multiple sclerosis. *Neurology 56:702.*
3. Liu, Z., C. M. Pelfrey, A. Cotleur, J. C. Lee, and R. A. Rudick. 2001. Immunomodulatory effects of interferon β-1a in multiple sclerosis. *J. Neuroimmunol. 112:153.*
4. Lu, H.-T., J. L. Riley, G. T. Babcock, M. Huston, G. R. Stark, J. M. Boss, and R. M. Ransohoff. 1995. Interferon (IFN) β acts downstream of IFN-γ-induced class II transactivator messenger RNA accumulation to block major histocompatibility complex class II gene expression and requires the 48-kD DNA-binding protein, ISGF3-γ. *J. Exp. Med. 182:1517.*
5. Teitelbaum, D., R. Aharoni, R. Arnon, and M. Sela. 1988. Specific inhibition of the T-cell response to myelin basic protein by the synthetic copolymer Cop 1. *Proc. Natl. Acad. Sci. USA 85:9724.*
6. Duda, P. W., M. C. Schmied, S. L. Cook, J. I. Krieger, and D. A. Hafler. 2000. Glatiramer acetate (Copaxone) induces degenerate, Th2-polarized immune responses in patients with multiple sclerosis. *J. Clin. Invest. 105:967.*
7. Genain, C. P., and S. S. Zamvil. 2000. Specific immunotherapy: one size does not fit all. *Nat. Med. 6:1098.*
8. Aharoni, R., D. Teitelbaum, M. Sela, and R. Arnon. 1998. Bystander suppression of experimental autoimmune encephalomyelitis by T cell lines and clones of the Th2 type induced by copolymer 1. *J. Neuroimmunol. 91:135.*
9. Kappos, L. 1996. Combinations of drugs. *Mult. Scler. 1:400.*
10. Lublin, F., G. Cutter, R. Elfont, O. Khan, R. Lisak, H. McFarland, H. Panitch, J. Noseworthy, S. Reingold, J. Whitaker, et al. 2001. A trial to assess the safety of combining therapy with interferon β-1a and glatiramer acetate in patients with relapsing MS. *Neurology 56(Suppl. 3):A148.*
11. Soos, J. M., and H. M. Johnson. 1999. Interferon-τ: prospects for clinical use in autoimmune disease. *BioDrugs 11:125.*
12. Soos, J. M., P. S. Subramaniam, A. C. Hobeika, J. Schiffenbauer, and H. M. Johnson. 1995. The IFN pregnancy recognition hormone, IFN-τ, blocks both development and superantigen reactivation of experimental allergic encephalomyelitis without associated toxicity. *J. Immunol. 155:2747.*
13. Soos, J. M., G. M. Mujtaba, P. S. Subramaniam, W. J. Streit, and H. M. Johnson. 1997. Oral feeding of interferon τ can prevent acute and chronic relapsing forms of experimental allergic encephalomyelitis. *J. Neuroimmunol. 75:43.*
14. Zamvil, S. S., and L. Steinman. 1990. The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol. 8:579.*
15. Mujtaba, M. G., W. J. Streit, and H. M. Johnson. 1998. IFN-τ suppresses both the autoreactive humoral and cellular immune responses and induces stable remission in mice with chronic experimental allergic encephalomyelitis. *Cell. Immunol. 186:94.*
16. Olek, M. J., D. J. Smith, S. L. Cook, S. J. Khoury, and H. L. Weiner. 2001. Phase 1 study of oral recombinant ovine interferon-τ in relapsing-remitting multiple sclerosis. *Neurology 56(Suppl. 3):A76.*
17. Hardardottir, F., J. L. Baron, and C. A. Janeway, Jr. 1995. T cells with two functional antigen-specific receptors. *Proc. Natl. Acad. Sci. USA 92:354.*
18. Slavin, A. J., J. M. Soos, O. Stuve, J. C. Patarroyo, H. L. Weiner, A. Fontana, E. K. Bikoff, and S. S. Zamvil. 2001. Requirement for endocytic antigen processing and influence of invariant chain and H-2M deficiencies in CNS autoimmunity. *J. Clin. Invest. 108:1133.*
19. Dayal, A. S., M. A. Jensen, A. Lledo, and B. G. Arnason. 1995. Interferon-γ-secreting cells in multiple sclerosis patients treated with interferon β-1b. *Neurology 45:2173.*
20. Porrini, A. M., D. Gambi, and A. T. Reder. 1995. Interferon effects on interleukin-10 secretion: mononuclear cell response to interleukin-10 is normal in multiple sclerosis patients. *J. Neuroimmunol. 61:27.*
21. Teitelbaum, D., R. Arnon, and M. Sela. 1999. Immunomodulation of experimental autoimmune encephalomyelitis by oral administration of copolymer 1. *Proc. Natl. Acad. Sci. USA 96:3842.*
22. Brod, S. A., J. W. Lindsey, and J. S. Wolinsky. 2000. Combination therapy with glatiramer acetate (copolymer-1) and a type I interferon (IFN-α) does not improve experimental autoimmune encephalomyelitis. *Ann. Neurol. 47:127.*
23. Brod, S. A., J. W. Lindsey, F. S. Vriesendorp, C. Ahn, E. Henninger, P. A. Narayana, and J. S. Wolinsky. 2001. Ingested IFN-α: results of a pilot study in relapsing-remitting MS. *Neurology 57:845.*