Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 421–425, January 1992
Immunology

Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor β after antigen-specific triggering

(tolerance/suppressor T cells/experimental autoimmune encephalomyelitis)

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Communicated by Barry R. Bloom, October 2, 1991 (received for review July 19, 1991)

ABSTRACT **Oral administration of myelin basic protein (MBP) is an effective way of suppressing experimental autoimmune encephalomyelitis (EAE). We have previously shown that such suppression is mediated by $CD8^+$ T cells, which adoptively transfer protection and suppress immune responses *in vitro*. In the present study we have found that modulator cells from animals orally tolerized to MBP produce a suppressor factor upon stimulation with MBP *in vitro* that is specifically inhibited by anti-transforming growth factor β (TGF-β) neutralizing antibodies. No effect was observed with antibodies to γ interferon, tumor necrosis factor α/β, or indomethacin. In addition, the active form of the type 1 isoform of TGF-β1 ($TGF\text{-}\beta_1$) can be directly demonstrated in the supernatants of cells from animals orally tolerized to MBP or ovalbumin after antigen stimulation *in vitro*. Antiserum specific for $TGF\text{-}\beta_1$ administered *in vivo* abrogated the protective effect of oral tolerization to MBP in EAE. Furthermore, injection of anti-$TGF\text{-}\beta_1$ serum to nontolerized EAE animals resulted in an increase in severity and duration of disease. These results suggest that immunomodulation of EAE induced by oral tolerization to MBP and natural recovery mechanisms use a common immunoregulatory pathway that is dependent on $TGF\text{-}\beta_1$. Implications of such an association are of therapeutic relevance to human autoimmune diseases and may help to explain one of the mechanisms involved in the mediation of active suppression by T cells.**

Immunological tolerance is the acquisition of unresponsiveness to self antigens and as such is essential for the preservation of the integrity of the host. A variety of mechanisms underly self-tolerance, including clonal deletion, clonal anergy, and active suppression (1), and its breakdown results in autoimmune diseases. The role and mechanism of action of active suppression in regulating the immune response are not well understood. One of the classic methods of inducing tolerance is via the oral administration of antigens, first described by Wells in 1911 (2) and subsequently by Chase in 1946 (3). We and others have been studying oral tolerance as a mechanism to suppress autoimmune processes in a number of experimental models (4–11, 49) and have found that oral administration of myelin basic protein (MBP) suppresses experimental autoimmune encephalomyelitis (EAE), a central nervous system autoimmune disease mediated by $CD4^+$ MBP-reactive cells (4–8). We have also found that this effect is mediated by active suppression. Specifically, $CD8^+$ T cells from animals orally tolerized to MBP suppress *in vitro* proliferative responses and adoptively transfer disease protection (5).

In the present investigation, we have found that T cells generated by oral tolerance mediate suppression both *in vitro* and *in vivo* via the release of the cytokine transforming growth factor-β (TGF-β). Our findings not only have relevance to orally induced tolerance but may help to explain one of the mechanisms by which active suppression mediated by T cells occurs.

MATERIALS AND METHODS

Animals. Female Lewis rats 6–8 weeks of age were obtained from Harlan–Sprague–Dawley. Animals were housed in Harvard Medical School animal care facilities and maintained on standard laboratory chow and water ad libitum.

Antigens. Guinea pig MBP was purified from brain tissue by the modified method of Deibler *et al.* (12). Protein content and purity were checked by gel electrophoresis and amino acid analysis.

Reagents. Commercial reagents used were as follows: mouse anti-rat γ interferon (INF-γ) neutralizing monoclonal antibody (mAb) (Amgen Biologicals); monoclonal hamster anti-murine TNF-α/β antibody (Genzyme); polyclonal rabbit neutralizing antibody against types 1 and 2 isoforms of TGF (anti$TGF\text{-}\beta_{1+2}$) (R & D Systems, Minneapolis), and indomethacin (Sigma). Turkey antiserum specific for $TGF\text{-}\beta_1$ was prepared as described (13).

Induction of Oral Tolerance. Rats were fed 1 mg of MBP dissolved in 1 ml of phosphate-buffered saline (PBS) or PBS alone by gastric intubation with a 18-gauge stainless steel animal feeding needle (Thomas Scientific). Animals were fed five times at intervals of 2–3 days with the last feeding 2 days before immunization.

***In Vitro* Suppression of Proliferative Responses by Supernatants.** Spleen cells were removed 7–14 days after the last feeding, and a single-cell suspension was prepared by pressing the spleens through a stainless steel mesh. Oral tolerance to MBP in the Lewis rat persists for ≈2 months after the last feeding (4). Thus, the 7- to 14-day period after feeding represents a relatively narrow window of time after oral tolerance is induced for performing the experiments. For preparation of supernatants, spleen cells at a concentration of 5×10^6 cells per ml were stimulated *in vitro* with MBP (50 μg/ml) in 10 ml of proliferation medium as described (20). Supernatants were harvested at 24 hr, and 100 μl was added

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Abbreviations: TGF, transforming growth factor; $TGF\text{-}\beta_1$ and $-\beta_2$, types 1 and 2 isoforms of TGF; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis; DTH, delayed-type hypersensitivity; INF-γ, γ interferon; mAb, monoclonal antibody; CFA, complete Freund's adjuvant.

to 2.5×10^4 MBP-specific T cells that had been raised and maintained as described (14) and cultured with 5×10^5 irradiated (2500 rad; 1 rad = 0.01 Gy) thymocytes in 100 μl of proliferation media. MBP (50 μg/ml) was added to the culture in a volume of 20 μl. Experiments were performed in triplicate in round-bottom 96-well plates (Costar). Cells were cultured for 72 hr at 37°C and harvested as described (20).

Purification of T-Cell Subsets. Depletion of lymphocyte subsets was performed by negative selection using magnetic beads according to a modified method of Cruikshank *et al.* (15). Spleen cells were incubated with a 1:10 dilution of mouse anti-rat CD8, CD4, or B-cell mAbs (clones OX/8, W3/25, or OX/33 respectively, Serotec Bioproducts) for 30 min on ice, washed twice, and then added to prewashed magnetic particles, with an average diameter of 4.5 μm (M-450) with goat anti-mouse IgG covalently attached (Dynal, Fort Lee, NJ). The cell–mAb–bead complexes were separated from unlabeled cells in a strong magnetic field with a magnetic-particle concentrator (Dynal MPC-1) for 2 min. The supernatant was removed, and the procedure was repeated twice to obtain the nonadherent fraction. The cells in the T cell- and B cell-depleted populations were >95% $CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$, or $CD4^+$ $CD8^+$ $OX/33^-$ (B cell-depleted) as demonstrated by indirect flow cytometry. Whole spleen populations (5×10^6 cells) from MBP-fed or control animals were cultured in the presence of MBP (50 μg/ml) in 1 ml of serum-free proliferation media. Depleted populations were cultured at a concentration of 2.5×10^6 cells per ml. Supernatants were collected at 24 hr, and 100 μl was added to responder cells as described above.

Treatment of Supernatants with Anti-Cytokine Antibodies. Spleen cells (5×10^6 cells per ml in proliferation media) from MBP-fed and control animals were incubated in the presence of MBP (50 μg/ml) and neutralizing antibodies against INF-γ, TGF-β, TNF-α/β, or with indomethacin for 72 hr. Antibodies were tested in a range of concentrations (1:250, 1:500, 1:1000), and indomethacin was tested at concentrations of 0.5–1 μg/ml. At 24 hr, supernatants were collected, and free antibody or antibody–cytokine complexes were removed by using magnetizable polymer beads (Dynabeads; Dynal). Beads coupled with anti-immunoglobulin antibodies were incubated at a concentration of 4×10^7 beads per ml for 30 min (done twice for each sample) and were removed by a modified method of Liabakk *et al.* (16) using a Dynal Magnetic Particle Concentrator (MPC-1).

Measurement of TGF-β Activity in Serum-Free Culture Supernatants. Serum-free culture supernatants were collected as described (17, 18). Briefly, modulator cells were first cultured for 8 hr with MBP (50 μg/ml) in proliferation medium. Thereafter, cells were washed three times, resuspended in serum-free medium for the remainder of the 72-hr culture, collected, and then frozen until assayed. Determination of TGF-β content and isoform type in supernatants was performed by using a mink lung epithelial cell line (CCL-64; American Type Culture Collection) as described by Danielpour *et al.* (13) and was confirmed by a sandwich ELISA assay as described (19). The percent active TGF-β was assayed without prior acid activation of the samples.

Immunization of Animals. Lewis rats were immunized by injection in the left foot pad with 25 μg of MBP in 50 μl of PBS emulsified in an equal volume of complete Freund's adjuvant (CFA) containing 4 mg of mycobacterium tuberculosis (Difco) per ml.

***In Vivo* Administration of Anti-TGF-β Antiserum and Control Sera.** Turkey antiserum specific for $TGF\text{-}\beta_1$ was used for *in vivo* experiments and had previously been prepared and characterized (13). Serum was heat-inactivated at 56°C for 30 min before injection. Animals (five per group) were injected i.p. with anti-TGF-β antiserum or control turkey serum at various concentrations (12.5, 25, or 50 μl diluted in PBS to a final volume of 100 μl), five times at days −2, 0, +2, +4, and +6 in relationship to MBP/CFA immunization. One microliter of the antiserum blocked the binding activity of ^{125}I-labeled $TGF\text{-}\beta_1$ at 4 ng/ml to A549 cells (13). *In vivo* treatment was given both to orally tolerized animals and to animals immunized for EAE without oral tolerization.

Clinical Evaluation. Animals were evaluated in a blinded fashion every day for evidence of EAE. Clinical severity of EAE was scored as follows: 0, no disease; 1, limp tail; 2, hind limb paralysis; 3, hind limb paraplegia and incontinence; 4, tetraplegia; and 5, death.

Delayed-Type Hypersensitivity (DTH) Testing. DTH was tested by injecting 25 μg of MBP in PBS subcutaneously in the ear. Thickness was measured by a blinded observer before and 48 hr after challenge with micrometer calipers (Mitutoyo, Utsunomia, Japan).

Statistical Analysis. Clinical scales were analyzed with a two-tailed Wilcoxon rank sum test for score samples, χ^2 analysis was used in comparing the incidence of disease between groups, and comparison of means was performed by using the Student *t* test.

RESULTS

***In Vitro* Suppression Is Mediated by Culture Supernatants from $CD8^+$ T Cells from MBP-Fed Animals.** In previous studies we have shown that modulator $CD8^+$ splenic T cells from MBP-fed animals suppress *in vitro* proliferation of an MBP-specific T-cell line (5). Furthermore, additional studies have demonstrated that such suppression occurs when modulator and responder cells are separated by a semipermeable membrane, suggesting the presence of a soluble factor as a mediator of the suppression (20). The generation of the soluble suppressor factor requires triggering of cells from orally tolerized animals with the oral tolerogen. Experiments were thus performed to determine whether supernatants collected from splenocytes depleted of T-cell subsets or B cells from rats orally tolerized to MBP and stimulated *in vitro*

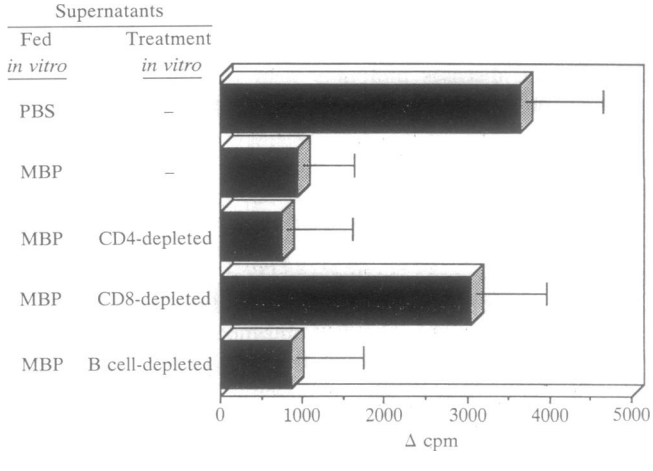

FIG. 1. *In vitro* suppression of proliferative response mediated by supernatants of lymphocyte subsets from orally tolerized animals. Whole spleen cells or depleted cell populations were stimulated *in vitro* with MBP (50 μg/ml). Spleen cells were depleted of B lymphocytes or $CD4^+$ or $CD8^+$ T lymphocytes by magnetized beads. One-hundred microliters of 24-hr culture supernatants of these cells was added to 2.5×10^4 MBP-specific T cells, cultured with 5×10^5 irradiated (2500 rad) thymocytes in proliferation medium containing MBP (50 μg/ml). Experiments were performed in triplicate in round-bottom 96-well plates. The proliferative response of the MBP-treated line in the presence of supernatants from MBP-stimulated spleen cells of nontolerized animals was 3654 ± 1651. Background counts of the MBP treated line in the absence of MBP were between 200 and 300 cpm.

with MBP could suppress an MBP line. A reduction in the proliferation of the MBP line occurred with the addition of supernatants from B cell-depleted or CD4-depleted splenocytes from animals fed MBP and stimulated *in vitro* with MBP (Fig. 1). No suppression occurred with supernatants from cells of PBS-fed animals or CD8-depleted splenocytes from MBP-fed animals.

Inhibition of *in Vitro* Suppression by Anti-TGF-β Antibodies. To determine whether a known cytokine was responsible for mediating the suppression, neutralizing antibodies to cytokines postulated to have suppressor activity were added to the supernatants in an attempt to abrogate the suppression. Rabbit anti-TGF-β antibody abrogated the suppression mediated by the supernatants in a dose-dependent fashion (Fig. 2). No effect on suppression was seen with neutralizing antibodies to INF-γ or TNF-α/β or when indomethacin, a prostaglandin blocker, was added. No suppression occurred when anti-TGF-β antibodies were added directly to the MBP-specific responder T-cell line (data not shown).

Demonstration of TGF-β in Culture Supernatants. To directly demonstrate the presence of TGF-β in supernatants of spleen cells from animals fed MBP and stimulated *in vitro* with MBP, we collected supernatants under serum-free conditions and assayed directly for TGF-β. TGF-β was secreted by spleen cells from MBP-fed animals stimulated *in vitro* in the presence but not in the absence of MBP (Fig. 3). Furthermore, TGF-β was also secreted when splenocytes from ovalbumin-fed animals are stimulated *in vitro* with ovalbumin. By using a specific sandwich ELISA assay with blocking antibodies specific for either TGF-β_1 or TGF-β_2 (13), it was further demonstrated that the TGF-β was of the TGF-β_1 isotype. In addition, the TGF-β secreted was in the active rather than latent form. The amount of TGF-β in the group fed and stimulated *in vitro* with MBP was 6.8 ± 1.7 ng/ml, with 68 ± 9% in the active form. In the ovalbumin-fed group, the amount of TGF-β was 6.1 ± 1.0 ng/ml, with 65 ± 9% in the active form. No active TGF-β was observed in supernatants from spleen cells of animals fed MBP and stimulated with concanavalin A, although small quantities (2.1 ± 0.45 ng/ml) of latent TGF-β were observed.

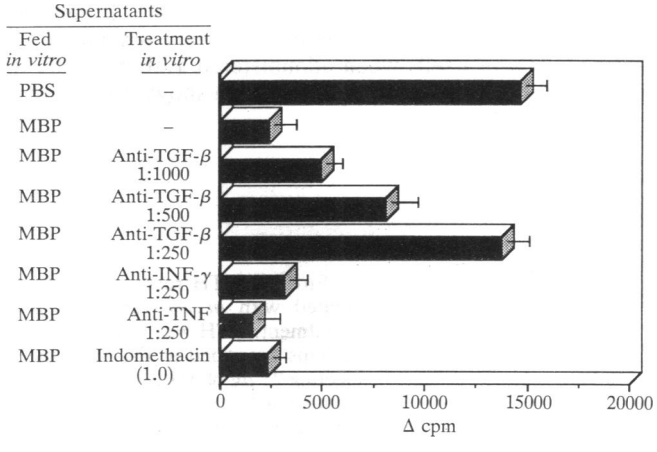

FIG. 2. Inhibition of *in vitro* suppression by anti-TGF-β antibody. Spleen cells from MBP-fed and control animals were incubated in the presence of MBP (50 μg/ml) and neutralizing antibodies against INF-γ, TGF-β, TNF-α/β, or with indomethacin for 72 hr. Free antibody or antibody–cytokine complexes from 24-hr supernatants of these cells were removed by using magnetizable polymer beads as described, and the suppressive effects of the treated supernatants were tested on an MBP-specific T-cell line. The proliferative response of the MBP line in the presence of supernatants from MBP-stimulated spleen cells of nontolerized animals was 18,995 ± 2395. Background counts of the MBP line in the absence of MBP were between 1000 and 2000 cpm.

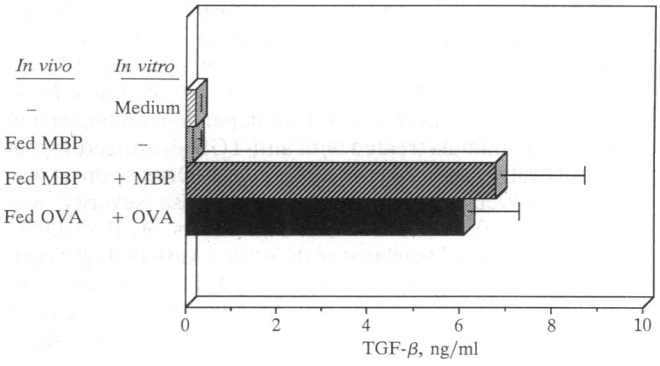

FIG. 3. TGF-β activity in serum-free culture supernatants. Spleen cells from MBP- or ovalbumin (OVA)-fed animals were incubated in the presence or absence of the homologous antigen (50 μg/ml). TGF-β content in the serum-free supernatants was assayed by using a CCL-64 assay as described.

Abrogation of Oral Tolerance by *in Vivo* Administration of Anti-TGF-β Antiserum. To determine whether TGF-β_1 also played a role in suppression of EAE by oral tolerization to MBP, we administered turkey anti-TGF-β_1 antiserum *in vivo*. Paralytic EAE developed in control animals with a maximal disease severity between 3.2 and 3.5 on day 13 regardless of whether animals were injected with PBS or control turkey serum (Fig. 4*A*). Oral tolerization with MBP markedly reduced the severity and duration of EAE (Fig. 4*C*) in animals injected with PBS or control turkey serum. Maximal disease severity in animals treated five times with 50 μl of control serum was 3.2 ± 0.2 and in orally tolerized animals treated five times with 50 μl of control serum was 1.0 ± 0.2 ($P < 0.05$). *In vivo* treatment with anti-TGF-β_1 antiserum abro-

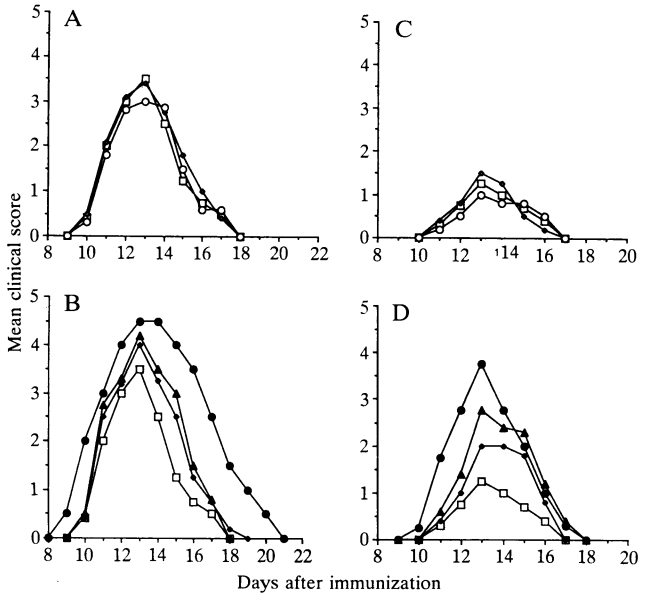

FIG. 4. The effect of anti-TGF-β and control sera on EAE. Animals received i.p. injections of turkey anti-TGF-β_1 antiserum or control preimmune turkey serum at various concentrations on days −2, 0, +2, +4, and +6 relative to the day of MBP/CFA challenge. Treatment was given both to orally tolerized animals (*C* and *D*) and to animals undergoing EAE without oral tolerance (*A* and *B*) (five animals per group). Maximal disease severity in orally tolerized animals treated five times with 50 μl of anti-TGF-β_1 antiserum was 3.7 ± 0.2 vs. 1.0 ± 0.2 in animals receiving control serum ($P < 0.05$) (*D* vs. *C*) and 4.5 ± 0.2 vs. 3.2 ± 0.2 in nontolerized animals ($P < 0.05$) (*B* vs. *A*). Treatments: □, PBS; ◇, control serum (12.5); ○, control serum (50); ◆, anti-TGF-β antiserum (12.5); ▲, anti-TGF-β antiserum (25); ●, anti-TGF-β antiserum (50).

gated protection induced by oral administration of MBP in a dose-dependent fashion; maximal disease severity in orally tolerized animals treated five times with 50 μl of anti-TGF-β_1 antiserum was 3.7 ± 0.2 vs. 1.0 ± 0.2 ($P < 0.05$; Fig. 4 *D* vs. *C*). Of note is that there was a dose-dependent enhancement of disease in animals treated with anti-TGF-β_1 antiserum that were not orally tolerized to MBP (Fig. 4*B*). Disease onset was earlier, recovery was delayed, and disease severity was greater (4.5 ± 0.2 vs. 3.2 ± 0.2, Fig. 4 *B* vs. *A*; $P < 0.05$).

DTH Responses Associated with *in Vivo* Anti-TGF-β Treatment. We have shown previously that DTH responses correlate with the clinical course of EAE and serve as a measure of *in vivo* cellular immunity to MBP (6, 7). In this study prominent DTH responses developed in animals undergoing EAE and DTH was suppressed by oral administration of MBP (Fig. 5). The suppressed DTH responses were abrogated by *in vivo* anti-TGF-β_1 treatment in a dose-dependent fashion (1.5 ± 0.5 vs. 0.5 ± 0.3; $P < 0.001$, in animals injected five times with 50 μl of anti-TGF-β vs. control serum). Furthermore, after the same *in vivo* treatment, there was enhancement of DTH responses to MBP in animals recovering from EAE that were not orally tolerized (2.1 ± 0.3 vs. 1.45 ± 0.3; $P < 0.01$, in animals injected five times with 50 μl of anti-TGF-β vs. control serum).

DISCUSSION

In the present study we found that T cells that mediate suppression of EAE after oral tolerization to MBP do so both *in vitro* and *in vivo* via the release of active TGF-β_1. *In vitro*, the release of TGF-β is dependent on antigen-specific triggering by the oral tolerogen whether it is MBP or ovalbumin. Among the mechanisms described for the maintenance of self-tolerance, active suppression mediated by T cells is probably the least well understood (21–23). Because oral tolerization represents a physiologic pathway by which the immune system is stimulated to generate suppression and because investigators have described active suppression mediated by T cells after oral tolerization (24, 25), an understanding of the mechanism by which suppressor cells act after stimulation of the gut-associated lymphoid tissue may provide insight into the mechanism of active suppression.

A number of cytokines exert suppressive activity on different aspects of the immune response such as cell growth, differentiation, and effector functions as well as the release of other cytokines. These include interferons, prostaglandins, tumor necrosis factor, and interleukin 10 (26). Our results show that $CD8^+$ suppressor T cells generated by oral tolerization, which are triggered in an antigen-specific fashion, act both *in vitro* and *in vivo* by the release of active TGF-β_1. In this regard they are analogous to the human $CD8^+$ suppressor cells associated with lepromatous leprosy, which act via an as-yet-unidentified antigen nonspecific factor after being triggered by a specific antigen (27). We recently have found that peripheral blood lymphocytes from humans orally tolerized to keyhole limpet hemocyaninin secrete a TGF-β-related suppressor factor after antigen-specific triggering (51). Because suppression generated by oral tolerance to autoantigens is antigen- and disease-specific, the secretion and action of TGF-β must occur in the local microenvironment of lymphoid tissue where the immune response is generated, along migratory pathways of the effector cells, and/or at the inflamed site in the target organ where the autoantigen is present. We recently have found specific elevation of TGF-β in the brains of EAE animals orally tolerized to MBP as compared to nontolerized animals (S. J. Khoury, W. W. Hancock, and H.L.W., unpublished observations). The ability of T cells to secrete TGF-β_1 in an active form may be important in this regard since active TGF-β has a short half-life and a small volume distribution, but latent TGF-β has an extended half-life and a larger volume of distribution (28).

We have also observed that injection of anti-TGF-β_1 serum to nontolerized animals immunized with MBP/CFA to induce EAE resulted in an increase in severity and duration of EAE. Other investigators have reported the presence of postrecovery $CD4^+$ suppressor cells in the Lewis rat EAE model that can adoptively transfer protection and suppress *in vitro* proliferative responses of an MBP line (29). *In vitro*, these cells have been shown to release TGF-β and to suppress interleukin-2 and IFN-γ production by encephalitogenic cells (29), although an *in vivo* effect of TGF-β by such cells has not been shown. Our *in vivo* results show a role for TGF-β in recovery from EAE. Thus, natural recovery in EAE and the induction of oral tolerance to MBP, though two distinct physiological processes, appear to involve a common immunoregulatory pathway in which TGF-β serves as an effector cytokine. The role of TGF-β-secreting $CD8^+$ T cells as opposed to $CD4^+$ T cells in natural recovery remains to be defined. TGF-β itself, when administered systematically in doses of 1–5 μg per treatment, has been shown to suppress

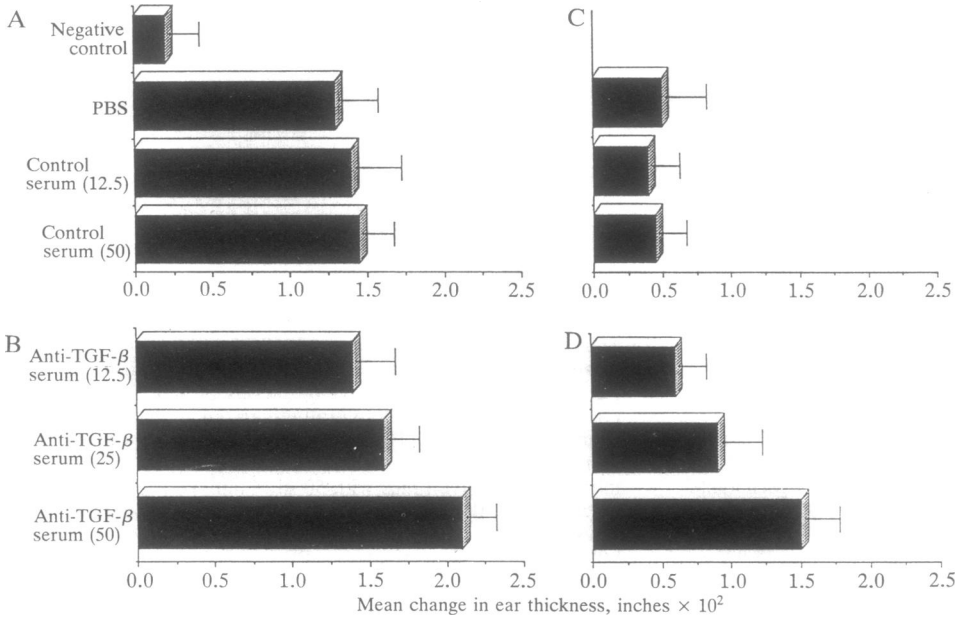

FIG. 5. DTH responses associated with *in vivo* anti-TGF-β treatment. DTH was tested in the groups described in Fig. 4 by injecting 25 μg of MBP in PBS subcutaneously in the ear. Thickness in inches (1 in. = 2.54 cm) was measured before and 48 hr after challenge. Change in ear thickness before and after challenge was recorded for each animal, and the results are expressed as the mean for each experimental group ± SEM. [$P < 0.001$, animals treated five times with 50 μl of anti-TGF-β_1 antiserum vs. control serum (*D* vs. *C*); $P < 0.01$, animals treated five times with 50 μl of anti-TGF-β_1 antiserum vs. control serum (*B* vs. *A*).]

animal models of autoimmunity both in the rat and mouse, including EAE (30, 31) and autoimmune arthritis (32, 33).

The mechanism by which TGF-β suppresses immune responses *in vitro* and down-regulates EAE and other autoimmune diseases *in vivo* is unknown. Recent studies have demonstrated multiple, sometimes contradictory, immunomodulatory effects of the TGF-β isoforms on various target cells and tissues (34, 35). Although initially identified as a growth factor, the immunoregulatory properties of TGF-β include inhibition of proliferation of B and T cells, (16, 36) affecting $CD4^+$ cells more than $CD8^+$ cells (37) in both rodent and human cells. TGF-β antagonizes inflammatory effector cytokines such as TNF-α and INF-γ (38, 39), blocks CTL activity (40, 50), and inhibits induction of receptors of interleukins 1 and 2 (41), rendering cells unresponsive to these cytokines. TGF-β inhibits *in vivo* T-cell and neutrophil adhesion to endothelial cells, which limits the migration and recruitment of inflammatory cells into the target organ (42, 43), downregulates class II expression on macrophages and astrocytes, and inhibits macrophage activation (40, 44).

TGF-β has been demonstrated to be secreted by a variety of cells including macrophages, natural killer cells, LAK cells, B cells, and both $CD4^+$ and $CD8^+$ T cells (45–47). The characteristics of the $CD8^+$ suppressor cells generated after oral tolerization are yet to be defined. Concanavalin A stimulation of spleen cells from animals tolerized with MBP, as opposed to MBP stimulation, did not lead to significant suppressor effects nor production of active TGF-β. This may be due to induction of proliferation rather than production of TGF-β after concanavalin A stimulation (48).

The results presented herein provide evidence for an immunoregulatory role played by endogenous TGF-β_1 in the spontaneously occurring recovery from EAE and in the suppression of EAE induced by oral tolerization to MBP. In view of its evolutionary high conserved features, it is likely that the immunosuppressive effects of TGF-β in experimental animals are similar to its effects in humans. Thus, our findings may point to the therapeutic potential of oral tolerization to MBP or other autoantigens as a source of endogenous TGF-β for the control of autoimmune diseases.

We thank David Danielpour for performing sandwich ELISA assays, Nan Roche and Ofer Caspi for technical support, and Amy Hostetter for secretarial support. A.M. is the recipient of a Public Health Service Fogarty International Research Fellowship (1F05TW04418, 1CP). This work was supported by National Institutes of Health Grant N529352, by a grant from Autoimmune, Inc., and by the MacArthur Foundation and was presented in part at the American Academy of Neurology, Boston, April, 1990.

1. Schwartz, R. H. (1989) *Cell* **57**, 1073–1081.
2. Wells, H. G. (1911) *J. Infect. Dis.* **9**, 147–171.
3. Chase, M. W. (1946) *Proc. Soc. Exp. Biol. Med.* **61**, 257–259.
4. Higgins, P. J. & Weiner, H. L. (1988) *J. Immunol.* **140**, 440–445.
5. Lider, O., Santos, L. M. B., Lee, C. S. Y., Higgins, P. J. & Weiner, H. L. (1989) *J. Immunol.* **142**, 748–752.
6. Brod, S. A., Al-Sabbagh, A., Sobel, R. A., Hafler, D. A. & Weiner, H. L. (1991) *Ann. Neurol.* **29**, 615–622.
7. Khoury, S. J., Lider, O., Al-Sabbagh, A. & Weiner, H. L. (1990) *Cell. Immunol.* **131**, 302–310.
8. Bitar, D. & Whitacre, C. C. (1988) *Cell. Immunol.* **112**, 364–370.
9. Nussenblatt, R. B., Caspi, R. R., Mahdi, R., Chan, C.-C., Roberge, F., Lider, O. & Weiner, H. L. (1989) *J. Immunol.* **144**, 1689–1695.
10. Zang, Z. J., Lee, C. S. Y., Lider, O. & Weiner, H. L. (1990) *J. Immunol.* **145**, 2489–2493.
11. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, F. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7443–7446.
12. Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) *Prep. Biochem.* **2**, 139–165.
13. Danielpour, D., Dart, L. L., Flanders, K. C., Roberts, A. B. & Sporn, M. B. (1989) *J. Cell. Physiol.* **138**, 79–86.
14. Ben-Nun, A., Wekerle, H. & Cohen, I. R. (1981) *Eur. J. Immunol.* **11**, 195–199.
15. Cruikshank, W. W., Berman, J. S., Theodor, A. C., Bernardo, J. & Center, D. M. (1987) *J. Immunol.* **138**, 3817–3823.
16. Liabakk, N.-B., Nustad, K. & Esekevik, T. (1989) *Scand. J. Immunol.* **30**, 641.
17. Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Jakowlew, S., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. S. (1986) *J. Exp. Med.* **163**, 1037–1050.
18. Wahl, S. M., Allen, J. B., Wong, H. L., Dougherty, S. F. & Ellingsworth, L. R. (1990) *J. Immunol.* **145**, 2514–2519.
19. Danielpour, D., Kim, K.-Y., Dart, L. L., Watanabe, S., Roberts, A. B. & Sporn, M. B. (1989) *Growth Factors* **2**, 61–71.
20. Miller, A., Lider, O. & Weiner, H. L. (1991) *J. Exp. Med.* **174**, 791–798.
21. Gershon, R. K. & Kondo, K. (1971) *Immunology* **21**, 903–914.
22. Dorf, M. E. & Benacerraf, B. (1984) *Annu. Rev. Immunol.* **2**, 127–158.
23. Tada, T., Asano, Y. & Sano, K. (1989) *Res. Immunol.* **140**, 291–294.
24. Miller, S. D. & Hanson, D. G. (1979) *J. Immunol.* **123**, 2344–2350.
25. Gautam, S. & Battisto, J. R. (1985) *J. Immunol.* **135**, 2975–2983.
26. Cohen, S., ed. (1990) *Lymphokines and the Immune Response* (CRC, Boca Raton, FL).
27. Salgame, P., Modlin, R. L. & Bloom, B. R. (1989) *Int. Immunol.* **1**, 121–129.
28. Wakefield, L. M., Winokur, T. S., Hollands, R. S., Christopherson, K., Levinson, A. D. & Sporn, M. B. (1990) *J. Clin. Invest.* **86**, 1976–1984.
29. Karpus, W. J. & Swanborg, R. H. (1991) *J. Immunol.* **146**, 1163–1168.
30. Racke, M. K., Dhib-Jalbut, S., Cannella, B., Albert, P. S., Raine, C. S. & McFarlin, D. E. (1991) *J. Immunol.* **146**, 3012–3017.
31. Johns, L. D., Flanders, K. C., Range, G. E. & Sriram, S. (1991) *J. Immunol.* **147**, 1792–1796.
32. Kuruvilla, A. P., Shah, R., Hochwald, G. M., Liggett, H. D., Palladino, M. A. & Thornbecke, G. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2918–2921.
33. Brandes, M. E., Allen, J. B., Ogawa, Y. & Wahl, S. M. (1991) *J. Clin. Invest.* **87**, 1108–1113.
34. Sporn, M. B., Roberts, A. B., Wakefield, L. M. & Assoian, R. K. (1986) *Science* **233**, 532–534.
35. Wahl, S. M., McCartney-Francis, N. & Mergenhagen, S. E. (1989) *Immunol. Today* **10**, 258–261.
36. Kehrl, J. H., Roberts, A. B., Wakefield, L. M., Jakowlew, S., Sporn, M. B. & Fauci, A. S. (1986) *J. Immunol.* **137**, 3855–3860.
37. Tada, T., Ohzeki, S., Utsumi, K., Takiuchi, H., Muramatsu, M., Li, X.-F., Shimizu, J., Fujiwara, H. & Hamaoka, T. (1991) *J. Immunol.* **146**, 1077–1082.
38. Espevik, T., Figaro, I. S., Shalaby, M. R., Lackides, G. A., Lewis, G. D., Shepard, H. M. & Palladino, M. A. (1987) *J. Exp. Med.* **166**, 571–576.
39. Schluesener, H. J. (1990) *J. Neuroimmunol.* **27**, 41–47.
40. Ranges, G. E., Figari, I. S., Espevik, T. & Palladino, M. A. (1987) *J. Exp. Med.* **166**, 991–998.
41. Dubois, C. M., Ruscetti, F. W., Palaszynski, E. W., Falk, L. A., Oppenheim, J. J. & Keller, J. R. (1990) *J. Exp. Med.* **172**, 737–744.
42. Gamble, J. R. & Vadas, M. A. (1991) *J. Immunol.* **146**, 1149–1154.
43. Gamble, J. R. & Vadas, M. A. (1988) *Science* **242**, 97–99.
44. Ding, A., Nathan, C. F., Graycar, J., Serynck, R., Stuehr, D. J. & Srimal, S. (1991) *J. Immunol.* **145**, 940–944.
45. Assoian, R. K., Fleurdelys, B. E., Stevenson, H. C., Miller, P. J., Madtes, D. K., Raines, E. W., Ross, R. & Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6020–6025.
46. Kasid, A., Bell, G. I. & Director, E. P. (1988) *J. Immunol.* **141**, 690–698.
47. Kim, S.-J., Kehrl, J. H., Burton, J., Tendler, C. L., Jeang, K.-T., Danielpour, D., Thevenin, C., Kim, K. Y., Sporn, M. B. & Roberts, A. B. (1990) *J. Exp. Med.* **172**, 121–129.
48. Bristol, L. A., Ruscetti, F. W., Brody, D. T. & Durum, S. K. (1990) *J. Immunol.* **145**, 4108–4114.
49. Zhang, Z. J., Davidson, L., Eisenbarth, G. & Weiner, H. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10252–10256.
50. Fontana, A., Frei, K., Bodmer, S., Hofer, E., Schreier, M. H., Pallidino, M. A. & Zinkernagel, R. M. (1989) *J. Immunol.* **143**, 3230–3234.
51. Matsui, M., Miller, A., Hafler, D. A. & Weiner, H. L. (1991) *J. Neuroimmunol.* (Suppl. 1), 85 (abstr.).