Immunomodulation of experimental autoimmune encephalomyelitis by oral administration of copolymer 1

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ABSTRACT The activity of copolymer 1 (Cop 1, Copaxone, glatiramer acetate) in suppressing experimental autoimmune encephalomyelitis (EAE) and in the treatment of multiple sclerosis patients when injected parenterally has been extensively demonstrated. In the present study we addressed the question of whether Cop 1 can induce oral tolerance to EAE similar to myelin basic protein (MBP). We now have demonstrated that oral Cop 1 inhibited EAE induction in both rats and mice. Furthermore, oral Cop 1 was more effective than oral MBP in suppressing EAE in rats. The beneficial effect of oral Cop 1 was found to be associated with specific inhibition of the proliferative and Th1 cytokine secretion responses to MBP of spleen cells from Cop 1-fed mice and rats. In all of these assays, oral Cop 1 was more effective than oral MBP. The tolerance induced by Cop 1 could be adoptively transferred with spleen cells from Cop 1-fed animals. Furthermore, Cop 1-specific T cell lines, which inhibit EAE induction in vivo, could be isolated from the above spleen cells. These T cell lines secrete the anti-inflammatory cytokines IL-10 and transforming growth factor type β, but not IL-4, in response to both Cop 1 and MBP. In conclusion, oral Cop 1 has a beneficial effect on the development of EAE that is associated with down-regulation of T cell immune responses to MBP and is mediated by Th2/3 type regulatory cells. These results suggest that oral administration of Cop 1 may modulate multiple sclerosis as well.

The mucous membranes covering the aerodigestive and urogenital tracts are endowed with a large and highly specialized immune system, mucosa-associated lymphoid tissues. Mucosal administration of antigens (for instance, by ingestion or inhalation) may result in the development of a state of peripheral immunological tolerance (1). Recently, oral and nasal administration of autoantigens were shown to suppress a variety of experimental autoimmune diseases, including experimental allergic encephalomyelitis (EAE) (2–4). Three distinct mechanisms have been elucidated for the systemic-antigen-specific immune suppression associated with oral tolerance: clonal deletion, clonal anergy, and active cellular suppression. The mechanism by which tolerance is generated depends on the amount of antigen administered. Feeding high doses of antigen induces either deletion or anergy of antigen-specific cells (5–7), whereas feeding multiple low doses of antigen induces regulatory T cells that mediate suppression by secreting the cytokines IL-4, IL-10, and transforming growth factor (TGF) β1. In the EAE system, feeding a low dose of myelin basic protein (MBP) induces cells that are structurally identical to Th1 encephalitogenic CD4+ clones in their T cell receptor usage, MHC class II restriction, and epitope recognition, but secrete the above suppressive cytokines. Such T cell clones isolated from mesenteric lymph nodes of mice orally tolerized with low doses of MBP were demonstrated to suppress ongoing EAE induced by either MBP or proteolipid protein (PLP) (8). This finding indicates that antigen-specific regulatory T cells generated in the gut can migrate to the target organ and suppress ongoing inflammatory reactions to a nonrelated antigen as well, a phenomenon termed bystander suppression (9).

The investigations in the experimental models of autoimmune diseases have led to a series of phase II/III double-blind clinical trials of oral tolerance in subjects with multiple sclerosis (MS), rheumatoid arthritis, and uveitis (10–13). The results of these trials were equivocal. Thus, although phase I/II trials in MS have shown some positive results, in a large phase III trial, patients fed with myelin showed no difference from placebo in the number of relapses. Double-blind studies of patients with rheumatoid arthritis treated with oral collagen showed positive effects that were dose dependent (11). In uveitis, oral administration of purified S antigen, but not the retinal mixture, showed positive effects (12, 13). These results highlight the need for further experimental studies to successfully apply oral tolerance as therapeutic modality for human autoimmune diseases.

Copolymer 1 (Cop 1, Copaxone, glatiramer acetate) is a synthetic amino acid copolymer composed of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine. It was demonstrated to suppress ongoing inflammatory reactions to a nonrelated antigen, resulting in inhibition of antigen-specific effector functions. In addition, complexes of Cop 1 with MHC class II molecules can compete with MBP complexes at the level of the T cell receptor to MBP p82–100 (20). Cop 1 binding to relevant MHC leads also to activation of T suppressor (Ts) cells of the Th2 type, which are activated by shared suppressive determinants between MBP and Cop 1 to secrete anti-inflammatory Th2 cytokines. These cytokines may mediate the therapeutic effect of Cop 1 in disease induced not only

Abbreviations: EAE, experimental autoimmune encephalomyelitis; Cop 1, copolymer 1; MBP, myelin basic protein; GP-MBP, guinea pig MBP; MS, multiple sclerosis; PLP, proteolipid protein; Ts, T suppressor; TGF, transforming growth factor.

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with MBP but also with PLP and MOG by the mechanism of bystander suppression (21, 22).

It was therefore of interest to test the effect of oral administration of Cop 1 on the development of EAE and the immune response to MBP. We report that oral Cop 1 exerts a beneficial effect on the development of EAE. This effect is associated with down-regulation of T cell immune responses to MBP and is mediated by Cop 1-specific suppressor cells secreting TGFβ.

MATERIALS AND METHODS

Antigens. Cop 1 b 0.05392 (used in the rat studies) and b 0.54496 (used in the mice studies) were obtained from TEVA Pharmaceutical Industries (Pethach-Tikva, Israel). Guinea pig myelin basic protein (GP-MBP) and mouse MBP were prepared from spinal cord by acid extraction and ammonium sulfate precipitation (23). The synthetic peptide p82–100 of MBP (DENVVHFKNVKTPRTTP) was synthesized by the Mer- rillfield solid-phase method (24), using the peptide synthesizer 430A of Applied Biosystems and purified by HPLC. Purified protein derivative of tuberculin was obtained from Statens Serum Institute (Copenhagen).

Animals. (SJL/J x BALB/c) F 1 female mice, 8–10 weeks old, and female Lewis rats, 8–12 weeks old, were obtained from Harlan Laboratories (Jerusalem).

Induction and Assessment of EAE. (SJL/J x BALB/c) F 1 mice were injected in all four footpads with 2 mg per mouse of spinal cord homogenate emulsified at a 1:1 ratio with complete Freund’s adjuvant (CFA) containing 1 mg/ml Mycobacteria H37Ra (Difco). Pertussis toxin (250 ng y Freund’s adjuvant (CFA) containing 1 mg y) was injected i.v. immediately afterward and 48 h later. EAE was induced 2 days after the last feeding, the rats were sacrificed, and spleen suspensions were prepared. Cells were activated with Con A (1.5 g/ml) or mouse splenocytes (5  10⁶) were injected i.p. into recipient rats.

Adoptive transfer with T cell lines. T cell lines from rat or mouse origin were incubated with Cop 1 (50–100 mg/ml) and irradiated (3,000 rad) rat thymocytes (1  10⁸) or mouse splenocytes (5  10⁶) were pooled and incubated (50  10⁶) or mouse spleen cells (5  10⁶) or mouse MBP (25). The results summarized in Table 1 demonstrate that suppression of EAE by Oral Administration of Cop 1.

The ability of orally administered Cop 1 to prevent clinical manifestations of EAE in Lewis rats was assayed under conditions previously reported to induce oral suppression by low doses of MBP (25). The results summarized in Table 1 demonstrate that Cop 1 could reduce both the incidence and the clinical signs of EAE in comparison to rats fed with PBS. A maximum effect was achieved with a dose of 1 mg of Cop 1, whereas a dose of 2 mg of Cop 1 was somewhat less efficient, although not significantly, in suppressing EAE. We then compared the efficacy of suppression induced by the most effective dose of Cop 1 (1 mg) to that of MBP. Feeding with either MBP or Cop 1 resulted in significant suppression of EAE with Cop 1 being somewhat more effective than MBP. It induced a 52% reduction in disease incidence and a 57% inhibition of disease severity as compared with PBS-fed control rats (Table 1).

The effect of oral Cop 1 on the development of EAE in mice was tested by using a protocol reported by Al-Sabbagh et al. (26) for MBP. As shown in Fig. 1, Cop 1 given orally was found to effectively suppress EAE, the most effective dose being 100–250 mg. The results in Fig. 1 demonstrate a representative experiment in which the most effective dose was 100 μg of Cop

RESULTS

Suppression of EAE by Oral Administration of Cop 1. The ability of orally administered Cop 1 to prevent clinical manifestations of EAE in Lewis rats was assayed under conditions previously reported to induce oral suppression by low doses of MBP (25). The results summarized in Table 1 demonstrate that Cop 1 could reduce both the incidence and the clinical signs of EAE in comparison to rats fed with PBS. A maximum effect was achieved with a dose of 1 mg of Cop 1, whereas a dose of 2 mg of Cop 1 was somewhat less efficient, although not significantly, in suppressing EAE. We then compared the efficacy of suppression induced by the most effective dose of Cop 1 (1 mg) to that of MBP. Feeding with either MBP or Cop 1 resulted in significant suppression of EAE with Cop 1 being somewhat more effective than MBP. It induced a 52% reduction in disease incidence and a 57% inhibition of disease severity as compared with PBS-fed control rats (Table 1).

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oral MBP, as regards both proliferation and IFN-

Table 1. Oral suppression of EAE in rats

<table>
<thead>
<tr>
<th>Fed Ag</th>
<th>Incidence</th>
<th>Mean max. score</th>
<th>Mean onset, days</th>
<th>Suppression of EAE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS, control</td>
<td>7/9</td>
<td>1.05 ± 0.7</td>
<td>11.2</td>
<td>Incidence</td>
</tr>
<tr>
<td>Cop 1, 0.5 mg</td>
<td>5/9</td>
<td>0.61 ± 0.6</td>
<td>11.6</td>
<td>29</td>
</tr>
<tr>
<td>Cop 1, 1 mg</td>
<td>3/9</td>
<td>0.33 ± 0.55 (P = 0.01)</td>
<td>13.0</td>
<td>57</td>
</tr>
<tr>
<td>Cop 1, 2 mg</td>
<td>4/9</td>
<td>0.44 ± 0.5</td>
<td>11.0</td>
<td>43</td>
</tr>
<tr>
<td>PBS, control</td>
<td>27/28</td>
<td>1.8 ± 0.5</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>MBP, 1 mg</td>
<td>10/17 (P = 0.0026)</td>
<td>0.9 ± 0.5 (P &lt; 0.0001)</td>
<td>11.4</td>
<td>39</td>
</tr>
<tr>
<td>Cop 1, 1 mg</td>
<td>13/28 (P = 0.00005)</td>
<td>0.78 ± 0.4 (P &lt; 0.00001)</td>
<td>12.6</td>
<td>52</td>
</tr>
</tbody>
</table>

Each incidence figure represents the cumulative results of 2–5 individual experiments.

1, (43% suppression, P = 0.03) whereas in other experiments
the most effective dose was 250 µg (data not shown). The dose
of 500 µg was always completely inefficient. The ability of oral
Cop 1 to suppress EAE also was tested when treatment began
only after EAE induction. Cop 1 was found effective under
these conditions as well, and a dose of 250 µg of Cop 1, which
was the most efficient one, decreased the incidence of disease
(40% reduction) and disease severity (44%) and delayed the
mean onset of disease from 12.3 days in the control group to
20.8 days in the Cop 1-fed group.

Down-Regulation of MBP-Specific Immune Responses by Oral Administration of Cop 1. The effect of oral administra-
tion of Cop 1 on the immune response to the disease-inducing
antigen MBP was evaluated in both rats and mice. We com-
pared the proliferative response and cytokine production of
spleen cells from animals receiving either PBS or Cop 1 orally
and then challenged with MBP.

Splenocytes from the control PBS-treated rats proliferated
in response to MBP and secreted high levels of IFN-γ, but not
IL-2 or the Th2 cytokines IL-4 and IL-10. Both proliferation
and IFN-γ secretion in response to MBP were markedly
inhibited in spleen cells from Cop 1-fed rats, i.e., 54% inhibition
of proliferation and 88% inhibition of IFN-γ secretion
were observed (Fig. 2). Maximum effect was obtained with 1
mg of Cop 1, a dose that also was found as most effective in
disease suppression (Table 1). As also shown in Fig. 2, the
inhibitory effect induced by oral Cop 1 was similar to that of
oral MBP, as regards both proliferation and IFN-γ secretion.

Similar studies also were conducted in mice that were fed
with either PBS or various doses of Cop 1. Control mice fed
with PBS proliferated in response to the encephalitogenic
epitope MBP p82–100 and secreted IL-2, IFN-γ, and IL-6,
whereas antigen-specific production of IL-4 or IL-10 could not
be detected. In mice fed with Cop 1, both the proliferation
and the secretion of the Th1 cytokines IL-2 and IFN-γ were
considerably reduced, whereas IL-6 secretion was only slightly
affected (Fig. 3). Thus 38% inhibition of proliferation, 74% inhibition of IL-2 secretion, 70% inhibition of IFN-γ secretion,
and only 16% inhibition of IL-6 secretion were observed. The
inhibition of MBP-specific responses by oral Cop 1 was a
dose-dependent bell-shaped curve and consistent with the
response inhibition of clinical manifestations of EAE. The
down-regulation of the immune response to MBP was
antigen specific, because in the Cop 1-fed mice no inhibition
was observed in the response to purified protein derivative, an
antigen that is included in the encephalitogenic inoculum,
compared with control mice (Fig. 3). Mice fed with MBP
produced reduced proliferation and cytokine secretion in
response to MBP p82–100 (Fig. 3, Insets). However, as shown,
Cop 1 was more effective in suppressing the proliferative
response and at least as effective in inhibiting the antigen-
specific cytokine secretion.

Adoptive Transfer of Protection Against EAE. To under-
stand the mechanism involved in the suppression of EAE after
oral administration of Cop 1, we tested whether this unre-
sponsiveness could be adoptively transferred by spleen cells
of Cop 1-fed donors or by Cop 1-specific T cell lines estab-
lished from such splenocytes. The results summarized in Table 2
indicate that Con A-activated splenocytes from rats fed
with Cop 1 significantly inhibited EAE manifestations as evidenced in two independent experiments
by reduction of both disease incidence and clinical score (Table
2). The less effective suppression obtained in experiment 2 may

Fig. 1. Oral suppression of EAE in mice by Cop 1. (SJL/JxBALB/c) F1 mice (10 per group) were fed with PBS or various doses
of Cop 1 and challenged for EAE induction by injection of mouse
spinal cord homogenate.

Fig. 2. Effect of antigen feeding on the immune response to MBP
in rats. Rats were fed with PBS, various doses of Cop 1, or GP-MBP
(1 mg/feeding), and primed with GP-MBP 2 days after the last feeding.
Spleens were removed 10 days later, and cells from three animals in
each group were pooled and tested for (A) proliferation and (B) IFN-γ
secretion, in response to GP-MBP.
be caused by either fewer cells that were transferred or to the longer interval between feeding and cell transfer. These results, however, clearly indicate that oral administration of Cop 1 induced regulatory cells that could adoptively transfer resistance to EAE induction.

We further addressed the issue of the type of cells involved in Cop 1-induced oral tolerance, by using Cop 1-specific T cell lines obtained from spleens of Cop 1-fed rats and mice, by repeated in vitro stimulations with Cop 1. The ability of these T cell lines to prevent EAE in vivo was followed. The results illustrated in Fig. 4 demonstrate that the disease was considerably inhibited in the recipient animals, as reflected in the clinical score, i.e., 44% inhibition in mice and 88% inhibition in rats. Thus, both the rat and murine Cop 1-specific T cell lines derived from splenocytes of Cop 1-fed donors are Ts cells capable of transferring the orally induced unresponsiveness to EAE.

Characterization of Ts Cell Lines from Cop 1 Oral Tolerized Animals. To characterize the Ts-Cop 1 lines, we studied their proliferation response and the pattern of cytokine secretion in response to Cop 1 and MBP. The detailed proliferation and cytokine secretion profile of the Ts line derived from rats is depicted in Fig. 5. The line proliferated in response to Cop 1 and secreted some IL-2, IFN-γ, and also IL-10 and TGFβ but not IL-4. A response to MBP could not be observed in the proliferation assay, neither by secretion of the Th1 cytokines IL-2 or IFN-γ. On the other hand, some IL-10 and large amounts of TGFβ were secreted in response to MBP, similar to the secretion induced by Cop 1 and Con A. A similar pattern of response also was demonstrated with the Ts derived from Cop 1-fed mice (Fig. 5B). The line proliferated in response to Cop 1, but Cop 1 could not induce either IL-2 or IFN-γ secretion. It induced, however, IL-10 and TGFβ, but not IL-4. MBP did not induce proliferation or secretion of Th1 cytokines (i.e., IL-2 and IFN-γ) and, as in the case of the rat T cell line, MBP triggered the secretion of IL-10 and TGFβ. Thus, considerable crossreaction between Cop 1 and MBP at the level of these anti-inflammatory cytokines IL-10 and TGFβ was demonstrated with both the rat and murine Ts lines.

**DISCUSSION**

Feeding autoantigens was shown to down-regulate autoimmune responses in a variety of autoimmune diseases. In the

![Fig. 3. Suppression of antigen-specific responses in mice by Cop 1 feeding. (SJL/JxBALB/c)F1 mice were fed with PBS various doses of Cop 1 or MBP and primed with MBP p82–100. Ten days later, spleens were removed, and cells of three animals in each group were pooled and tested for (A) proliferation, (B) IL-2 secretion, (C) IFN-γ secretion, and (D) IL-6 secretion, in response to (□) MBP p82–100 and (□) purified protein derivative. (Insets) Feeding with PBS (empty columns), Cop 1 (250 μg) (filled columns), and MBP (250 μg) (striped columns).](image-url)

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**Table 2. Adoptive transfer of protection against EAE in rats by splenocytes from Cop 1-fed rats**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>EAE in recipients</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Incidence</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>4/5</td>
</tr>
<tr>
<td>200 × 10⁶ SPC from BSA-fed donors</td>
<td>3/5</td>
</tr>
<tr>
<td>200 × 10⁶ SPC from Cop 1-fed donors</td>
<td>0/5</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6/6</td>
</tr>
<tr>
<td>100 × 10⁶ SPC from BSA-fed donors</td>
<td>5/6</td>
</tr>
<tr>
<td>100 × 10⁶ SPC from Cop 1-fed donors</td>
<td>2/6</td>
</tr>
</tbody>
</table>

In experiment 1 spleen cells were taken 2 days after last feeding and in experiment 2 after 16 days. SPC, spleen cells.
EAE system, several studies have demonstrated the effectiveness of orally administered myelin antigens (MBP and PLP) in suppressing disease in both rat and mouse models (12). Recently there has been a renewed interest in the potential of this method for modulating human autoimmune diseases, including MS (12). In the present study, we addressed the question of whether Cop 1, which had been found effective in suppressing EAE and MS when injected parenterally, is also effective when given orally. The results presented in this paper demonstrate that feeding both rats and mice with Cop 1 effectively inhibited subsequent EAE induction (Table 1, Fig. 1). Furthermore, oral Cop 1 was more effective than oral MBP in suppressing EAE in rats (Table 1). The activity of Cop 1 in both mice and rats is dose dependent, and exceeding the effective dose in mice resulted in inefficient suppression of EAE. It is interesting that the optimal dose for inducing oral tolerance by Cop 1 in both species was very similar to that reported previously for MBP (25, 26). The effective dose found is consistent with low dose tolerance. Whether high dose tolerance also could be induced by Cop 1 is an open question.

The beneficial effect of oral Cop 1 was found to be paralleled with specific down-regulation of T cell immune responses to MBP as demonstrated by the inhibition of the ex vivo proliferative and Th1 cytokine secretion responses of spleen cells from Cop 1-fed mice and rats (Figs. 2 and 3). The response of these lymphocytes to purified protein derivative, which is one of the components in the EAE inducing inoculum, was not affected, indicating that feeding with Cop 1 resulted in an antigen-specific tolerance to MBP. Similar inhibition of Th1 responses also was obtained with MBP. Cop 1, however, was somewhat superior to MBP in down-regulating the immune response to MBP in both rats and mice (Figs. 2 and 3). Similar results recently were reported by Maron et al. (27) in MBP T cell receptor-transgenic mice, where Cop 1 feeding resulted in inhibition of EAE and decreased proliferation and IL-2, IL-6, and IFN-γ production. Interestingly, in this system, too, Cop 1 was more efficient than MBP in inducing oral tolerance. It is noteworthy that there is a good correlation between the

Fig. 4. Inhibition of EAE by T cell lines derived from Cop 1-fed donors. Activated Ts cell lines derived from spleens of Cop 1-fed donors were injected 3 days after stimulation with Cop 1 to naive (A) rats (20 × 10⁶/rat i.p.) and (B) to mice (15 × 10⁶/mouse) i.v. Recipient animals then were challenged for EAE induction.

Fig. 5. Proliferation and cytokine secretion profile of rat (A) and murine (B) Cop 1-specific Ts cell lines. Cells were cultured with no antigen, Cop 1 (50 μg/ml), GP-MBP (100 μg/ml), and Con A (5 μg/ml). (A) Proliferation and cytokine secretion, (B) IL-2, (C) IFN-γ, (D) IL-4, (E) IL-10, and (F) TGFβ were measured.
dose-response range for disease suppression and inhibition of the immune response to MBP by Cop 1, indicating that the two activities of Cop 1 are intimately associated.

The inhibition of EAE and the specific immune response to MBP by oral Cop 1 can result from different mechanisms, such as induction of anergy, deletion state, or regulatory cells. To study which of the above mechanisms is involved, we tested whether resistance to EAE induced by oral Cop 1 could be adoptively transferred by spleen cells of Cop 1-fed donors and by Cop 1-specific T cell lines originating from these spleen cells. Our results (Table 2, Fig. 4) indicate that regulatory T cells that can suppress EAE are induced by Cop 1 feeding, in accordance with the finding that low doses of antigen favor the generation of regulatory cells (12). The regulatory cells induced by Cop 1 were demonstrated to be of the Th2/3 type as revealed by the cytokine secretion profile of Cop 1-specific T cell lines isolated from spleens of Cop 1-fed donors (Fig. 5). Both the rat and the murine T cell lines secreted large amounts of IL-10 and TGFβ, but not IL-4, in response to Cop 1. Although the rat line also secreted some Th1 cytokines (IL-2 and IFN-γ) in response to Cop 1, the murine line was confined to the secretion of IL-10 and TGFβ. Both lines also could be stimulated by MBP to secrete those anti-inflammatory cytokines, but did not proliferate or secrete Th1 cytokines. These results indicate that feeding with Cop 1 induces regulatory cells that can suppress not only MBP-induced responses but also other encephalitogenic responses (e.g., to PLP and myelin oligodendrocyte glycoprotein) involved in EAE and MS, by bystander suppression, a phenomenon demonstrated by regulatory cells induced by MBP feeding (9). Indeed, we have demonstrated in mice that both Cop 1 feeding and injection of Cop 1-specific T cell lines originating from Cop 1-fed mice were capable of suppressing EAE induced by whole spinal cord tissue as induction of anergy, deletion state, or regulatory cells. To adoptively transferred by spleen cells of Cop 1-fed donors and whether resistance to EAE induced by oral Cop 1 could be bystander suppression, a phenomenon demonstrated by regulatory cells induced by MBP feeding (9). Indeed, we have demonstrated in mice that both Cop 1 feeding and injection of Cop 1-specific T cell lines originating from Cop 1-fed mice were capable of suppressing EAE induced by whole spinal cord homogenate that contains several autoantigens, suggesting that bystander suppression actually occurs in vivo. We previously have demonstrated that similar Cop 1-specific Th2 type T cells are induced by parenteral administration of Cop 1. These T cell lines crossreacted with MBP at the level of Th2 cytokine secretion and mediated in vitro and in vivo bystander suppression of encephalitogenic responses (21, 22). A major difference, however, between these T cell lines and those induced by Cop 1 feeding is in the IL-4 secretion activity. Whereas the latter cells secreted almost no IL-4 while secreting IL-10 and TGFβ, the Cop 1 T cells induced by s.c. injection secreted large amounts of IL-4 and the crossreactivity with MBP was most prominent at the level of IL-4 secretion. It thus can be concluded that Cop 1 induces Th2/3 type responses irrespective of the route of administration; however, the spectrum of the cytokines they secrete depends on the site of their induction. Indeed, it was demonstrated that gut-associated lymphoid tissue favors the induction of unique T cell lines (termed Th3) secreting primarily TGFβ (8). On the other hand, Th2 cells induced in the periphery secrete high levels of IL-4 (28).

Mucosal (oral or nasal) tolerance induction offers several important advantages over the parenteral route, such as minimization of adverse effects and easy delivery. However, it may represent a two-edged sword (29) when an autoantigen is used, because the development of active immune response may follow mucosal processing of antigen. Thus, oral administration of MBP to neonatal animals resulted in enhanced disease expression during adulthood (30) and nasal administration of MBP enhanced clinical signs of ongoing EAE induced in DA rats (29). In this respect, the use of Cop 1, which is devoid of any encephalitogenic activity (14), yet is at least as effective as MBP in inducing oral tolerance to EAE, may be advantageous as a potential treatment of MS.

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