Oral tolerance to copolymer 1 in myelin basic protein (MBP) TCR transgenic mice: cross-reactivity with MBP-specific TCR and differential induction of anti-inflammatory cytokines

Ruth Maron, Anthony J. Slavin, Ethan Hoffmann, Yoshinori Komagata and Howard L. Weiner

Center for Neurologic Diseases, Brigham and Women’s Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA

Keywords: anti-inflammatory cytokines, copolymer 1, experimental allergic encephalomyelitis, myelin basic protein, oral tolerance, transforming growth factor-β

Abstract

Oral tolerance to myelin basic protein (MBP) is an effective antigen-specific method to suppress experimental allergic encephalomyelitis (EAE). Glatiramer acetate [copolymer 1 (Cop1)] is a synthetic copolymer designed to mimic MBP which suppresses EAE, is used parenterally to treat multiple sclerosis (MS) and is being tested orally for efficacy in MS. We investigated the immunologic properties of Cop1 to determine the degree to which its effects were antigen specific using MBP TCR transgenic mice. Immunization of MBP TCR transgenic mice fed Cop1, MBP or MBP Ac1–11 resulted in decreased proliferation, and IL-2, IL-6 and IFN-γ production, and increased secretion of IL-10 and transforming growth factor (TGF)-β in Cop1-fed animals. IFN-γ was decreased, and IL-10 and TGF-β were increased in non-immunized mice fed Cop1 and stimulated in vitro with MBP. No such effects were observed in ovalbumin TCR transgenic mice. To determine if the effects of Cop1 were specific to MBP TCR-bearing cells, MBP TCR transgenic Rag2−/− mice were immunized and re-stimulated in vitro with Cop1. We found a marked increase in IL-4 and similar increases in IL-4 after feeding Cop1. In disease models, feeding Cop1 suppressed EAE in MBP TCR transgenic mice, (PL/J × SJL)F1 mice, and in myelin oligodendrocyte glycoprotein-induced EAE in NOD mice. Oral Cop1 had no effect on collagen-induced arthritis. These results demonstrate that Cop1 is active orally in an antigen-specific fashion, and may function as an altered peptide ligand for MBP-specific TCR-bearing cells by decreasing pro-inflammatory cytokines (IFN-γ) and increasing anti-inflammatory cytokines (IL-4, IL-10 and TGF-β).

Introduction

Oral tolerance refers to the long-held observation of systemic hyporesponsiveness following orally administered antigen. Orally administered antigen can suppress autoimmune diseases in animal models including experimental allergic encephalomyelitis (EAE), arthritis, uveitis, and diabetes, and oral autoantigens are being tested in human autoimmune diseases (reviewed in 1). Different mechanisms of immune tolerance are induced by oral antigen and are related to the dose of antigen fed. Low doses favor induction of Treg,2-type regulatory T cells that secrete IL-4 and IL-10, and Treg,3-type regulatory cells that secrete transforming growth factor (TGF)-β (2–4), whereas higher doses induce anergy or deletion (5,6).

Glatiramer acetate [copolymer 1 (Cop1)] is a synthetic basic copolymer effective in the suppression of EAE (7) and is an approved drug (Copaxone) for the treatment of multiple sclerosis (MS) by s.c. injection (8). A number of properties of Cop1 have been described: (i) Cop1 exhibits cross-reactivity on the cellular (9) and humoral (10) level with myelin...
basic protein (MBP), although the cross-reactivity is partial; (ii) Cop1 binds in a promiscuous fashion to HLA-DR molecules (11) and can serve as a TCR antagonist of the 82–100 epitope of MBP (12); and (iii) in animals Cop1 induces regulatory cells that can adaptively transfer protection not only to MBP-induced EAE (13) but EAE induced by proteolipid protein (14) and spinal cord homogenate (15). It has recently been demonstrated that Cop1 can suppress EAE when given orally to conventional animals (16) and oral Cop1 is being tested in human trials of MS. We investigated the immunologic effects and specificity of oral Cop1 utilizing MBP transgenic mice, which provides a unique in vivo model for delineating the biologic properties of orally administered Cop1.

Methods

Animals

Female (PL/J×SJL)F1 and DBA/1 male mice were purchased from the Jackson Laboratory (Bar Harbor, ME). NOD mice were purchased from Taconic Farms (Germantown, NY). Mice transgenic for a TCR specific for Ac1–11 peptide of MBP were generated by introducing the rearranged α and β TCR chain genes into the germline of C57BL/6 mice (17). The transgenic-positive mice were extensively backcrossed to B10.PL mice. Mice were screened for the expression of MBP-specific TCR by PCR analysis as previously described (17). All mice used were between 7 and 9 weeks of age.

Antigens

Mouse MBP was prepared from brain tissue by a modified method of Deibler et al. (18). The purity of the MBP preparation was confirmed by gel electrophoresis and amino acid analysis. myelin oligodendrocyte glycoprotein (MOG) 35–55 and Ac1–11 peptide of MBP (Ac-ASQKRPSQRHG) were made using a peptide synthesizer and purified through HPLC. Cop1 manufactured for TEVA Marion Partners (Kansas City, MO) was obtained from the Brigham and Women’s Hospital pharmacy. Chicken type II collagen (Arthrogen-CIA) was purchased from Chondrex (LLC, Seattle, WA). Ovalbumin (OVA) was purchased from Sigma (St Louis, MO).

Oral administration of MBP, Cop1, collagen or OVA

MBP TCR transgenic and (PL/J×SJL)F1 mice were fed with mouse MBP (500 µg/feeding), MBP Ac1–11 (250 µg/feeding), Cop1 (250 µg/feeding) or OVA (1 mg/feeding) dissolved in 0.2 ml PBS by gastric intubation with an 18-gauge stainless steel feeding needle (Thomas Scientific, Swedesboro, NJ). DBA/1 mice were fed with collagen type II (30 µg/feeding) or Cop1 (250 µg/feeding). NOD mice were fed with MOG35–55 (250 µg/feeding) or Cop1 (250 µg/feeding). Animals were fed 5 times and immunized 2 days after the last feeding.

Induction and clinical evaluation of EAE and collagen-induced arthritis (CIA)

MBP TCR transgenic mice were immunized with 100 µg mouse MBP emulsified with complete Freund’s adjuvant (CFA) containing 200 µg of Mycobacterium tuberculosis H37RA (Mt) (Difco, Detroit, MI). Innoculum was injected into the footpads in a volume of 100 µl and boosted 1 week later with the same innoculum injected into the flank. (PL/J×SJL)F1 mice received a s.c. injection in the flank of 100 µg of mouse MBP in 0.2 ml of PBS emulsified in an equal volume of CFA containing 200 µg of Mt (Difco). NOD mice were immunized in the hind footpads with 100 µg MOG35–55 in CFA. Immediately thereafter and again 48 h later mice also received an i.v. injection of 150 ng of pertussis toxin in 0.2 ml of PBS. Animals were monitored for symptoms of EAE beginning 7 days after the induction and scored as follows: 0, no disease; 1, tail paralysis; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb plus forelimb paralysis; 5, moribund.

DBA/1 mice were immunized with 100 µg of chicken collagen type II emulsified with CFA containing 50 µg of Mt (Difco). The intradermal injection was divided between five sites on the back and at the base of the tail. Twenty-one days later the mice received a booster of 100 µg of collagen type II given by i.p. injection. Mice were observed twice a week for the presence of distal joint swelling and erythema. Each limb was scored on a scale from 0 to 4 (0, absence of arthritis; 1, erythema and mild swelling of the tarsus; 2, moderate erythema and swelling of tarsus and ankles; 3, severe swelling of tarsus and ankle; 4, ankylosis and bony deformity). A maximum arthritic index (MAI) was obtained by summing the greatest score recorded for each limb (0, no disease; 16, highest possible score).

In vitro analysis.

MBP TCR transgenic mice were treated orally with OVA, mouse MBP, MBP Ac1–11 or Cop1 and 2 days after the last treatment were immunized in the foot pads with 100 µg of antigen (mouse MBP, MBP Ac1–11 or Cop1) emulsified in CFA containing 50 µg of Mt. Ten days post-immunization lymphocytes from the draining lymph nodes and spleens were harvested and tested in vitro for proliferation and cytokine production by [³H]thymidine uptake and ELISA respectively.

Cell culture, proliferation and ELISA for cytokines.

For proliferation assays or cytokine analysis, lymphocytes were cultured in 96-well plates at 5×10⁶ or 10×10⁶ cells/ml respectively in X-Vivo 20 (Biowhittaker, Walkersville, MD). For proliferation assays, cells were pulsed with [³H]thymidine 72 h later and radioactivity determined 16 h later. For cytokine assays, culture supernatants were collected at 24 h for IL-2 and IL-4, at 40 h for IL-6, IL-10 and IFN-γ, and at 72 h for TGF-β. Quantitative ELISA for IL-2, IL-4, IL-10, IL-6 and IFN-γ was performed using paired mAb specific for corresponding cytokines per the manufacturer’s recommendation (PharMingen, San Diego, CA). TGF-β (R & D Systems, Minneapolis, MN) was determined as previously described (19).

Statistical analysis

Maximum disease scores, cytokine concentrations and thymidine incorporation were analyzed by Student’s t-test or ANOVA.

Results

Suppression of MBP- or MOG35–55-induced EAE by oral Cop1

We first tested oral Cop1 as a treatment for EAE in (PL/J×SJL)F1 and NOD mice, susceptible to MBP- or MOG35–
Oral tolerance to Cop1 in MBP TCR transgenic mice

Fig. 1. Oral administration of Cop1 suppresses the severity of EAE in conventional mice. (A) (PL/J×SJL)F1 mice were fed with OVA (1 mg/feeding), MBP (500 µg/feeding) or Cop1 (250 µg/feeding) every day for a total of five feedings. Two days after the last feeding mice were immunized s.c. in the flank with 100 µg of mouse MBP emulsified in CFA containing 200 µg of MT. NOD mice were fed with OVA (1 mg/feeding), MOG35–55 (250 µg/feeding) or Cop1 (250 µg/feeding) every day for a total of five feedings. Two days after the last feeding, mice were immunized in the footpads with 100 µg of mouse MOG35–55 emulsified in CFA. Immediately thereafter and again 48 h later mice received an i.v. injection of 150 ng of pertussis toxin in 0.2 ml of PBS. Mice (10 mice/group) were monitored and scored for EAE as described in Methods.

55-induced EAE respectively. Mice were fed MBP (500 µg), MOG35–55 (250 µg) or Cop1 (250 µg) prior to EAE induction by immunization with 100 µg of MBP or MOG35–55 emulsified in CFA. Both oral Cop1 and MBP suppressed the severity of MBP-induced EAE compared to control OVA-fed mice (P ≤ 0.006 day 22–30) (Fig. 1A). Oral Cop1 also inhibited EAE induced by MBP Ac1–11 in (PL/J×SJL)F1 mice (not shown). As shown in Fig. 1(B), oral Cop1 suppressed MOG35–55-induced EAE in NOD mice (P ≤ 0.005 by day 12) even more effectively than feeding MOG35–55 (P ≤ 0.04 day 20–46). At day 45 both Cop1- and MOG-fed mice were significantly different from OVA-fed mice (P ≤ 0.001). In these studies, between 90 and 100% of animals develop EAE. These results demonstrate that oral Cop1 suppresses EAE induced both by MBP and by MOG, an encephalitogenic antigen different from MBP.

Fig. 2. Cytokine secretion of spleen cells from MBP and OVA TCR transgenic mice fed MBP, OVA or Cop1. MBP Ac1–11 peptide-specific TCR transgenic mice were fed with OVA as control (1 mg/feeding), MBP (5 mg/feeding) or Cop1 (2.5 mg/feeding) every day for a total of five feedings. OVA DO11.10 TCR transgenic mice were fed with insulin as control (1 mg/feeding), OVA (20 mg/feeding) or Cop1 (2.5 mg/feeding) every day for a total of five feedings. Two days after the last feeding spleens were harvested and stimulated in vitro with MBP (10 µg/ml) for the MBP TCR transgenic mice or OVA (100 µg/ml) for the OVA TCR transgenic mice. Cytokines were determined as described in Methods.

Immunologic effects of oral Cop1 in MBP TCR transgenic mice without subsequent immunization

By utilizing MBP TCR transgenic mice, we were able to determine whether oral Cop1 had any immunologic effects as compared to orally administered MBP. OVA TCR transgenic mice were used as a control. As shown in Fig. 2(A), IFN-γ production was significantly (P ≤ 0.0001) reduced in splenocytes from both MBP- and Cop1-fed MBP TCR transgenic animals upon re-stimulation in vitro with MBP (Fig. 2A). IL-10 was significantly increased (P ≤ 0.0009) in both MBP- and Cop1-fed mice compared to OVA-fed controls (Fig. 2B), whereas TGF-β was significantly increased (P ≤ 0.0044) only in Cop1-fed mice, a trend which was observed in MBP-fed animals (Fig. 2C). As a control, we fed OVA TCR transgenic mice and re-stimulated them in vitro with OVA. As shown in Fig. 2(D), IFN-γ was decreased after feeding OVA compared to control (insulin)-fed mice (P ≤ 0.015), whereas oral Cop1 increased IFN-γ in these mice. There was no effect on IL-10...
production by any of the oral antigens, whereas TGF-β was significantly increased in OVA TCR transgenic mice fed OVA ($P \leq 0.011$) but not in mice fed Cop1 (Fig. 2F). OVA TCR transgenic mice are BALB/c which have an innate tendency towards Th2-type responses. Unlike MBP, Cop1 had no effect in vitro when cultured with cells from MBP TCR transgenic animals. These results demonstrate that Cop1 is active orally and in vitro when cultured with cells from MBP TCR transgenic animals and does not affect cytokine production in MBP TCR transgenic mice.

**Immunologic effect of oral Cop1 in MBP TCR transgenic mice followed by immunization with Cop1**

We then tested the specificity of oral Cop1 in MBP TCR transgenic mice following immunization with Cop1 in CFA and in vitro stimulation with either Cop1 or MBP. Following oral Cop1 and Cop1 immunization, popliteal draining lymph nodes proliferated significantly less ($P \leq 0.001$) in vitro stimulation with either Cop1 or MBP compared to control (OVA)-fed animals (Fig. 3A). There was also less IL-2 and IL-6 ($P \leq 0.03$) production to in vitro stimulation with Cop1 (Fig. 3B and C) and a marked increase in TGF-β secretion ($P \leq 0.0014$) (Fig. 3D). Increased TGF-β was observed whether cells were stimulated in vitro with either Cop1 or MBP, whereas effects on IL-2 were more pronounced with Cop1 in vitro (Fig. 3B) and more IL-6 was produced with MBP in vitro (Fig. 3C). As a control, we fed and immunized OVA TCR transgenic animals with Cop1 and re-stimulated them in vitro with either Cop1 or OVA. OVA TCR transgenic animals fed and immunized with Cop1 showed no difference in proliferation when challenged with OVA in vitro, but proliferated less when stimulated with Cop1 in vitro ($P \leq 0.005$) (Fig. 3E). Unlike the MBP TCR transgenic mice, oral Cop1 had no effect on cytokine secretion (IL-2, IL-10, IL-6) in OVA TCR transgenic mice stimulated in vitro with OVA (Fig. 3F and H). In contrast, when stimulated in vitro with Cop1, there was decreased IL-2 ($P \leq 0.002$) (Fig. 3F) and increased IL-10 production ($P \leq 0.0017$) (Fig. 3H). TGF-β production was not observed in OVA TCR transgenic animals. These results demonstrate that feeding and immunization with Cop1 induced a pronounced immune deviation involving suppression of T1,1 responses and induction of T1,2/T1,3 responses.

**Immunologic effect of oral Cop1 or oral MBP Ac1–11 in MBP TCR transgenic mice followed by immunization with MBP Ac1–11**

Since MBP TCR transgenic mice are specific for an acetylated peptide of MBP (MBP Ac1–11) we tested the specificity of oral Cop1 in the MBP TCR transgenic mice fed Cop1 or MBP Ac1–11 and immunized with MBP Ac1–11. There was a marked reduction in IL-2, IFN-γ and IL-6 production to Ac1–11 in both Cop1- and MBP Ac1–11-fed mice compared to OVA-fed mice ($P \leq 0.0001$) (Fig. 4A–C). Concomitantly, IL-10 was increased following both Cop1 and MBP Ac1–11 feeding with a greater increase in Ac1–11-fed animals (Fig. 4D). No increase in TGF-β production was observed following in vitro stimulation with MBP Ac1–11. However, increased TGF-β (500 pg/ml) was observed in lymphocytes from mice fed either Cop1 or MBP Ac1–11 and stimulated in vitro with Cop1, demonstrating a preferential induction of TGF-β by activation with Cop1.

**Immunologic effect of oral Cop1 in MBP TCR transgenic mice on a Rag−/− background**

In order to investigate whether cross-reactivity of Cop1 with MBP-specific transgenic T cells is due to the expression of endogenous TCR α chain in the MBP-specific T cells, we tested MBP TCR transgenic mice on a Rag−/− background. Such animals do not have endogenous α chains and thus all T cells express only MBP Ac1–11-specific transgenic TCR. Animals were immunized either with MBP or Cop1 and re-stimulated in vitro with the immunizing antigens, or fed with Ac1–11 or Cop1- with no immunization and stimulated in vitro with Ac1–11. Immunization with MBP and re-stimulation with MBP in vitro resulted in increased secretion of IFN-γ and IL-6, whereas there was no IFN-γ or IL-6 after MBP immunization upon re-stimulation in vitro with Cop1 (Fig. 5A and B). After Cop1 immunization, IFN-γ was observed upon in vitro stimulation with MBP and to a lesser extent with Cop1; no IL-6 was detected (Fig. 5A and B). In contrast, IL-4 was produced.
Oral tolerance to Cop1 in MBP TCR transgenic mice

Fig. 4. Feeding MBP Ac1–11 or Cop1 inhibits T:\textsubscript{h}1 and induces T:\textsubscript{h}2/3-like responses in MBP Ac1–11 peptide-specific TCR transgenic mice immunized with MBP Ac1–11. MBP Ac1–11 peptide-specific TCR transgenic mice were fed with OVA (1 mg/feeding), MBP Ac1–11 (250 µg/feeding) or Cop1 (250 µg/feeding) every day for a total of five feedings. Two days after the last feeding, mice were immunized intradermally in the hind footpads with 100 µg of MBP Ac1–11 emulsified in CFA containing 50 µg of MT. Ten days later popliteal and inguinal lymph nodes were harvested and cultured in vitro with MBP Ac1–11 (10 µg/ml). IL-2, IFN-γ, IL-6 and IL-10 were determined as described in Methods.

Fig. 5. Cytokine secretion of lymphocytes from MBP TCR transgenic mice on the Rag-deficient background immunized with MBP or Cop1 or fed with no immunization, with MBP Ac1–11 or Cop1. MBP TCR transgenic Rag-deficient mice were immunized in the footpads with 100 µg of MBP or Cop1 emulsified in CFA and 10 days later the draining lymph nodes excised and stimulated in vitro with either MBP or Cop1. In parallel, MBP TCR transgenic Rag-deficient mice were fed with OVA (1 mg/feeding) as control, MBP Ac1–11 (250 µg/feeding) or Cop1 (250 µg/feeding) every day for a total of five feedings. Two days after the last feeding, spleens were excised and stimulated in vitro with MBP Ac1–11; cytokines were determined as described in Methods.

Differential effect of oral Cop1 on EAE in MBP TCR transgenic mice and on CIA in DBA/1 mice

After demonstrating in vitro immunologic effect of oral Cop1 in MBP TCR transgenic mice, we tested the ability of oral Cop1 to suppress EAE in these animals. A dose–response curve with oral Cop1 (50–1000 µg per feeding) revealed that the optimal dose to suppress EAE in these animals was 250 µg/feeding. As shown in Fig. 6(A), oral MBP and oral Cop1 significantly suppressed EAE in the MBP TCR transgenic mice (P \leq 0.0007 by day 13). To further test the in vivo disease specificity of oral Cop1 we fed Cop1 to mice susceptible to induction of collagen arthritis, as it had been reported that Cop1 can inhibit type II collagen-reactive clones in vitro (20). As shown in Fig. 6(B), feeding type II collagen to DBA/1 mice significantly suppressed arthritis (P \leq 0.031 by day 11), whereas oral Cop1 had no effect compared to control fed mice.

Discussion

Cop1 is a synthetic basic copolymer that has been shown in an extensive series of animal studies to inhibit EAE. Its immunologic effects are multiple, and include binding to several different HLA-DR molecules and induction of regulatory cells that can adoptively transfer protection in EAE models. Given its demonstrated efficacy in MS and its use as injectable therapy, studies in humans have begun to further define its mechanism of action (21). Although originally designed as a copolymer to mimic MBP, it has been shown to function as a universal antigen in humans stimulating proliferation independent of any prior exposure to Cop1 in MS or healthy subjects (22,23). These responses were HLA class II restricted. The repeated injection of Cop1 preferentially generates T:\textsubscript{h}2 and T:\textsubscript{h}3 cells (24). Cop1 has been shown to have partial cross-reactivity with human MBP reactive clones,
Although this is not complete as not all MBP-reactive clones react with Cop1 (21).

Although Cop1 has been shown to cross-react with MBP-specific T cell clones, it is not clear whether this is true cross-reactivity of Cop1 with the MBP-specific TCR or due to expression of hybrid receptors expressed by MBP-specific T cell clones. This question has not been addressed directly either in animals or in human T cell clones. The data presented in this paper demonstrates that Cop1 can indeed activate MBP-specific TCR transgenic Rag2<sup>−/−</sup> T cells to produce anti-inflammatory cytokines since these T cells do not express other endogenous TCR. In addition we have shown that feeding Cop1 (i) markedly inhibits EAE in MBP TCR transgenic mice, (ii) increases production of anti-inflammatory cytokines (IL-10 and TGF-β) from the transgenic T cells, (iii) has immunologic effects that are specific to MBP TCR transgenic T cells but not to OVA TCR transgenic T cells and (iv) specifically inhibits EAE but does not affect CIA, another autoimmune disease.

Orally administered antigens can induce tolerance by a number of mechanisms including anergy, deletion and the induction of regulatory cells that secrete IL-4, IL-10 and TGF-β (1). Oral Cop1 has been studied in the EAE model, and has been shown to suppress disease and to generate T<sub>H</sub>2- and T<sub>H</sub>3-type responses (16), and a clinical trial of oral Cop1 in MS patients is in progress. By utilizing MBP TCR transgenic mice we were able to investigate the in vivo immunologic effects of oral Cop1 and to shed new light on its immunologic mechanism of action. Orally administered Cop1 in MBP TCR transgenic mice suppressed IFN-γ and increased IL-10 and TGF-β independent of immunization. These effects were seen when either MBP or Cop1 was fed and cells re-stimulated in vitro with MBP. These effects were not seen in OVA TCR transgenic mice. Cop1 itself did not induce and expand proliferation of cells from MBP TCR transgenic mice unless animals had previously been fed or immunized with Cop1, suggesting that Cop1-reactive cells need to be expanded in vivo for them to be immunologically active. Cross-reactivity was observed when animals were immunized with Cop1 and re-stimulated in vitro either with Cop1 or MBP. These effects included increased secretion of TGF-β and decreased proliferation, IL-2 and IL-6 production. These changes were not observed in OVA TCR transgenic animals stimulated in vitro with OVA. However, if lymphocytes from OVA TCR transgenic mice fed and immunized with Cop1 were re-stimulated in vitro with OVA, immune modulation occurred, resulting in decreased proliferation and IL-2 secretion and increased IL-10 secretion. This we believe is because of other endogenous TCR that are expanded by OVA-specific transgenic T cells and are cross-reactive with Cop1. Because the transgene in the MBP TCR mouse recognizes Ac1–11, we also tested the effect of oral Cop1 in animals immunized and stimulated in vitro with MBP Ac1–11. Similar results were obtained as when animals were immunized with Cop1. These results demonstrate that Cop1 is immunologically active when orally administered in an antigen-specific fashion, and preferentially stimulates T<sub>H</sub>2 and T<sub>H</sub>3 responses in vitro and in vivo.

Because MBP TCR transgenic mice have a rearranged α chain we also tested the effect of Cop1 on MBP TCR transgenic mice on the Rag-deficient background. This addresses the issue of cross-reactivity in vivo between Cop1 and MBP, as these animals' T cells have TCR specific only for MBP. We found that immunization of these animals with MBP induced IFN-γ and IL-6 when cells were re-stimulated in vitro with MBP, whereas if they were stimulated in vitro with Cop1 there was no IFN-γ or IL-6. Surprisingly, however, MBP-immunized TCR transgenic Rag2<sup>−/−</sup> T cells stimulated in vitro with Cop1 produced IL-4. If Rag-deficient mice were immunized with Cop1 and stimulated in vitro with MBP there was a marked increase in IL-4, which was not seen if animals were re-stimulated with Cop1. Thus, it appears that the cytokine pattern is skewed towards a T<sub>H</sub>1 response (IFN-γ) when animals are immunized and stimulated in vitro.
with the same antigen, and a T\(_\text{H}2\)-type response (IL-4) if the immunizing antigen is different from the antigen used in \textit{vivo}. When Rag-deficient mice were fed either Cop1, MBP or Ac1–11 and re-stimulated \textit{in vitro} with Ac1–11, in addition to an increase in IL-4, IL-6 was also increased associated with a small increase in IFN-\(\gamma\). These results establish \textit{in vivo} immunologic cross-reactivity between Cop1 and clones reactive to Ac1–11 when Cop1 is either fed or given parenterally. Of note, in the Teitelbaum et al. study of oral Cop1 in conventional mice, they did not observe increased IL-4 following feeding, whereas increased IL-4 was observed when Cop1 was given by injection (16). We have previously hypothesized that Cop1 may behave as an altered peptide ligand for MBP-specific T cells, resulting in skewing the cytokine profile of responding T cells by either changing the activation thresholds or by acting as a partial agonist of MBP-specific T cells. Thus Cop1 induces some but not all effector cytokines from the MBP-specific T cells. Our studies begin to define the mechanisms by which Cop1 may regulate EAE (and potentially MS) \textit{in vivo}.

To further study the specificity of Cop1 in disease models we found that oral Cop1 suppressed EAE in MBP TCR transgenic mice as efficiently as oral MBP and more efficiently than MOG in the NOD model. Cop1 treatment does not suppress other autoimmune diseases in animals including myasthenia gravis, thyroiditis, diabetes and lupus models. Recently it has been reported to prevent graft-versus-host disease \textit{in vivo}, but in much higher doses (25), and uveitis, but only if the antigen was mixed with Cop1 in the injected solution (26). Cop1 has also been reported to inhibit type II collagen-reactive T cell clones \textit{in vitro} (20), but we did not observe an effect of oral Cop1 in the collagen arthritis model. To our knowledge there are no previous reports of the effect of Cop1 in the collagen arthritis model.

In summary, our studies demonstrate that Cop1 is active orally in an antigen-specific fashion, and appears to function as an altered peptide ligand capable of triggering \textit{in vivo} MBP-reactive cells that preferentially secrete IL-4, IL-10 and TGF-\(\beta\) that are capable of down-regulating EAE.

Acknowledgements

Supported by NIH grants AI43458 and NS38037.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA</td>
<td>complete Freund’s adjuvant</td>
</tr>
<tr>
<td>CIA</td>
<td>collagen-induced arthritis</td>
</tr>
<tr>
<td>Cop1</td>
<td>copolymer 1</td>
</tr>
<tr>
<td>MBP</td>
<td>myelin basic protein</td>
</tr>
<tr>
<td>MOG</td>
<td>myelin oligodendrocyte glycoprotein</td>
</tr>
<tr>
<td>MS</td>
<td>multiple sclerosis</td>
</tr>
<tr>
<td>MT</td>
<td>Mycobacterium tuberculosis H37RA</td>
</tr>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
</tr>
<tr>
<td>TGF</td>
<td>transforming growth factor</td>
</tr>
</tbody>
</table>

References


