Protective Effect of the Type IV Phosphodiesterase Inhibitor Rolipram in EAU: Protection Is Independent of IL-10–Inducing Activity

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PURPOSE. Experimental autoimmune uveoretinitis (EAU) is a T-cell-mediated model of retinal autoimmunity that is negatively regulated by interleukin (IL)-10. The antidepressant drug rolipram, a type IV phosphodiesterase inhibitor, enhances IL-10 production by monocyte/macrophages. The effect of rolipram on induction of EAU and its associated immunologic responses was investigated.

METHODS. Mice were challenged for EAU induction by immunization with the retinal antigen interphotoreceptor retinoid-binding protein (IRBP) or by adoptive transfer of uveitogenic T cells and were treated with rolipram. EAU severity and immunologic responses to IRBP were analyzed. In addition, the effect of rolipram added to the culture on antigen-driven responses of primed lymph node cells was tested.

RESULTS. Rolipram treatment from days −1 to 7 after immunization (afferent phase) was not protective, but severity of EAU was reduced to 50% by treatment from days 8 to 16 after immunization or when EAU was induced by adoptive transfer (efferent phase). Antigen-specific proliferation and interferon (IFN)-γ production ex vivo by lymph node cells of protected mice were not reduced. However, the addition of rolipram directly to the culture suppressed IRBP-driven proliferation and IFN-γ production by primed lymph node cells. Freshly explanted lymph node cells of treated mice showed inhibition of IFN-γ mRNA but no parallel enhancement of IL-10 mRNA by quantitative polymerase chain reaction. Rolipram inhibited EAU in IL-10 knockout mice equally well compared with controls and suppressed their primed lymph node cells in culture.

CONCLUSIONS. Rolipram appears to inhibit the expansion and effector function of uveitogenic T cells, raising the possibility that it may be useful for treatment of established disease. Contrary to expectations based on in vitro studies, the protective effects in vivo appear to be independent of IL-10. The observation that suppression of antigen-specific responses is demonstrable only in the physical presence of the drug suggests that, in a clinical setting, continuous administration of rolipram might be needed to sustain its therapeutic effect. (Invest Ophthalmol Vis Sci. 1999;40:942-950)

Experimental autoimmune uveoretinitis (EAU) is a T-cell-mediated autoimmune disease that is caused by an immune response to retinal antigens.1-3 EAU in mice and rats appears to share essential immunologic mechanisms with human uveitis and has successfully served as a model for understanding the mechanisms of the disease and for development of immunotherapeutic strategies.4 We have previously shown that T helper type 1 (Th1) cells and Th1-type cytokines are involved in the pathogenesis of EAU.5-7 The Th1-inducing cytokine interleukin (IL)-12 is able to augment the pathogenicity of ocular antigen–primed lymphocytes.8 In contrast, an adoptive transfer of a Th2-like cell line suppresses EAU development in the rat model.8 Finally, treatment with IL-10 inhibits EAU, and IL-10 inhibits interferon (IFN)-γ production and proliferation of uveitogenic effector T cells in culture.8,10

Rolipram, a type IV phosphodiesterase inhibitor that is currently in use clinically as an antidepressant, recently has been shown to have anti-inflammatory effects. It suppresses tumor necrosis factor-α and IL-6 release by macrophages,11-13 inhibits migration of leukocytes,14 downregulates Th1 function (as represented by production of IFN-γ), and upregulates the lipopolysaccharide (LPS)-induced production of IL-10 in cultured peritoneal macrophages.11,15 Because our previous data indicated that IL-10 has a negative regulatory role in EAU,10 we decided to investigate the effects of rolipram on EAU development and on the associated immunologic responses to the uveitogenic antigen interphotoreceptor retinoid-binding protein (IRBP). Our data show that rolipram has a protective effect when administered during the expression phase of EAU but not if given during the induction phase. Furthermore, although rolipram treatment upregulates monocyte/macrophage IL-10 mRNA, the inhibitory effects of rolipram on EAU appear not to depend on IL-10.
MATERIALS AND METHODS

Mice

B10.A and B10.RIII mice were purchased from Jackson Laboratories (Bar Harbor, ME). C57BL/6 wild-type and IL-10 knockout mice (on C57BL/6 background) were from the stock originally developed at the Genetics Institute, Köln University. Males and females between the ages of 6 to 12 weeks were used. All mice were kept under specific pathogen-free conditions. Under these conditions IL-10 knockout mice remained healthy until at least 4 months of age. The use of laboratory animals conformed to institutional guidelines and to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Reagents and Antigens

Rolipram was purchased from Biomol Research Laboratories (Cat. No. PD-175, Lot No. W1037; Plymouth Meeting, PA). Dimethyl sulfoxide (DMSO) was purchased from Sigma Chemical Company (St. Louis, MO). Complete Freund’s adjuvant was purchased from Sigma and was supplemented with Mycobacterium tuberculosis strain H37RA to the final concentration of 2.5 mg/ml. Bovine IRBP was prepared from bovine retinal tissue by Con A Sepharose 4B (Pharmacia, Piscataway, NJ) affinity chromatography and fast-performance liquid chromatography as described previously.

Antibodies

Capture antibodies and biotinylated antibodies for IL-4, IL-10, and IFN-γ enzyme-linked immunosorbent assay (ELISA) were purchased from Pharmingen (San Diego, CA; rat anti-mouse IL-4 monoclonal antibodies, Cat. No. 18031D and Cat. No. 18191D; biotinylated rat anti-mouse IL-4 monoclonal antibodies, 18042D; purified rat anti-mouse IL-10 monoclonal antibodies, Cat. No. 18141D; biotinylated rat anti-mouse IL-10 monoclonal antibodies, Cat. No. 18152D; purified rat anti-mouse interferon-γ monoclonal antibody, 18181D; biotinylated rat anti-mouse IFN-γ monoclonal antibody, 18112D). Horseradish peroxidase-labeled streptavidin was obtained from Southern Biotechnology Associates (Birmingham, AL).

Uveitogenic Mouse T-Cell Line

A uveitogenic Th1 cell line specific to a major H-2-restricted uveitogenic epitope of IRBP was derived and propagated as described previously. Briefly, B10.RIII mice were immunized with human IRBP peptide 161-180, and draining lymph node cells from the immunized mice were primed in vitro in the presence of antigen, IL-12, and anti-IL-4. The cell line thus derived was fed with IL-2 (20 U/ml) every 2 to 3 days and was restimulated with 1 μg/ml of antigen every 2 to 3 weeks in the presence of irradiated antigen-presenting cells.

Induction and Scoring of EAU

Mice were immunized with 50 μg bovine IRBP in a 1:1 (vol/vol) emulsion with Complete Freund’s adjuvant in a total volume of 0.2 ml divided among base of tail and both hind thighs. Bordetella pertussis toxin (0.5 μg in a volume of 100 μl) was injected intraperitoneally at the same time. In most experiments, delayed-type hypersensitivity (DTH) challenge was performed on day 17 and was scored after 48 hours. Eyes were collected for histopathology on day 19 after immunization.

In the adoptive transfer model, 1 × 10^6 freshly stimulated T cells from the uveitogenic T-cell line described above were injected intraperitoneally into syngeneic mice. Eyes were collected 5 days after adoptive transfer for EAU scoring.

Severity of disease was quantitated by histopathology. Freshly enucleated eyes were fixed for 1 hour in 4% buffered glutaraldehyde and transferred into 10% buffered formaldehyde until processing. Fixed and dehydrated tissue was embedded in methacrylate and 4- to 6-μm sections were stained by standard hematoxylin and eosin. Eight to 10 sections cut at different planes were examined for each eye. EAU was scored in a masked fashion on an arbitrary scale of 0 to 4 by an independent observer using a semiquantitative system described previously.

Rolipram Treatment

Rolipram was dissolved in DMSO at 1 mg/10 μl, and then adjusted to 1 mg/ml in phosphate-buffered saline (PBS; yielding 1% final concentration of DMSO). Mice were injected intraperitoneally with 3 mg/kg rolipram (or another dose where indicated) twice daily on days −1 through 7 to cover the afferent phase of EAU, or on days 8 through 16 to cover the efferent phase of EAU. Control mice were injected with the same volume of vehicle containing 1% DMSO.

Proliferation Assay

Draining lymph nodes were harvested from immunized mice and were dispersed into a single-cell suspension by pressing through a stainless steel mesh. Washed cells were suspended at 5 × 10^6 cells/ml in DMEM (HyClone, Logan, UT) supplemented with 2 mM glutamine, 50 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 5 × 10^-5 M 2-mercaptoethanol and containing 1.5% mouse serum. Triplicate 100-μl aliquots of the cell suspension were placed into 96-well U-bottomed culture plates, and another 100 μl of medium containing different stimulants, as specified, was then added to the wells. After the plates were incubated for 48 hours at 37°C, 10% CO_2, each well was pulsed with 1 μCi of [3H]thymidine for 16 to 18 hours. [3H]Thymidine incorporation was determined by standard liquid scintillation counting.

Delayed-Type Hypersensitivity

Ten micrograms of IRBP in 10 μl PBS was injected into the left ear pinna, and the same volume of PBS was injected into the right ear pinna as a control. Ear thickness was measured 48 hours later with a spring-loaded micrometer. The specific response was calculated as the difference of ear thickness between the IRBP-injected ear and the control ear.

Cytokine Measurement

Interleukin-4, IL-10, and IFN-γ in culture supernatants were analyzed by ELISA. Briefly, 96-well ELISA plates were coated overnight with anti-IL-4 (100 μl containing 1 μg/ml of 18031D and 1 μg/ml of 18191D), anti-IL-10 (100 μl of 3 μg/ml), or anti-IFN-γ (100 μl of 2 μg/ml) in 0.1 M NaHCO_3, pH 8.2. The plates were rinsed with washing buffer (PBS, pH 7.4, with 0.05% Tween-20 and 0.05% merthiolate) and blocked with blocking buffer (PBS–Tween with 0.1% bovine serum albumin and 0.05% merthiolate) for 2 hours at room temperature. After 2 washes, duplicates of serially diluted supernatant samples or standards were added to the plates. After 2 hours at room temperature, plates were washed and horseradish peroxidase-labeled antibody was added. After 2 hours, plates were washed again and the peroxidase activity was measured by standard liquid scintillation counting.
Preparation of Peritoneal Cells

Washes. Color reaction was performed with o-phenylenediamine dihydrochloride substrate and was terminated by the addition of 2N H2SO4. The plates were read at 490 nm with an ELISA reader (Molecular Devices, Menlo Park, CA).

The washed cell pellets were processed for reverse transcription for IFN-γ mRNA and PCR with competitor-specific primers. Tested samples were coamplified with IL-10 competitor using mouse IL-10 primers and HPRT, hypoxanthine phosphoribosyl transferase. Tested samples were electrophoresed on 2% agarose gel in presence of ethidium bromide. Bands were photographed with Polaroid 665 film and quantitated with a 300B densitometer (Molecular Dynamics, Sunnyvale, CA).

Reproducibility and Data Presentation

Experiments were repeated at least twice, and usually 3 times. Group size was routinely 5 mice. Data were highly reproducible. Graphs show representative experiments. Statistical analysis for parametric data (DTH) was by independent t-test, and analysis for nonparametric data (EAU scores) was by Snedecor and Cochran's analysis for linear trend in proportions.21

RESULTS

Suppression of IRBP-Specific DTH Response and Inhibition of EAU Development by Rolipram

In the mouse B10.A EAU model, clinical onset of the disease usually occurs from days 12 to 14 after mice are challenged with a uveitogenic regimen of IRBP. Retinal damage is maximal by days 19 to 21 and includes infiltration of inflammatory cells into the uvea and retina, disorganization of retinal architecture, retinal folding and detachment, and photoreceptor damage. The first week after immunization is considered as the afferent phase of the disease, or the priming period. The second and third weeks represent the efferent phase, or expression stage, of the disease and include migration of effector cells, infiltration of the target tissues, and photoreceptor damage. EAU induced by adoptive transfer of immune lymphocytes (onset day 3-4) represents a “pure” efferent phase disease.

To address the question of whether rolipram affects the afferent phase or the efferent phase of the disease, B10.A mice were treated twice daily with 3 mg/kg of rolipram on days −1 through 7 or on days 8 through 16, respectively. Rolipram treatment during the afferent phase alone was not effective; however, treatment during the efferent phase suppressed IRBP-specific DTH responses and lowered EAU scores by up to 50% (Fig. 1). At the level of histopathology, treated mice had fewer infiltrating cells in the vitreous, less vasculitis, a better preserved photoreceptor cell layer, and markedly reduced choroiditis (Fig. 2). Dose-response experiments showed that the

### Table 1. Sequence of PCR Primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product Size (bp)</th>
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<tbody>
<tr>
<td>IFN-γ Sense</td>
<td>5'-TGG AGG AAC TGG CAA AAG GAT GGT-3'</td>
<td>245</td>
</tr>
<tr>
<td>IL-10 Sense</td>
<td>5'-ACC TGG TAG AAG TGA TGC CCC AGG CA-3'</td>
<td>237</td>
</tr>
<tr>
<td>IL-10 competitor Sense</td>
<td>5'-ACC TGG TAG AAG TGA TGC CCC AGG CAT GGG TGA GAA GCT GAA GA-3'</td>
<td>197</td>
</tr>
<tr>
<td>HPRT Sense</td>
<td>5'-GAT GGA TAC AGG CCA GAC TTT GTT G-3</td>
<td>163</td>
</tr>
<tr>
<td>HPRT Antisense</td>
<td>5'-GAT TCA ACT TGC GCT CAT CTT AGG C-3'</td>
<td></td>
</tr>
</tbody>
</table>

HPRT, hypoxanthine phosphoribosyl transferase.
Rolipram Protects from EAU

Rolipram is a phosphodiesterase-4 inhibitor that has been shown to have immunosuppressive effects. In this study, the protective effect of rolipram on EAU was dose dependent (Fig. 3). The effectiveness of rolipram on effector phase EAU was also confirmed in adoptive transfer experiments, in which (unimmunized) B10.RIII mice were injected with a syngeneic uveitogenic T-cell line and were treated with rolipram. The results showed that rolipram reduced EAU scores in mice infused with a uveitogenic T-cell line to one half the scores of vehicle-treated control mice (Fig. 4). These results suggest that rolipram may protect from EAU by inhibiting the function of uveitogenic effector cells but may be unable to inhibit their priming.

Requirement for Continuous Presence of Rolipram for Suppression of IRBP-Specific Responses

It has been well documented that the pathogenesis of EAU is associated with Th1-type cellular response to ocular antigens, as represented by high levels of antigen-specific IFN-γ production. In the present study, although the DTH response and EAU were reduced after effector rolipram treatment (Fig. 1), explanted lymph node cells from the protected groups that were stimulated with antigen in culture were fully able to proliferate (Fig. 5) and to produce IFN-γ (Table 2). Furthermore, there was no detectable difference between the groups in their ability to produce IL-4 and IL-10 (not shown). This was an apparent contradiction to the in vivo suppression of disease scores. We therefore set out to determine whether rolipram could affect antigen-specific responses of primed lymph node cells when it was added directly to the culture. Draining lymph node cells were harvested from IRBP-immunized mice and were restimulated with antigen in the presence of rolipram or solvent control. Rolipram suppressed antigen-driven proliferation of those cells in a dose dependent manner. Furthermore, 10 μM rolipram dramatically inhibited IRBP-induced IFN-γ production (Fig. 6). The inhibition did not seem to involve a toxic effect on the cells, because under microscopic examination the
FIGURE 3. Dose response of rolipram-induced protection from EAU. B10.A mice were immunized with a uveitogenic regimen of IRBP and were treated with graded doses of rolipram from day 8 to 16. EAU was analyzed on day 19 after immunization. The probability value between 0 mg/kg and 3 mg/kg rolipram was 0.065.

Cells at all rolipram concentrations appeared viable. These data suggest that whatever inhibitory effect rolipram may have on antigen-specific T cells in vivo, it is fully reversible when the cells are explanted into culture, and that the physical presence of rolipram is required for maintaining suppression of IRBP-specific cellular responses.

Lack of Requirement for IL-10 in Rolipram-Induced Suppression of IRBP-Specific Responses

Interleukin-10 expression in peritoneal cells is enhanced by rolipram treatment, but IL-10 is not required for suppression of IRBP-specific cellular responses. Rolipram is able to upregulate LPS-induced IL-10 production in cultured macrophages. Because our previous studies showed that IL-10 is able to suppress EAU induction and because it is one of few cytokines that suppress antigen-specific responses of mature uveitogenic Th1 effector cells, we looked for evidence of IL-10 upregulation in mice immunized with antigen and treated with rolipram. IL-10 protein could not be detected by ELISA in the sera of treated mice or in culture supernatants of their antigen-stimulated lymph node cells. However, competitive PCR for IL-10 mRNA in peritoneal lavage cells from mice immunized with IRBP and injected with rolipram showed that IL-10 mRNA expression was enhanced, peaking 18 hours after initiation of treatment (Fig. 7). This in vivo response was similar to our previous data obtained with cultured macrophages. However, when draining lymph node cells extracted 12 hours after the last rolipram treatment were analyzed for IFN-γ and IL-10 mRNA expression, it was found that although IFN-γ mRNA was depressed, IL-10 mRNA in the same lymph node did not appear to be upregulated (Fig. 8). Furthermore, IL-10 knockout mice

FIGURE 4. Rolipram protects from EAU induced by a uveitogenic T-cell line. B10.RIII mice were injected intraperitoneally with 1 × 10⁶ freshly stimulated uveitogenic T cells and were treated with rolipram from days −1 to 5 (3 mg/kg, 2 times per day). Eyes were analyzed 5 days after adoptive transfer. *Statistically significant difference from control; P < 0.05.

FIGURE 5. Rolipram treatment in vivo does not result in suppression of antigen-specific proliferation in explanted lymph node cells. B10.A mice were immunized with a uveitogenic regimen of IRBP and were treated with 3 mg/kg rolipram or vehicle twice daily from days −1 to 7 or from days 8 to 16. Lymph node cells were harvested on day 19 and were stimulated in culture with IRBP. Shown is [³H]thymidine uptake after subtraction of background counts (background in unstimulated cultures ranged from 7 × 10³ cpm to 12 × 10³ cpm). Cont., control; Rolipr., rolipram.
**TABLE 2.** Antigen-Specific IFN-γ Production in Culture by Lymph Node Cells of IRBP-Immunized Mice Treated with Rolipram

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Afferent Treatment (Days -1-7)</th>
<th>Efferent Treatment (Days 8-16)</th>
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<tbody>
<tr>
<td></td>
<td>Rolipram</td>
<td>Vehicle</td>
</tr>
<tr>
<td>18</td>
<td>0.54</td>
<td>1.22</td>
</tr>
<tr>
<td>36</td>
<td>2.68</td>
<td>3.38</td>
</tr>
<tr>
<td>72</td>
<td>6.06</td>
<td>5.68</td>
</tr>
</tbody>
</table>

Values are expressed as ng/ml of IFN-γ. Lymph node cells were harvested on day 19. Lower limit of detection was 0.312 ng/ml.

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933211/)

**Figure 6.** Rolipram present in the culture inhibits antigen-driven proliferation and IFN-γ production. Triplicate lymph node cell cultures from IRBP-immunized B10.A mice were stimulated with IRBP in the presence of rolipram or solvent control. (A) Proliferation (in counts per minute); (B) IFN-γ production at 18, 36, and 72 hours (ELISA).

![Figure 7](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933211/)

**Figure 7.** Rolipram treatment enhances IL-10 mRNA expression in peritoneal cells. B10.A mice were immunized with a uveitogenic regimen of IRBP and were treated with a single dose of 10 mg/kg rolipram (R) or with vehicle (C). Peritoneal cells were recovered after 6, 18, and 36 hours, and IL-10 mRNA expression was analyzed by competitive RT-PCR. Naive, mice not immunized or treated. HPRT, hypoxanthine phosphoribosyl transferase.

immunized with IRBP and treated with rolipram were protected equally as well as their wild-type controls (Fig. 9). This did not support a significant role for IL-10 in the protective effects of rolipram.

To address the possibility that at least the suppression in culture of IFN-γ production by rolipram was mediated by IL-10, we tested the effect of rolipram on the IRBP-primed lymph node cells from IL-10-deficient mice. IRBP-specific IFN-γ production of lymph node cells from IL-10-deficient mice, typically higher than that of the wild type,16 was still inhibited by rolipram to the same level as that in the wild-type mice (Fig.

![Figure 8](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933211/)

**Figure 8.** Rolipram treatment reduces IFN-γ mRNA in the draining lymph node, without a parallel enhancement in IL-10 mRNA. B10.A mice were immunized with a uveitogenic regimen of IRBP and were treated with 3 mg/kg rolipram or vehicle twice daily. Lymph node cells were harvested on day 17 (12 hours after the last rolipram treatment). HPRT, hypoxanthine phosphoribosyl transferase.
expression after IRBP immunization is augmented by rolipram, Figure 9. Rolipram protects IL-10-deficient mice from EAU. IL-10-deficient mice on C57BL/6 background and wild-type C57BL/6 controls were immunized with a uveitogenic dose of IRBP and were treated with rolipram or vehicle on days 8 to 16 after immunization. Eyes were collected for histopathology on day 19.

These data suggest that, although monocyte IL-10 mRNA expression after IRBP immunization is augmented by rolipram, the inhibitory effects on disease, and on antigen-driven proliferation and IFN-γ production, are not dependent on IL-10.

**DISCUSSION**

Rolipram has been approved for use in humans, which gives it a practical advantage as a potential therapeutic agent. Rolipram therapy was explored by others in several autoimmune and inflammatory disease models. In experimental autoimmune encephalomyelitis, a Th1-dependent disease that shares essential mechanisms with EAU, rolipram was effective in preventing the clinical onset of disease and in ameliorating pathogenesis in rodents and primates. It was therefore attractive to explore its ability to inhibit EAU.

In the present study, mice were treated so as to affect either the induction (afferent) or the expression (efferent) phase of EAU. The protection was effective only if treatment was given during the efferent phase of the disease, whether induced by active immunization or by adoptive transfer of uveitogenic T cells. The ability of rolipram to inhibit the effector phase of the uveitic response suggests that it might be useful clinically. Interestingly, even in protected mice, we could not demonstrate suppression of antigen-specific responses (proliferation and production of IFN-γ) in lymph node cells that were explanted into culture. This is consistent with a similar observation by Jung et al. However, if rolipram was added directly to the culture, suppression was observed in primed lymph node cells even if the mice did not receive rolipram treatment in vivo. This leads to the interpretation that the effect of rolipram administered in vivo is fully reversible, and once the cells are removed from its presence, they recover their function. If this interpretation is correct, it might also provide an explanation for the lack of efficacy of rolipram in the inhibition of EAU induction when administered only during the afferent stage. It is possible that priming can take place in the presence of rolipram, and once the drug was withdrawn, effector function could be expressed. Alternatively, the generation of uveitogenic cells could have occurred after drug withdrawal, because the antigen depot is present at the site of injection for several weeks. Either way, the need for the physical presence of rolipram to affect suppression of immunologic functions suggests that if used clinically continuous treatment would be required to maintain therapeutic efficacy.

The mechanism by which rolipram might exert its immunosuppressive and anti-inflammatory effects is not completely understood. Our in vitro data, showing that rolipram suppressed antigen-induced proliferation and IFN-γ production and inhibited IFN-γ mRNA levels in the draining lymph nodes of treated mice, suggest that protection from EAU may involve inhibition of the expansion of IRBP-specific lymphocytes and the suppression of IFN-γ production by those cells, curtailing the number and function of uveitogenic Th1 effector cells. Inhibition by rolipram of T-cell proliferation and of IFN-γ production, and the inhibition of LPS-induced tumor necrosis factor-α and upregulation of LPS-induced IL-10, have also been reported by others.

Because rolipram augments IL-10 production in LPS-stimulated macrophages in culture, we concentrated our efforts on investigating the possible role of IL-10 as a mediator of the therapeutic effects of rolipram in EAU. IL-10 is a major Th2-type cytokine that suppresses Th1-type immunity (which is involved in EAU), inhibits macrophage activation, and ameliorates some types of cell-mediated autoimmunity. Recently, we found that administration of recombinant IL-10 protects against EAU. Notably, IL-10 can inhibit antigen-driven proliferation and IFN-γ production of mature uveitogenic Th1 effector cells, which is in line with the observed protective effects of rolipram in the efferent stage of EAU. However, the present data do not support a major role for IL-10 as a mediator of the protective and inhibitory effects of rolipram, either in vivo or in vitro, in the EAU model. This is indicated by several lines of evidence. Although IL-10 mRNA was elevated in peritoneal cells from immunized mice that were injected with rolipram, confirming the data of Kambayashi et al. in LPS-stimulated macrophages, IL-10 mRNA was not upregulated in the draining lymph nodes of rolipram-treated mice despite reduction in the levels of IFN-γ mRNA in the same lymph node. This may reflect a functional difference
but also did not require the induction of IL-10. Taken together, the in vivo and in vitro data point to the conclusion that the protective and inhibitory effects of rolipram in the EAU model occur through an IL-10-independent mechanism. The possibility that a combination therapy with rolipram and IL-10 might have an additive or a synergistic effect as a result of eliciting separate inhibitory pathways, should be investigated.

We did not find evidence for a possible skewing of the response toward the Th2 pathway as a result of rolipram treatment, because there was no elevation of antigen-driven IL-4 production by lymph node cells in any of the experiments (data not shown). Others have reported that in a Th2-type antigen-driven response of peripheral blood mononuclear cells, IL-4 gene expression was unaffected by rolipram; proliferation of human T cells to allergen was also unaffected.

In summary, the administration of rolipram during the effrse phase of EAU induction reduces the subsequent severity of EAU. The suppressive mechanism appears to involve downregulation of type 1 cellular immune responses, as manifested by the suppression of DTH and inhibition of antigen-driven proliferation and IFN-γ production. The suppression of cellular function is fully reversible on removal of the drug and is effected through an IL-10-independent mechanism. The reversibility of the suppressive effect of rolipram suggests that in a clinical situation chronic treatment would be required to sustain a therapeutic effect.

References


