Original Article

Suppressive effects of a novel compound on interphotoreceptor retinoid-binding protein-induced experimental autoimmune uveoretinitis in rats

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ABSTRACT

The immunosuppressive effect of ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate(CAM) was examined in interphotoreceptor retinoid-binding protein (IRBP)-induced experimental autoimmune uveoretinitis (EAU) in rats. Lewis rats immunized with bovine IRBP were treated with various oral doses of CAM postimmunization. The degree of inflammation was assessed clinically each day and histologically on day 14 or day 20. Production of various cytokines and IRBP-specific antibody, as well as IRBP-specific proliferation response, was assessed. Complete inhibition of EAU in rats, both by clinical and histologic criteria, was achieved with 50 mg/kg CAM when administered daily for 14 days following IRBP immunization. Partial inhibition was observed at lesser doses of CAM. This CAM-mediated response was accompanied by diminished production of cytokines interleukin-2, interferon-γ and tumor necrosis factor-α, as well as a reduction in IRBP-specific antibody production. Furthermore, administration of CAM either in the induction phase only (days 0–7) or in the effector phase only (days 9 or 11 to day 20) was also capable of suppressing EAU, as assessed histopathologically on day 20. We conclude that CAM is effective in suppressing EAU in rats and its mechanism of action appears to involve modulation of T cell function.

INTRODUCTION

Experimental autoimmune uveoretinitis (EAU) has been extensively studied as a model for human ocular inflammatory diseases, such as birdshot retinochoroidopathy, sympathetic ophthalmia and Behçet's disease.[1–3] In animals, EAU can be induced by immunization with interphotoreceptor retinoid-binding protein (IRBP), an eye-specific retinal antigen, or by transfer of IRBP-specific T cells.[4,5] We have previously found that type 1 T helper (Th1) cells are activated following IRBP immunization and that transfer of these activated T cells can induce EAU development.[6] Furthermore, such Th1 cell-mediated induction of EAU is counteracted by the activation of type 2 T helper (Th2) cells.[6] These results have suggested that EAU may be treated or its development prevented by the manipulation of T cell activation.

Immunosuppressive drugs that impair T cell function, such as cyclosporine A, FK506 and rapamycin, have been used for the treatment of severe ocular inflammatory diseases.[7–10] However, these drugs are limited in their use because of unwanted side effects, in particular the hepatic and renal toxicity caused by cyclosporine A and FK506.[11,12] To achieve therapeutic results with minimal side effects, an immunosuppressive drug with selective action on lymphocytes would be useful. Ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate (CAM), a derivative of mycophenolic acid (MPA) produced by *Penicillium brevicompactum*, has been found to inhibit immune responses as well as tumor cell growth.[13]

Specifically, CAM has been shown to prolong cardiac allografts in rats and to suppress the onset of experimental allergic encephalomyelitis (EAE) in rats.[14,15] Following *in vivo* administration, CAM is converted to mycophenolic acid, which in turn exerts its effect by impairing the activity of inosine monophosphate dehydrogenase (IMPDH), an enzyme involved in purine metabolism. Decreased IMPDH activity leads to depletion of GTP, which is essential for lymphocyte activation.[16]

In the present study, we examined the effect of CAM on IRBP-mediated EAU in rats. Severity of EAU inflammation was assessed by clinical and histopathologic criteria and production of various cytokines and IRBP-specific antibody, as well as the IRBP-specific proliferation response, were measured.

METHODS

Rats

Male Lewis rats of 6–8 weeks of age were obtained from Charles River Japan (Atsugi, Japan) and housed in our specific pathogen-free animal facility. The animals were treated in accordance with Guide for the Care and Use of Laboratory Animals.[17]

Reagents

The CAM was obtained in powder form from Ajinomoto Co. (Osaka, Japan). Bovine IRBP was purified using the method described by Redmond *et al*.[18] Other reagents were all standard grade unless otherwise stated.

Induction and assessment of EAU

Rats were immunized with 50 μg bovine IRBP emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA), in one hind footpad in a total volume of 0.1 mL. An additional adjuvant, *Bordetella pertussis* bacteria (Wako Pure Chemical Industries, Osaka, Japan), was concurrently injected intraperitoneally at a dose of 10^{10} /rat. Following immunization, the rats were examined daily by an independent observer using slit-lamp biomicroscopy (Kowa, Tokyo, Japan). Eyes were removed on day 14 or 20 after immunization and fixed in 10% buffered neutral formalin. Sections of samples were embedded in paraffin and stained with hematoxylin and eosin for histologic study. The clinical severity of EAU was assessed by scoring three different criteria for a maximum total score of 7 (Table 1). Similarly, the histologic severity of EAU was assessed by scoring cellular infiltration in four

Table 1 Scoring of clinical and histologic severity

Clinical scoring	
Symptom	Scale
Mydriasis after tropicamide instillation	0–2
0: Complete	
1: Partial	
2: Absent	
Fibrin exudates in the anterior chamber	0–2
0: None	
1: Limited to the pupillary margin	
2: Covering the iris and pupil	
Hypopyon	0–3
0: None	
1: Mild	
2: Moderate	
3: Severe	
Maximum total score	7
Histologic scoring	
Cellular infiltration	
Ciliary body	0–2
Retina	0–2
Choroid	0–2
Vitreous	0–2
Tissue destruction	
Ciliary body	0–2
Retina	0–2
Choroid	0–2
Maximum total score	14

different areas and tissue destruction in three different areas for a maximum total score of 14. Incidence was expressed as number of EAU-positive rats out of the total number of rats in each group, based on both clinical and histologic examinations.

Administration of CAM

Suspensions of CAM were made in a 0.9% NaCl solution containing 0.5% sodium carboxymethyl-cellulose, protected from light and preserved at 4°C. Aliquots from this stock suspension of CAM were sonicated and diluted in NaCl solution for each administration. Rats received a daily oral gavage of 0, 10, 30 or 50 mg/kg CAM for different periods of time depending on the experiment as follows: (i) entire observation period (day 0, day of IRBP immunization, to day 14 or 20); (ii) induction phase (days 0–7); (iii) effector phase 1 (day 9, 1 day before the usual onset of iridocyclitis in EAU, to day 20); and (iv) effector phase 2 (day 11, after the onset of iridocyclitis had been confirmed, to day 20). Control rats received equivalent volumes of phosphate-buffered solution (PBS).

Measurement of cytokine production

Spleen cells (2×10^6/2 mL) from control rats and from IRBP-immunized rats on day 20 that had or had not been treated with CAM were cultured in the presence of 5 μg/mL IRBP for 24 h. Interleukin-2 and IL-6 activities were then measured by biological assay using the murine CTLL-2 and murine hybridoma MH60.BSF2 cell lines (gift from Drs T Hirano and T Kishimoto), respectively.[19] One unit of IL-2 activity was defined as the concentration by which a half-maximal [^{3}H]-thymidine (TdR) uptake by CTLL was induced. Concentrations of tumor necrosis factor (TNF)-α and interferon (IFN)-γ were measured by specific enzyme-linked immunosorbent assay (ELISA) kits (GIBCO Life-Technologies, Tokyo, Japan and COSMO Bio., Tokyo, Japan).

Measurement of IRBP-specific lymphocyte proliferation

Spleens were removed on day 14 postimmunization and single-cell suspensions were prepared. Cells at a density of 2×10^5/well in 96-well plates were incubated in triplicate in RPMI-1640 medium containing 10% fetal calf serum, 5×10^{-5} mol/L 2-mercaptoethanol, and 50 μg/mL kanamycin, with or without 5 μg/mL IRBP. [^{3}H]-Thymidine was added for the final 16 h of the 90 h incubation period, followed by harvesting of cells and measurement of [^{3}H]-TdR uptake using a liquid scintillation counter. Results were expressed as c.p.m., representing the mean of at least three experiments with similar results.

Measurement of IRBP-specific serum IgG antibody formation by ELISA

Serum obtained from blood taken from the tail vein of IRBP-immunized rats on day 14 was diluted 1:80 and layered on to plates coated with 1 μg/mL IRBP (in carbonate buffer, pH 9.5). Peroxidase-conjugated goat antirat IgG antibody (Cappel, Organo Teknika Corp., West Chester, PA, USA) was used as the secondary antibody. Color was developed by adding o-phenylenediamine in a

0.01% H_2O_2 buffer and was assessed at 492 nm using an ELISA autoreader (Colona, Tokyo, Japan).

Statistics

The Mann–Whitney *U*-test and Student's *t*-test were used for statistical analysis. Data are represented as the mean ± SEM.

RESULTS

Inhibition of IRBP-induced EAU mediated by CAM

Lewis rats were immunized with 50 μg IRBP and then administered either 50 mg/kg CAM or PBS (control) each day by oral gavage from day 0 to day 14. Clinical examination was performed daily and eyes were removed on day 14 for histologic examination. Generally, both eyes of each rat developed EAU at about the same time and with the same degree of severity. When a difference was noted between the two eyes, the eye with the earlier onset or greater severity was selected for use in evaluation. Nine of nine control rats developed EAU, observed clinically as iridocyclitis, on approximately day 10 (mean 9.8 ± 0.9 days) after IRBP immunization (Table 2). This inflammation was subsequently confirmed histopathologically after enucleation on day 14 (Fig. 1a). In contrast, treatment with CAM completely inhibited EAU development, both clinically and pathologically, in 10 of 10 rats (Fig. 1b).

Dose–response profile of CAM

In order to determine the dose–response profile of CAM in inhibiting IRBP-mediated EAU, various doses of the drug were administered from day 0 to day 20 (Table 3). A significant delay in onset (10.3 ± 1.1 days) was accompanied by a decrease in inflammation (clinical score 3.8 ± 1.0) with 10 mg/kg CAM, compared with control (onset at 8.0 ± 0.5 days and clinical score 6.0 ± 0.9). Further improvement was obtained with 30 mg/kg CAM,

Table 2   Inhibition of IRBP-induced EAU by CAM

Treatment	EAU* (day of onset)	Clinical score†	Pathologic score
PBS (control)	9/9 (9.8 ± 0.9)	5.5 ± 0.6	10.0 ± 0.6
CAM	0/10	0	0

Rats received a daily oral gavage of phosphate-buffered saline (PBS) or 50 mg/kg ethyl *O*-(*N*-(*p*-carboxyphenyl)-carbamoyl)-mycophenolate (CAM) from day 0, the day of interphotoreceptor retinoid-binding protein (IRBP) immunization, to day 14. Eyes were removed on day 14 after immunization. *Number of rats that developed experimental autoimmune uveoretinitis (EAU). †The maximum score at the peak of inflammation is shown.
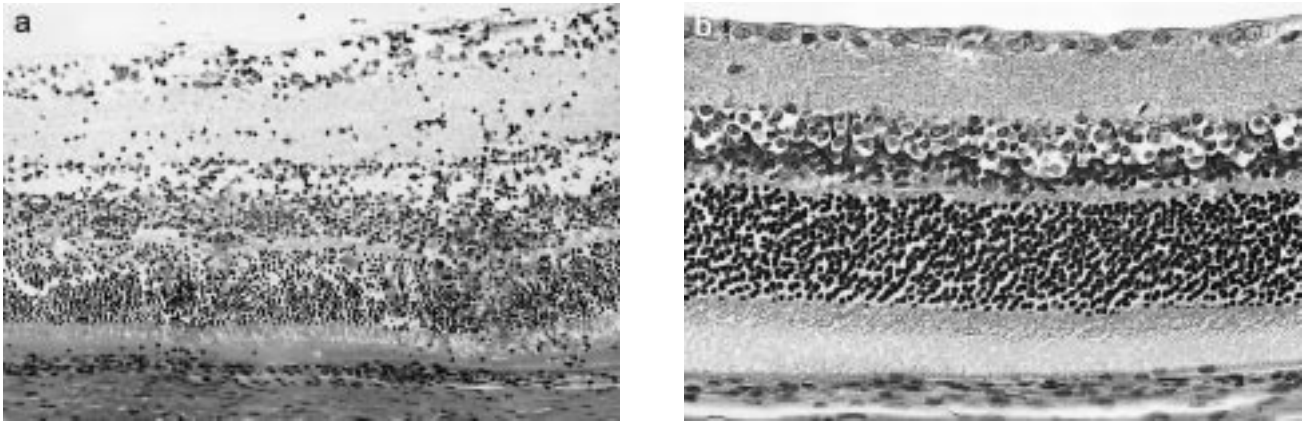
Fig. 1 Histopathology, on day 14, of the retina, of (a) a rat immunized with interphotoreceptor retinoid-binding protein (IRBP) that did not receive ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate (CAM) treatment (control), showing widespread disorganization with marked inflammatory cell infiltration, and (b) a rat immunized with IRBP that received 50 mg/kg CAM from days 0 to 14, showing no evidence of inflammation (original magnification × 130).

Table 3   Dose–response scores for CAM

Dose (mg/kg)	EAU* (day of onset)	Clinical score†	Pathologic score
PBS (control)	6/6 (8.0 ± 0.5)	6.0 ± 0.9	11.4 ± 0.8
10	4/4 (10.3 ± 1.1)	3.8 ± 1.0‡	6.8 ± 0.6‡
30	4/4 (13.5 ± 0.5)	1.3 ± 0.5‡	2.0 ± 1.0‡
50	0/5	0	0

Rats received a daily oral gavage of phosphate-buffered saline (PBS) or ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate (CAM) from day 0 to day 20. Eyes were removed on day 20 after immunization. *Number of rats that developed experimental autoimmune uveoretinitis (EAU). †The maximum score at the peak of inflammation is shown. ‡$P < 0.01$ compared with the control group, using the Mann–Whitney U-test.

with complete suppression of EAU development achieved at a dose of 50 mg/kg. Pathologic scores correlated well with clinical scores. These results indicate that CAM inhibits EAU development in a dose-dependent manner.

Kinetics of CAM administration

To examine whether CAM acts in the early phase or in the late phase of EAU development, rats were administered 50 mg/kg CAM at various intervals (Table 4). Concomitant treatment with CAM during the first 8 days (induction phase) caused a significant delay in onset (13.8 ± 1.7 days) accompanied by a moderately diminished maximal clinical score (2.8 ± 0.5) compared with the control (onset of 9.8 ± 0.9 days and clinical score 5.2 ± 0.9). Histopathologic examination of these rats on day 20 showed little evidence of inflammation (Fig. 2a). When rats that developed iridocyclitis by approximately day 10 were treated with CAM during only the effector phase (days 11–20), clinical inflammation was moderately decreased (clinical score 3.0 ± 0.5) compared with the control group (clinical score 5.2 ± 0.9), with almost

no inflammation observed on histopathologic examination. To further investigate the action of CAM on the effector phase of EAU development, IRBP-immunized rats were treated with 50 mg/kg CAM from days 9–20. Treatment with CAM during this period of time resulted in almost complete inhibition of uveoretinitis development, although the clinical effect appeared to be less pronounced (Table 4; Fig. 2b). These findings, taken together, suggest that CAM affects both the effector and induction phases of EAU development.

Inhibition of IRBP-induced cytokine production *in vitro* mediated by CAM

T helper 1 cells or the cytokines they produce have been reported to play a pivotal role in IRBP-induced EAU in mice and rats.[20,21] We, therefore, investigated whether CAM affected such cytokine production in our experiments. Treatment with 10 mg/kg CAM during the entire observation period significantly suppressed production of the cytokines IL-2, IFN-γ and TNF-α, with more pronounced suppression at a dose of 30 mg/kg and

Table 4   Kinetics of CAM administration

Period of CAM treatment	EAU* (day of onset)	Clinical score†	Pathologic score
None (control)	13/13 (9.8 ± 0.9)	5.2 ± 0.9	8.6 ± 0.7
Day 0–7	6/7 (13.8 ± 1.7)	2.8 ± 0.5‡	0.5 ± 0.5‡
Day 9–20	4/6 (10.5 ± 0.8)	2.5 ± 0.4‡	0.5 ± 0.5‡
Day 11–20	5/5 (9.8 ± 0.7)	3.0 ± 0.5‡	0.5 ± 0.5‡

Dose (50 mg/kg) was administered daily by oral gavage. Eyes were removed on day 20 after immunization. *Number of rats that developed experimental autoimmune uveoretinitis (EAU). †The maximum score at the peak of inflammation is shown. ‡$P < 0.01$ compared with the control group, using the Mann–Whitney U-test. CAM, ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate.
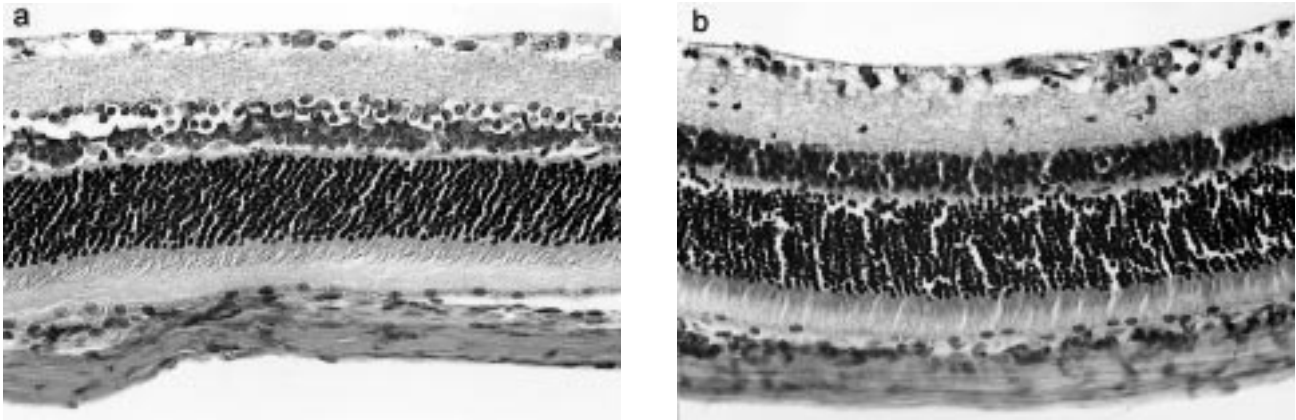
Fig. 2   Histopathology, on day 20, of the retina of (a) a rat immunized with interphotoreceptor retinoid-binding protein (IRBP) and treated with 50 mg/kg ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate (CAM) from days 0 to 7 (induction phase), showing little evidence of inflammation, and (b) a rat immunized with IRBP and treated with 50 mg/kg CAM from days 9 to 20 (effector phase), showing a few inflammatory cells infiltrating the retina and choroid (original magnification × 130).

Table 5   Dose–response for CAM inhibition of IRBP-induced cytokine production *in vitro*

Dose (mg/kg)	Cytokine		
	IL-2 (U/mL)	IFN-γ (ng/mL)	TNF-α (ng/mL)
PBS (control)	43 ± 4	222.0 ± 26.0	10.8 ± 1.0
10	11 ± 1*	128.9 ± 47.1†	7.8 ± 1.4
30	< 1*	60.4 ± 10.5*	4.0 ± 0.7†
50	< 1*	< 1.0*	< 1.0*

Rats received a daily oral gavage of phosphate-buffered saline (PBS) or ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate (CAM) from day 0 to day 20. Eyes were removed on day 20 after immunization. *$P < 0.01$, †$P < 0.05$ compared with the control group using the Student's t-test. IRBP, interphotoreceptor retinoid-binding protein; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.

complete suppression at a dose of 50 mg/kg (Table 5). Moreover, the production of IL-6 was also suppressed by CAM treatment (Table 6). This CAM-mediated inhibition of cytokine production correlates well with the inhibition of EAU development clinically. Treatment with CAM during the effector phase only also inhibited IRBP-mediated IL-2 and IFN-γ production, although treatment during the induction phase alone did not show any significant inhibition.

Inhibition of IRBP- but not Con A-induced spleen cell proliferation *in vitro* mediated by CAM

We examined whether CAM also influences IRBP-induced spleen cell proliferation *in vitro*. Spleen cells from IRBP-immunized rats treated with 50 mg/kg CAM from days 0–14 were cultured in the presence of 5 μg/mL IRBP or 2.5 μg/mL concanavalin A (Con A; control). As shown in

Table 6 *In vitro* inhibition by CAM of IRBP-induced cytokine production

Period of CAM treatment	Cytokine		
	IL-2 (U/mL)	IFN-γ (ng/mL)	IL-6 (ng/mL)
Control	85 ± 6	82.4 ± 5.3	18.4 ± 2
Days 0–20 (entire period)	4 ± 1*	15.6 ± 3.2*	2.1 ± 1*
Days 0–7 (induction phase only)	83 ± 7	65.0 ± 5.1	15.8 ± 3
Days 11–20 (effector phase only)	< 1*	< 1.0*	Not done

Dose (50 mg/kg) was administered daily by oral gavage. *$P < 0.01$ compared with the control group using the Student's *t*-test.
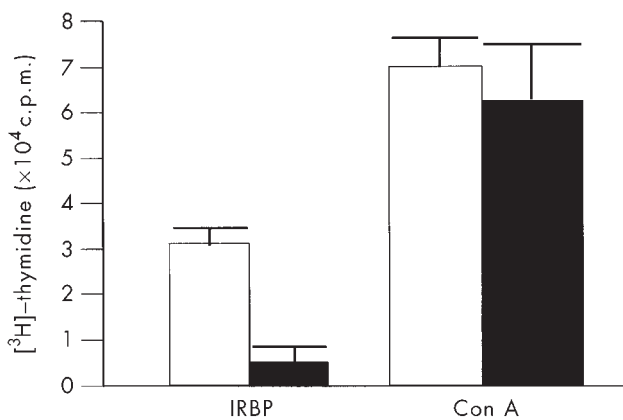

Fig. 3 Lymphocyte proliferation response under the stimulation of interphotoreceptor retinoid-binding protein (IRBP), 5 μg or concanavalin A (Con A), 2.5 μg in experimental autoimmune uveoretinitis rats with (■; $n = 5$) or without (□; $n = 6$) ethyl O-(N-(p-carboxyphenyl)-carbamoyl)-mycophenolate treatment. Spleen cells were obtained on day 14 after immunization. Bars represent one standard error.

Fig. 3, IRBP-induced spleen cell proliferation was dramatically inhibited by CAM, whereas Con A-induced spleen cell proliferation was unchanged. These results suggest that CAM suppression of IRBP-induced lymphocyte activation is not due to non-specific inactivation of T cell function.

Inhibition of anti-IRBP antibody production by CAM *in vivo*

To examine whether CAM also inhibits anti-IRBP antibody production *in vivo*, sera obtained from mice immunized with IRBP and then treated with 50 mg/kg CAM from days 0–14 were assayed by ELISA. As shown in Fig. 4, CAM completely suppressed IRBP-specific IgG antibody production in rats, suggesting that the drug inhibits humoral immune responses in EAU as well.
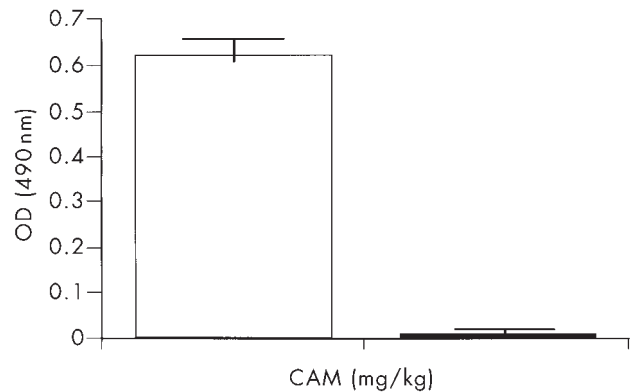
Fig. 4 Interphotoreceptor retinoid-binding protein-specific serum immunoglobulin G antibody was measured by enzyme-linked immunosorbent assay on day 14 with (□; $n = 6$) or without (■; $n = 5$) CAM treatment. Bars represent one standard error.

DISCUSSION

In the present study, the oral administration of CAM was shown to suppress IRBP-mediated EAU in rats in a dose-dependent manner. Cytokine production, which appears to play an important role in IRBP-mediated EAU,[22] is inhibited by CAM treatment given during the effector phase only, as well as for the entire period. As expected, partial treatment with CAM was somewhat less effective than treatment for the entire period, especially when assessed by the clinical degree of iridocyclitis. Suppression of IRBP-induced production of cytokines, such as IL-2 and IFN-γ, by CAM correlated well with the suppression of EAU, although no measurable effect on IL-2 and IFN-γ production was detected with CAM administered during the induction phase only. Treatment with CAM suppressed IRBP-induced spleen cell proliferation, while Con A-induced proliferation remained largely intact, suggesting that CAM does not affect lymphocyte function non-specifically.

Following oral administration, CAM is immediately metabolized to MPA.[23] To check whether the MPA concentration achieved *in vivo* is therapeutically effective, plasma MPA levels have previously been measured at various times following CAM administration. The plasma level of MPA reaches 10 μg/mL at 1 h after administration and remains above this level for the first 8 h. At 24 h, the MPA concentration was still greater than 4 μg/mL, a level reported to be sufficient for inhibiting induction of hemolytic plaque-forming cells against sheep red blood cell antigen *in vitro*.[23] This indicates that an effective concentration of CAM is, indeed, obtained through oral administration.

Originally, CAM was developed for the treatment of tumors,[23] but because of its immunosuppressive activity[24] it has recently been used for the prevention of graft rejection in cardiac transplantation.[14] The action of CAM appears to be relatively restricted to lymphocytes, because these cells mainly use the *de novo* pathway of purine synthesis, which is inhibited by the CAM metabolite MPA.[16] Cells other than lymphocytes can use both the salvage and *de novo* pathways for DNA synthesis and therefore are not affected by MPA. Because of its selective effects on lymphocytes, CAM is an ideal agent to be used in autoimmune disease processes without causing serious side effects. Indeed, no apparent adverse effects have been observed in this study. Furthermore, in a phase I clinical trial for the treatment of gastric cancer and leukemia, the only side effect of CAM observed was mild gastrointestinal discomfort; there was no adverse effect on hepatic or renal function.[25] A water-soluble form of CAM has also been developed and tested in a separate study as a topical ophthalmic solution for the treatment of uveitis, with encouraging results.[26] Mycophenolate mofetil (MM), another derivative of MPA, has also been shown to inhibit the development of EAU.[27] However, CAM may be more effective than MM in EAU, because EAU could be completely inhibited by CAM at high doses in our present study. In addition, CAM has previously been found to be more effective than MM in a study of rat cardiac transplantation.[14] It has been suggested that the increased bioavailability of CAM contributes to a prolonged effective plasma level when compared with MM.

CD4^{+} T helper cells are classified into Th1 and Th2 cells according to their profile of cytokine production.[28] The balance between Th1 and Th2 cells plays a major role in determining the outcome of immune responses. Activation of Th1 cells promotes a cell-mediated immune response, while activation of Th2 cells promotes a humoral immune response. We have shown that IRBP-specific Th1 cells play a pivotal role in EAU development in mice and that these cells are down-regulated by IRBP-specific Th2 cells.[6] In the present study, we have found that CAM inhibits the cytokine production profiles of both types of cells, because IL-2 and IFN-γ are produced by Th1 cells, while IL-6 is produced by Th2 cells. Furthermore, we have found that anti-IRBP-specific antibody production is also inhibited by CAM treatment, consistent with the inhibition of Th2 cells. In the future, it is likely that CAM will become available for use as an immunosuppressive agent with efficacy in both Th1-type autoimmune diseases (e.g. organ-specific autoimmune disease) and Th2-type autoimmune diseases (e.g. allergy).

Treatment with CAM during only the induction phase or only the effector phase dramatically abolishes the development of IRBP-induced EAU, as assessed histologically. The extent of this inhibition of EAU is almost the same as that for CAM treatment during the entire period. Inhibition of EAU mediated by CAM correlates well with the suppression of IRBP-induced cytokine production and lymphocyte proliferation. These results are consistent with the finding that cytokines produced by uveitogenic T cell lines play a pivotal role in EAU development.[6] Curiously, although rats administered CAM during only the induction phase achieved inhibition of EAU, the production of IL-2, IFN-γ or IL-6 was not markedly suppressed when assayed using spleen cells taken on day 20 after IRBP immunization. These results suggest that IRBP-specific T cells are not deleted by CAM treatment. Rather, it may be that IRBP-specific T cells are being recruited from a precursor pool. This would agree with the finding that the action of CAM is reversible. In data not shown, EAU development in three of three rats was completely inhibited by CAM at day 20. However, after stopping CAM treatment and re-immunizing with IRBP on day 20, two of three rats developed inflammation. This suggests that CAM cannot induce immune tolerance in this EAU model. It appears that EAU is largely inhibited by treatment with CAM during only the induction phase, with lymphocyte activation during this induction phase being essential to the pathogenesis of EAU. Mizoguchi *et al*. have reported that CAM administered during only the induction phase inhibits EAE in rats induced by spinal cord homogenates.[15]

Iridocyclitis is observed at approximately day 10 following IRBP immunization, both clinically and histopathologically. However, no evidence of posterior pole

inflammation is found at this point. Retinitis is first observed at around day 14, suggesting that further cellular events take place between day 10 and day 14.[29] These events may be sensitive to CAM, because treatment during the effector phase only (days 11–20) still inhibits the development of retinitis. Treatment with CAM during only the effector phase also suppresses both IL-2 and IFN-γ production, further supporting a role for Th1-derived cytokines in the development of EAU. It has been suggested that CAM may inhibit lymphocyte proliferation, cytokine production and antibody formation under stimulation by specific antigen only during the period of drug administration. Based on the data of the present study, it is unclear whether CAM suppression of IRBP-induced lymphocyte activation is antigen specific, although from Fig. 3 we speculate that it is. Moreover, antigen specificity is supported by work in the rat cardiac transplantation model, which has shown the development of CAM-induced antigen-specific tolerance.[14]

In summary, our results indicate that the novel immunosuppressive drug CAM inhibits IRBP-mediated EAU development and that this is accompanied by a decrease in cytokine production. It appears that CAM has only minor adverse side effects, due to its relatively specific action on lymphocytes, and thus has the potential for long-term usage. Furthermore, the finding that CAM also acts in the effector phase of EAU suggests that CAM may be useful in the clinical setting as a therapy for ocular inflammatory diseases.

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