JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 877–882
© 2004 The Authors
Received January 15, 2004
Accepted March 25, 2004
DOI 10.1211/0022357023655
ISSN 0022-3573

Effects of the phosphodiesterase IV inhibitor rolipram on Th1 and Th2 immune responses in mice

Kouya Yamaki, Xiaojuan Li, Hiroyuki Uchida, A. H. M. Khurshid Alam, Md. Aslam Hossain, Rie Yanagisawa, Hirohisa Takano, Shinji Taneda, Hideyuki Hayashi, Yoki Mori and Shin Yoshino

Department of Pharmacology, Kobe Pharmaceutical University, Kobe, Hyogo 658-8558, Japan

Kouya Yamaki, Xiaojuan Li, Hiroyuki Uchida, Shin Yoshino

Department of Pharmacy, Rajshahi University, Rajshahi-6205, Bangladesh

A. H. M. Khurshid Alam, Md. Aslam Hossain

Pathophysiology Research Team, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan

Rie Yanagisawa, Hirohisa Takano

Air Pollutants Health Effects Research Team, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan

Shinji Taneda

Department of Clinical Toxicology and Metabolism, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Tobetsu, Hokkaido 061-02, Japan

Hideyuki Hayashi

Department of Immunology and Microbiology, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Tobetsu, Hokkaido 061-02, Japan

Yoki Mori

Correspondence: Shin Yoshino, Department of Pharmacology, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan. E-mail: yoshino@kobepharma-u.ac.jp

Abstract

The present study was designed to investigate the effect of the phosphodiesterase IV inhibitor rolipram on Th1 and Th2 immune responses in mice. Mice were immunized subcutaneously at the base of the tail with ovalbumin (OVA) emulsified with complete Freund's adjuvant (day 0) and were treated daily with oral administration of various doses of rolipram from days 0 to 20. On day 21, production of anti-OVA IgG and proliferative responses to the antigen were determined. Anti-OVA IgG2a and interferon-γ (IFN-γ), as indicators of Th1 responses, and anti-OVA IgG1 and interleukin-10 (IL-10), as indicators of Th2 responses, were also measured. The results showed that treatment with rolipram failed to affect the production of OVA-specific IgG but decreased the proliferation of spleen cells to the antigen. Its inhibitory effect on these immune responses was correlated with a marked decrease in IFN-γ but not IL-10 production, although neither anti-OVA IgG2a nor IgG1 production was affected by rolipram. These results suggest that rolipram may preferentially inhibit Th1 responses more effectively than Th2 responses. Administration of rolipram resulted in suppression of antigen (OVA)-induced arthritis in mice. The suppression of joint inflammation by rolipram was associated with the inhibition of the OVA-specific proliferative responses of spleen cells and IFN-γ secretion. These results indicate that rolipram may be effective in regulating Th1-mediated diseases such as rheumatoid arthritis.

Introduction

$CD4^+$ T-cells are classified into two major types, T-helper type 1 (Th1) and type 2 (Th2), on the basis of their distinct pattern of cytokine profiles and roles in the immune system (Mossmann et al 1986; Mossmann & Coffman 1989). Th1 cells produce predominately Th1 cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ), which are involved in cellular immunity, whereas Th2 cells produce predominately Th2 cytokines such as IL-4, -5 and -6, which are implicated in humoral responses. Th1 and Th2 cytokines counteract each other, namely IFN-γ suppresses Th2 immune responses (Dickensheets et al 1999), while IL-4 (van Roon et al 1995) and IL-10 (Yin et al 1997) downregulate Th1 responses. Th1 and Th2 cytokines have been shown to play an important role in a number of diseases in humans. For example, IFN-γ is produced in the joints of patients with rheumatoid arthritis (RA) (Liblau et al 1995) and neutralization of IFN-γ attenuates the severity of the disease (Sigidin et al 2001). On the other hand, an increased expression of IL-4 mRNA is observed in the sputum of patients with asthma (Oliverstein et al 1999) and neutralization of IL-4 blocks the development of acute airway hypersensitivity in a murine asthma model (Corry et al 1996).

Phosphodiesterases (PDEs), including PDE IV, are key enzymes that degrade cAMP and play a role in inflammatory and immune reactions. The prevention of cAMP degradation by PDE IV inhibitors elevates the level of cAMP in the cells followed by suppression of inflammation and immune responses (Kammer 1998). It has been reported that PDE IV inhibitors preferably inhibit the production of Th1 cytokines more effectively than of Th2 cytokines (Ross et al 1997; Navikas et al 1998;

Dinter et al 2000). In contrast, several studies have demonstrated that PDE IV inhibitors have a greater suppressive effect on Th2 responses than on Th1 (Essayan et al 1994, 1997; van Wauwe et al 1995). Since PDE IV inhibitors have an inhibitory effect on both Th1 and Th2 immune responses, we were interested in determining the selectivity of the inhibitory effect of the PDE IV inhibitor rolipram on Th1 and/or Th2 immune responses.

In the present study, we found that treatment of mice with rolipram was followed by the marked suppression of secretion of the Th1 cytokine IFN-γ but weak suppression of the Th2 cytokine IL-10, although antigen-specific IgG, IgG1 and IgG2a production was not affected by the PDE IV inhibitor. In addition, treatment with rolipram significantly suppressed the severity of antigen (OVA)-induced arthritis (AIA) in mice, a Th1-dependent murine model of RA. The attenuation of AIA by rolipram was associated with a decrease in Th1 immune response, including IFN-γ production.

Materials and Methods

Materials

Female DBA/1J mice, 7–9 weeks of age, were used in all experiments. The animals were maintained in a temperature-controlled environment with free access to standard rodent chow and water. All procedures in animal experiments were approved by the Animal Ethics Committee of Kobe Pharmaceutical University.

Immunization with OVA

One hundred micrograms of OVA (Sigma-Aldrich Fine Chemicals, St Louis, MO) was dissolved in 50 μL of phosphate-buffered saline (PBS) and emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, MI). One hundred microlitres of the emulsion was injected s.c. at the base of the tail (day 0).

Administration of rolipram

Various doses of rolipram, including 0.2, 1 and 5 mg kg^{-1}, were dissolved in 500 μL of saline containing 10% chremophor EL (Sigma-Aldrich Fine Chemicals, St Louis, MO) and orally administered daily from days 0 to 20. Five hundred microlitres of saline containing 10% chremophor EL alone was given as a control. There was no significant difference in gaining body weights between control and rolipram-treated mice (Table 1).

Measurement of antibodies to OVA

Sera were obtained on day 21 and heat inactivated at 56°C for 30 min. Anti-OVA IgG, IgG1 and IgG2a were measured using an ELISA as described previously (Yoshino et al 1999). In brief, 96-well flat-bottom microlitre plates were incubated with 100 μL well^{-1} of OVA (100 μg mL^{-1}) at 37°C for 1 h and washed three times with PBS containing 0.05% Tween-20. The wells were then blocked by incubation with 100 μL of PBS containing 1% casein (Sigma-Aldrich Fine Chemicals, St Louis, MO) at 37°C for 30 min. After washing, the plates were incubated with 100 μL of a 1:5000 (for IgG and IgG1 measurement) or 1:500 (for IgG2a measurement) dilution of each serum sample at 37°C for 30 min. The plates were washed and 100 μL well^{-1} of a 1:2000 dilution of alkaline phosphatase-labelled rat anti-mouse IgG (Sigma-Aldrich Fine Chemicals, St Louis, MO) or a 1:1000 dilution of alkaline phosphatase-labelled anti-mouse IgG1 and IgG2a (Phar Mingen, San Diego, CA) was added and incubated at 37°C for 1 h. After washing, 100 μL of 3 mM *p*-nitrophenylphosphate (Bio-Rad, Richmond, CA) was added per well and the plates were incubated in the dark at room temperature for 15 min. The absorbance was then measured at 405 nm by IMMUNO-MINI NJ-2300 (Nalge Nunc International K.K., Tokyo, Japan). The results were expressed as absorbance units at 405 nm (A405) $\pm$ s.e.m.

Measurement of cytokines

Spleens were removed on day 21 and cell suspensions were prepared (Yoshino et al 1998). The erythrocytes in the cells were lysed with Tris-NH_4Cl. A total of 5×10^6 cells in 1 mL of RPMI1640 (Sigma-Aldrich Fine Chemicals, St Louis, MO) containing 1 mM L-glutamine, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 5×10^{-5} M 2-mercaptoethanol and 2% heat-inactivated autologous mouse serum were cultured in 24-well tissue culture plates either in medium alone or with 50 μg mL^{-1} OVA. Forty-eight hours later supernatants were harvested and stored at −20°C until assayed. Cytokine production was quantified by a commercially available ELISA kit (Endogen, Inc., Woburn, MA) for IFN-γ and IL-10.

Proliferation assay

A total of 5×10^5 spleen cells in 0.1 mL of RPMI1640 (Sigma-Aldrich Fine Chemicals, St Louis, MO) containing 1 mM L-glutamine, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 5×10^{-5} M 2-mercaptoethanol and 2% heat-inactivated autologous mouse serum were cultured with 50 μg mL^{-1} of OVA in each well of 96-well tissue culture plates. Seventy-two hours later, each well was pulsed with 0.5 μCi of tritiated thymidine, and the cells were cultured for another 17 h. The cultures were harvested onto fibreglass filters using a multi harvester and counted using standard liquid scintillation techniques. Results, expressed in cpm, are the average of triplicate cultures of cells pooled from 5–6 mice.

Induction of AIA

Mice were immunized with OVA as described above and 21 days later the animals were intra-articularly challenged by injection of 25 μL of PBS containing 50 μg of OVA into left ankle joints (Yoshino 1995). The right ankle joints were injected with 25 μL of PBS alone as a control. To evaluate the severity of the arthritis, the thickness of both ankles was measured using a dial gauge caliper calibrated

with 0.01 mm graduations. The net increase in joint thickness attributable to the antigenic challenge was calculated by subtracting the thickness of the right ankle from that of the left. There was no net joint swelling after injection of OVA in non-immunized mice.

Statistics

The data obtained were statistically evaluated using one-way analysis of variance followed by Bonferroni's test for comparison of individual treatments or using a Mann–Whitney *U*-test. In this study, $P < 0.05$ was accepted to denote significance.

Results

Effect of rolipram on OVA-specific immune responses

As shown in Table 2, treatment with rolipram failed to affect the production of anti-OVA IgG antibodies in sera. On the other hand, proliferative responses of spleen cells to OVA were dose-dependently suppressed in mice treated with rolipram compared to control mice. Approximately 54% inhibition (vs control) of the cell proliferation was observed in mice treated with 5 mg kg^{-1} of rolipram.

Table 1 Effect of rolipram on gaining body weights

Rolipram (mg kg^{-1})	Body weight (g)	
	Day 0	Day 21
0 (saline)	22.1 ± 0.3	23.4 ± 0.4
0.2	21.9 ± 0.6	23.8 ± 0.5
1	22.4 ± 0.5	24.2 ± 0.5
5	22.1 ± 0.4	23.6 ± 0.4

Mice were treated with oral administration of rolipram daily up to 20 days from the time of immunization with OVA (day 0). Values are the mean ± s.e.m. of 5–6 mice.

Table 2 Effects of rolipram on the production of anti-OVA IgG and proliferative responses of spleen cells to the antigen

Rolipram (mg kg^{-1})	Anti-OVA IgG (A405)[a]	Proliferation (cpm)[b]
0 (saline)	0.70 ± 0.06	59 616 ± 17 009
0.2	0.72 ± 0.02	42 118 ± 10 258
1	0.74 ± 0.07	34 495 ± 10 618
5	0.70 ± 0.04	27 525 ± 12 131

Mice were treated with oral administration of rolipram daily up to 20 days from the time of immunization with OVA (day 0). On day 21, anti-OVA IgG in sera and proliferative responses of spleen cells to OVA were measured as described in Materials and Methods. [a]Mean ± s.e.m. of 5–6 mice. Data are representative of three experiments. [b]Mean ± s.e.m. of average of triplicated cultures of cells pooled from 5–6 mice.

Effect of rolipram on Th1 and Th2 immune responses

As shown in Table 3, administration of rolipram resulted in no changes in the production of OVA-specific IgG2a and IgG1. To investigate the effect of rolipram on the secretion of Th1 and Th2 cytokines, IFN-γ and IL-10, respectively, were measured from the OVA-stimulated spleen cells. Spleen cells from rolipram-treated mice showed marked suppression of IFN-γ secretion (up to 69% inhibition), while only mild inhibition of IL-10 production by rolipram was observed.

Effect of rolipram on AIA

In order to study the effect of rolipram on AIA, mice were orally treated with rolipram daily up to 20 days from the time of immunization with OVA (day 0) and were challenged with intra-articular injection of OVA on day 21. As shown in Figure 1, treatment with 5 mg kg^{-1} of rolipram significantly suppressed the severity of AIA. The suppressive effect of rolipram on joint inflammation was associated with the marked decrease in proliferative responses (49% vs control) and in IFN-γ production (30% vs control) from OVA-specific splenocytes (Table 4). There was mild suppression of anti-OVA IgG, IgG2a and IgG1 production in the rolipram-treated mice. The secretion of IL-10 was not affected by rolipram.

Discussion

The present study demonstrates that rolipram may have a relatively more selective inhibitory effect on Th1 responses than on Th2 responses since spleen cells from rolipram-treated mice showed marked inhibition of the Th1 cytokine IFN-γ secretion but very mild inhibition of the Th2 cytokine IL-10 production, compared to those of a control. The selective inhibitory effect of rolipram on Th1 responses was also observed in its significant suppressive effect on AIA in mice (Th1-mediated murine model of RA) and the suppression of AIA was associated with the decreased production of IFN-γ but not of IL-10 from the spleen cells.

The present findings show that treatment with rolipram inhibited the proliferation of spleen cells to OVA (Table 2), which is similar to the previously reported inhibitory effect of rolipram on non-specific proliferation of T-cells to Con-A (Lewis et al 1993). The inhibitory effect of rolipram in both types of proliferation is supposed to be mediated by multiple mechanisms, including modulation of inositol metabolism (Park et al 1992; Tamir & Isakov 1994), Ca^{2+} mobilization (van Tits et al 1991), phosphorylation of the T-cell receptor (Patel et al 1987) and modulation of IL-2 receptor expression (Krause & Deutsch 1991), but not inhibition of IL-2 production (Lewis et al 1993; Giembycz et al 1996).

Table 3 Effects of rolipram on production of anti-OVA IgG2a and IgG1, and IFN-γ and IL-10 production of spleen cells to the antigen

Rolipram (mg kg^{-1})	Th1 responses		Th2 responses	
	Anti-OVA IgG2a (A405)[a]	IFN-γ (pg mL^{-1})[b]	Anti-OVA IgG1 (A405)[a]	IL-10 (pg mL^{-1})[b]
0 (saline)	0.69 ± 0.03	1739 ± 403	0.63 ± 0.02	261 ± 101
0.2	0.72 ± 0.12	1352 ± 216	0.62 ± 0.04	248 ± 104
1	0.72 ± 0.14	559 ± 54	0.63 ± 0.01	236 ± 108
5	0.75 ± 0.09	545 ± 144	0.64 ± 0.01	225 ± 108

Mice were treated with oral administration of rolipram daily up to 20 days from the time of immunization with OVA (day 0). On day 21, anti-OVA IgGs in sera and cytokine productions of spleen cells to OVA were measured as described in Materials and Methods. [a]Mean ± s.e.m. of 5–6 mice and the representative of three experiments. [b]Mean ± s.e.m. of average of triplicated cultures of cells pooled from 5–6 mice.

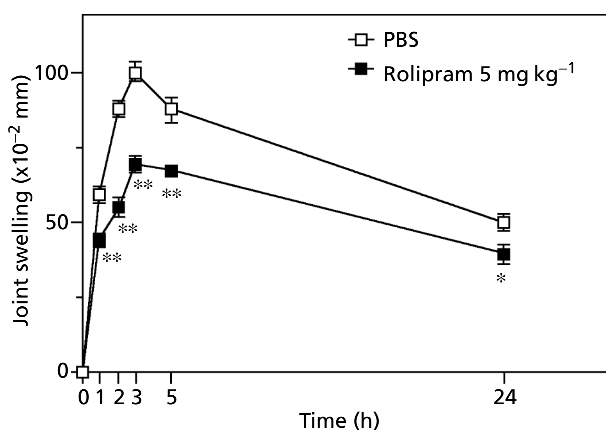

Figure 1 Effect of rolipram on AIA. Mice were immunized with OVA on day 0. Five hundred microlitres of saline with or without the indicated doses of rolipram were orally administered daily from days 0 to 20 for control and experimental groups, respectively. On day 21, AIA was induced by intra-articular injection of OVA into ankle joints as described in Materials and Methods. □, saline alone (control); ■, saline containing 5 mg kg^{-1} of rolipram. Bars show s.e.m. of 5–6 mice. *$P < 0.05$, **$P < 0.01$ vs control, Mann–Whitney analysis.

Our studies also demonstrated that spleen cells from rolipram-treated mice showed marked inhibition of IFN-γ production but very mild inhibition of IL-10 production (Table 3). As with the similar preferable inhibitory effect of rolipram on Th1 response in the present study, in-vivo (Ross et al 1997; Dinter et al 2000) and in-vitro (Navikas et al 1998) studies have demonstrated that PDE IV inhibitors selectively inhibit the Th1 responses rather than the Th2 responses. In contrast to the selective inhibitory effect of PDE IV inhibitors on Th1 response, Essayan et al (1994) demonstrated using in-vitro studies that rolipram strongly inhibits ragweed-induced Th2 cell proliferation, but moderately inhibits tetanus toxoids-induced Th1 cell proliferation. Similarly, another in-vitro study reported that rolipram inhibits the production of the Th2 cytokine IL-5 more efficiently than the production of Th1 cytokines such as IFN-γ and IL-2 (van Wauwe et al 1995). In terms of the mild inhibition of IL-10 production by rolipram shown in the present study, contradictory effects of rolipram on IL-10 production have also been reported. For example, Souza et al (2001) demonstrated that PDE IV inhibitor decreases IL-10 production in mice with ischaemia and reperfusion injury, whereas Ross et al (1997) reported that PDE IV inhibitor increases IL-10 production in mice with collagen-induced arthritis (CIA). The present findings of the marked inhibition of IFN-γ but only mild inhibition of IL-10 production by rolipram suggest that rolipram

Table 4 Effect of rolipram on Th1 and Th2 responses in mice with AIA

Rolipram (mg kg^{-1})	Anti-OVA IgG (A405)[b]	Proliferation (cpm)[a]	Th1 responses		Th2 responses	
			Anti-OVA IgG2a (A405)[b]	IFN-γ (pg mL^{-1})[a]	Anti-OVA IgG1 (A405)[b]	IL-10 (pg/ml)[a]
0 (saline)	0.98 ± 0.05	55 656 ± 14 659	0.73 ± 0.09	1817 ± 456	0.68 ± 0.01	261 ± 94
5	0.86 ± 0.04	27 318 ± 11 039	0.66 ± 0.07	551 ± 152	0.61 ± 0.01	241 ± 99

Mice were treated with oral administration of rolipram daily up to 20 days from the time of immunization with OVA (day 0). On day 21, AIA was induced by intra-articular injection of OVA into ankle joints. On day 22, anti-OVA IgGs in sera and proliferative responses and cytokine production of spleen cells to the antigen were measured as described in Materials and Methods. [a]Mean ± s.e.m. of triplicated cultures of cells pooled from 5–6 mice. [b]Mean ± s.e.m. of 5–6 mice.

preferentially inhibits the Th1 responses more effectively than the Th2 responses. The preferential inhibition of Th1 by rolipram may be due to an increase in cAMP in the cell since PDE inhibitors, including rolipram, are known to elevate the level of the nucleotide that suppresses Th1 (Munoz et al 1990; Novak & Rothenberg 1990; Betz & Fox 1991; van der Poow-Kraan et al 1992). Alternatively, the preferential inhibition of Th1 by rolipram might be explained by the more selective death of Th1 cells since rolipram has been shown to induce apoptosis of HL-60 (Zhu et al 1998). We are continuing studies to determine the effect of rolipram on Th2 responses.

Unexpectedly, rolipram failed to inhibit Th1-dependent IgG2a production (Table 3) in contrast to its inhibitory effect on Th1 responses such as IFN-γ production (Table 3). The similar discrepancy between the inhibitory effect of PDE IV inhibitor on Th1 cytokine production and its lack of effect on Th1-dependent antibody production has also been reported in collagen-specific IgG2a production in mice with CIA (Ross et al 1997). Despite the inhibitory effect of rolipram on Th1 response, the mechanism of the inability of rolipram to produce antibody is not fully understood, but the following mechanisms might be involved. Rolipram should decrease anti-OVA antibody production by B-lymphocytes through the inhibition of T-cell function because the antibody production is T-cell-dependent (Shapira-Nahor et al 1987). However, rolipram can enhance IL-4 plus lipopolysaccharide-induced CD19^{+} B-lymphocyte proliferation (Gantner et al 1998), which in turn increases antibody production. These two mechanisms might compensate for each other, and it would then be possible that rolipram does not have an effect on antibody production.

Finally, treatment with rolipram significantly ($P < 0.05$) suppressed the severity of AIA (Figure 1), which is associated with the marked inhibition of proliferative responses and of IFN-γ production but not with the inhibition of anti-OVA IgG, IgG2a, IgG1 and IL-10 production (Table 4). These results suggest that the downregulation of Th1 response, especially IFN-γ production by rolipram, might be critically involved in the suppression of joint inflammation. As with the role of IFN-γ in the synthesis of tumour necrosis factor-α (TNF-α), it has been reported that recombinant IFN-γ can prime macrophages to produce TNF-α (Lorence et al 1990). On the other hand, TNF-α has also been proven to have a pivotal role in the pathogenesis of experimental arthritis and RA. Thus, suppression of TNF-α by rolipram can inhibit the development of CIA in mice (Ross et al 1997). In addition, repeated treatment with monoclonal antibody on TNF-α can flare the disease in patients with RA (Elliott et al 1994). Taken together, the suppressive effect of rolipram on AIA can be explained by the possible mechanism that rolipram might inhibit the production of IFN-γ, followed by the decreased production of TNF-α from the macrophages at the inflammatory sites, resulting in the suppression of AIA. Since RA is a Th1-mediated disease (McIntyre et al 1996) and rolipram showed a relatively selective inhibitory effect on Th1 response, PDE IV inhibitors might be attractive therapeutic agents for the treatment of RA.

Conclusion

Rolipram inhibited the production of the Th1 cytokine IFN-γ more effectively than the Th2 cytokine IL-10 from OVA-stimulated spleen cells, whereas it failed to inhibit the production of OVA-specific IgG, IgG2a and IgG1 in sera. Treatment with rolipram was followed by significant suppression, which is associated with a marked decrease in Th1 responses, including IFN-γ production, suggesting that PDE IV inhibitors may be effective in treating Th1-mediated diseases such as RA.

References

Betz, M., Fox, B. S. (1991) Prostaglandin E_2 inhibits production of Th-1 lymphokines but not of Th-2 lymphokines. *J. Immunol.* **146**: 108–113

Corry, D. B., Folkesson, H. G., Warnock, M. L., Erle, D. J., Matthay, M. A., Wiener-Kronish, J. P., Locksley, R. M. (1996) Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hypersensitivity. *J. Exp. Med.* **183**: 109–117

Dickensheets, H. L., Venkataraman, C., Schindler, U., Donnelly, R. P. (1999) Interferons inhibit activation of STAT6 by interleukin 4 in human monocytes by inducing SOCS-1 gene expression. *Proc. Natl. Acad. Sci. USA.* **96**: 10800–10805

Dinter, H., Tse, J., Halks-Miller, M., Asarnow, D., Onuffer, J., Faulds, D., Mitrovic, B., Kirsch, G., Laurent, H., Esperling, P., Seidelmann, D., Ottow, E., Schneider, H., Tuohy, V. K., Wachtel, H., Perez, H. D. (2000) The type IV phosphodiesterase specific inhibitor mesopram inhibits experimental autoimmune encephalomyelitis in rodents. *J. Neuroimmunol.* **108**: 136–146

Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Bijl, H., Woody, J. N. (1994) Repeated therapy with monoclonal antibody to tumour necrosis factor (cA2) in patients with rheumatoid arthritis. *Lancet* **344**: 1125–1127

Essayan, D. M., Huang, S. K., Undem, B. J. (1994) Modulation of antigen- and mitogen-induced proliferative responses of peripheral blood mononuclear cells by non-selective and isozyme-selective cyclic nucleotide phosphodiesterase inhibitors. *J. Immunol.* **153**: 3408–3416

Essayan, D. M., Huang, S. K., Kabey-Sobatka, A., Lichtenstein, L. M. (1997) Differential efficacy of lymphocyte- and monocyte-selective pretreatment with a type 4 phosphodiesterase inhibitor on antigen-driven proliferation and cytokine gene expression. *J. Allergy Clin. Immunol.* **99**: 28–37

Gantner, F., Gotz, C., Gekeler, V., Schudt, C., Wendel, A., Hatzelmann, A. (1998) Phosphodiesterase profile of human B-lymphocytes from normal and atopic donors and the effects of PDE inhibition on B-cell proliferation. *Br. J. Pharmacol.* **123**: 1031–1038

Giembycz, M. A., Corrigan, C. J., Seybold, J., Newton, R., Barnes, P. J. (1996) Identification of cyclic AMP phosphodiesterase 3, 4 and 7 in human CD4^{+} and CD8^{+} T-lymphocytes: role in regulating proliferation and biosynthesis of interleukin-2. *Br. J. Pharmacol.* **118**: 1945–1958

Kammer, G. M. (1998) The adenylate cyclase cAMP-protein kinase A pathway and regulation of the immune responses. *Immunol. Today* **9**: 222–229

Krause, D. S., Deutsch, C. (1991) Cyclic AMP inhibits IL-2 receptor expression in human T-cells: expression of both p55 and p75 subunits is affected. *J. Immunol.* **146**: 2285–2296

Lewis, G. M., Caccese, R. G., Heaslip, R. L., Bansbach, C. C. (1993) Effects of rolipram and CI-930 on IL-2 mRNA transcription in human Jurkat cells. *Agents Actions* **39**: C89–C92

Liblau, R. S., Singer, S. M., McDevitt, H. O. (1995) Th1 and Th2 $CD4^+$ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today* **16**: 34–38

Lorence, R. M., Edward, C. K. 3rd., Walter, R. J., Kelley, K. W., Greager, J. A. (1990) In vivo effects of recombinant interferon-γ: augmentation of endotoxin-induced necrosis of tumors and priming of macrophages for tumor necrosis factor-α production. *Cancer Lett*. **53**: 223–229

McIntyre, K. W., Shuster, D. J., Gillooly, K. M., Warrier, R. R., Connaughton, S. E., Hall, L. B., Arp, L. H., Gately, M. K., Magram, J. (1996) Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice. *Eur. J. Immunol*. **26**: 2933–2938

Mossmann, T. R., Coffman, R. L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol*. **7**: 145–173

Mossmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., Coffman, R. L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol*. **136**: 2348–2357

Munoz, E., Zubiaga, A. M., Merrow, M., Sauter, N. P., Huber, B. T. (1990) Cholera toxin discriminates between T helper 1 and 2 cells in T cell receptor-mediated activation: role of cAMP in T cell proliferation. *J. Exp. Med*. **172**: 95–103

Navikas, V., Matusevicius, D., Soderstrom, M., Pirskanen, R., Fredrikson, S., Link, H. (1998) The phosphodiesterase i.v. inhibitor rolipram in vitro reduces the numbers of MBP-reactive IFN-γ and TNF-α mRNA expressing blood mononuclear cells in patients with multiple sclerosis. *Clin. Neuropharmacol*. **21**: 236–244

Novak, T. J., Rothenberg, E. V. (1990) Cyclic AMP inhibits induction of interleukin-2 but not of interleukin-4 in T-cells. *Proc. Natl. Acad. Sci. USA*. **87**: 9353–9357

Oliverstein, R., Taha, R., Minshall, E. M., Hamid, Q. A. (1999) IL-4 and IL-5 mRNA expression in induced sputum of asthmatic subjects: comparison with bronchial wash. *J. Allergy Clin. Immunol*. **103**: 238–245

Park, D. J., Min, H. K., Rhee, S. G. (1992) Inhibition of CD3-linked phospholipase C by phorbol ester and by cAMP is associated with decreased phosphotyrosine and increased phosphoserine contents of PLCγ1. *J. Biol. Chem*. **27**: 1469–1501

Patel, M. D., Samuelson, L. E., Klausner, R. D. (1987) Multiple kinases and signal transduction: phosphorylation of the T-cell antigen receptor complex. *J. Biol. Chem*. **262**: 5831–5838

Ross, S. E., Williams, R. O., Mason, L. J., Mauri, C., Marinova-Mutafchieva, L., Malfait, A. M., Maini, R. N., Feldmann, M. (1997) Suppression of TNF-α expression, inhibition of Th1 activity, and amelioration of collagen-induced arthritis by rolipram. *J. Immunol*. **159**: 6253–6259

Shapira-Nahor, O., Eynat, E., Ben-Sasson, S. Z. (1987) Isotype specificity of antigen-specific helper clone in vivo. *J. Immunol*. **139**: 35–41

Sigidin, Y. A., Loukina, G. V., Skurkovich, B., Skurkovich, S. (2001) Randomized, double-blind trial of anti-interferon-γ antibodies in rheumatoid arthritis. *Scand. J. Rheumatol*. **30**: 203–207

Souza, D. G., Cassali, G. D., Poole, S., Teixeira, M. M. (2001) Effects of inhibition of PDE4 and TNF-α on local and remote injuries following ischaemia and reperfusion injury. *Br. J. Pharmacol*. **134**: 985–994

Tamir, A., Isakov, N. (1994) Cyclic AMP inhibits phosphatidylinositol-coupled and -uncoupled mitogenic signals in T-lymphocytes. Evidence that cyclic AMP alters PKC-induced transcription regulation of members of the *jun* and *fos* family of genes. *J. Immunol*. **152**: 3391–3399

van der Poow-Kraan, T., van Kooten, C., Rensink, I., Aarden, L. (1992) Interleukin (IL)-4 production by human T cells: differential regulation of IL-4 vs. IL-2 production. *Eur. J. Immunol*. **22**: 1237–1241

van Roon, J. A., van Roy, J. L., Duits, A., Lafeber, F. P., Bijilsma, J. W. (1995) Proinflammatory cytokine production and cartilage damage due to rheumatoid synovial T helper-1 activation is inhibited by interleukin-4. *Ann. Rheum. Dis*. **54**: 836–840

van Tits, L. J. H., Michel, M. C., Motulsky, H. J., Maisel, A. S., Brodde, O.-E. (1991) Cyclic AMP counteracts mitogen-induced inositol phosphate generation and increases in intracellular Ca^{2+} concentrations on human leukocytes. *Br. J. Pharmacol*. **103**: 1288–1294

van Wauwe, J., Aerts, F., Walter, H., de Boer, M. (1995) Cytokine production by phytohemagglutinin-stimulated human blood cells: effects of corticosteroids, T-cell immunosuppressants and phosphodiesterase IV inhibitors. *Inflamm. Res*. **44**: 400–405

Yin, Z., Braun, J., Neure, L., Wu, P., Liu, L., Eggens, U., Sieper, J. (1997) Crucial role of interleukin-10/interleukin-12 balance in the regulation of the type 2 T helper cytokine response in reactive arthritis. *Arthritis Rheum*. **40**: 1788–1797

Yoshino, S. (1995) Antigen-induced arthritis in rats is suppressed by the inducing antigen administered orally before, but not after immunization. *Cell. Immunol*. **163**: 55–58

Yoshino, S., Murata, Y., Ohsawa, M. (1998) Successful induction of adjuvant arthritis in mice by treatment with a monoclonal antibody against IL-4. *J. Immunol*. **161**: 6904–6908

Yoshino, S., Sasatomi, E., Mori, Y., Sagai, M. (1999) Oral administration of lipopolysaccharide exacerbates collagen-induced arthritis in mice. *J. Immunol*. **163**: 3417–3422

Zhu, W. H., Majluf-Cruz, A., Omburo, G. A. (1998) Cyclic AMP-specific phosphodiesterase inhibitor rolipram and RO-20-1724 promoted apoptosis in HL60 promyelocytic leukemic cells via cyclic AMP-independent mechanism. *Life Sci*. **63**: 265–274