

Phosphodiesterase type 4 inhibitors, but not glucocorticoids, are more potent in suppression of cytokine secretion by mononuclear cells from atopic than nonatopic donors

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Background: Both glucocorticosteroids and phosphodiesterase (PDE) type 4 inhibitors have modulatory effects on PBMC cytokine secretion. In this study we compared the effect of glucocorticoids and PDE inhibitors on IL-10 and TNF- α production by PBMCs from nonatopic versus atopic individuals.

Methods: PBMCs were incubated with glucocorticoids (beclomethasone dipropionate and mometasone furoate) or media alone for 24 hours. PDE type 4 inhibitors (Ro20-1724 and rolipram) were then added to the cells preincubated with media. After stimulation with PHA, incubation was continued for 48 hours. The cytokine content of the cell supernatants was determined by ELISA.

Results: PDE-4 inhibitors and glucocorticoids caused a concentration-dependent inhibition of the secretion of both TNF- α and IL-10. PDE-4 inhibitors were over 20 times more potent in suppressing cytokine secretion by PBMCs from atopic than nonatopic donors, and approximately 5 times more potent in preventing TNF- α than IL-10 secretion. In cells from nonatopic donors, glucocorticoids inhibited the production of TNF- α to a greater extent than IL-10, but these drugs were more potent in cells from nonatopic than atopic persons.

Conclusion: In conclusion, both PDE-4 inhibitors and glucocorticoids suppress secretion of TNF- α and IL-10. However, because PDE-4 inhibitors are more potent in suppressing cytokine secretion by PBMCs from atopic individuals but less potent in inhibiting production of IL-10, PDE-4 inhibitors may have greater therapeutic potential than glucocorticoids in allergic diseases. (*J Allergy Clin Immunol* 1998;102:797-804.)

Key words: Atopic, cytokines, glucocorticoids, IL-10, phosphodiesterase inhibitors, TNF- α

Both phosphodiesterase (PDE)-4 inhibitors and glucocorticoids are potent suppressors of inflammatory cell activation and cytokine secretion. PDE-4 inhibitors increase intracellular cyclic adenosine monophosphate

Abbreviations used

BDP:	Beclomethasone dipropionate
cAMP:	Cyclic adenosine monophosphate
IC ₅₀ :	Inhibitory concentration of 50%
MF:	Mometasone furoate
NF- κ B:	Nuclear factor- κ B
PDE:	Phosphodiesterase

(cAMP) levels.¹ This leads to decreases in proliferation of PBMCs, inhibition of release of IL-4 and IL-5 by T_{H2}-like cells,² and even suppression of chemotaxis by eosinophils and neutrophils.³ In comparison, glucocorticoids act through a specific cytoplasmic receptor to downregulate cell function at the gene level. Analogues of cortisol potently suppress many aspects of inflammation, including T-cell proliferation, cytokine secretion, and leukotriene production.⁴⁻⁷

IL-10, an 18-kd immunosuppressive cytokine, is secreted by both T_{H1} and T_{H2} cells in humans.^{8,9} The major mechanisms whereby inhibition of the immune response is achieved by IL-10 include downregulation of the MHC II complex on the surface of antigen-presenting cells¹⁰ and decreased production of cytokines by macrophages,¹¹ T cells,^{12,13} and monocytes.¹⁴

TNF- α differs from IL-10 in that it is proinflammatory and is secreted primarily by neutrophils and monocytes. Low concentrations of TNF- α cause local inflammation because of expression of endothelial cell adhesion molecules and activation of neutrophils. As a result, mononuclear phagocytes are stimulated to secrete IL-1, IL-6, TNF, and chemokines. Finally, the cascade initiated by TNF- α results in potentiation of cytolytic T cell-mediated lysis. In higher concentrations TNF- α acts as an endocrine hormone, leading to production of fever, activation of the coagulation system, and initiation of a cascade of cytokine secretion.

Pharmacologic agents having a differential effect on the release of TNF- α versus IL-10 could be of therapeutic importance in the treatment of immune-mediated diseases. Some investigators have reported that PDE-4 inhibitors¹¹ and glucocorticoids¹⁵ suppress TNF- α secretion while enhancing the production of IL-10. In com-

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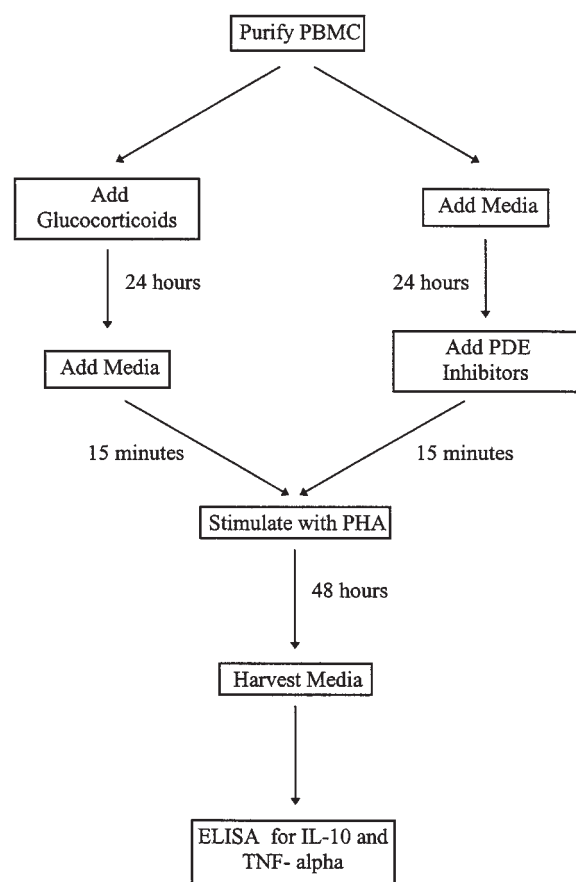


FIG 1. Method used to determine comparative effect of study drugs on PBMC cytokine secretion.

parison, other groups claim that these drugs inhibit production of both cytokines.^{16,17} The first aim of this study was therefore to provide a direct comparison between the effects of PDE-4 inhibitors and glucocorticoids on secretion of TNF- α and IL-10 by PBMCs.

It is unclear whether there are differences in the ability of PDE-4 inhibitors to suppress cytokine secretion by PBMCs from nonatopic individuals versus atopic persons. There is evidence that monocytes from patients with atopic dermatitis have more PDE-4 than those from normal individuals.¹⁸ The effect of altered levels of PDE-4 on the sensitivity of lymphocytes to PDE-4 inhibitors is, however, controversial. Ravid et al¹⁹ reported that lymphocytes from allergic donors are less sensitive to agents that elevate intracellular cAMP, such as PDE-4 inhibitors, than those from normal individuals. In contrast, rolipram has been shown to be more potent in inhibiting ragweed-driven than tetanus toxoid-driven proliferative responses in cells from atopic donors.²⁰ Chan and Hanifin²¹ reported that the PDE-4 isoenzyme found in cells from patients with atopic dermatitis is more sensitive to PDE-4 inhibition than the isoenzymes found in cells from nonatopic donors. To resolve this controversy, we compared the

TABLE I. Demographic details of the atopic study population and which cytokines were studied

Patient	IL-10	TNF- α	Disease	Allergy
1	X		AR, AD*	Dust, weeds
2	X		AR†	June grass
3	X		AR	Weeds, animals, venoms
4	X	X	AR	Trees, weeds, animals
5	X	X	AR	Grass, animals, weeds
6	X	X	AR	Grass
7	X	X	AR	Molds
8	X	X	AR	Dust
9	X	X	AR	Dust, foods

*Atopic dermatitis.

†Allergic rhinitis.

effect of PDE-4 inhibitors on PHA-activated PBMCs derived from patients with allergic rhinitis with their effect on cells from nonatopic persons.

It has been shown that exposure of PBMCs to allergen or IL-2 and IL-4 reduces the antiproliferative effects of glucocorticoids²² by decreasing glucocorticoid-receptor affinity.²³ Because alteration of glucocorticoid-receptor activity has also been detected in vivo,²⁴ it is feasible that glucocorticoids may differentially modify cytokine secretion by PBMCs from atopic versus nonatopic persons. Therefore our final aim was to directly compare the effects of glucocorticoids on TNF- α and IL-10 secretion by PBMCs from both types of patients. A preliminary report of some of the results in this paper have been presented in abstract form.^{7,25}

METHODS

Materials

Centrifuge tubes for cell purification were purchased from Fisher Scientific (Fairlawn, NJ). Ficol-Hypaque, used in PBMC separation, was purchased from Sigma (St. Louis, Mo). Cell culture media RPMI-1640 was purchased from Gibco (Grand Island, NY). RPMI-1640 was supplemented with 1.7 mmol/L L-glutamine, 0.087 mmol/L nonessential amino acids, 0.87 mmol/L sodium pyruvate, penicillin (100 U/mL), and streptomycin (100 μ g/mL) (media additives or MA), also purchased from Gibco, and 10% FCS purchased from Atlanta Biologicals (Atlanta, Ga). Cell culture plates were purchased from Fisher Scientific. PHA was purchased from Gibco Laboratories. Ro20-1724 was obtained from Sigma, and rolipram was a kind gift from Schering AG (Berlin, West Germany). Mometasone furoate (MF) and beclomethasone dipropionate (BDP) were donated by Schering-Plough Research Institute (Kenilworth, NJ). High-binding ELISA plates were purchased from Fisher Scientific. Purified anticytokine mAbs, biotinylated anticytokines, and recombinant human IL-10 and TNF- α were purchased from PharMingen (San Diego, Calif). The chemicals used in these immunoassays were purchased from Sigma Laboratories. The substrate (ABTS) for the ELISA assays was made by adding 150 mg of 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to 500 mL of 0.1 mol/L citric acid in distilled water (pH 4.35). Aliquots of the substrate were frozen (-20°C) until immediately before use when 10 μ L of 30% hydrogen peroxide was added.

Experimental subjects

Nonatopic and atopic volunteers, aged 18 to 40 years, had 100 mL of blood drawn (0.2% EDTA) between 7 and 11 AM after

TABLE II. IC₅₀ (nmol/L) of the PDE-4 inhibitors in suppressing TNF- α and IL-10 secretion by PHA-stimulated PBMCs

Patient type	Ro20-1724		Rolipram	
	TNF- α	IL-10	TNF- α	IL-10
Atopic	219 $\pm$	1260 $\pm$	10.2 $\pm$	81.3 $\pm$
	262	1010	89.9	110
Nonatopic	4900 $\pm$	28,600 $\pm$	300 $\pm$	41,200 $\pm$
	1720	12,600	218	15,000

overnight avoidance of caffeine. Donors were not receiving antihistamines at the time of phlebotomy, and use of theophylline and systemic corticosteroids had been discontinued 4 weeks before the study. The mean age of the atopic persons (5 men and 4 women) was 38 ± 3.4 years (mean $\pm$ SEM), whereas the normal donors (6 men and 2 women) were 35 ± 3.9 years old. All atopic persons had medically diagnosed allergic rhinitis, and 1 also had atopic dermatitis. Demographic details of allergic individuals are shown in Table I.

Determination of the effect of the study drugs on cytokine secretion

To investigate the effects of the glucocorticoids and PDE-4 inhibitors on IL-10 and TNF- α secretion, PBMCs were suspended at 5×10^5 and 10^6 cells/mL, respectively, and incubated with media, BDP, or MF (10^{-12} to 10^{-7} mol/L) for 24 hours as shown in Fig 1. Various concentrations of either media, rolipram, or Ro20-1724 (10^{-10} to 10^{-4} mol/L) were added to the wells that had been preincubated with media. Then all the wells, except controls, were stimulated with 5 μ g/mL PHA. That is, the negative control or baseline was cells that were not exposed to drug or PHA, and the positive control was cells that were stimulated with PHA in the absence of drug. After 48 hours of continued incubation at 37°C, which is when the levels of cytokines were readily detectable, cytokine secretion levels were determined with ELISA. Cells from atopic and nonatopic donors were treated identically.^{26,27}

ELISA

Calculation of all cytokine secretion levels was based on the difference between the amount of cytokine measured in the media from the cells that received a stimulus of PHA (positive control) and media from wells that had not (baseline). Cytokine quantities are expressed as picograms/10⁶ cells and graphed as the mean percent of inhibition $\pm$ SEM.

Quantification of IL-10 and TNF- α in the cell culture supernatants was achieved with an ELISA system described by Schumacher et al²⁸ with their recommended antibodies and cytokine standards. Enhanced protein binding ELISA plates were coated with purified anticytokine mAb (2 μ g/mL) in sodium bicarbonate buffer (pH 8.2) by overnight incubation at 4°C. The plates were then washed twice with PBS/Tween, and the wells were blocked with 10% FCS in PBS (pH 7.0) (PBS/FCS) for 2 hours at room temperature. Standards and samples were added to the wells in duplicate or triplicate (100 μ L/well), and the plates were incubated overnight (4°C). After washing the plates 4 additional times with PBS/Tween, 100 μ L of biotinylated anticytokine mAb (2 μ g/mL) in PBS/FCS was added to each well. Incubation was at room temperature for 45 minutes. Plates were washed 6 more times and then incubated at room temperature for 30 minutes with 100 μ L of 2.5 μ L/mL avidin-peroxidase in PBS/FCS. Immediately after washing 8 additional times, 100 μ L of the ABTS substrate (see Materials section) was

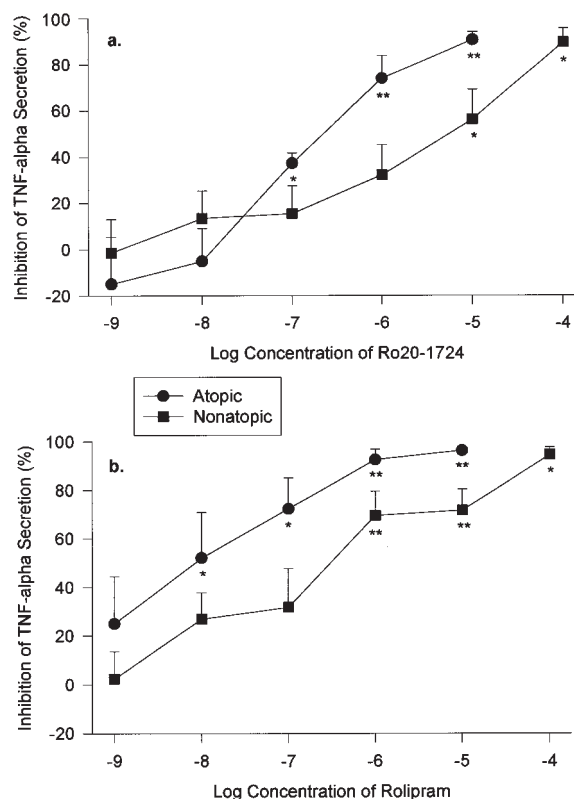


FIG 2. Percent inhibition of TNF- α secretion achieved by Ro20-1724 (a) and rolipram (b). Data represent mean $\pm$ SEM of PBMCs from 6 atopic and 8 nonatopic donors. Single asterisk denotes $P < .05$ and double asterisk denotes $P < .01$ when TNF- α secretion by PDE-4 inhibitor-treated PBMCs was compared with positive control.

added to each well, and the color reaction was allowed to develop for 40 minutes. OD was read at 405 nm with a Biorad 3550 microplate reader (Richmond, Calif).

Data analysis

Inhibition of cytokine secretion was calculated as a percentage of that found in an aliquot of PBMCs stimulated with PHA but not incubated with the test drugs after subtraction of the background secretion of cells in the absence of PHA.

The computer packages, Excel (Word) and Statview (Abacus Concepts), were used to analyze the effect of PDE-4 inhibitors versus glucocorticoids on TNF- α and IL-10 secretion. Descriptive data were generated from the experimental results (means, SEM) and simple graphs were constructed. The data followed a normal distribution pattern (Kolmogorov-Smirnov test), and therefore a 1-way repeated measures ANOVA test was used to ascertain whether there was a difference between the drug groups. Pairwise comparisons were then made with the Student-Newman-Keuls test to determine which drug or concentration groups were significantly different and whether the effect of the drugs was different in the 2 patient populations.

RESULTS

Effect of the study drugs on PBMCs

Previous studies have shown that incubation of PBMCs with PDE-4 inhibitors and glucocorticoids suppresses proliferation of PBMCs from atopic donors after 72 hours of

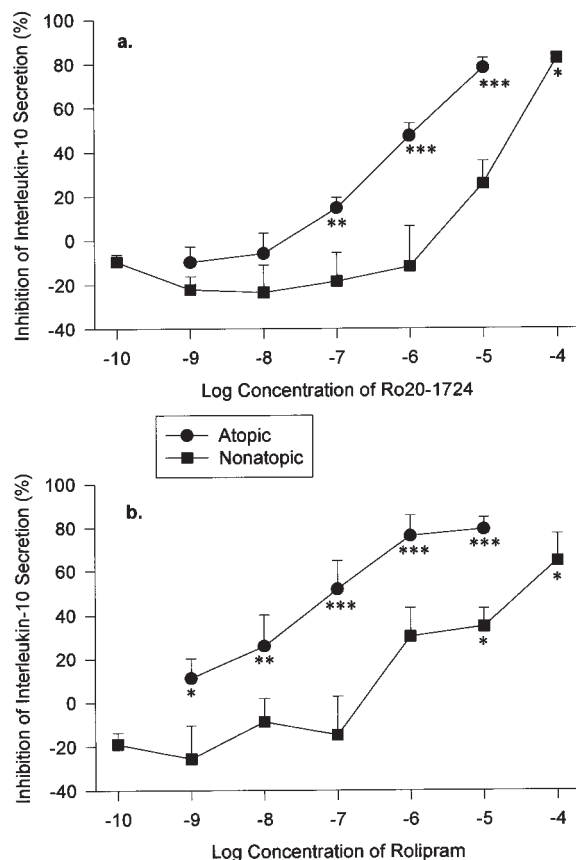


FIG 3. Percent inhibition of IL-10 secretion achieved by Ro20-1724 (a) and rolipram (b). Data represent mean $\pm$ SEM of PBMCs from 9 atopic donors and 8 nonatopic donors. Single asterisk denotes $P < .05$, double asterisk denotes $P < .01$, and triple asterisk denotes $P < .001$ when IL-10 secretion by PDE-4 inhibitor-treated PBMCs was compared with positive control.

stimulation with phorbol myristate acetate and anti-CD3 or PHA.^{2,6} It was established in those studies, and confirmed in this study, that at the concentrations tested, these drugs do not affect cell viability (trypan blue exclusion).

Effect of PDE-4 inhibitors on TNF- α secretion by PBMCs

After incubation of PBMCs from nonatopic donors with PHA for 48 hours in the absence of drug, the amount of TNF- α secreted was 786.8 ± 198.0 pg/ 10^6 cells (range, 60.6 to 1660.0 pg/ 10^6 cells). In comparison, the concentration of TNF- α secreted by positive control cells from atopic persons was 263.9 ± 78.6 pg/ 10^6 cells (range, 28.6 to 525.2 pg/ 10^6 cells).

The effect of the specific PDE-4 inhibitors on TNF- α secretion was evaluated in PBMCs from nonatopic and atopic individuals. Production of this cytokine was suppressed in a concentration-dependent manner by Ro20-1724 (10^{-10} to 10^{-4} mol/L) and rolipram (10^{-10} to 10^{-4} mol/L) in cells from both populations (Fig 2). However, concentration-response curves in cells from atopic

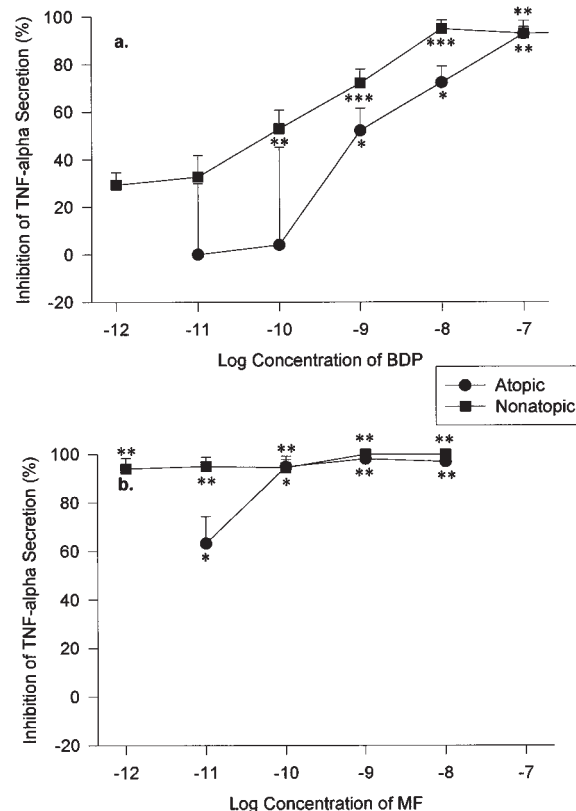


FIG 4. Percent inhibition of TNF- α secretion achieved by BDP (a) and MF (b). Data represent mean $\pm$ SEM of PBMCs from 6 atopic and 8 nonatopic donors. Single asterisk denotes $P < .05$, double asterisk denotes $P < .01$, and triple asterisk denotes $P < .001$ when TNF- α secretion by glucocorticoid-treated PBMCs was compared with positive control.

patients lay to the left of those from nonatopic donors, yielding the inhibitory concentration of 50% (IC_{50}) values shown in Table II. There was a statistically significant difference ($P < .05$) in the inhibition of TNF- α secretion by rolipram (10^{-5} mol/L) and Ro20-1724 (10^{-5} mol/L) in atopic versus nonatopic patients (Fig 2). It is also pertinent to note that rolipram (10^{-7} mol/L) was significantly more potent than Ro20-1724 (10^{-7} mol/L) in cells from both populations ($P < .05$).

Effect of PDE-4 inhibitors on IL-10 secretion by PBMCs

It was of interest to compare the effect of the PDE-4 inhibitors on IL-10 secretion with that on TNF- α secretion because the 2 cytokines have opposite effects; the former is antiinflammatory, whereas the latter is proinflammatory. It is therefore possible that secretion of IL-10 and TNF- α may be differentially affected by PDE inhibition. The concentration of IL-10 measured after 48 hours of PHA stimulation was 406.4 ± 40.3 pg/ 10^6 cells and 645.7 ± 104.4 pg/ 10^6 cells in PBMCs from atopic

TABLE III. IC₅₀ (nmol/L) of BDP in suppressing TNF- α and IL-10 secretion by PHA-stimulated PBMCs

Patient type	Beclomethasone	
	TNF- α	IL-10
Atopic	0.661 $\pm$ 0.24	0.490 $\pm$ 0.46
Nonatopic	0.042 $\pm$ 0.086	4.700 $\pm$ 6.31

and nonatopic donors, respectively. Both rolipram (10^{-9} to 10^{-4} mol/L) and Ro20-1724 (10^{-9} to 10^{-4} mol/L) caused a concentration-dependent inhibition of IL-10 production (Fig 3). At 10^{-9} mol/L, both PDE-4 inhibitors were significantly ($P < .05$) more potent in cells from atopic than nonatopic donors. Rolipram was significantly more potent than Ro20-1724 in inhibiting IL-10 secretion by PBMCs from atopic donors in the concentration range 10^{-7} to 10^{-6} mol/L ($P < .05$).

Effect of glucocorticosteroids on IL-10 and TNF- α secretion by PBMCs

Because it has been suggested that PDE-4 inhibitors are antiinflammatory,²⁹ it was of interest to compare their effect on cytokine secretion with that of glucocorticoids. Studies evaluating the effects of incubating PBMCs from atopic and nonatopic donors with glucocorticosteroids on IL-10 and TNF- α secretion were performed in the same series of experiments as those with PDE-4 inhibitors. In this system BDP (10^{-12} to 10^{-7} mol/L) and MF (10^{-12} to 10^{-8} mol/L) both inhibited TNF- α (Fig 4) and IL-10 (Fig 5) secretion in a concentration-dependent manner. It is of interest to note that concentration-response curves for TNF- α by glucocorticoids of nonatopic individuals lay to the left of those obtained with cells from atopic individuals. That is, at 10^{-11} mol/L inhibition of TNF- α secretion by BDP and MF in cells from nonatopic persons was $67.2\% \pm 8.9\%$ and $5.0\% \pm 3.8\%$, respectively. In comparison, the same concentrations of the glucocorticoids caused no inhibition and $27.4\% \pm 19.9\%$ inhibition in PBMCs from atopic donors. TNF- α secretion inhibition by both glucocorticoids was significant at 10^{-11} mol/L ($P < .05$). As shown in Table III, there was no significant difference between the 2 populations in suppression of IL-10 by BDP. In addition, in PBMCs from atopic donors BDP also suppressed secretion of the 2 cytokines, TNF- α and IL-10, to an equivalent degree (Table III). Finally, at 10^{-10} mol/L, MF was significantly more potent than BDP in inhibiting TNF- α and IL-10 production in cells from both populations ($P < .05$).

DISCUSSION

We have demonstrated that the PDE-4 inhibitors Ro20-1724 and rolipram and the glucocorticoids MF and BDP significantly inhibited TNF- α and IL-10 secretion by PBMCs from nonatopic and atopic donors in a concentration-dependent manner. In PHA-stimulated PBMCs from normal donors, PDE-4 inhibitors and glucocorticoids were both more potent in suppressing TNF-

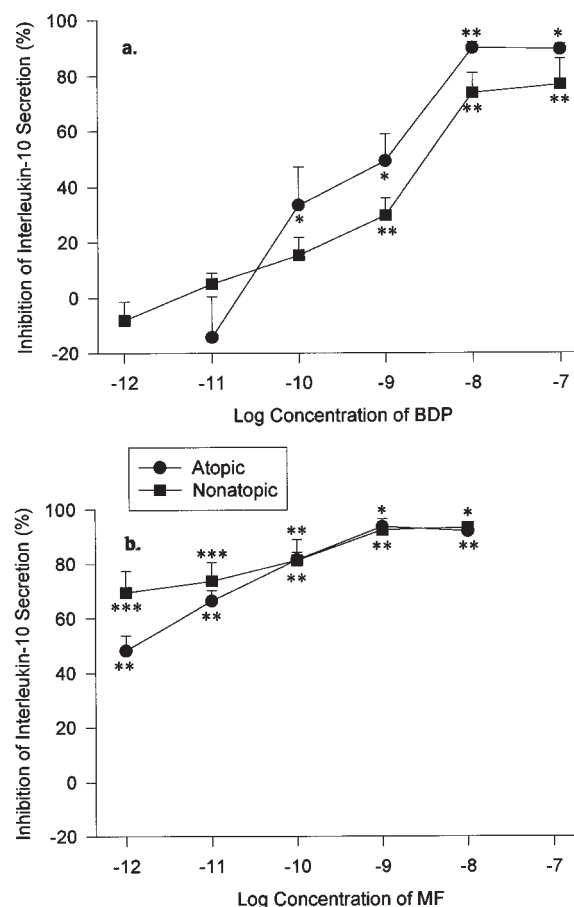


FIG 5. Percent inhibition of IL-10 secretion achieved by BDP (a) and MF (b). Data represent mean $\pm$ SEM of PBMCs from 9 atopic and 8 nonatopic donors. Single asterisk denotes $P < .05$, double asterisk denotes $P < .01$, and triple asterisk denotes $P < .001$ when IL-10 secretion by glucocorticoid-treated PBMCs was compared with positive control.

α than IL-10 production. In comparison, PDE-4 inhibitors, but not glucocorticoids, were more potent in preventing TNF- α secretion in cells from atopic persons. Although PDE-4 inhibitors were 20 times more potent in suppressing TNF- α and IL-10 secretion by PBMCs from atopic versus nonatopic donors, glucocorticoids were more potent in inhibiting TNF- α production by cells from nonatopic individuals.

In monocytes PDE-4 inhibitors, such as Ro20-1724 and rolipram, suppress TNF- α secretion and mRNA expression,^{18,30-32} an effect mediated by an increase in intracellular cAMP.¹⁸ In this study both Ro20-1724 and rolipram caused a concentration-related suppression of TNF- α secretion. It is pertinent to note that when compared with those reported by Hartman et al³³ (IC₅₀ for TNF- α and rolipram, 11 μ mol/L), we found that rolipram had lower IC₅₀ values in nonatopic (0.3 μ mol/L) and atopic (0.01 μ mol/L) patients. The discrepancy between our results with PBMCs from nonatopic patients and those observed by Hartman et al³³ may be due to the fact

that they used whole blood and only incubated for 4 hours, whereas we exposed isolated PBMCs to rolipram for 48 hours. The IC_{50} for Ro20-1724 obtained with PBMCs from nonatopic donors in this study is consistent with that reported by Brackeen et al³² for lipopolysaccharide-activated cells incubated for 18 to 24 hours.

To the best of our knowledge, this is the first report of a direct comparison of the effect of PDE-4 inhibitors on TNF- α secretion in PBMCs from atopic versus nonatopic individuals. It is feasible that the increased sensitivity of PBMCs from atopic persons to PDE-4 inhibitors may be due to the expression of a different subtype of PDE-4.^{21,34-37} Indeed, this has been demonstrated in monocytes from patients with atopic dermatitis, which express a cytosolic PDE-4 subtype in addition to the 3 PDE-4 subtypes expressed by cells from nonallergic persons.³⁸ Hanifin et al³⁹ showed that this PDE subtype is more sensitive to PDE inhibition than the PDE expressed in cells from normal control subjects using IL-4 and IL-10 secretion as parameters. Proliferation of T cells is also more potently suppressed by PDE-4 inhibitors in cells from patients with atopic dermatitis than in those from normal control subjects.²⁶ In addition, PDE-4 inhibitors are known to suppress ragweed-driven proliferative responses by PBMCs from atopic individuals more potently than tetanus toxoid-stimulated mitogenesis.²⁰ Although Essayan et al²⁰ speculate that this is due to a difference in the stimuli used, both ragweed and tetanus act through the T-cell receptor complex.⁴⁰ Finally, it is possible that IL-4, which is secreted to a greater degree by PBMCs from atopic than nonatopic persons,⁴¹ may cause an alteration in the function of a transcription factor, rendering the cells more sensitive to PDE-4 inhibition. Indeed, Ravid et al¹⁹ showed that lymphocytes from normal donors are less sensitive to the antiproliferative effects of cAMP-elevating agents than those from allergic persons but that sensitivity could be restored with dexamethasone. Because glucocorticoids downregulate nuclear factor (NF)- κ B,^{42,43} whereas IL-4 increases its activation,⁴⁴ it is possible to speculate that the differences observed in this study between the response of PBMCs from atopic versus nonatopic individuals may therefore be due to an IL-4-mediated alteration in the activity of NF- κ B.

IL-10 secretion by PBMCs was studied because in contrast to TNF- α this cytokine has potent antiinflammatory effects. It is therefore possible that immunosuppressive agents could affect IL-10 production differently from TNF- α secretion. Our results show that both rolipram and Ro20-1724 significantly inhibited IL-10 secretion by PHA-stimulated PBMCs, an effect that was much more marked in cells from atopic than nonatopic persons. However, the PDE-4 inhibitors are less potent in the prevention of IL-10 than TNF- α production. Van Furth et al¹⁶ demonstrated that the xanthine derivatives pentoxifylline, caffeine, and theophylline suppressed IL-10 secretion in whole blood stimulated with *Streptococcus pneumoniae* for 4 hours. In contrast, Kambayashi et al¹¹ showed that rolipram and Ro20-1724 increased both

IL-10 secretion and mRNA expression by murine macrophages. This augmentation of IL-10 protein and message synthesis by a PDE-4 inhibitor has also been observed in lymphocytes from patients with multiple sclerosis.³¹ The above discrepancies in results may be explained by the use of different methods (eg, cell types, species, or even lengths of incubation) used by the various investigators.

In this study IL-10 secretion was less sensitive to modulation by PDE-4 inhibitors than TNF- α by a factor greater than 5 in PBMCs from both atopic and nonatopic individuals. Both cytokines were quantified simultaneously in cells from the same donors, and therefore the differences in the observed inhibition of TNF- α and IL-10 production by rolipram and Ro20-1724 were not due to variations in the cell populations. It is known that PDE-4 inhibitors increase the activation of protein kinase A (PKA) by increasing intracellular cAMP. PKA potently inhibits TNF- α secretion through an effect on NF- κ B. Although it has not yet been elucidated which transcription factor regulates IL-10 secretion, it is reasonable to speculate that this antiinflammatory cytokine would be differently regulated than the inflammatory TNF- α . Because the pathways whereby secretion of the 2 cytokines are distinct, the effect of PDE-4 inhibitors on them may be also. Alternatively, IL-10 has previously been shown to inhibit secretion of TNF- α in other systems.^{11,14} Thus it is possible that the observed suppression of TNF- α secretion could be due to concomitant PHA-stimulated IL-10 release, as well as inhibition of PDE-4.

Secretion of TNF- α was inhibited in a concentration-dependent fashion by both BDP and MF, with MF being significantly more potent than BDP. Suppression of TNF- α secretion by the glucocorticoid dexamethasone has also been observed by Kunicka et al¹⁷ in anti-CD3-treated murine lymphocytes. Interestingly, they found a difference in the IC_{50} for dexamethasone in inhibition of TNF- α in comparison with IL-4 production. We also found that the IC_{50} of BDP for TNF- α was approximately 50 times less than that reported for IL-4 in a similar system.⁶ Although we tested out to 10^{-12} mol/L, we did not reach 50% inhibition of cytokine secretion with MF, indicating that the IC_{50} was less than 10^{-12} mol/L. However, there did appear to be a difference in drug potency according to cytokine type, which is in accord with results we obtained in the past when studying the effect of MF on IL-4 and IL-5 secretion by T_H2 cell lines (IC_{50} 7×10^{-12} mol/L)⁴⁵ and those of Umland et al⁴⁶ who report an IC_{50} of 10^{-10} mol/L for inhibition of IL-4 and IL-5 secretion by PBMCs.

The glucocorticoid BDP was found to be more potent in inhibiting TNF- α secretion in PBMCs from nonatopic than atopic donors. This may be a function of the documented reduction in glucocorticoid-receptor affinity by the allergic process.^{22,23} Indeed, it has been shown that allergen treatment of PBMCs from atopic donors renders cells less sensitive to the inhibitory effects of hydrocortisone and dexamethasone on proliferation.²³ To the best of our knowledge, this is the first report of a differential

effect of BDP and MF on TNF- α secretion by PBMCs derived from atopic versus nonatopic donors.

In cells from nonatopic donors only, IL-10 secretion was less potently inhibited by the glucocorticoids than TNF- α . This may be due to these 2 cytokines having opposite effects and their secretion being mediated by different pathways. Therefore the complex effects of glucocorticoids on nuclear transcription factors may have dissimilar effects on production of these 2 cytokines. Our study indicates that in PBMCs from nonatopic donors TNF- α is more sensitive to modulation by glucocorticoids than IL-10. It is possible that this effect was not duplicated in PBMCs from atopic donors because of their generalized reduced responsiveness to glucocorticoids.

Asthmatic persons have less IL-10, an antiinflammatory cytokine, in their bronchoalveolar lavage fluid than normal individuals.⁴⁷ Because glucocorticoids are effective in asthma, 1 mechanism of action of the steroids may be to increase of secretion of IL-10. In support of this hypothesis, Tabardel et al¹⁵ noted that administration of dexamethasone or methylprednisolone during cardiopulmonary bypass surgery prevents the usual rise in TNF- α and increases the IL-10 levels in the blood 90 minutes after declamping. This effect has also been reported in vitro where dexamethasone treatment increases IL-10 secretion by whole blood after a 4-hour stimulus with *S pneumoniae*.¹⁶ In contrast, ex vivo experiments in mice and in vitro work with human PBMCs demonstrate that dexamethasone and methylprednisolone inhibit IL-10 secretion.^{17,47} Our work also indicates that IL-10 production is inhibited after treatment of PBMCs from both atopic and nonatopic donors with glucocorticoids (48 hours). Because glucocorticoid therapy is usually long-term, it is possible that treatment of atopy with glucocorticoids decreases the natural secretion of antiinflammatory cytokines, such as IL-10, and thus may have detrimental effects. Therefore it is tempting to speculate that the addition of IL-10 to a glucocorticoid treatment regimen may improve patient response and even have glucocorticoid-sparing effects.

Results from this study suggest that PDE-4 inhibitors have potential as drugs selective for cells active in allergy. First, they may *self-adjust* to the degree of allergic activation, as is indicated by their suppression of cytokine secretion by PBMCs from atopic donors to a greater extent than from nonatopic persons. In addition, PDE-4 inhibitors do not appear to suppress IL-10 secretion to the same extent as TNF- α in cells from both types of donors. In comparison, glucocorticoids are more potent in, and are only selectively more potent in suppression of TNF- α secretion in, cells from nonatopic donors. In the future it will be of interest to distinguish the effect of both classes of drug on cytokine secretion by lymphocytes in comparison with monocytes from atopic and nonatopic donors.

In conclusion, both PDE-4 inhibitors and glucocorticoids suppress secretion of TNF- α and IL-10. However, because PDE-4 inhibitors are more potent in suppressing cytokine secretion by PBMCs from atopic individuals

but less potent in inhibiting production of IL-10, PDE-4 inhibitors may have advantages over glucocorticoids for use in treatment of atopic diseases.

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