

Oral corticosteroids decrease eosinophil and CC chemokine expression but increase neutrophil, IL-8, and IFN- γ -inducible protein 10 expression in asthmatic airway mucosa

Motonori Fukakusa, MD, PhD,^a Celine Bergeron, MD, MSc,^a Meri K. Tulic, PhD,^a Pierre-Olivier Fiset, BSc,^a Oday Al Dewachi, MSc,^a Michel Laviolette, MD,^b Qutayba Hamid, MD, PhD,^a and Jamila Chakir, PhD^b *Montreal and Ste-Foy, Quebec, Canada*

Background: Cytokines and chemokines have been implicated in the pathogenesis of asthma. Inhaled corticosteroids have been shown to decrease the number of eosinophils and to downregulate T_H2 cytokines but to increase neutrophils. The effect of corticosteroids on chemokine expression in asthma has not yet been investigated.

Objective: We sought to investigate the effect of a 2-week course of oral corticosteroid (methylprednisolone, 40 mg/d) on the expression of CXC chemokines (IL-8 and IFN- γ -inducible protein 10 [IP-10]) and CC chemokines (eotaxin and monocyte chemotactic proteins [MCPs] 1-4) in endoscopic biopsy specimens of 13 patients with moderate-to-severe asthma.

Methods: CD3, major basic protein, and elastase immunoreactivities were monitored before and after treatment by using immunocytochemistry. Eotaxin, IL-8, IP-10, MCP-1, MCP-2, MCP-3, and MCP-4 mRNA expression in epithelium and submucosa were studied by using *in situ* hybridization.

Results: Corticosteroids reduced the number of CD3-positive T cells and major basic protein-positive eosinophils ($P < .05$), whereas the number of neutrophils were increased ($P < .05$). Corticosteroids also reduced the number of eotaxin ($P < .05$), MCP-3, and MCP-4 mRNA-positive cells ($P < .001$) in the epithelium and subepithelium. However, corticosteroids caused a significant increase in the epithelial expression of IL-8 ($P < .001$), IP-10 ($P < .05$), and MCP-2 mRNAs ($P < .01$). Corticosteroids had no effects on MCP-1 mRNA expression.

Conclusion: Our results demonstrate the dual nature of corticosteroids. Although corticosteroids can downregulate the expression of some asthma-associated chemokines, such as eotaxin, MCP-3, and MCP-4, they can also upregulate the expression of other chemokines, including IL-8, IP-10, and MCP-2. The failure of oral corticosteroids to inhibit IL-8 mRNA expression might contribute to persistent airway neutrophilia observed in patients with moderate-to-severe

asthma, despite treatment with corticosteroids. (*J Allergy Clin Immunol* 2005;115:280-6.)

Key words: Asthma, chemokines, IL-8, eotaxin, bronchial epithelial cell, corticosteroids

Asthma is a chronic disease of the airways associated with severe inflammation caused by inflammatory cells and potent proinflammatory mediators. The asthmatic inflammatory response is orchestrated by T_H2-type cytokines and small-molecular-weight cytokines called chemokines.¹ Chemokines are involved in the recruitment of cells to the site of inflammation, and these chemokines, including eotaxin, IL-8, IFN- γ -producing protein (IP-10), and monocyte chemotactic proteins (MCPs) 1 to 4, are thought to be involved in the pathology of asthma.^{2,3} Airway epithelial cells are thought to be the major producers of chemokines; however, inflammatory cells themselves have also been shown to be a source of these chemokines.¹ IL-8 and IP-10 chemokines are capable of attracting neutrophils,² whereas eotaxin, MCP-3, and MCP-4 chemokines attract eosinophils.¹ MCP-1 and MCP-2 are chemoattractants for monocytes, lymphocytes, and basophils.³ MCP-2 has also been shown to be chemotactic for eosinophils.⁴

Patients with difficult or severe asthma have reduced lung function and usually receive high doses of inhaled corticosteroids (ICSs) or oral corticosteroids.⁵ Corticosteroids have many immunomodulatory effects. They have been shown to decrease T_H2 and increase T_H1 cytokine levels in the bronchial biopsy specimens of human bronchial epithelial cells.⁶ Although corticosteroids are known to effectively abolish eosinophilia in asthmatic patients, they have been shown to increase neutrophilia in the serum and tissue of these patients.⁷⁻¹⁰

The airway epithelial cells are an obvious target for ICSs. In human cultured airway epithelial cells, corticosteroids significantly abolish the production of CC chemokines, and IL-8 can be significantly downregulated by corticosteroids.¹¹ Epithelial cells from bronchoalveolar lavage fluid and biopsy specimens from asthmatic patients produce more MCP-1, MCP-3, MCP-4, and eotaxin compared with levels seen in control subjects.¹²⁻¹⁴

From ^aMeakins-Christie Laboratories, McGill University, Montreal and ^bUnité de Recherche en Pneumologie de l'Hôpital Laval, Institut Universitaire de Cardiologie et de Pneumologie, Ste-Foy.

Received for publication May 10, 2004; revised October 18, 2004; accepted for publication October 25, 2004.

Available online December 22, 2004.

Address for reprints: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, 3626 St Urbain, Montreal, Quebec H2X 2P2, Canada. E-mail: qutayba.hamid@mcgill.ca.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.10.036

Abbreviations used

GR: Glucocorticoid receptor
ICS: Inhaled corticosteroids
IP-10: IFN- γ -inducible protein 10
MBP: Major basic protein
MCP: Monocyte chemotactic protein

Epithelial cells from the nasal mucosa of patients with seasonal allergy show decreased eotaxin immunoreactivity in patients treated with topical corticosteroids.¹⁵ Studies in our own laboratory have previously shown that topical budesonide effectively abolished allergen-induced epithelial MCP-3 and MCP-4 protein expression in patients with allergic rhinitis.¹⁶

Although the effect of corticosteroids on chemokine expression has previously been studied both *in vitro* and *in vivo* in patients with allergic rhinitis, there is no comprehensive study that examines the effect of corticosteroids on chemokine production in asthmatic patients *in vivo*. In the present study our aim was to investigate the effects of 2-week oral treatment with corticosteroids on the production of both T_H1- and T_H2-associated chemokines in patients with moderate-to-severe asthma and to correlate these differences with inflammatory changes seen in the biopsy specimens. Our results show that although corticosteroids are effective in reducing eosinophilia and expression of eosinophil-associated chemoattractants, they are not capable of reducing neutrophilia and the expression of neutrophil-associated chemokines in the airways of asthmatic patients. These results might explain why neutrophilia persists in patients with moderate-to-severe asthma, despite treatment with high doses of oral corticosteroids.

METHODS

Subjects

Thirteen patients with moderate-to-severe asthma, as defined by the American Thoracic Society criteria,¹⁷ were recruited from the Asthma Clinic at the Laval Hospital (Quebec, Canada). Atopy was confirmed with a positive skin reaction to at least one common allergen. All patients had a baseline FEV₁ value of less than 80% of predicted value with a significant bronchodilator response (>12% and 180 mL of FEV₁ improvement after bronchodilator, Table I). None of these subjects were receiving oral prednisone during the 6 months preceding the study. The patients had an ICS and leukotriene receptor antagonist washout period of 1 month. During this washout period, the subjects were treated with inhaled β_2 -agonists only. After washout, all patients were treated for 2 weeks with oral corticosteroid (methylprednisolone, 40 mg/d). Corticosteroid compliance was monitored by observing morning plasma cortisol levels. All subjects were nonsmokers. Subjects who had a history of respiratory tract infection within 6 weeks before therapy or patients who had immunotherapy within the previous 12 months were excluded. The study was approved by the Ethics Committee of the Laval Hospital, and written consent was obtained from all subjects before the start of the study.

TABLE I. FEV₁ response to oral corticosteroid treatment

	FEV ₁ before BD	FEV ₁ after BD	% Change in FEV ₁ after BD
Before oral CS	58 $\pm$ 6	93 $\pm$ 1	63 $\pm$ 16
After oral CS	71 $\pm$ 3	92 $\pm$ 1	35 $\pm$ 4
% Change in FEV ₁ after oral CS	22 $\pm$ 7		

Results are expressed as percent predicted.

BD, Bronchodilator (inhaled salbutamol, 200 μ g); CS, corticosteroid.

Bronchoscopy and tissue processing

Bronchial biopsy specimens were obtained from all patients, as per the American Thoracic Society guidelines.¹⁸ Biopsy specimens were taken at baseline and again 2 weeks after steroid treatment from the bronchial segmental divisions. Tissues were blocked in optimal cutting temperature embedding media (Sakura Finetech, Tokyo, Japan) and snap-frozen in liquid nitrogen-cooled isopentane (for immunocytochemistry) or fixed in 4% paraformaldehyde and embedded in paraffin (for *in situ* hybridization). All blocks were stored at -80°C until use.

Immunocytochemistry

Sections (5 μ m) were cut from the frozen biopsy specimens, air-dried for 1 hour, fixed in acetone/methanol solution (60:40), blocked with Protein Block Serum-Free solution (DAKO Diagnostics, Mississauga, Ontario, Canada), and incubated with mouse anti-human CD3 (1:100, DAKO Diagnostics), mouse anti-human neutrophil elastase (1:300, DAKO), or mouse anti-human major basic protein (MBP; 1:50, BD Biosciences, Mississauga, Ontario, Canada) primary antibody overnight at 4°C in a humidified chamber. The next day, slides were washed and incubated with the secondary and tertiary antibodies. Immunostaining was developed with Fast Red (1 mg/mL; Sigma Chemical Company, Toronto, Ontario, Canada). All slides were counterstained with Gill II Haematoxylin, and positive cells appeared red under bright field illumination. For negative controls, the primary antibody was replaced by an isotype-matched control antibody.

Probe preparation

Digoxigenin-labeled complementary RNA probes coding for IL-8, eotaxin, IP-10, MCP-1, MCP-2, MCP-3, and MCP-4 mRNA were prepared from complementary DNA (cDNA), as previously described.^{19,20} Briefly, cDNA was inserted into PGEM vectors, linearized, and transcribed *in vitro* in the presence of digoxigenin-11-UTP and either SP6 or T7 polymerases. Antisense (complementary to mRNA) and sense probes (identical to mRNA) were prepared.

In situ hybridization

Sections of bronchial biopsy tissue were processed for *in situ* hybridization for IL-8, eotaxin, IP-10, MCP-1, MCP-2, MCP-3, and MCP-4 mRNA, as previously described.^{19,20} Briefly, sections were permeabilized with 0.3% Triton (BDH, Toronto, Ontario, Canada) and proteinase K (1 μ g/mL, Sigma Chemical Co), washed, and prehybridized in 50% formamide (Sigma) in 2 $\times$ standard sodium citrate solution (BDH) for 15 minutes at 37°C . The samples were subsequently fixed in 4% paraformaldehyde, washed, and air-dried. Hybridization was carried out with the hybridization mixture containing the appropriate sense control or antisense. Each section was then covered and incubated overnight at 42°C in a humid chamber. After incubation, slides were subjected to a series of high-stringency washes in decreasing concentrations of standard sodium citrate solution at 42°C to remove nonspecific binding. Unbound RNA

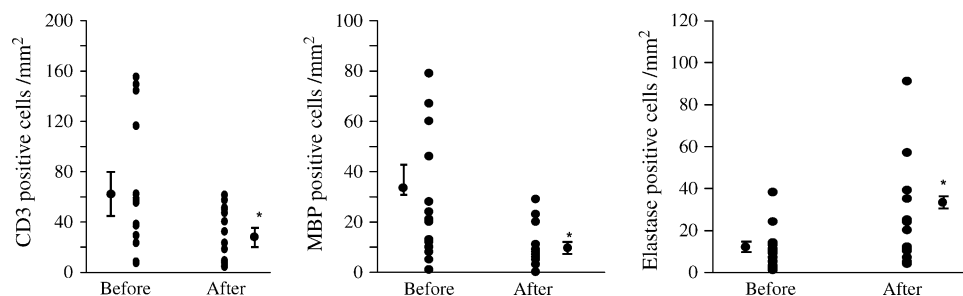


FIG 1. Effect of oral corticosteroids on cellular inflammation. The effect of oral corticosteroids on the number of CD3-positive T cells (*left*), MBP-positive eosinophils (*middle*), and elastase-positive neutrophils (*right*) in the bronchial biopsy specimens of patients with moderate-to-severe asthma is shown. Results are expressed as means $\pm$ SEM. * $P < .05$ versus before steroid treatment.

probes were removed by washing the slides with RNase (20 μ g/mL) for 20 minutes at 42°C. Positive hybridization signal was visualized by incubating the sections for 4 hours with sheep polyclonal anti-digoxigenin antibody (1:1000) conjugated with alkaline phosphatase. Color development was achieved by adding the freshly prepared substrate (X-phosphate-5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium). Once the reaction was complete, tissue sections were counterstained with hematoxylin, mounted with a cover slip, and examined with a graduated microscope for positive signals.

Quantitation

All cells were counted blindly by 2 observers using an Olympus light microscope (Carson Group Inc, Markam, Ontario, Canada) at 400 $\times$ magnification. The number of CD3-, MBP-, or elastase-positive cells in the epithelium and submucosa, as well as the number of chemokine mRNA-positive cells in the submucosa, was averaged from 6 to 8 random, nonoverlapping grids and expressed as the mean number of positive cells per square millimeter of submucosa. Chemokine mRNA-positive staining within the epithelium was expressed as the percentage of the epithelium (0.5-mm basement membrane length) positively stained. Staining was scored from 0 (no expression) to 8 (all cells positive), and the results were expressed as a percentage (where a score of 0 is 0% positivity and a score of 8 corresponds to 100% of epithelium stained).

Statistical analysis

Significant differences among groups of immunocytochemistry and *in situ* hybridization experiments were determined by using the paired nonparametric Kruskal-Wallis test and the Mann-Whitney *U* test. A *P* value of less than .05 was considered statistically significant.

RESULTS

Clinical characteristics of subjects

The study included 5 women and 8 men with a mean age of 31 ± 3 years and a mean asthma duration of 16 ± 3 years. All subjects were atopic. Table I shows the physiologic characteristics of subjects before and after oral corticosteroids. Before treatment, asthmatic patients had low lung function, with an average FEV₁ value of $58\% \pm 6\%$ of predicted value (Table I). Their FEV₁ values, however, improved after bronchodilator administration to more than $93\% \pm 1\%$ of predicted value. Treatment with oral corticosteroids improved baseline

FEV₁ values by $22\% \pm 7\%$ when compared with baseline FEV₁ values before corticosteroids ($P < .05$). Corticosteroids significantly reduced morning cortisol levels in all asthmatic patients from 512 ± 61 nmol/L at baseline to 24 ± 4 nmol/L after corticosteroid treatment.

Effect of oral corticosteroids on airway inflammation

Inflammation was present in the airways of patients with moderate-to-severe asthma, as demonstrated by the large number of CD3-positive T cells, MBP-positive eosinophils, and elastase-positive neutrophils (Fig 1). Corticosteroids significantly reduced the number of CD3-immunoreactive ($P < .05$) and MBP-immunoreactive ($P < .05$) cells in the tissue; however, significantly, the number of elastase-positive neutrophils was increased ($P < .05$, Fig 1).

Effect of oral corticosteroids on chemokine expression

A significant baseline mRNA expression of chemokines was detected in the epithelium, as well as the submucosa, of patients with moderate-to-severe asthma (Figs 2-5). Corticosteroids significantly reduced the expression of eotaxin mRNA-positive ($P < .01$; Fig 2, A), MCP-3 mRNA-positive ($P < .05$; Fig 2, B), and MCP-4 mRNA-positive ($P < .001$; Fig 2, C) cells in the airway epithelium of asthmatic patients when compared with baseline epithelial expression. A similar decrease in the expression of these eosinophil-associated chemokines was also seen in the submucosa (Fig 2, second column). However, treatment with corticosteroids significantly increased the expression of IL-8 mRNA-positive ($P < .01$; Fig 3, A) and IP-10 mRNA-positive ($P < .05$; Fig 3, B) cells in the airway epithelium. An increase in IP-10 levels ($P < .05$; Fig 3, B) and a decrease in IL-8 levels ($P < .05$; Fig 3, A) were found in the submucosa of the tissue. The expression of MCP-2 mRNA significantly increased with corticosteroid treatment only in the airway epithelium ($P < .05$; Fig 5, B, second column) but not in the submucosa ($P = .12$; Fig 5, B). MCP-1 mRNA-positive cells did not change in the airway epithelium ($P = .06$) or the submucosa with steroid treatment ($P = .45$; Fig 5, A).

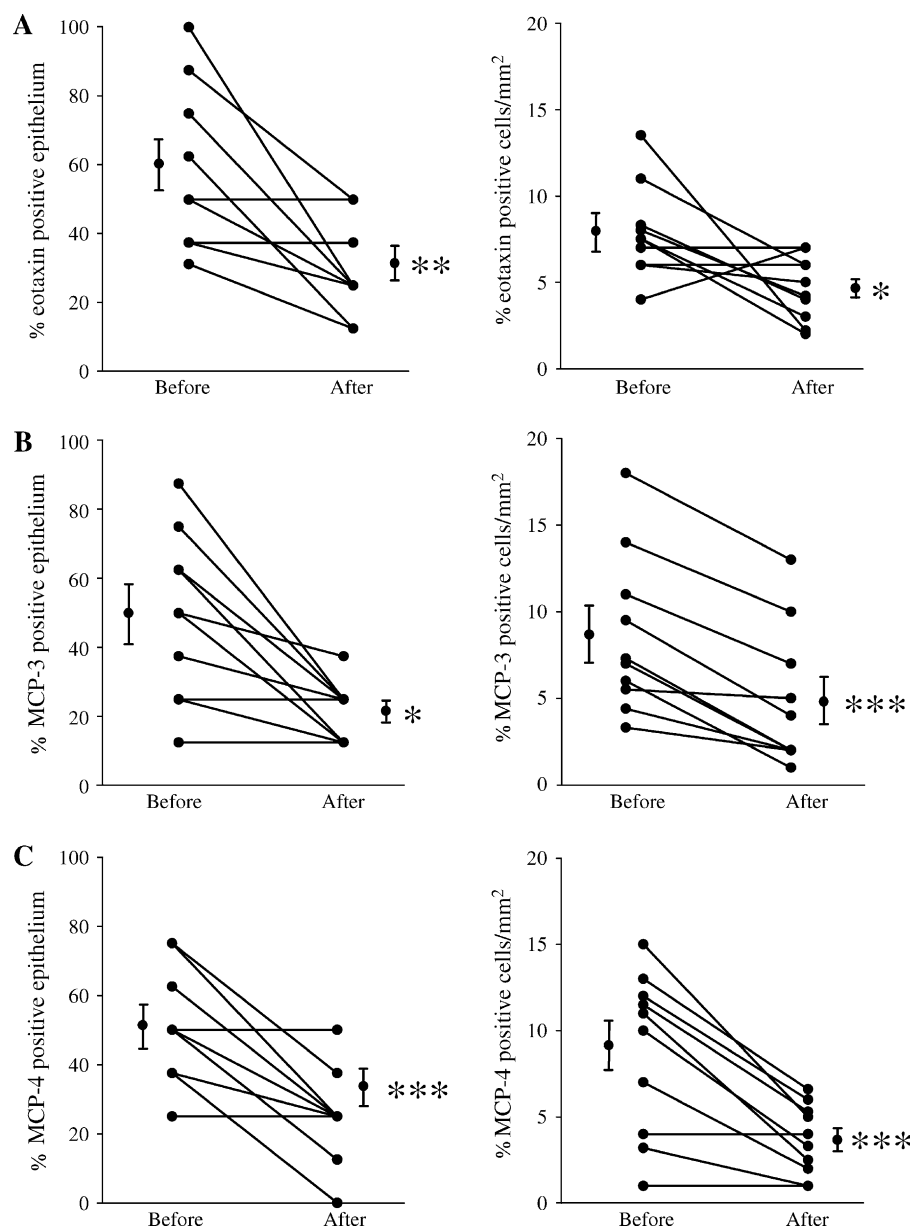


FIG 2. Effect of corticosteroids on eosinophil-associated chemokines. The effect of oral corticosteroids on the number of eotaxin mRNA-positive cells (**A**), MCP-3 mRNA-positive cells (**B**), and MCP-4 mRNA-positive cells (**C**) in the bronchial epithelium (*first column*) and subepithelium (*second column*) of patients with moderate-to-severe asthma is shown. Results are presented as means $\pm$ SEM. * $P < .05$, ** $P < .01$, and *** $P < .001$ versus expression before steroid treatment.

DISCUSSION

It is widely accepted that corticosteroids effectively reduce eotaxin expression and lung eosinophilia in asthmatic patients and that this is usually associated with favorable clinical response. However, in these groups of patients, corticosteroids have previously been reported to have no effect on neutrophilia²¹ or even to promote it.^{7,8} This has been a controversial area of debate over the last decade, and the mechanisms involved in such persistence of neutrophilia are currently unknown. Chemokines,

which are small cytokines involved in the recruitment of cells to the site of inflammation, might play an important role in this process. Whether corticosteroids have a differential regulation of eosinophil- or neutrophil-associated chemokines or whether they are pansuppressors of all chemokines in asthmatic patients, in particular in those patients with the moderate-to-severe stage of the disease, is currently unknown.

In this study we demonstrated for the first time that corticosteroids do differentially regulate eosinophil- or neutrophil-associated chemokines in the airway mucosa of

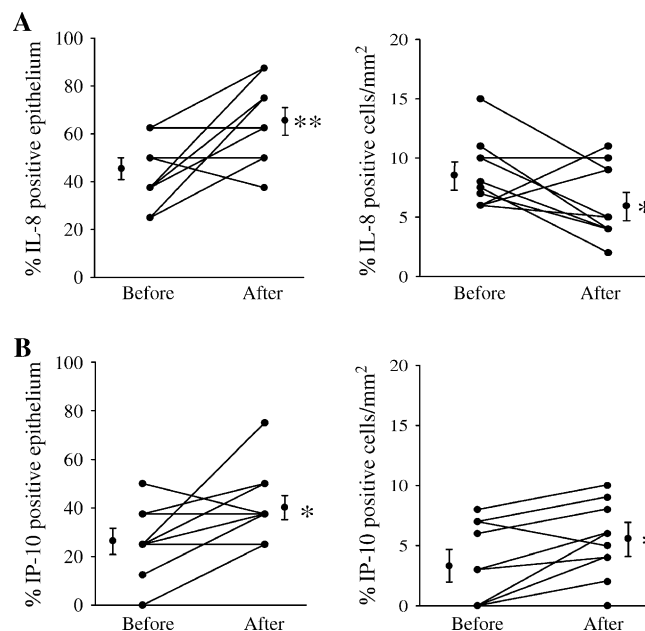


FIG 3. Effect of corticosteroids on neutrophil-associated chemokines. The effect of oral corticosteroids on the number of IL-8 mRNA-positive cells (**A**) and IP-10 mRNA-positive cells (**B**) in the bronchial epithelium (*first column*) and subepithelium (*second column*) of patients with moderate-to-severe asthma is shown. Results are presented as means $\pm$ SEM. * $P < .05$ and ** $P < .01$ versus expression before steroid treatment.

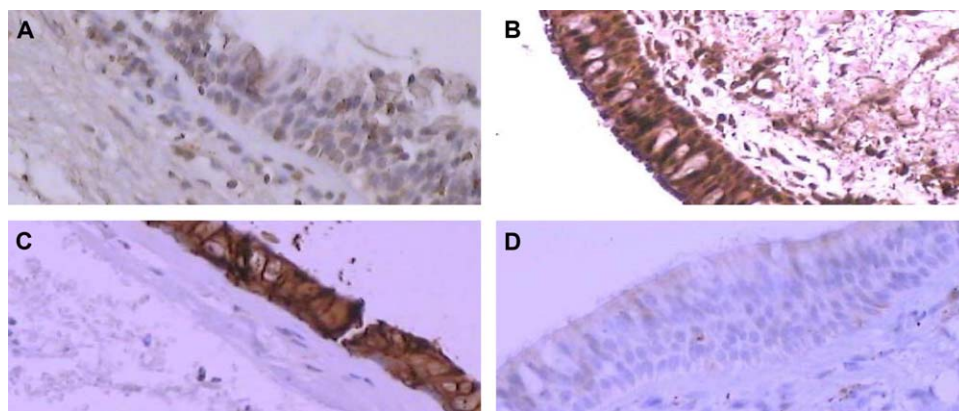


FIG 4. *In situ* hybridization of asthmatic airways using digoxigenin-labeled chemokine probes. A representative example of *in situ* hybridization is shown: **A**, baseline *in situ* hybridization for IL-8; **B**, IL-8 expression after corticosteroid treatment. Baseline *in situ* hybridization for eotaxin (**C**) and after corticosteroid treatment (**D**) is also indicated.

patients with moderate-to-severe asthma. Corticosteroids are simply not pansuppressors of all chemokines. Although a 2-week steroid treatment effectively abolished eosinophil-associated chemokine expression, including eotaxin, MCP-3, and MCP-4 (Fig 2), expression of the neutrophil-associated chemokines IL-8 and IP-10 was significantly increased (Fig 3) when compared with baseline expression before corticosteroid treatment. The changes in chemokine expression were seen in the airway epithelium, as well as in the submucosa, with the exception of IL-8, for which a submucosal decrease was observed. Furthermore, increased expression of MCP-3 and MCP-4 protein has been documented in the nasal

mucosa of ragweed-sensitive allergic patients after allergen challenge, and this expression was abolished after topical treatment with corticosteroids.¹⁶ These data, combined with our results, suggest that corticosteroids are effective in reducing MCP-3 and MCP-4 levels in both the upper and the lower airways in response to oral or topical corticosteroids.

Eotaxin, MCP-3, and MCP-4 mRNA expression and eosinophilia were decreased with corticosteroid treatment, whereas there were significant increases in IL-8 and IP-10 mRNA-positive cells and neutrophilia. These results are of significant interest because steroid-induced neutrophilia was associated with not only increased epithelial

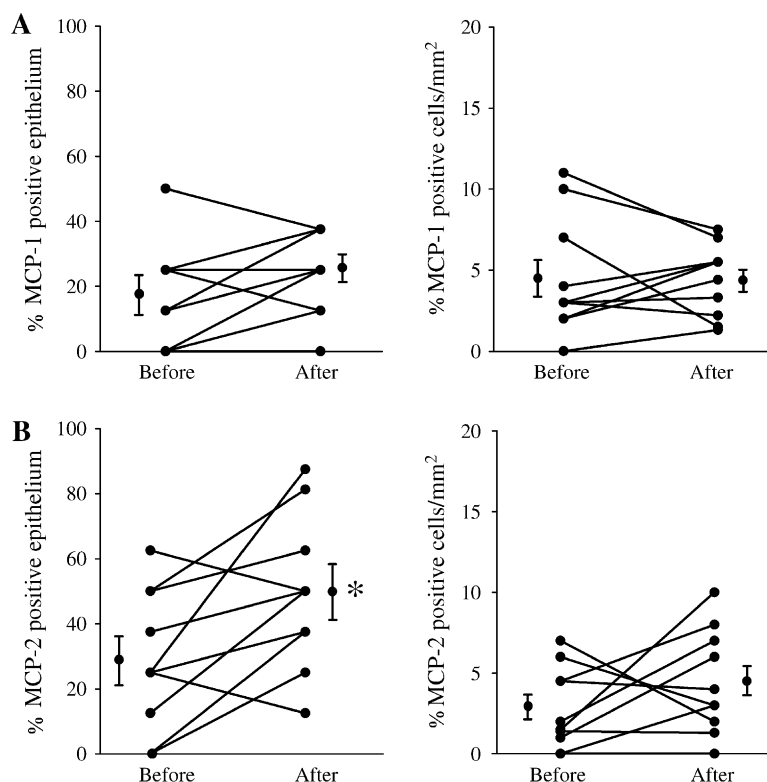


FIG 5. Effect of corticosteroids on MCP-1 and MCP-2 expression. The effect of oral corticosteroids on the number of MCP-1 mRNA-positive cells (**A**) and MCP-2 mRNA-positive cells (**B**) in the bronchial epithelium (first column) and subepithelium (second column) of patients with moderate-to-severe asthma is shown. Results are presented as means $\pm$ SEM. * $P < .05$ versus expression before steroid treatment.

IL-8 but also increased IP-10 mRNA expression in both the airway epithelium and the submucosa. Although there was an upregulation of epithelial IL-8 expression with corticosteroid treatment, a significant decrease of IL-8 expression was seen in the submucosa, suggesting that corticosteroids have a cell-specific regulatory effect. However, Wilson et al²² demonstrated a significant reduction in IL-8 protein expression in the epithelium of patients with mild asthma and no change in submucosal IL-8 immunoreactivity after 8 weeks of ICS treatment. This discrepancy could be due to a difference in the severity of the disease in the 2 studies and the difference in mRNA and protein expression. Both IL-8 and IP-10 are neutrophil-associated chemokines, and upregulation of their expression by corticosteroids might help to explain the augmentation of neutrophilia in this group of patients. Steroid-induced neutrophilia was recently documented by our group, showing that although new hydrofluoroalkane formulations of glucocorticoids effectively reduce eosinophils, IL-5, and eotaxin expression in both peripheral and central airways, neutrophil numbers were increased.⁸ In the present study we do not know whether infiltrating neutrophils are activated and have not examined any marker of apoptosis. Work by Meagher et al¹⁰ shows that dexamethasone is a potent inhibitor of neutrophil apoptosis and suggests that this can explain the poor effective-

ness of corticosteroids in clearing neutrophilia from the airways. A differential expression of glucocorticoid receptor (GR) β might also explain the differences of corticosteroid effects on inflammatory versus epithelial cells. Poor responsiveness to corticosteroids has been related to increase expression of GR β , an isoform of GR α , which induces a competition between corticosteroids and their functional receptor, GR α .²³⁻²⁵ An increased expression of GR β has been reported in asthma.

MCP-1 expression in the epithelium and submucosa of the patients was not different before and after corticosteroid treatment, despite the significant decrease in CD3 immunoreactivity. This chemokine expression is increased in asthmatic patients compared with that seen in healthy subjects,²⁶ but corticosteroid treatment does not appear to affect MCP-1 mRNA expression in our study. On the other hand, corticosteroid treatment significantly increased MCP-2 mRNA expression in the epithelium. However, dexamethasone can have an inhibitory effect on human airway smooth muscle cell production of MCP-2,²⁷ suggesting a difference of regulation of this chemokine by epithelial cells. Despite being a T-cell and eosinophil chemoattractant, the level of these cells was decreased, suggesting that upregulation of MCP-2 is insufficient and that other factors inhibited by corticosteroids are more important.

Although we did not measure chemokine expression at the protein level, previous studies on chemokines showed good correlation between transcription and translation products.^{12,13} The main weakness of this article is the inability to include a nonasthmatic group of control patients or a group of patients with moderate-to-severe asthma who were treated with placebo. It would have been unethical to give an oral corticosteroid to healthy subjects or to keep patients with moderate-to-severe asthma out of standard recommended treatment for a longer time period. The patients in this study have moderate-to-severe asthma, with FEV₁ values of less than 80% of predicted value after an ICS or leukotriene receptor antagonist washout period of 1 month. This observation suggests that the airway inflammation was back in the absence of anti-inflammatory treatment. Each patient acted as his or her own control subject because biopsy specimens were taken both at baseline and after corticosteroid treatment. The baseline inflammation was characterized by a predominant airway infiltration of T cells and eosinophils. After a corticosteroid course, neutrophil numbers increase in airways, whereas T-cell and eosinophil numbers significantly decrease. In parallel to the reduction of the eosinophilic and lymphocytic inflammation, an increase in lung function is observed. This observation confirms the physiologic implication of both cell types in asthma.

In conclusion, we have shown that corticosteroids differentially regulate the expression of chemokines in the bronchial biopsy specimens of patients with moderate-to-severe asthma. Our results suggest that the steroid-mediated inhibition of eosinophilia might occur through steroid-induced inhibition of eotaxin, MCP-3, and MCP-4 production in the airway epithelium, inflammatory cells in the submucosa, or both. We propose steroid-associated neutrophilia to be mediated by IL-8 and IP-10.

REFERENCES

- Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998;50:515-96.
- Gangur V, Birmingham NP, Thanavorakul S. Chemokines in health and disease. *Vet Immunol Immunopathol* 2002;86:127-36.
- Rose CE Jr, Sung SS, Fu SM. Significant involvement of CCL2 (MCP-1) in inflammatory disorders of the lung. *Microcirculation* 2003;10:273-88.
- Garcia-Zepeda EA, Combadiere C, Rothenberg ME, Sarafi MN, Lavigne F, Hamid Q, et al. Human monocyte chemoattractant protein (MCP)-4 is a novel CC chemokine with activities on monocytes, eosinophils, and basophils induced in allergic and nonallergic inflammation that signals through the CC chemokine receptors (CCR)-2 and -3. *J Immunol* 1996;157:5613-26.
- Stirling RG, Chung KF. Severe asthma: definition and mechanisms. *Allergy* 2001;56:825-40.
- Meyer-Hoffert U, Lezcano-Meza D, Bartels J, Montes-Vizuet AR, Schroder JM, Teran LM. Th2- and to a lesser extent Th1-type cytokines upregulate the production of both CXC (IL-8 and gro- α) and CC (RANTES, eotaxin, eotaxin-2, MCP-3 and MCP-4) chemokines in human airway epithelial cells. *Int Arch Allergy Immunol* 2003;131:264-71.
- Bentley AM, Hamid Q, Robinson DS, Schotman E, Meng Q, Assoufi B, et al. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon- γ cytokine gene expression within the bronchial mucosa. *Am J Respir Crit Care Med* 1996;153:551-6.
- Hauber HP, Gotfried M, Newman K, Danda R, Servi RJ, Christodoulou P, et al. Effect of HFA-flunisolide on peripheral lung inflammation in asthma. *J Allergy Clin Immunol* 2003;112:58-63.
- Miyamasu M, Misaki Y, Izumi S, Takaishi T, Morita Y, Nakamura H, et al. Glucocorticoids inhibit chemokine generation by human eosinophils. *J Allergy Clin Immunol* 1998;101:75-83.
- Meagher LC, Cousin JM, Seckl JR, Haslett C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J Immunol* 1996;156:4422-8.
- Kwon OJ, Au BT, Collins PD, Baraniuk JN, Adcock IM, Chung KF, et al. Inhibition of interleukin-8 expression by dexamethasone in human cultured airway epithelial cells. *Immunology* 1994;81:389-94.
- Miotto D, Christodoulou P, Olivenstein R, Taha R, Cameron L, Tsiopoulos A, et al. Expression of IFN- γ -inducible protein; monocyte chemoattractant proteins 1, 3, and 4; and eotaxin in TH1- and TH2-mediated lung diseases. *J Allergy Clin Immunol* 2001;107:664-70.
- Ying S, Meng Q, Zeibecoglou K, Robinson DS, Macfarlane A, Humbert M, et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1999;163:6321-9.
- Lamkhieou B, Renzi PM, Abi-Younes S, Garcia-Zepeda EA, Allakhverdi Z, Ghaffar O, et al. Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics contributes to the chemotaxis of eosinophils to the site of inflammation. *J Immunol* 1997;159:4593-601.
- Greiff L, Petersen H, Mattsson E, Andersson M, Erjefalt JS, Linden M, et al. Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment. *Clin Exp Allergy* 2001;31:1321-7.
- Christodoulou P, Wright E, Frenkel S, Luster A, Hamid Q. Monocyte chemoattractant proteins in allergen-induced inflammation in the nasal mucosa: effect of topical corticosteroids. *J Allergy Clin Immunol* 1999;103:1036-44.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;136:225-44.
- Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway diseases. *J Allergy Clin Immunol* 1991;88:808-14.
- Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541-6.
- Hamid Q, Wharton J, Terenghi G, Hassall CJ, Aimi J, Taylor KM, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci U S A* 1987;84:6760-4.
- Chakir J, Hamid Q, Bosse M, Boulet LP, Laviolette M. Bronchial inflammation in corticosteroid-sensitive and corticosteroid-resistant asthma at baseline and on oral corticosteroid treatment. *Clin Exp Allergy* 2002;32:578-82.
- Wilson SJ, Wallin A, Della-Cioppa G, Sandstrom T, Holgate ST. Effects of budesonide and formoterol on NF- κ B, adhesion molecules, and cytokines in asthma. *Am J Respir Crit Care Med* 2001;164:1047-52.
- Leung DY, Bloom JW. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol* 2003;111:3-23.
- Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. *J Allergy Clin Immunol* 2000;105:943-50.
- Leung DY, Hamid Q, Vottero A, Szefer SJ, Surs W, Minshall E, et al. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J Exp Med* 1997;186:1567-74.
- Sousa AR, Lane SJ, Nakhosteen JA, Yoshimura T, Lee TH, Poston RN. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am J Respir Cell Mol Biol* 1994;10:142-7.
- Pyle JL, Dupont LJ, Menten P, Van Coillie E, Opendakker G, Van Damme J, et al. Expression of monocyte chemoattractant protein (MCP)-1, MCP-2, and MCP-3 by human airway smooth-muscle cells. Modulation by corticosteroids and T-helper 2 cytokines. *Am J Respir Cell Mol Biol* 1999;21:528-36.