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

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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]

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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 FEV_1 value of less than 80% of predicted value with a significant bronchodilator response (>12% and 180 mL of FEV_1 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. FEV_1 response to oral corticosteroid treatment

	FEV_1 before BD	FEV_1 after BD	% Change in FEV_1 after BD
Before oral CS	58 ± 6	93 ± 1	63 ± 16
After oral CS	71 ± 3	92 ± 1	35 ± 4
% Change in FEV_1 after oral CS	22 ± 7		

Results are expressed as percent predicted.
BD, Bronchodilator (inhaled salbutamol, 200 μg); 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 Finetechnical, 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 μm) 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 μg/mL, Sigma Chemical Co), washed, and prehybridized in 50% formamide (Sigma) in 2× 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