

Monocyte chemotactic proteins in allergen-induced inflammation in the nasal mucosa: Effect of topical corticosteroids

Pota Christodoulouopoulos, BSc,^a Erin Wright, MD,^b Saul Frenkiel, MD,^{b,c} Andrew Luster, MD, PhD,^d and Qutayba Hamid, MD, PhD^a Montreal, Quebec, Canada, and Charlestown, Mass

Background: Human allergen-induced rhinitis is associated with the recruitment and activation of inflammatory cells, particularly eosinophils and CD4⁺ T cells, in the nasal mucosa. Chemokines are inflammatory mediators capable of attracting specific inflammatory cell populations. Monocyte chemotactic proteins (MCPs), a subfamily of CC chemokines, have been shown to induce chemotactic activity particularly in eosinophils, T cells, and monocytes under in vitro assay conditions.

Objective: To assess the contribution of MCPs in the recruitment of inflammatory cells in vivo, we investigated the allergen-induced late response in subjects with allergic rhinitis.

Methods: Patients were randomized to receive a 6-week treatment with either topical corticosteroid (n = 6) or a matched placebo (n = 6). Nasal inferior turbinate biopsy specimens were obtained from all subjects before and during allergen-induced late responses. By using immunocytochemistry, tissue sections were examined for the presence of MCP-1, MCP-3, and MCP-4 and for the phenotype of infiltrating cells within the nasal mucosa. In addition, double sequential immunocytochemistry was used to confirm the phenotype of MCP-immunoreactive positive cells. Furthermore, the effect of topical corticosteroids on the expression of MCPs and on the cellular infiltrate was also examined.

Results: MCP-1, MCP-3, and MCP-4 were expressed in all the baseline samples, with prominent staining observed within the nasal epithelium. Biopsy specimens taken after challenge exhibited significant upregulation in the expression of MCP-3 and MCP-4 ($P < .001$). On the other hand, this increase in response to allergen was reduced in patients pretreated with topical corticosteroids. Colocalization experiments revealed that the majority of MCP⁺ cells in the subepithelium were macrophages, followed by T cells and eosinophils.

Conclusion: Our results demonstrate that allergen-induced rhinitis is associated with an increased expression of MCP-3 and MCP-4, which may be closely related to the influx of

inflammatory cells and may thus contribute to the pathogenesis of allergic rhinitis. (*J Allergy Clin Immunol* 1999;103:1036-44.)

Key words: Allergic rhinitis, chemokines, monocyte chemotactic proteins, eosinophils, topical corticosteroids

The local accumulation of inflammatory cells in the nasal mucosa is a feature of allergic rhinitis. Immunohistologic analysis of nasal biopsy specimens from patients with allergic rhinitis have shown that after local allergen challenge there is infiltration of the mucosa with inflammatory cells, particularly CD4⁺ T cells, eosinophils, and macrophages.¹ It has been proposed that chemokines may play an important role in late-phase nasal responses by regulating the influx of leukocytes at the site of inflammation.²

Members of the chemokine gene superfamily of cytokines share homologous sequences and a highly conserved cysteine motif in their primary amino acid structure and can be subdivided into 4 groups (ie, C, C-X₃-C, C-X-C, and CC) depending on the number and spacing of conserved cysteines.^{2,3} CC chemokines were initially thought to act specifically on monocytes. However, recent studies have demonstrated that CC chemokines may regulate immune responses through a broader spectrum of mechanisms because they are also capable of activating T lymphocytes, basophils, and eosinophils.⁴ It has previously been shown that allergen-induced rhinitis is associated with the release and upregulation of RANTES in the nasal mucosa and that this is related to the increasing number of infiltrating eosinophils.⁵ In addition, we demonstrated a local upregulation of eotaxin expression in the nasal mucosa after allergen challenge,⁶ which has previously been reported to be highly selective in its actions on eosinophil recruitment.^{7,8}

To date, the role of monocyte chemotactic proteins (MCPs) has not been studied in the nasal inflammatory response. This subfamily of chemokines includes MCP-1, MCP-2, MCP-3, and MCP-4, which show significant structural similarity and which share greater than 60% amino acid homology.⁹ In vitro, MCPs are produced by mononuclear cells, fibroblasts, eosinophils, endothelial cells, and epithelial cells.⁹⁻¹² These chemokines were initially described as macrophage chemoattractants but subsequently were shown to exert chemotactic properties on other leukocytes. MCP-1 activity is restricted to monocytes,

From ^aMeakins-Christie Laboratories, Montreal; ^bthe Department of Otolaryngology—Head and Neck Surgery, SMBD-Jewish General Hospital, Montreal; ^cMcGill Nasal Research Group, McGill University, Montreal; and ^dMassachusetts General Hospital Infectious Disease Division, Charlestown.

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Reprint requests: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, 3626 St Urbain St, Montreal, Quebec, Canada, H2X 2P2.

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Abbreviations used

MBP: Major basic protein

MCP: Monocyte chemotactic protein

basophils, and lymphocytes, whereas MCP-2, MCP-3, and MCP-4 have been reported to be chemotactic for monocytes, basophils, lymphocytes, and eosinophils.^{9,13-15} These *in vitro* findings are of particular interest because these inflammatory cells are predominant in allergic rhinitis, and MCPs may have the potential to play a role in attracting these cells to the nasal mucosa in response to antigen challenge.

In this study we tested the hypothesis that MCPs play a role in the recruitment of inflammatory cells into the nasal mucosa after allergen challenge. Furthermore, given that prolonged treatment with topical corticosteroids has been shown to inhibit allergen-induced late nasal responses,^{16,17} we hypothesized that the inhibition of this response after steroid therapy may be partly attributed to the inhibition of MCP expression. The specific objectives of this study were to examine the differential expression of MCP-1, MCP-3, and MCP-4 immunoreactivity in the nasal mucosa of patients with allergic rhinitis after nasal allergen provocation, to determine their relationship to the infiltrating inflammatory cells in the nasal mucosa, and to investigate the cellular source of MCPs. We also proposed to investigate the influence of topical corticosteroids on the cellular infiltrate and MCP expression in allergen-induced rhinitis.

METHODS

Study design

A total of 12 patients with allergic rhinitis were recruited from the Nasal Clinic at the Sir Mortimer B. Davis-Jewish General Hospital. Hospital ethics committee approval was obtained before patient recruitment, and all participating subjects gave informed consent before commencement of the study. Before consideration for the study, patients underwent epicutaneous testing with a panel of common seasonal and perennial aeroallergens. A positive histamine control is a standard part of the study. Inclusion criteria for the subjects included (1) a history of seasonal rhinitis and (2) a positive skin prick test response (>5 mm wheal) to ragweed extract. Patients were excluded if they had perennial allergies (eg, dust or pets), had previously received immunotherapy, or were taking oral anti-inflammatory medication. Baseline inferior nasal turbinate biopsy specimens were taken out of season at a time when the subjects were asymptomatic. All patients were treated in a double-blind fashion with either topical nasal corticosteroids (budesonide 200 µg) or a matched placebo for 6 weeks. After the 6 weeks of treatment, all subjects underwent a nasal allergen challenge with ragweed extract (1000 PNU aerosol/nostil) followed by a second nasal turbinate biopsy performed 24 hours after challenge. To confirm clinically the adequacy of the challenge, patient symptoms after challenge were noted, specifically the number of sneezes and the degree of nasal obstruction (previously assessed 4-point scale). Nasal biopsy specimens were taken from the inferior turbinate just beyond the anterior tip. Anesthesia was obtained by using a mixture

of oxymetazoline and xylocaine applied topically. Biopsy specimens were placed in OCT medium, snap-frozen in liquid nitrogen-cooled isopentane, and stored at -80°C before immunohistology.

Tissue preparation

In preparation for immunocytochemistry, the nasal biopsy tissue was immersed in 15% PBS and cooled on ice for 15 to 20 minutes. After the tissue was blocked in OCT medium, cryostat sections (5 µm) were cut, air-dried for 1 hour, and then fixed by immersion in a mixture of acetone-methanol (60/40) for 7 minutes, again air-dried, and stored at -20°C until further use.

Immunocytochemistry

Immunostaining was performed by using the alkaline phosphatase-anti-alkaline phosphatase method as previously described.¹⁸ To phenotype the inflammatory cells within the nasal biopsy specimens, specific mAbs detecting total eosinophils (anti-major basic protein [anti-MBP], a gift from Dr Redwan Moqbel) and macrophages (anti-CD68, Dako Diagnostics) were used. MCP-1, MCP-3, and MCP-4 immunoreactivity was detected by the use of specific mAbs, anti-MCP-1 (Cedar Lane, Canada), anti-MCP-3 (Cedar Lane), and anti-MCP-4 (supplied by Dr Andrew Luster). An antibody to MCP-2 was unable to be procured either through commercial or noncommercial sources. For negative control preparations, the primary antibody was replaced by either nonspecific mouse immunoglobulin or Tris-buffered saline.

Double immunocytochemistry

To confirm the phenotype of MCP-3 and MCP-4 immunoreactive positive cells, we undertook double sequential immunocytochemistry as previously described.¹⁹ Briefly, endogenous peroxidase activity in cryostat sections of nasal biopsy specimens was blocked by using 1% H₂O₂ (plus 0.02% sodium azide in Tris-buffered saline) for 30 minutes. A mixture of primary antibodies was then applied consisting of either a polyclonal anti-MCP-3 antibody or a monoclonal anti-MCP-4 antibody to detect MCP-3 and MCP-4 immunoreactivity and the appropriate mAb to determine the presence of eosinophils (anti-MBP antibody), macrophages (anti-CD68 antibody), and T cells (anti-CD3 antibody).

Quantitation

For immunocytochemical analysis, sections were coded and counted in a blinded, random fashion with an Olympus microscope with an eyepiece graticule at 200× magnification. At least 2 sections were immunostained, from which 6 to 8 fields were evaluated. Epithelial staining was assessed by using morphometry, and the results were expressed as a semiquantitative score from 0 to 4 on the basis of the percentage of epithelium that expressed immunoreactivity (0, no staining; 0.5, <12.5%; 1, 12.5% to 25%; 1.5, 25% to 37.5%; 2, 37.5% to 50%; 2.5, 50% to 62.5%; 3, 62.5% to 75%; 3.5, 75% to 87.5%; and 4, 87.5% to 100%). Subepithelial immunostaining was presented as the mean counts of cells expressing protein per high-power field (0.202 mm²).

Statistics

Data analysis was performed with a statistical package (Systat v 6.0, Evanston, Ill). To compare the number of cells expressing MCP-1, MCP-3, and MCP-4 immunoreactivity and the number of MBP⁺, CD68⁺, and CD4⁺ cells at baseline and after allergen challenge, we used a paired Student *t* test. The mean Δ values for the steroid- and placebo-treated patients were compared with the use of an unpaired Student *t* test. Correlation coefficients and significance values were obtained by the use of Pearson's moment correlation

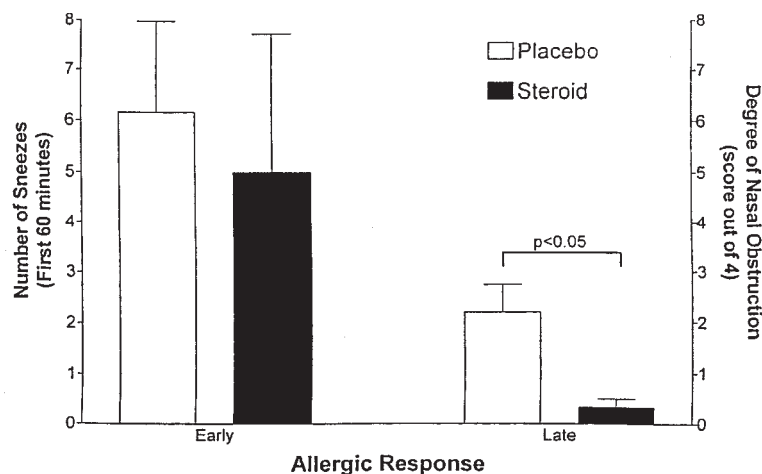


FIG 1. Clinical responses to nasal ragweed challenge. Early response was estimated by recording the number of sneezes during the 60 minutes after challenge. Late response was estimated by the degree of nasal blockage (subjective score out of 4) from 1 to 24 hours after challenge. Late response was significantly suppressed by steroid pretreatment.

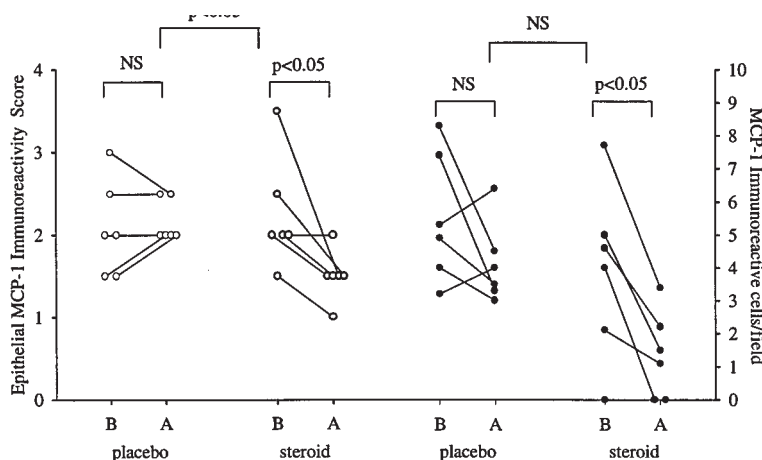


FIG 2. Epithelial MCP-1 immunoreactivity (open circles) and subepithelial MCP-1 immunoreactivity (filled circles) in nasal biopsy specimens obtained before (B) and 24 hours after (A) challenge from placebo- and steroid-treated subjects with allergic rhinitis. Epithelial MCP-1 immunoreactivity is expressed as a score from 0 to 4 on the basis of the percentage of epithelium showing positive signal per total epithelium. Subepithelial immunoreactivity is expressed as the mean number of MCP-1+ cells per high-power field.

with a subsequent Bonferroni correction factor. Results were considered statistically significant at *P* values less than .05.

RESULTS

Clinical observations

Patients were requested to note the number of sneezes and the degree of nasal blockage during the 24-hour period after nasal ragweed challenge. Although steroids were not effective in suppressing the early response as assessed by the number of sneezes, there was a significant reduction in the degree of nasal obstruction between the time points of 1 hour and 24 hours. Thus there was a clearly observable difference between the placebo- and steroid-

treated groups, which validated the adequacy of challenge and the efficacy of our topical corticosteroid (Fig 1).

MCP-1 expression

At baseline, MCP-1 immunoreactive cells were identified at high levels in both epithelial and subepithelial areas. Within the epithelium, MCP-1 immunoreactivity was localized predominantly in the apical layer. In the placebo-treated subjects, there was no significant increase either in the epithelial or subepithelial MCP-1 immunoreactivity 24 hours after antigen provocation compared with baseline values. Within the budesonide-treated group, there was a significant decrease in the

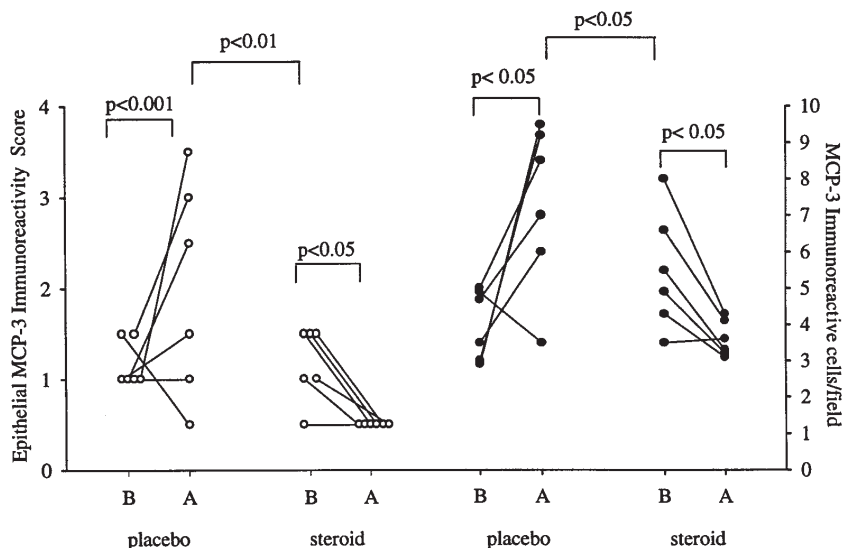


FIG 3. Epithelial MCP-3 immunoreactivity (open circles) and subepithelial MCP-3 immunoreactive (filled circles) in nasal biopsy specimens obtained before (B) and 24 hours after (A) challenge from placebo- and steroid-treated subjects with allergic rhinitis. Epithelial MCP-3 immunoreactivity is expressed as a score from 0 to 4 on the basis of the percentage of epithelium showing positive signal per total epithelium, and subepithelial immunoreactivity is expressed as the mean number of MCP-3⁺ cells per high-power field.

MCP-1 immunoreactive positive cells ($P < .05$), both in the epithelium and subepithelium (Fig 2).

MCP-3 expression

MCP-3 immunoreactivity was identified at baseline in all subjects with rhinitis, and 24 hours after allergen challenge, MCP-3 was increased significantly both in the epithelium ($P < .001$) and subepithelium ($P < .05$) of the nasal mucosa in subjects given placebo. In contrast, in the budesonide-treated group there was a significant decrease in the epithelial expression of MCP-3 ($P < .05$) and in the number of MCP-3 immunoreactive cells within the subepithelium ($P < .05$) after challenge compared with baseline values (Fig 3).

MCP-4 expression

At baseline, MCP-4 immunoreactivity was identified predominantly within the epithelium, with some staining detected in the subepithelium of the nasal mucosa. In subjects treated with placebo, immunohistologic examination of nasal biopsy specimens that were obtained 24 hours after antigen challenge revealed a substantial augmentation in MCP-4 immunoreactivity distributed particularly along the epithelial cell layer ($P < .001$), as shown in Fig 4, A. In addition, there was a statistical increase in the number of cells expressing MCP-4 after challenge in the submucosal layer. As shown in Fig 4, B, in patients treated with corticosteroid, MCP-4 immunoreactivity was decreased after challenge both in the epithelium and subepithelium ($P < .05$). Epithelial counts failed to reach statistical significance, however, although when comparing the Δ values between the placebo- and steroid-treated groups, there was a significant decrease in MCP-4

immunoreactivity in the epithelium and subepithelium after steroid treatment (Fig 5).

Phenotype of cells expressing MCP-3 and MCP-4

Colocalization studies were performed in nasal biopsy specimens from 3 subjects with rhinitis given placebo after challenge by using double immunocytochemistry. The majority of MCP-3 and MCP-4 within the nasal mucosa was associated with CD68⁺ macrophages (mean $\pm$ SD [$n = 3$], $64.8\% \pm 10.2\%$ and $67.3\% \pm 6.4\%$, respectively). Significant numbers of cells expressing MCP-3 and MCP-4 also expressed eosinophil and T-cell markers (mean $\pm$ SD [$n = 3$], MBP⁺ eosinophils, $14.2\% \pm 3.05\%$ expressing MCP-3 and $12\% \pm 2.8\%$ expressing MCP-4; CD3⁺ T lymphocytes, $15.83\% \pm 6.6\%$ expressing MCP-3 and $15.5\% \pm 3.9\%$ expressing MCP-4) (Tables I and II). The remainder of the MCP-3⁺ and MCP-4⁺ cells within the submucosa (approximately 5%) did not colocalize to any of the inflammatory markers used.

Assessment of inflammatory cell infiltrate

In patients treated with placebo, there was a statistically significant increase in the number of MBP⁺ cells in the nasal submucosa after nasal allergen challenge compared with baseline biopsy specimens ($P < .05$). This increase was not observed when patients were pretreated with topical corticosteroids, and there was actually a significant difference between the Δ values (difference between after and before challenge) for MBP immunoreactivity between the placebo- and steroid-treated groups ($P < .05$) (Fig 6, A).

The number of CD4⁺ cells also increased significantly

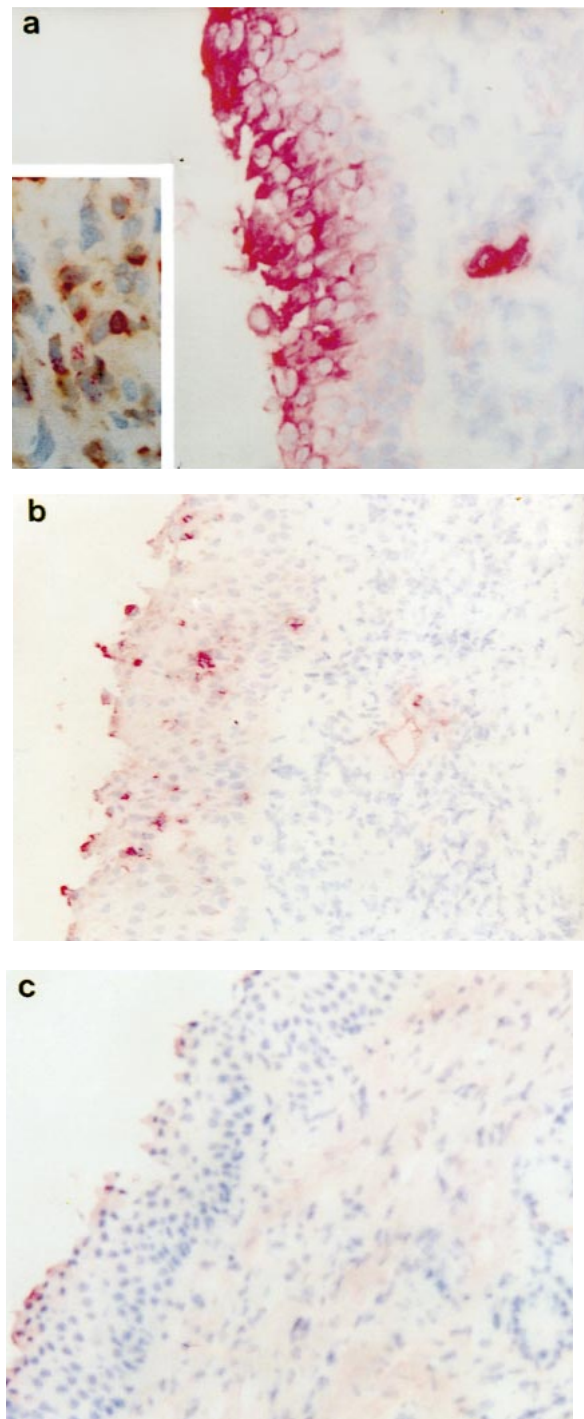


FIG 4. Representative examples of immunostaining for MCP-4 in nasal biopsy specimens from placebo-treated (**a**) and steroid-treated (**b**) patients with allergic rhinitis after allergen challenge. Note a high expression of MCP-4 after allergen challenge in the nasal biopsy section from a subject given placebo (**a**). In comparison, the expression of MCP-4 in the nasal mucosa after challenge was inhibited in subjects pretreated with steroids (**b**). The **insert** in panel **a** shows the colocalization of MCP-4 immunoreactivity (brown) to CD3+ T cells (red) in the nasal tissue after challenge. Nasal biopsy specimen after allergen challenge stained with a nonspecific mouse Ig (negative control), showing no cellular staining (**c**).

after allergen challenge in the placebo-treated group ($P < .05$). This increase in CD4 immunoreactivity observed 24 hours after challenge was reversed by pretreatment with topical corticosteroids ($P < .05$). In addition, the Δ values between the 2 groups were also significantly different ($P < .05$) (Fig 6, B).

Finally, the expression of CD68⁺ cells in the subepithelium after allergen challenge showed no significant difference when compared with baseline biopsy specimens; however, the number of positive cells was significantly decreased after allergen provocation in the steroid-treated group ($P < .05$), and there was also a statistical difference between the Δ values of the placebo- and steroid-treated groups ($P < .05$) (Fig 6, C).

To determine whether the expression of the MCPs in the epithelial or subepithelial cell layers was associated with the infiltration of inflammatory cells after challenge, we performed correlation analyses. There were no significant correlations between MCP-1 and any of the inflammatory cells, and although both MCP-3 and MCP-4 moderately correlated with MBP⁺, CD4⁺, and CD68⁺ cells, this failed to achieve statistical significance.

DISCUSSION

The aims of this study were to examine the expression of MCPs in allergen-induced rhinitis and to investigate their possible contribution to the late nasal response with the use of the nasal allergen challenge model. Furthermore, we assessed whether the late nasal response could be inhibited by pretreatment with topical glucocorticoid steroids. Our results demonstrate that exposure to allergen increases the expression of MCP-3 and MCP-4 within the nasal mucosa of patients with allergic rhinitis and that these effects are abrogated by topical steroid treatment.

It has previously been shown that allergen-induced late nasal responses are accompanied by an influx of inflammatory cells, particularly eosinophils and CD4⁺ T cells, which is characteristic of allergic rhinitis.^{1,6,20} The mechanisms responsible for the recruitment of these cells to the site of inflammation are not clearly understood; however, it has been proposed that one possibility may be the local generation of chemotactic agents that regulate this influx of leukocytes.⁴ In particular, CC chemokines have been implicated in allergic diseases because they have been shown to act on the inflammatory cells associated with allergic inflammation. Although several chemokines, such as eotaxin and RANTES, have been shown to be associated with the recruitment of eosinophils to the nasal mucosa in allergen-induced rhinitis,^{3,6} the factors responsible for the infiltration of such leukocytes in disease states remain to be clarified. Although the expression of MCPs have previously been detected in allergic tissues isolated from patients with chronic allergic sinusitis, asthma, and atopic dermatitis,^{9,21,22} apart from a single report detecting MCP-1 in nasal secretions after challenge in patients with rhinitis,²³ there is no previous evidence in vivo for a role of MCPs

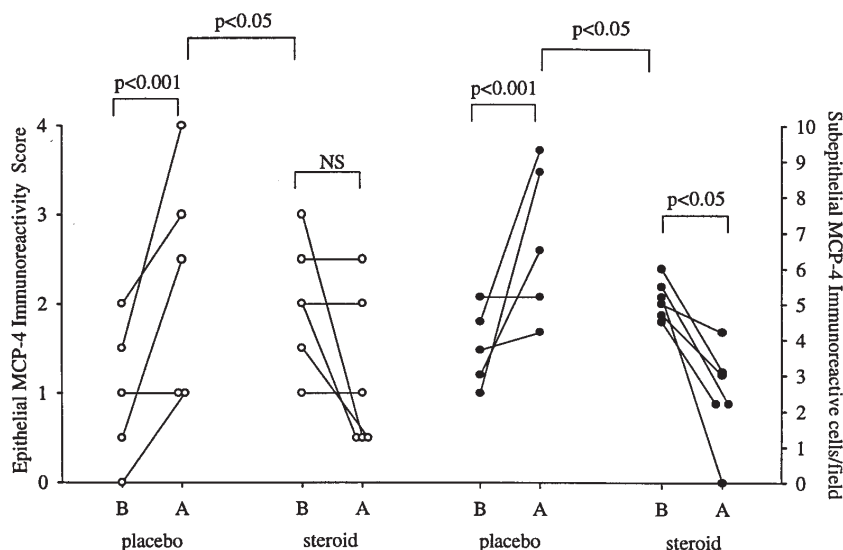


FIG 5. Epithelial MCP-4 immunoreactivity (*open circles*) and subepithelial MCP-4 immunoreactivity (*filled circles*) in nasal biopsy specimens obtained before (*B*) and 24 hours after (*A*) challenge from placebo- and steroid-treated subjects with allergic rhinitis. Epithelial MCP-4 immunoreactivity is expressed as a score from 0 to 4 on the basis of the percentage of epithelium showing positive signal per total epithelium, and subepithelial immunoreactivity is expressed as the mean number of MCP-4+ cells per high-power field.

TABLE I. Phenotype of MCP-3 immunoreactive cells

Patient	Percent of MCP-3 immunoreactive cells		
	CD3 ⁺	CD68 ⁺	MBP ⁺
1	23	53	17.5
2	10	70	13.5
3	14.5	71.5	11.5
Mean $\pm$ SD	15.83 $\pm$ 6.6	64.8 $\pm$ 10.2	14.2 $\pm$ 3.05

Percent of CD3⁺, CD68⁺, and MBP⁺ cells expressing MCP-3 immunoreactivity in nasal biopsy specimens obtained from 3 randomly selected placebo-treated patients after allergen challenge.

TABLE II. Phenotype of MCP-4 immunoreactive cells

Patient	Percent of MCP-4 immunoreactive cells		
	CD3 ⁺	CD68 ⁺	MBP ⁺
1	20	60	11.5
2	13.5	72	9.5
3	13	70	15
Mean $\pm$ SD	15.5 $\pm$ 3.9	67.3 $\pm$ 6.4	12 $\pm$ 2.8

Percent of CD3⁺, CD68⁺, and MBP⁺ cells expressing MCP-4 immunoreactivity in nasal biopsy specimens obtained from 3 randomly selected placebo-treated patients after allergen challenge.

in human allergic rhinitis. Therefore our findings of an increased expression of MCP-3 and MCP-4 immunoreactive cells in the nasal mucosa as a result of allergen challenge is novel and may partly account for the inflammatory cell accumulation seen in allergen-induced late nasal responses.

We demonstrated a constitutive expression of MCP-1, MCP-3, and MCP-4 immunoreactivity, particularly in the epithelial cell layer of the nasal mucosa, suggesting a role for MCPs in baseline leukocyte trafficking through epithelial tissue as part of the natural host defense against pathogens as previously suggested by Garcia-Zepeda et al.⁹ MCP-4 has previously been shown to be expressed in several other normal tissues, particularly with large epithelial surfaces in contact with the environment, such as the intestines and the lung.²⁴ Because eosinophils in particular are not normally found within the tissues of normal individuals, the action of MCPs within these tissues needs to be further investigated.

Although constitutively expressed at high levels within the nasal mucosa, MCP-1 immunoreactivity was not

increased as a result of allergen provocation as seen with MCP-3 and MCP-4. In contrast, MCP-1 protein has been shown to be particularly abundant in mononuclear cell-rich inflammatory conditions, such as granulomatous inflammation, and in macrophage-rich regions of atherosclerotic lesions.^{25,26} Interestingly, few eosinophils are present in these conditions, unlike in allergic diseases. Our finding may support in vitro studies demonstrating that MCP-1 lacks chemotactic activity for eosinophils.

To determine whether the local upregulation of MCP-3 and MCP-4 play a role in cellular recruitment, we examined the numbers and phenotype of cells infiltrating the nasal submucosa. Our data demonstrated a significant eosinophil and CD4⁺ T-cell infiltration after allergen challenge, which may be associated with the increase in MCP-3 and MCP-4 within the nasal epithelium and subepithelium. In contrast, no change in CD68⁺ cells were observed in the nasal mucosa after challenge when compared with baseline biopsy specimens, which is consistent with our previous findings.⁶ The fact that MCP-1

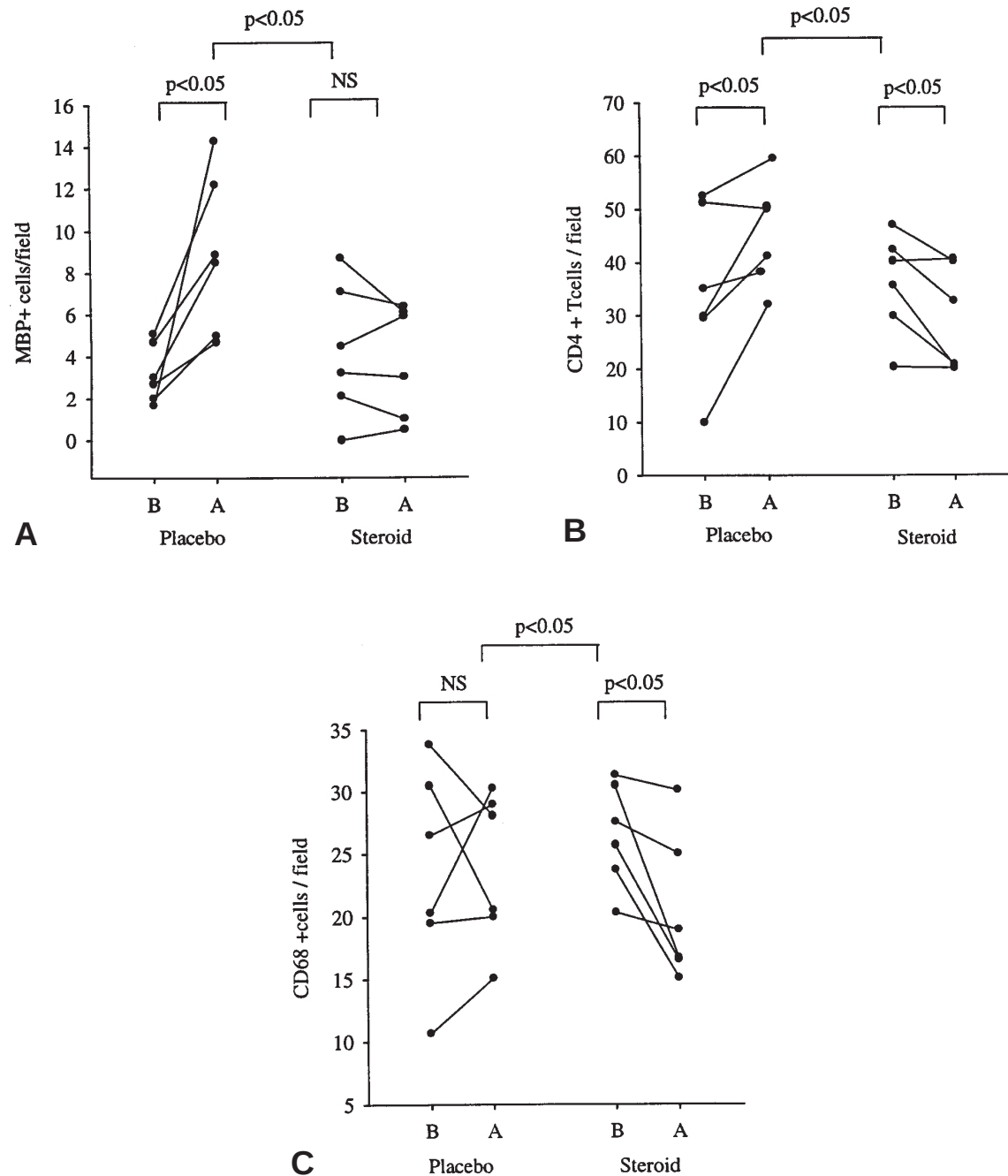


FIG 6. The number of MBP+ (**A**), CD4+ (**B**), and CD68+ (**C**) cells within the submucosa of nasal biopsy specimens obtained before (**B**) and 24 hours after (**A**) challenge in placebo- and steroid-treated patients with allergic rhinitis. Results are expressed as the mean number of positive cells per high-power field.

immunoreactivity was not increased in the nasal mucosa after challenge may account for this finding, given that MCP-1 levels seem to be enhanced, particularly in macrophage-rich conditions.²⁶

To determine whether the expression of MCP-3 and MCP-4 immunoreactivity in the epithelial or subepithelial cell layers was quantitatively associated with the infiltration of inflammatory cells, we performed correlation analyses. The fact that there were no significant correla-

tions between the infiltration of inflammatory cells and MCP expression may suggest that other chemokines play a substantial role in the acute inflammatory cell influx after allergen provocation. The lack of correlation observed may also be due to the time course of chemokine production and its influence on the kinetics of leukocyte recruitment. A disadvantage of the nasal biopsy technique is that it is not amenable to the performance of time-course studies. We chose the 24-hour time point on

the basis of previous studies showing identifiable changes in inflammatory cells at this particular time point.¹

In our tissue specimens we observed MCP-3 and MCP-4 expression within the epithelium and the resident inflammatory cells in the submucosa. It is well known that chemokines are often secreted from activated leukocytes, presumably as part of amplification and desensitization mechanisms. Phenotyping the cells expressing MCP-3 and MCP-4 demonstrated that macrophages, eosinophils, and T cells were significant cellular sources of these chemokines, with macrophages being a prominent source within the submucosa. However, the expression of MCP-3 and MCP-4 was most strongly localized to the ciliated pseudostratified epithelium in the nasal mucosa in our clinical specimens, as seen with other CC chemokines such as eotaxin.^{6,27}

We demonstrated that pretreatment with topical steroids abrogates the increase of MCP-1, MCP-3, and MCP-4 expression both in the epithelium and subepithelium, as well as the increase in MBP⁺ and CD4⁺ cells, as a result of allergen provocation in the placebo-treated subjects. These data are consistent with previous studies showing that topical corticosteroid treatment leads to the inhibition of the late nasal response¹⁶ and with previous in vitro studies showing that budesonide may be an effective inhibitor of MCP-4 mRNA in cultured BEAS-2B epithelial cells activated with cytokines.²⁸ We postulate that the suppression of cellular infiltrate after allergen challenge in subjects undergoing glucocorticoid therapy occurs partly through the inhibition of MCPs, effectively attenuating leukocyte transendothelial migration. Epithelial cells are the first cell type to come in contact with inhaled glucocorticoids, and because we and others have shown that epithelial cells are the main source of chemokines,²⁸ this cell type may be an important target of the anti-inflammatory effects of inhaled glucocorticoids.

In summary, we have demonstrated a constitutive expression of MCP-1, MCP-3, and MCP-4 in the nasal mucosa of allergic patients, with a substantial increase in MCP-3 and MCP-4 immunoreactivity after allergen challenge. The increase in these chemokines was accompanied by an upregulation of inflammatory cells, particularly eosinophils, within the nasal submucosa. Furthermore, prolonged treatment with topical corticosteroids was associated with a significant attenuation of allergen-induced increases in MCP-1, MCP-3, and MCP-4 immunoreactivity and in MBP⁺, CD68⁺, and CD4⁺ cells. These data suggest that the increased expression of MCP-3 and MCP-4 may be relevant to the pathophysiology of late-phase reactions in allergen-induced rhinitis through their ability to recruit leukocytes into the nasal mucosa in response to antigen. Inhibition of MCP-3 and MCP-4 production may thus serve as a favorable approach for the treatment of allergic diseases.

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