

Expression of IL-16 in allergen-induced late-phase nasal responses and relation to topical glucocorticosteroid treatment

Sophie Laberge, MD,^{a, b} Stephen R. Durham, MD,^c Omar Ghaffar, BSc,^a Sabina Rak, MD,^d David M. Center, MD,^e Mikila Jacobson, PhD,^c and Qutayba Hamid, MD, PhD^a Montreal, Quebec, Canada, London, England, Gottenburg, Sweden, and Boston, Mass.

Allergen-induced late nasal responses (LNRs) are associated with a cellular infiltrate in which CD4⁺ cells are prominent. These cells have been shown to be the major cellular source of Th2-type cytokines. Mechanisms responsible for the local accumulation of CD4⁺ cells in the nasal mucosa after allergen exposure are unclear. IL-16 is a potent chemoattractant for CD4⁺ cells in vitro and may play a significant role in recruiting CD4⁺ cells in LNRs. We investigated the expression of IL-16 messenger RNA and immunoreactivity in nasal biopsy specimens from 17 subjects with allergic rhinitis. A biopsy specimen of the nasal inferior turbinate was obtained before and 24 hours after local nasal provocation with grass pollen extract after 6 weeks of treatment with either topical fluticasone propionate ($n = 9$) or placebo ($n = 8$) nasal spray twice daily. IL-16 mRNA-positive cells and IL-16-immunoreactive cells were identified in both the epithelium and the subepithelial tissue at baseline. Within the placebo-treated group, the numbers of epithelial and subepithelial IL-16 mRNA-positive cells and IL-16-immunoreactive cells were significantly increased 24 hours after challenge compared with baseline ($p < 0.001$). Topical glucocorticoid therapy resulted in a decrease in allergen-induced epithelial immunoreactive cells and subepithelial IL-16 mRNA-positive cells. The numbers of CD4⁺ cells increased after antigen challenge compared with baseline ($p < 0.05$), and this increase was inhibited by glucocorticoid treatment. There were significant correlations between epithelial and subepithelial IL-16 immunoreactivity and CD4⁺ cell infiltration after antigen challenge. The upregulation of IL-16 expression in allergic nasal mucosa after antigen challenge may have critical implications in the accumulation of CD4⁺ cells in response

to antigen exposure. Steroid-mediated inhibition of IL-16 may be partly responsible for the decrease in local CD4⁺ cells after topical glucocorticoid therapy. (*J Allergy Clin Immunol* 1997;100:569-74.)

Key words: Interleukin, late nasal response, messenger RNA, inflammation, immunoreactivity

Late nasal response (LNR) has been used as a valuable model for the study of the inflammatory mechanisms involved in allergic types of inflammation. Immunocytochemical studies of nasal biopsy specimens have shown increases in eosinophils and CD4⁺ cells in the nasal mucosa of subjects with allergic rhinitis 24 hours after local antigen provocation.^{1,2} CD4⁺ cells have received much attention because these cells are a major source of cytokines that regulate the allergic immune response. We and others have previously shown that the LNR is associated with an increase in cells expressing cytokine messenger RNA for IL-4, IL-5, and IL-13 but not interferon- γ and IL-2, suggesting local activation of Th2-type cells in the nasal mucosa.³⁻⁶ Inhibition of the LNR by topical glucocorticoid therapy is associated with downregulation of IL-4 and IL-13 mRNA expression.^{6,7} Th2-type cytokines are critical to IgE synthesis and eosinophil accumulation into sites of inflammation.⁸ T lymphocytes are the major cellular source of Th2-type cytokines during the LNR, although other types of cells may also contribute.⁵ Despite considerable improvement in our understanding of the pathogenesis of the late-phase response, little is known about the events that initiate the T-cell inflammatory response in allergic types of inflammation.

IL-16, a newly characterized cytokine, has biologic properties that are pertinent to the development of the LNR. IL-16 is a 56 kd homotetrameric cytokine that uses CD4 as its receptor.⁹ Unlike other cytokines, IL-16 has specific activities on CD4⁺ cells including induction of cell migration.^{9,10} Because IL-16 is a potent chemoattractant factor for CD4⁺ cells, it may play a role in late-phase inflammation by attracting CD4⁺ cells into inflamed tissues. However, little is known about the presence or significance of IL-16 at sites of inflammation. We recently showed increased expression of IL-16 mRNA and immunoreactivity in the airway walls of patients with asthma.¹¹ To further investigate the potential role of IL-16 in allergic types of inflammation, we

From ^aMeakins-Christie Laboratories, Royal Victoria Hospital, McGill University; ^bSte-Justine Hospital, University of Montreal; ^cUpper Respiratory Medicine, Imperial College School of Medicine at the National Heart & Lung Institute, London; ^dSahlgren Hospital, Gottenburg; and ^ePulmonary Center, Boston University School of Medicine.

Supported by the Medical Research Council of Canada and UK Glaxo group research.

Sophie Laberge is a recipient of an MRC Scholarship, Canada. Qutayba Hamid is a Research Scholar of the Fonds de la Recherche en Santé du Québec. David Center is supported by grant no. AI 35680 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Received for publication Feb. 26, 1997; revised May 30, 1997; accepted for publication May 30, 1997.

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

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/83668

Abbreviations used

LNR: Late nasal response

mAb: Monoclonal antibody

have examined the expression of IL-16 mRNA and immunoreactivity in the nasal mucosa in biopsy specimens obtained before and 24 hours after antigen challenge. We also investigated, in the context of a double-blind trial, the effects of topical glucocorticoids on IL-16 expression and CD4⁺ cell infiltration.

METHODS**Patients and study protocol**

The study was approved by the Ethics Committees of the Sahlgren Hospital, Gottenburg, Sweden, and The Royal Brompton National Heart and Lung Hospital, London, England. Seventeen subjects with a clinical history of seasonal hay fever were recruited, and informed written consent was obtained from all subjects. Inclusion criteria included: (1) a history of summer hay fever for at least 2 years and (2) a positive skin prick test response (>5 mm) to timothy grass pollen extract (Soluprick, *Phleum pratense*; ALK, Denmark). Patients were excluded from the study if they had received topical or oral medication in the 6 months before the study or immunotherapy within the previous 5 years. Baseline nasal biopsy specimens were obtained from all subjects outside the grass pollen season at a time when they were free of symptoms. Subjects were randomized in a double-blind fashion to receive 6-week treatment with either topical fluticasone propionate, 200 µg twice daily, or matched placebo nasal spray. After the 6-week treatment period, local nasal allergen provocation was performed by applying a 4 mm filter paper disk containing normal saline-diluted grass pollen extract to the undersurface of the nasal inferior turbinate for 10 minutes as previously described.¹ Nasal biopsy specimens (2.5 mm, taken with Geritsma forceps after induction of local anesthesia with 3% cocaine) were obtained 24 hours after the allergen challenge. Biopsy specimens were fixed immediately and prepared for immunocytochemistry and in situ hybridization procedures.

Immunocytochemistry

We used the following antibodies. An anti-human CD4 monoclonal antibody (mAb) was obtained from Dako Canada Inc. (Mississauga, Ontario, Canada). Mouse anti-human IL-16 mAb (IgG2a kappa) raised against human recombinant (r)IL-16 molecule was prepared by AGMED Inc. (Bedford, Mass.) and has been previously characterized.⁹ IL-16 immunoreactivity was assessed by using the avidin-biotin-peroxidase complex method. Briefly, cryostat sections were first incubated with anti-IL-16 mAb at a dilution of 1:30 in automated diluting buffer (Dimension Laboratories Inc., Mississauga, Ontario, Canada) at 43° C for 30 minutes. Further staining was completed as a titration protocol with the avidin-biotin-horseradish peroxidase method according to the manufacturer's recommendations on an automated immunostainer (Ventana Medical Systems Automated Immunostainer ES series). Sites of immunoreaction were visualized by using diaminobenzidine. Omission of the primary anti-IL-16 mAb resulted in absence of

staining. CD4 immunoreactivity was assessed by using the alkaline phosphatase-anti-alkaline phosphatase technique as previously described¹ and an anti-CD4 mAb (1:10 dilution). As control, sections were processed in the absence of the primary antibody.

In situ hybridization

Nonradiolabeled IL-16-specific complementary RNA probe was produced from a 379 bp fragment of the human IL-16 complementary DNA corresponding to the 436 to 815 bp of the published sequence.⁹ The DNA template was inserted into an SPT19 vector and linearized with the appropriate enzymes before transcription. Linearized templates were labeled with digoxigenin-11-uridine triphosphate by transcription with the RNA polymerases SP6 or T7 to generate anti-sense or sense probes, respectively. In situ hybridization was performed as previously described.⁵ Cryostat sections were first permeabilized by immersion in 0.3% Triton X-100 in phosphate-buffered saline, followed by exposure to proteinase K solution (1 µg/ml in 20 mmol/L Tris-HCl and 1 mmol/L ethylenediaminetetraacetic acid, pH 7.2) for 30 minutes at 37° C. Concentrations of digoxigenin-labeled IL-16 probe were adjusted to 250 ng per section in hybridization buffer, which contains 50% formamide, 5× Denhart's solution, 5× standard saline citrate buffer, and 500 µg/ml denatured salmon sperm DNA. The sections were incubated with the hybridization mixture overnight at 40° C. Posthybridization washings were performed by using standard saline citrate solutions with increasing stringency at 45° C, followed by RNase treatment. After a brief wash in Tris-buffered saline, sections were incubated with 3% bovine serum albumin-Tris-buffered saline for 10 minutes to reduce nonspecific background, then incubated with 1:1000 dilution of digoxigenin-alkaline phosphatase conjugate. Color development was achieved with nitroblue tetrazolium salt in equalization buffer. Sections were counterstained with hematoxylin. The corresponding sense probe and RNase pretreatment were used as controls, and in all cases this produced negative results.

Counting and data analysis

IL-16 mRNA-positive cells and IL-16-immunoreactive cells in the epithelium were counted in blinded fashion by optical analysis and expressed as a semiquantitative score on the basis of the percentage of the epithelium showing positive signal/total epithelium (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%; 4, 87.5% to 100%). In the subepithelial tissue, IL-16 mRNA-positive and IL-16-immunoreactive cells, as well as CD4⁺ cells, were counted with the use of an eyepiece graticule at 200× magnification. The results were expressed as mean counts ± SD per field. The within-observer coefficient of variation for repeated counts was less than 5%.¹²

Data were analyzed by using a statistical package (Systat v 5.1; Systat Inc., Evanston, Ill.). Within-group comparisons were performed by using the Wilcoxon matched-pairs signed-rank test. Changes in the mean counts of IL-16-immunoreactive or IL-16 mRNA-positive cells observed after challenge compared with baseline values were expressed as delta values, and between-group comparisons were performed by using the Mann-Whitney U test. Correlations were determined by using the Pearson correlation coefficient. Significance was accepted at the 5% level of confidence.

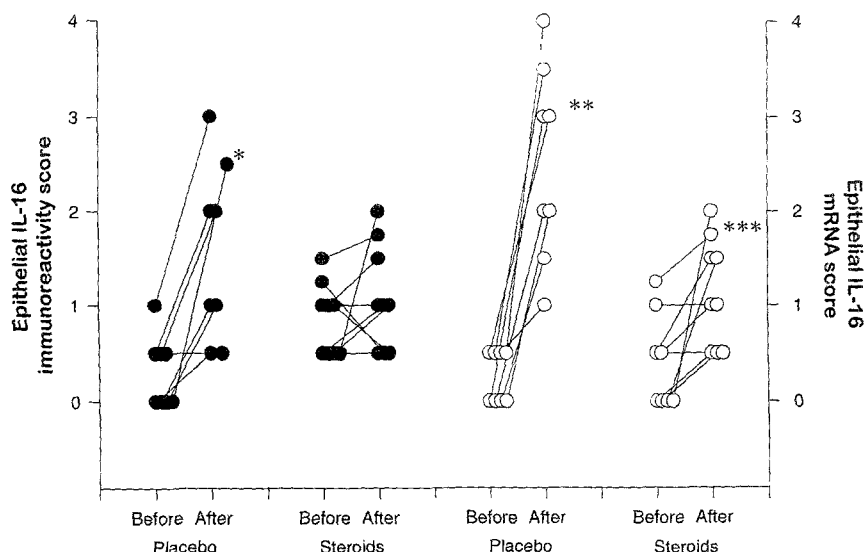


FIG. 1. Effects of allergen challenge on epithelial IL-16 immunoreactivity (filled circles) and mRNA (open circles) expression in nasal biopsy specimens from placebo- and steroid-treated subjects with allergic rhinitis. Biopsy specimens were obtained at baseline (before) and 24 hours after allergen challenge. Results are expressed as a percentage of positive cells per total cells (score, 0 to 4; see Methods). * $p < 0.01$, ** $p < 0.001$, *** $p = 0.01$ compared with baseline values.

RESULTS

Epithelial IL-16 expression

At baseline, IL-16-immunoreactive and IL-16 mRNA-positive cells were identified in both the epithelial and subepithelial areas. Within the epithelium, IL-16-immunoreactive cells and IL-16 mRNA-positive cells localized predominantly in cells within the basal layer of the epithelium. In the placebo-treated subjects, significant increases in epithelial IL-16 immunoreactivity ($p < 0.01$) and IL-16 mRNA ($p < 0.001$) scores were observed 24 hours after antigen provocation compared with baseline values (Fig. 1). There was a significant correlation between the IL-16 immunoreactivity score and the IL-16 mRNA score observed 24 hours after antigen provocation ($r = 0.91$, $p < 0.01$). Within the fluticasone-treated group, the epithelial immunoreactivity score did not increase significantly after antigen challenge. There was a slight, although significant, increase in the number of epithelial IL-16 mRNA-positive cells after challenge compared with baseline ($p = 0.01$). However, the increase in the epithelial mRNA score was significantly greater in the placebo-treated subjects (delta value, 2.25 ± 0.59) compared with the steroid-treated subjects (delta value, 0.50 ± 0.44 ; $p < 0.01$).

Subepithelial IL-16 expression

Within the subepithelial area, significant increases in the numbers of IL-16-immunoreactive cells ($p < 0.01$) and IL-16 mRNA-positive cells ($p < 0.001$) were observed 24 hours after allergen challenge compared with baseline in the placebo-treated subjects (Fig. 2). There was a significant correlation between the num-

bers of IL-16-immunoreactive cells and the numbers of IL-16 mRNA-positive cells at 24 hours after antigen provocation ($r = 0.84$, $p < 0.01$). Within the fluticasone-treated group, the numbers of subepithelial IL-16 mRNA-positive cells did not increase significantly after challenge, whereas the number of subepithelial IL-16-immunoreactive cells increased slightly, although significantly ($p < 0.01$), after challenge. However, the increase in the numbers of IL-16-immunoreactive cells in the subepithelial area was significantly greater in the placebo-treated subjects (delta value, 4.35 ± 3.2) compared with that of the steroid-treated subjects (delta value, 1.5 ± 0.7 ; $p < 0.001$). The morphology of the nonepithelial IL-16-immunoreactive cells and IL-16 mRNA-positive cells was consistent with that of small mononuclear cells.

CD4 immunoreactivity

In subjects treated with placebo, immunohistologic examination of nasal biopsy specimens that were obtained 24 hours after antigen challenge revealed an increase in the mean counts of CD4⁺ cells compared with baseline values (before challenge, 10.9 ± 2.3 cells/field; after challenge, 15.7 ± 5.4 cells/field; $p = 0.01$). In contrast, in patients treated with fluticasone, the number of CD4⁺ cells did not increase significantly after challenge (before challenge, 11.7 ± 2.2 cells/field; after challenge, 12.4 ± 2.4 cells/field; $p = 0.42$). Within the placebo-treated group, there were significant correlations between CD4⁺ cell infiltration and epithelial ($r =$

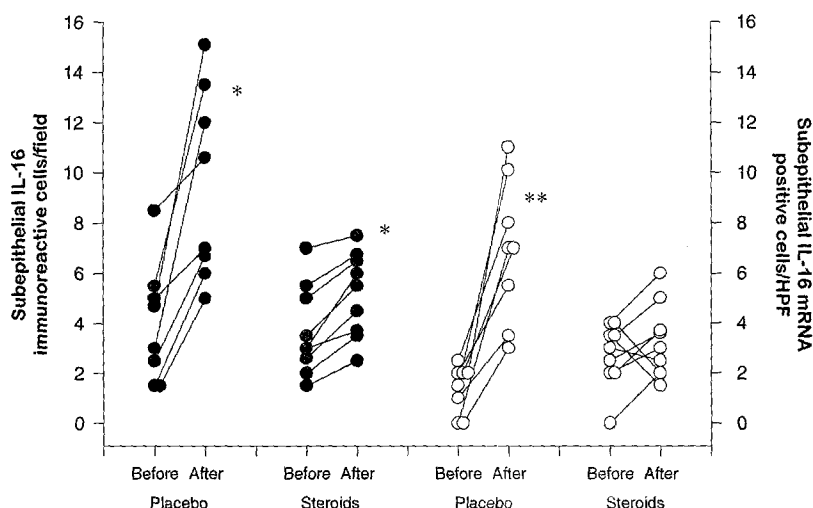


FIG. 2. Effects of allergen challenge on subepithelial IL-16 immunoreactivity (filled circles) and mRNA (open circles) expression in nasal biopsy specimens from placebo- and steroid-treated subjects with allergic rhinitis. Biopsy specimens were obtained at baseline (before) and 24 hours after allergen challenge. Results are expressed as the number of positive cells per high-power field (HPF). * $p < 0.01$ and ** $p < 0.001$ compared with baseline values.

0.76, $p = 0.03$) and subepithelial IL-16 immunoreactivity ($r = 0.75$, $p = 0.03$) after antigen challenge.

DISCUSSION

Prior studies have shown that IL-16 is a specific chemotactic factor for CD4⁺ cells in vitro.⁹ These observations raised the hypothesis that IL-16 may play a role in the immune regulation of CD4-mediated processes such as those associated with allergic types of inflammation. To investigate the potential contribution of IL-16 to the LNR, we have examined the in vivo expression of IL-16 in nasal biopsy specimens obtained from subjects with allergic rhinitis at 24 hours after local allergen challenge and its relationship to CD4⁺ cell infiltration. We have demonstrated that the numbers of IL-16 mRNA-positive cells are significantly increased in the nasal mucosa at 24 hours after antigen provocation. Immunocytochemical analyses revealed a corresponding upregulation of translated IL-16 protein. There was a significant correlation between the numbers of IL-16-immunoreactive cells and the numbers of IL-16 mRNA-positive cells observed after antigen provocation, suggesting that antigen-induced IL-16 upregulation occurred at both the mRNA and protein levels. We also found evidence of a correlation between the numbers of infiltrating CD4⁺ cells and IL-16 immunoreactivity, consistent with the view that synthesis of IL-16 may be implicated in the recruitment of these cells in response to antigen provocation in vivo.

Several lines of evidence suggest that IL-16 may play a role in the allergic types of inflammation. First, IL-16 is a chemoattractant factor for CD4⁺ cells with an ED₅₀ (half the maximal effective dose) of recombinant protein in the 10⁻¹¹ mol/L range.¹⁰ On the basis of this biologic activity, we hypothesized that IL-16 might play a role in

the recruitment of CD4⁺ cells in the inflamed nasal mucosa in vivo. Our findings show that antigen provocation results in upregulation of IL-16 expression and an increase in the numbers of CD4⁺ cells, which are both inhibitable by topical glucocorticoid therapy. Although our data do not prove this hypothesis, it is strongly supported by the significant correlation between IL-16 expression and the numbers of CD4⁺ cells in the nasal mucosa. Second, similar elevated levels of IL-16 immunoreactivity and mRNA expression have been recently reported by our laboratory in bronchial biopsy specimens from patients with stable asthma compared with those from normal control subjects.¹¹ Third, secreted IL-16 is detected in bronchoalveolar lavage fluid obtained after segmental antigen bronchoprovocation in patients with asthma.¹³ The current data extend these previous observations and further strengthen the association between IL-16 expression, CD4⁺ infiltration, and development of atopic inflammation.

In vitro IL-16 can be produced by a variety of cell types including lymphocytes,^{10, 14} eosinophils,¹⁵ and epithelial cells.^{16, 17} In this study IL-16 immunoreactivity and gene transcripts were identified in both the epithelium and the subepithelial area. These findings are consistent with our previous observations identifying IL-16 expression in airway epithelium of bronchial biopsy specimens from patients with asthma.¹¹

The cellular source of nonepithelial IL-16 in the nasal mucosa remains to be determined. The morphology of subepithelial IL-16-positive cells is consistent with that of mononuclear cells, although combined in situ hybridization and immunocytochemistry must be performed before a definitive statement can be made. Interestingly, at baseline the numbers of subepithelial IL-16-immuno-

reactive cells were greater than the numbers of IL-16 mRNA-positive cells. This may be explained by the fact that we used a specific antibody that was raised against the C-terminal peptide of the molecule and that recognizes both the precursor and the secreted form of IL-16. This finding might also suggest that IL-16 identified in nonepithelial IL-16-immunoreactive cells may represent a rapidly available local source of preformed IL-16 as opposed to increased synthesis of IL-16 from resident and/or recruited inflammatory cells after antigen stimulation. Although lymphocytes are not generally considered to have significant storage capacity, preformed IL-16 has been detected in unstimulated CD8⁺ T cells,¹⁴ and more recently, in eosinophils.¹⁵

The relative contribution of epithelial and nonepithelial cells in the synthesis of IL-16 in response to antigen is unknown. However, both types of cells are likely to be a significant source of IL-16 in vivo because IL-16 immunoreactivity and mRNA expression increased in both the epithelial and subepithelial layers of the nasal mucosa 24 hours after antigen provocation. We cannot exclude the possibility that the increase in the numbers of epithelial IL-16-positive cells observed 24 hours after antigen challenge may be partly attributed to proliferation of epithelial cells, as opposed to induction of cytokine synthesis per se. The effects of antigen challenge on epithelial cell proliferation are unclear. However, some preliminary data suggest that IL-16 gene expression is upregulated in bronchial epithelial cell lines after stimulation with histamine, IL-1 β , or tumor necrosis factor- α , mediators that can be released in the allergic response.^{16, 17} Whether these effects occur in vivo in the nasal mucosa remains to be established.

Corticosteroids are potent inhibitors of LNR, presumably through suppression of specific cytokine gene expression.⁷ We hypothesized that glucocorticoids may act in part by suppressing IL-16. We have demonstrated here that allergen-induced IL-16 upregulation in the nasal mucosa is significantly reduced in both the epithelium and the subepithelial layer after 6 weeks of treatment with topical glucocorticosteroids compared with placebo. Although we observed a statistically significant increase in the numbers of epithelial IL-16 mRNA-positive cells after challenge in the fluticasone-treated subjects, the magnitude of this response was small and significantly less compared with the placebo-treated subjects. Glucocorticoids, however, ablated the allergen-induced upregulation of epithelial IL-16 immunoreactivity, suggesting that steroids may act not only to reduce transcription but also at the posttranscriptional level. It is not possible from this study to determine whether complete inhibition of allergen-induced epithelial IL-16 gene expression may occur at a different time point.

Consistent with previous observations,⁷ the numbers of CD4⁺ cells in response to allergen challenge were not increased after corticosteroid therapy. These findings further support the notion that IL-16 expression and CD4⁺ cell infiltration are linked phenomena and suggest

that the effects of corticosteroids on cellular infiltration may be mediated by inhibition of IL-16.

In summary, we have shown that the numbers of IL-16 mRNA-positive and IL-16-immunoreactive cells are increased in nasal biopsy specimens from subjects with allergic rhinitis 24 hours after antigen provocation in vivo. Prolonged treatment with topical corticosteroids was associated with significant attenuation of allergen-induced increases in IL-16 mRNA and immunoreactivity. These data suggest that increased expression of IL-16 may be relevant to the pathophysiology of late-phase reactions in mucosal tissues, including the nasal mucosa, through its ability to selectively recruit CD4⁺ cells in response to antigen.

We thank Dr. O. Lowhagen, Ms. Barbara Baldo, and Mr. R. Sudderick for their help with the clinical studies and Ms. Marie Ploude and Ms. Zivart Yasruel for their technical assistance.

REFERENCES

1. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils and neutrophils. *Am Rev Respir Dis* 1992;146:170-6.
2. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon- γ . *J Allergy Clin Immunol* 1996;97:1356-65.
3. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and GM-CSF in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
4. Ying S, Durham SR, Barkans J, Masuyama K, Jacobson M, Rak S, et al. T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. *Am J Respir Cell Mol Biol* 1993;9:356-60.
5. Ying S, Durham SR, Jacobson MR, Rak S, Matsuyama K, Lowhagen O, et al. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994;82:200-6.
6. Ghaffar O, Durham S, Laberge S, Jacobson M, Rak S, Lowhagen O, et al. Expression of IL-13 in allergen-induced late nasal reaction and in response to topical steroids [abstract]. *J Allergy Clin Immunol* 1996;97:194.
7. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, et al. Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 (IL-4) in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994;82:192-9.
8. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996;383:787-93.
9. Cruikshank WW, Center DM, Nisar N, Natke B, Theodore AC, Kornfeld H. Molecular and functional analysis of a lymphocyte chemoattractant factor: association of biologic function with CD4 expression. *Proc Natl Acad Sci USA* 1994;91:5109-13.
10. Center DM, Kornfeld H, Cruikshank WW. Interleukin-16 and its function as a CD4 ligand. *Immunol Today* 1996;17:476-81.
11. Laberge S, Ernst P, Ghaffar O, Cruikshank WW, Center DM, Hamid Q. Increased expression of interleukin-16 in bronchial mucosa of subjects with atopic asthma. *Am J Respir Cell Mol Biol* 1997. In press.
12. Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, et al. Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510-3.
13. Cruikshank WW, Long A, Tarpy H, Kornfeld H, Carrol MP, Teran L, et al. Early identification of lymphocyte chemoattractant

- factor (IL-16) and macrophage inflammatory protein 1 α (MIP-1 α) in BAL of antigen challenged asthmatics. *Am J Respir Cell Mol Biol* 1995;13:738-47.
14. Laberge S, Cruikshank WW, Kornfeld H, Center DM. Histamine-induced secretion of lymphocyte chemoattractant factor from CD8⁺ T cells is independent of transcription and translation: evidence for constitutive protein synthesis and storage. *J Immunol* 1995;155:2902-10.
 15. Lim KG, Wan HC, Bozza PT, Resnick MB, Wong DT, Cruikshank WW, et al. Human eosinophils elaborate the lymphocyte chemoattractants. IL-16 (lymphocyte chemoattractant factor) and RANTES. *J Immunol* 1996;156:2566-70.
 16. Arima M, Plitt JR, Stellano C, Schwiebert LM, Schleimer RP. The expression of lymphocyte chemoattractant factor (LCF) in human bronchial epithelial cells [abstract]. *J Allergy Clin Immunol* 1996;97:443.
 17. Wu D, Cruikshank WW, Kornfeld H, Cook J, Chupp G, Center DM. Detection of IL-16 in normal bronchial epithelial cell line after cytokine modulation [abstract]. *Am J Respir Cell Mol Biol* 1996;153:A208.