

Identification of IL-16 as the lymphocyte chemotactic activity in the bronchoalveolar lavage fluid of histamine-challenged asthmatic patients

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Objective: We have previously demonstrated that the earliest lymphocyte chemotactic factors present in bronchoalveolar lavage fluid (BALF) of subjects with atopic asthma after subsegmental antigen challenge are IL-16 and MIP-1 α , of which IL-16 appears to contribute a majority of the chemotactic activity. Because IL-16 is released *in vitro* after histamine stimulation of CD8⁺ T cells and epithelial cells, we evaluated the potential role of histamine in the release of IL-16 into the airways of allergic asthmatics *in vivo*.

Methods: Eight allergic asthmatic subjects, six normal subjects, and six atopic nonasthmatic subjects were challenged with saline in the lingula and with serial concentrations of histamine (1×10^{-7} to 5×10^{-5} mol/L) in the right middle lobe followed by bronchoalveolar lavage (BAL) 15 minutes and 6 hours later.

Results: The BALF from saline- and histamine-challenged lobes of normal subjects and atopic nonasthmatic subjects contained no significant lymphocyte chemoattractant activity. In six of the eight atopic asthmatic subjects, the histamine-challenged but not saline-challenged segment contained IL-16 chemotactic activity but no other identifiable lymphocyte chemoattractant activities at 6 hours.

Conclusions: IL-16 appears in the airways after histamine challenge and therefore could contribute to the earliest infiltration of CD4⁺T cells and eosinophils observed after antigen challenge due to histamine release from mast cells. (*J Allergy Clin Immunol* 1998;101:786-92.)

Key words: Asthma, bronchoalveolar lavage, IL-16, lymphocyte chemotaxis, histamine

Asthma is characterized by reversible airflow obstruction and airway inflammation. Numerous studies have demonstrated an influx of eosinophils, activated lymphocytes, and neutrophils in the airways of atopic

Abbreviations used

BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
PBS:	Phosphate-buffered saline

asthmatic subjects after antigen challenge.¹⁻⁷ CD4⁺ T cells are increased in the bronchoalveolar lavage fluid (BALF) of asthmatic subjects 48 hours after antigen challenge,³ and there are increased numbers of T cells in the submucosa of asthmatic subjects compared with normal subjects.⁴ Furthermore, the T cell cytokines believed to be products of infiltrating CD4⁺ T_{H2}-type cells (e.g., IL-3, IL-4, IL-5, and GM-CSF) have been implicated in asthma.⁸⁻¹⁰

A number of the functional characteristics of IL-16 led us to study its presence in atopic asthma. It is secreted by CD8⁺ T cells and asthmatic airway epithelial cells after stimulation with histamine.¹¹⁻¹³ IL-16 stimulates the migration of CD4⁺ T lymphocytes, eosinophils, and monocytes dependent on cell surface expression of CD4.¹⁴⁻¹⁶ IL-16 induces the expression of high-affinity IL-2 receptors on a percentage of CD4⁺ T cells over a period of 24 to 72 hours.^{14, 15} *In vitro*, IL-16 is released from CD8⁺ T cells 4 hours after histamine stimulation, a process that does not require protein synthesis or generation of new IL-16 mRNA.¹² The mechanisms of its synthesis and release from cultured respiratory epithelial cells from asthmatic subjects after stimulation with histamine are not known. Recently, Laberge et al.¹⁷ demonstrated IL-16 mRNA and immunoreactivity in airway epithelium to be significantly higher in bronchial biopsies from asthmatic subjects compared with both atopic nonasthmatic subjects and normal subjects. Epithelial expression of IL-16 immunoreactivity and mRNA correlated with CD4⁺ cell infiltration.

Along those lines, we have previously described the release *in vivo* of IL-16 and MIP-1 α into BALF of asthmatic subjects but not normal subjects or atopic nonasthmatic subjects 6 hours after segmental challenge with allergen.¹⁸ The purpose of this study was to elucidate the role of histamine in the release of lymphocyte chemoattractant factor in general and IL-16 in particular

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into the airways of asthmatic subjects with allergies *in vivo*. Normal volunteers, atopic subjects without asthma, and asthmatic subjects with allergies underwent local segmental histamine challenge followed 15 minutes and 6 hours later by bronchoalveolar lavage (BAL). Lymphocyte chemoattractant activity was significantly increased after histamine challenge in asthmatic subjects but not normal subjects or atopic nonasthmatic subjects. These findings indicate that in asthmatic subjects, histamine alone is sufficient to induce release of IL-16 into the airways and that all the chemoattractant activity in the BALF was due to IL-16.

METHODS

Subjects

Twenty nonsmoking volunteers were evaluated in this study (Table I). Eight were atopic with mild asthma (seven men), six were healthy normal volunteers (five men), and six were atopic without clinical evidence of asthma (four men). After a detailed explanation of the study, informed consent was obtained from each subject before the initial screening. The study was approved by both the Institutional Review Board at Boston University Medical Center Hospital and the Human Subjects Committee at Boston City Hospital (now Boston Medical Center). Each subject underwent a screening history and physical examination with specific attention to allergic symptoms. An electrocardiogram, posteroanterior chest film, spirometry, and standard methacholine challenge were performed. Subjects without a history of asthma were screened for atopy with intradermal skin tests to a standard battery of allergens found commonly in the northeast United States. Subjects were classified as atopic if a wheal of 3×3 mm or greater developed to any of the antigens. Normal subjects were asymptomatic, nonsmoking, with negative skin test results with an FEV₁ greater than 80% and a negative methacholine challenge. Asthmatic subjects required a history of symptoms, skin test positivity, and a positive methacholine challenge with PC20 10 mg/ml or less. In addition, the asthmatic subjects were required to have an FEV₁ greater than 60% of predicted on the day of challenge and could not have used either oral or inhaled corticosteroids in the preceding 8 weeks. Atopic nonasthmatic subjects were required to be without clinical evidence of asthma and to have a positive skin test result and negative methacholine challenge. We excluded from examination any subject with an upper respiratory infection within 6 weeks before the study.

Bronchoscopy with segmental histamine challenge

Each subject arrived the morning of the bronchoscopy and had a baseline spirometry performed. Asthmatic subjects were allowed to use a β -agonist by metered dose inhaler if needed. Only one subject used oral theophylline, which was held for 48 hours before the study, and no subject used either H₁ or H₂ antihistamines. Asthmatic subjects 3 and 6 used albuterol within 1 hour of challenge. An intravenous catheter was placed for hydration with 0.9% saline, and each subject was premedicated with 0.6 to 0.8 mg atropine sulfate subcutaneously. Lidocaine (4%) was aerosolized to provide anesthesia of the upper airway, and lidocaine (2%) was given through the bronchoscope directly onto the vocal cords. All subjects were monitored by continuous electrocardiography and pulse oximetry while receiving oxygen at 2 L/min by nasal cannula. Fiberoptic bronchoscopy was performed with an Olympus BF type P10 flexible

TABLE I. Subject characteristics

Subject	Age ($\pm$ SD)	FEV ₁ ($\pm$ SD)* (% predicted)	PC20 ($\pm$ SD) (mg/ml)
Normal subjects	31 (5.7)	97 (10.6)	>25
Atopic subjects	26 (4.1)	99 (12.1)	>25
Asthmatic subjects	29 (9.7)	102 (16.1)	3.2 (4.2)

*FEV₁ percent predicted on the basis of forced expiratory volume in the first second.

fiberoptic bronchoscope (Olympus Corp. of America, New Hyde Park, N.Y.). The bronchoscope was initially wedged into a subsegment of the lingula for sham challenge with 0.9% saline. Saline (5 ml) was injected through the bronchoscope every 60 seconds for a total of five doses while maintaining wedge position. Two minutes later, BAL was performed with 150 ml of 0.9% saline divided into three aliquots and removed by gentle aspiration with a syringe. The bronchoscope was then wedged into a subsegment of the right middle lobe for local histamine challenge. Histamine dichloride (Sigma Chemical Company Inc., St. Louis, Mo.) was prepared with phosphate-buffered saline (PBS) at pH 7.4 at concentrations of 1×10^{-7} , 5×10^{-7} , 1×10^{-6} , 5×10^{-6} , 1×10^{-5} , and 5×10^{-5} mol/L. Histamine (5 ml) was then injected through the bronchoscope every 2 to 3 minutes at increasing concentrations while monitoring closely for signs of a local or systemic response. One asthmatic subject (subject 4) had the challenge terminated after the 1×10^{-6} mol/L dose because of complaints of nasal congestion. The remainder of the subjects received all concentrations. Two minutes later, BAL was performed as previously described.¹⁸ Subjects were monitored for at least 1 hour after the bronchoscopy. Six hours after the initial histamine challenge, a second bronchoscopy with BAL of the sham-challenged and histamine-challenged subsegments was performed. Subjects were discharged from the bronchoscopy suite once they were deemed clinically stable.

Analysis of bronchoalveolar lavage fluid

The BALF was initially filtered through a layer of sterile gauze and then centrifuged at 600g for 10 minutes. The supernatant was removed and then concentrated to a volume of to 1 to 2 ml by Amicon Filtration (Amicon, Beverly, Mass.) at 20° C before analysis for IL-16 bioactivity. The cell pellet was resuspended in PBS before total cell count on a Neubauer hemocytometer. Cytoentrifuged specimens were made with a Shandon Cytospin (Shandon Southern Instruments, Runcorn, U.K.). Cells were then stained with Leuko-Stat (Fisher Scientific, Pittsburgh, Pa.), and differential cell counts were performed on 200 cells.

Lymphocyte chemotaxis assay

Cell migration was performed as previously described¹⁴ with a modification of the Boyden Chamber assay with a microchemotaxis chamber (Neuroprobe, Cabin John, Md.) Briefly, normal human T lymphocytes were isolated with Hypaque-Ficoll separation of peripheral blood mononuclear cells followed by nylon wool adherence and then cultured overnight in RPMI 1640 containing 10% bovine fetal serum. The upper well was loaded with 50 μ l of the T cell suspension (10×10^6 /ml) and 30 μ l of the concentrated BALF was placed in the bottom well. The two wells were separated by 8 μ m micropore nitrocellulose filter paper and incubated at 37° C for 3 hours. The filters were

TABLE II. BALF cellular composition

	Cells/ml	AM	L	N	E
Normal subjects					
A	1.26 (0.61)	85 (10.4)	12.6 (11.0)	1.8 (1.5)	0.50 (0.84)
B	0.92 (0.74)	80 (11.0)	12.5 (8.1)	7.3 (4.2)	0.08 (0.20)
C	0.93 (0.49)	86 (10.0)	11.6 (9.5)	1.7 (1.3)	0.33 (0.51)
D	1.04 (0.78)	52 (21.2)	14.0 (9.0)	33.5 (26.4)	0.40 (0.63)
Atopic subjects					
A	5.78 (5.2)	93 (2.1)	6.3 (2.07)	1.1 (0.86)	0 (0)
B	3.41 (3.3)	80 (13.9)	6.8 (3.5)	12.7 (12.7)	0.17 (0.41)
C	4.24 (3.1)	90 (4.1)	7.6 (4.1)	1.7 (0.98)	0.41 (0.49)
D	4.10 (3.5)	62 (22.4)	6.6 (1.9)	34.8 (22.7)	1.20 (1.03)
Asthmatic subjects					
A	0.60 (0.32)	82 (14.9)	14.4 (11.3)	0.69 (0.95)	0.86 (1.07)
B	1.21 (1.04)	77 (22.7)	10.9 (6.0)	11.7 (22.7)	0.92 (1.43)
C	0.53 (0.30)	84 (8.0)	12.1 (7.2)	1.60 (1.7)	0.99 (1.43)
D	1.55 (1.99)	58 (13.7)	10.0 (4.1)	29.1 (16.0)	3.42 (3.64)

Data are expressed as mean for each condition with standard deviation in parentheses.

Cell counts are expressed as cells $\times 10^5$ /ml in recovered BALF.

AM, Alveolar macrophages; L, lymphocytes; N, neutrophils; E, eosinophils; A, 15 minutes after saline; B, 6 hours after saline; C, 15 minutes after histamine; D, 6 hours after histamine.

then fixed and stained with hematoxylin. Migration was assessed by counting the number of cells that had migrated beyond a certain depth into the filter (50 μ m). All migration is expressed as percentage values of cell migration in control buffer.

Inhibition experiments were performed by mixing BALF with a monoclonal, IL-16 neutralizing antibody (clone 14.1), sufficient to neutralize bioactivity of 50 ng/ml of specific protein for 30 minutes before loading the chemotaxis chamber.¹⁸ For all experiments, between 10 and 15 cells/hpf were counted in the control wells. All subjects had inhibition experiments performed with anti-IL-16. In selected individuals, inhibition experiments were also performed with neutralizing antibody to MIP-1 α , IL-8, or an isotype control (Biosource International, Carmarillo, Calif.). We have previously described that the lack of detectable cross-neutralization of anti-IL-16 with MIP-1 α or IL-8.^{12, 18} In addition, our laboratory has investigated the effect of IL-16 monoclonal antibody on the chemotactic dose-response curve for MCP-1 and MCP-2. Lymphocyte chemotaxis for these cytokines was not affected by the anti-IL-16 antibody (data not shown), indicating no cross-reactivity for these cytokines as well.

Statistical analyses

Comparisons between the means of asthmatic subjects, atopic nonasthmatic subjects, and normal subjects were made by Student's unpaired *t* test. The comparisons within groups or on individual subjects were made with Student's paired *t* test. A probability (*p*) value less than 0.05 was considered significant.

RESULTS

Cell counts and cell differentials in BAL samples

Total and differential cell counts were performed on BAL obtained 10 to 15 minutes and 6 hours after histamine and saline challenge. Cell counts were expressed as the total number of cells per milliliter in the recovered BAL. The total number of cells per milliliter

in the recovered BAL varied from individual to individual in normal subjects, atopic nonasthmatic subjects, and atopic asthmatic subjects (Table II). The number of cells was unchanged in normal subjects and atopic nonasthmatic subjects 15 minutes after saline as compared with 6 hours after saline challenge (1.26 ± 0.61 versus $0.92 \pm 0.74 \times 10^5$ and 5.78 ± 5.21 versus $3.41 \pm 3.30 \times 10^5$ cells/ml). There was also no change in total cell numbers 15 minutes and 6 hours after histamine challenge (0.93 ± 0.49 versus $1.04 \pm 0.78 \times 10^5$ and 4.24 ± 3.08 versus $4.10 \pm 3.53 \times 10^5$ cells/ml). Although there was a trend toward an increase in the number of cells 6 hours after saline (0.6 ± 0.32 versus $1.21 \pm 1.04 \times 10^5$ cells/ml) and histamine ($0.53 \pm 0.3 \times 10^5$ versus $1.55 \pm 1.99 \times 10^5$ cells/ml) in asthmatic subjects, this was not statistically significant. In addition, there was no difference in the total number of cells at either interval among normal subjects, atopic nonasthmatic subjects, or asthmatic subjects.

Similarly, the BAL cell differentials (Table II) 15 minutes after saline and histamine was the same in normal subjects, atopic nonasthmatic subjects, and asthmatic subjects, with a predominance of alveolar macrophages (saline: $85\% \pm 10\%$, $93\% \pm 2.1\%$, and $82\% \pm 15\%$; histamine: $86\% \pm 10\%$, $90\% \pm 4.1\%$, and $84\% \pm 8\%$, respectively). Six hours after either saline or histamine challenge there was a significant increase in the percentage of neutrophils in normal subjects (saline: $1.8\% \pm 1.5\%$ versus $7.3\% \pm 4.2\%$; histamine: $1.7\% \pm 1.3\%$ versus $33\% \pm 26\%$; $p < 0.05$), atopic nonasthmatic subjects (saline: $1.19\% \pm 0.86\%$ versus $12.7\% \pm 12.7\%$; histamine: $1.70\% \pm 0.98\%$ versus $34.7\% \pm 22.7\%$; $p < 0.05$), and asthmatic subjects (saline: $0.69\% \pm 0.95\%$ versus $11.7\% \pm 23\%$; histamine: $1.6\% \pm 1.7\%$ versus $29\% \pm 16\%$; $p > 0.05$). The percentage of lymphocytes and eosinophils remained unchanged.

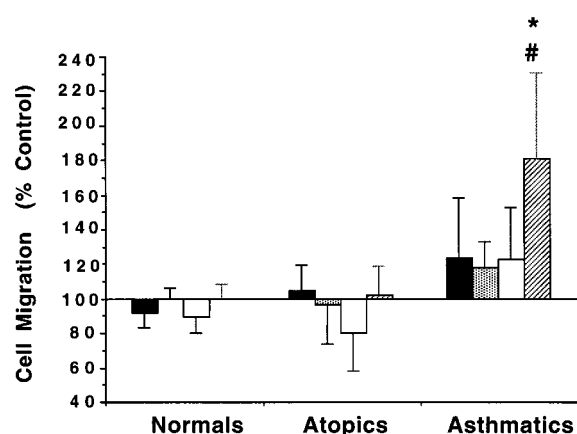


FIG. 1. Mean values for lymphocyte migration induced by concentrated BALF obtained from six normal subjects, six atopic nonasthmatic subjects, and eight asthmatic subjects after segmental challenge with saline or histamine. Cell migration is expressed as percentage of control cell migration in presence of PBS alone. BALF obtained 15 minutes after saline challenge is depicted in *solid bars*; 6 hours after saline challenge in *shaded bars*; 15 minutes after histamine challenge in *open bars*; and 6 hours after histamine challenge in *striped bars*. Asterisks denote cell migration induced by histamine-challenged BALF from asthmatic subjects at 6 hours, which was statistically different from migration induced by histamine-challenged BALF from normal subjects and atopic nonasthmatic subjects at 6 hours ($p < 0.05$). Pound sign (#) denotes cell migration induced by histamine-challenged BALF from asthmatic subjects at 6 hours, which was statistically different from migration induced by histamine-challenged BALF from asthmatic subjects at 15 minutes ($p < 0.005$).

Lymphocyte chemoattractant activity in BALF of normal subjects

The BALF was concentrated approximately 100-fold to a final volume of 1 to 2 ml before analysis for chemotactic activity. None of the normal subjects had evidence of chemoattractant activity compared with chemotaxis assay buffer alone 15 minutes after challenge with saline or histamine (Figs. 1 and 2, *A*). In fact, most of the normal subjects had evidence of inhibitory activity at this time point after saline and histamine ($90\% \pm 7\%$, $88\% \pm 9\%$, respectively), similar to prior observations.¹⁸ Identification and characterization of the inhibitory activity in the lower respiratory tract of normal subjects are topics of separate investigation. Six hours after saline and histamine challenge, normal subjects showed less inhibitory activity (cell migration approximately 99% of control for both conditions). However, there was no increase over chemotactic buffer control migration.

Lymphocyte chemoattractant activity in BALF from atopic nonasthmatic subjects

The BALF of all seven atopic nonasthmatic subjects contained no significant chemoattractant activity at either 15 minutes or 6 hours after challenge with saline or histamine (Figs. 1 and 2, *B*). Similar to the normal subjects, most individual BALF was inhibitory. Subject 2 had minimal (statistically insignificant) lymphocyte che-

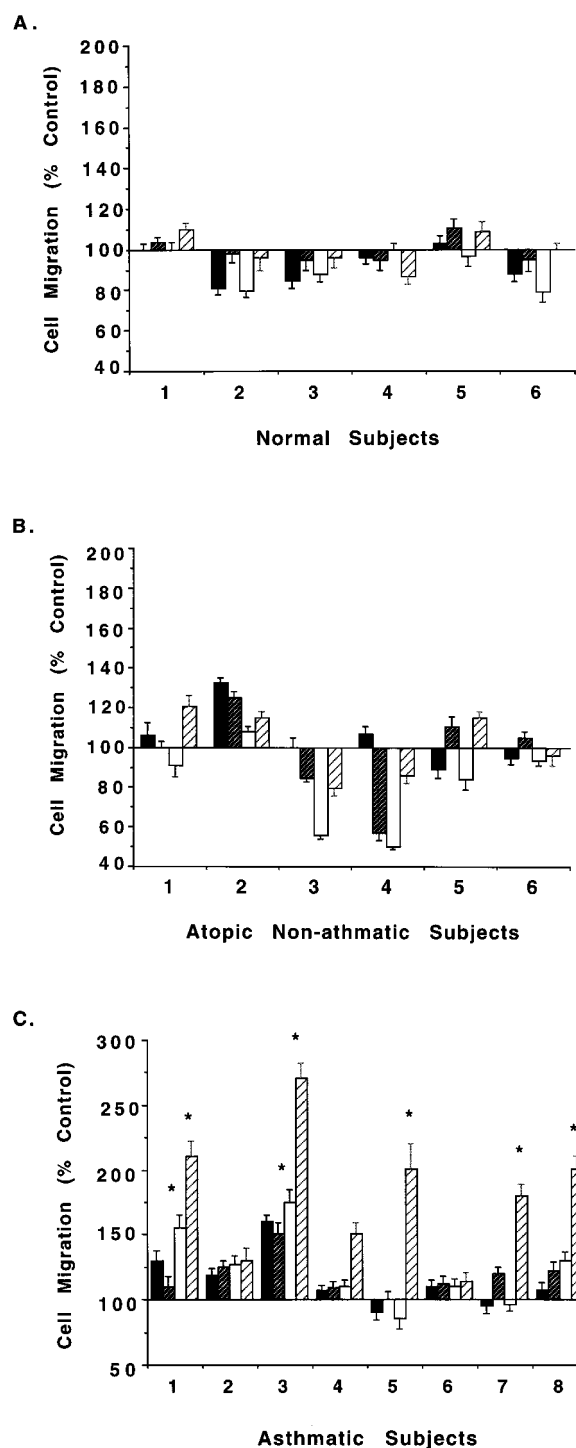


FIG. 2. Lymphocyte migration induced by concentrated BALF obtained from six normal subjects (*A*), six atopic nonasthmatic subjects (*B*), or eight asthmatic subjects (*C*) after segmental challenge with saline or histamine. Cell migration is expressed as percentage of control cell migration in presence of PBS alone. For each subject, chemotactic activity in BALF obtained 15 minutes after saline challenge is depicted in *solid bars*; 6 hours after saline challenge in *shaded bars*; 15 minutes after histamine challenge in *open bars*; and 6 hours after histamine challenge in *hatched bars*. Asterisks denote statistically significant cell migration compared with control chemotactic buffer ($p < 0.05$).

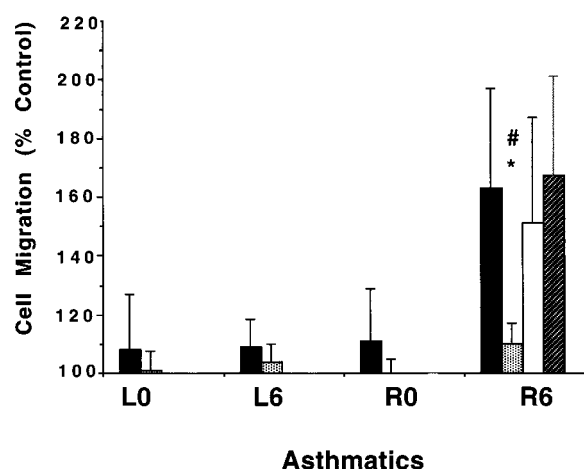


FIG. 3. Effects of neutralizing anti-IL-16 antibody, anti-MIP-1 α antibody, and IgG_{2a} isotype control antibody on BALF-induced T cell migration. BALF obtained from asthmatic subjects after saline and histamine challenge at 15 minutes and 6 hours was assessed in presence of anti-IL-16 antibody sufficient to neutralize 50 ng/ml of recombinant human IL-16 bioactivity. Samples were first assessed after 1:1 dilution of PBS (*solid bars*), then assessed 1:1 in presence of anti-IL-16 antibody (*shaded bars*). Induction of cell migration was then assessed for BALF obtained from six asthmatic subjects 6 hours after histamine challenge in presence of anti-MIP-1 α antibody (*open bar*) and isotype control antibody (*striped bar*) sufficient to neutralize 50 ng/ml of bioactivity. Mean value for cell migration was calculated for each time point. Data are expressed as percentage of control cell migration in presence of PBS alone, with *asterisks* denoting inhibition of migration that was statistically different from BAL-induced migration in absence of blocking antibodies ($p < 0.05$) and *pound sign* denoting inhibition of migration that was statistically different from BAL-induced migration in presence of anti-MIP-1 α and isotype control antibodies.

motactic activity present in both lobes at both 15 minutes and 6 hours.

Lymphocyte chemoattractant activity in BALF from asthmatic subjects

Unlike the normal subjects and atopic nonasthmatic subjects, the BALF of most of the asthmatic subjects did not contain chemoattractant inhibitory activity 15 minutes after saline and histamine challenge (Figs. 1 and 2, C). In fact, low levels of chemotactic activity were observed (saline challenge, 15-minute lavage: 120% $\pm$ 27%; histamine challenge, 15-minute lavage: 119% $\pm$ 31%). This represented a significant difference compared with normal subjects and atopic asthmatic subjects at this time point ($p < 0.05$). There was no increase in the mean lymphocyte chemoattractant activity in the BALF of asthmatic subjects 6 hours after saline challenge compared with the 15-minute time point (15 minutes: 120% $\pm$ 27%; 6 hours: 116% $\pm$ 18%). In contrast, the BALF from the histamine-challenged lobes at 6 hours contained significantly more lymphocyte chemoattractant activity ($p < 0.005$) than the corresponding BALF at 15 minutes (15 minutes: 119% $\pm$ 27%; 6 hours: 165% $\pm$ 46%) (Figs. 1 and 2, C). When

each individual was assessed independently, we found that the BALF of the histamine challenged lobe at 6 hours in five of the eight asthmatic subjects contained significantly more chemotactic activity than the BALF from the saline-challenged lobe (Fig. 2, C). The BALF of the histamine-challenged lobe of subject 4 at 6 hours contained increased chemotactic activity compared with the saline lobe; however the magnitude of the difference did not quite reach statistical significance (Fig. 2, C). Finally, the BALF of the histamine-challenged lobes of subjects 2 and 6 at 6 hours contained no demonstrable chemotactic activity (Fig. 2, C). The mean chemotactic activity in the 6-hour posthistamine BALF of the asthmatic subjects was significantly greater ($p < 0.05$) than the activity present in the 6 hour postsaline challenge and when compared with the group of normal subjects and atopic nonasthmatic subjects ($p < 0.05$) at this time point.

Characterization of lymphocyte chemoattractant activity

To determine the identity of the chemoattractant activity from the BALF 15 minutes and 6 hours after saline and histamine challenge, chemotaxis assays were done in the presence of neutralizing antibodies to lymphocyte chemotactic factors known to be present after antigen challenge (i.e., IL-16, MIP-1 α). Fig. 3 illustrates the effect of an IL-16 monoclonal neutralizing antibody on asthmatic BALF-induced T cell migration from the saline and histamine challenge at both 15 minutes and 6 hours. Coincubation of the 6-hour histamine BALF (from 6 asthmatic subjects) with neutralizing antibodies for MIP-1 α or IL-8 bioactivity and IgG_{2a} isotype control antibodies had no effect on the lymphocyte chemoattractant activity (Fig. 3, IL-8 data not shown). Because there was no significant lymphocyte chemoattractant activity in the 15-minute or 6-hour saline BALF or in the 15-minute histamine BALF, these samples were not coincubated with MIP-1 α , IL-8, or isotype control antibodies. A mean value was calculated for each time point. No significant chemoattractant activity was found in the BALF 15 minutes after saline and histamine and 6 hours after saline challenge (Fig. 3). However, by 6 hours after histamine challenge, a significant amount of the lymphocyte chemotactic activity observed in the BALF was attributable to IL-16. This is in contrast to our findings with antigen challenge in which 10% to 15% of the lymphocyte chemotactic activity was due to MIP-1 α at 6 hours.

DISCUSSION

In prior studies, we demonstrated that IL-16 was detectable in the BALF of asthmatic subjects but not normal subjects or atopic nonasthmatic subjects 6 hours after segmental challenge with antigen *in vivo*.¹⁸ *In vitro* IL-16 is released from CD8⁺ T cells within 4 hours after histamine stimulation¹² by H₂ receptors,¹⁹ a process that does not require new protein synthesis or generation of IL-16 mRNA.¹² Bellini et al.¹³ have shown that tracheal

epithelial cells from asthmatic subjects but not normal subjects release IL-16-like bioactivity 24 hours after histamine challenge in vitro. Furthermore, Laberge et al.¹⁷ have identified greater IL-16 immunoreactivity and mRNA expression in airway epithelial cells of asthmatic subjects obtained by bronchial biopsies compared with both normal subjects and atopic nonasthmatic subjects. Epithelial and subepithelial expression of IL-16 in biopsies of asthmatic subjects correlated with the increased number of submucosal CD4⁺ T cells.

Taken together, these findings prompted us to undertake this study, which was designed to determine whether the lack of release of IL-16 in vivo in normal subjects challenged with antigen represented a lack of mast cell-derived mediator release^{20, 21} or whether asthmatic subjects are phenotypically different from normal subjects and atopic nonasthmatic subjects in their ability to release IL-16 into the airways after exposure to products of activated mast cells, for example, histamine.

In this study we demonstrated the presence of IL-16 in the BALF of atopic asthmatic subjects but not normal subjects or atopic nonasthmatic subjects 6 hours after segmental challenge with histamine. We chose to look for lymphocyte chemotactic activity at 6 hours after histamine challenge because it is a time point that precedes the influx of eosinophils and CD4⁺ T cells, which might contribute nonresident cell sources of IL-16 or other chemotactic factors after antigen challenge.^{22, 23} In fact, in this study, although the total number of cells increased in both normal subjects and asthmatic subjects 6 hours after saline and histamine challenge, there was not a statistically significant difference between the two groups. The increased cell numbers after both histamine and saline challenge in asthmatic subjects and nonasthmatic subjects could be largely attributed to neutrophils, probably caused by release of cytokines such as IL-8. Of importance for the present studies, neutrophils are not a confounding source of IL-16 (W. Cruikshank, unpublished observation).

Taken together with the endobronchial biopsy data of Laberge et al.,¹⁷ it is likely that the source of IL-16 in histamine-stimulated airways is the epithelium, and that the reason we observe no IL-16 after histamine challenge of normal subjects or atopic nonasthmatic subjects is that their epithelium does not express sufficient amounts of IL-16 protein to be released and measured in BALF. Another possible source for the increased levels of IL-16 in the BALF of asthmatic subjects after histamine challenge could be the stimulation of resident CD8⁺ T cells in the mucosal or submucosal surfaces of asthmatic subjects. However, based on the relatively low numbers of resident CD8⁺ T cells present in the mucosal and submucosal layer in the lung, it is unlikely that the CD8⁺ T cells is a significant source of IL-16 after histamine challenge.

Because histamine is known to increase vascular permeability, it could be argued that the increase in IL-16 we observe in airways is of serum origin. This is unlikely because we observe no IL-16 in normal subjects chal-

lenged with histamine and we have not detected IL-16 activity in the serum of asthmatic subjects. Furthermore, we cannot identify differences in the histamine-induced release of IL-16 from peripheral blood CD8⁺ T cells between the normals and atopic asthmatic subjects in the current study (data not shown).

Interestingly, two asthmatic subjects (Nos. 2 and 6) showed no evidence of IL-16 release. Subject 6 had used a β_2 -agonist just before the initial histamine challenge. It is possible the β_2 -agonist played a role in inhibition of IL-16 release in vivo, a possibility currently being studied in vitro. Subject 2 had mild chronic airflow obstruction and a high PC20 for methacholine responsiveness. We do not know the significance of these differences on the generation of IL-16 protein. At 15 minutes after saline and histamine challenge, asthmatic subjects 1 and 3 had IL-16 present in their airways on the basis of inhibition of the chemotactic activity by IL-16 neutralizing antibodies (data not shown). It is possible that these subjects released IL-16 before challenge as a result of an independent stimulus before arriving in the laboratory. Possibilities include environmental antigens, exercise, or cold air.

In conclusion, we found that we could attribute all of the T lymphocyte chemoattractant activity in the BALF of asthmatic subjects but not normal or atopic nonasthmatic subjects 6 hours after local subsegmental challenge with histamine to IL-16. A reasonable hypothesis for this finding is that after exposure of atopic asthmatic airways to antigen, mast cell-derived histamine is released, which can act in a paracrine fashion to induce release of IL-16 from resident CD8⁺ T cells (recruited as part of the atopic state) or more likely, epithelial cells (changed phenotypically to synthesize and constitutively express IL-16 protein). The release of IL-16 within hours would permit it to participate in the inflammatory cascade of events that follow, characterized by recruitment of CD4⁺ T cells, eosinophils, and monocytes. The ability of IL-16 to activate CD4⁺ T cells¹⁴ to express IL-2R and MHC class II molecules is in concert with the activation state of CD4⁺ T cells identified in the mucosa of asthmatic subjects.⁵ Last, the constitutive storage of bioactive IL-16, which is susceptible to secretion after histamine challenge,^{12, 13} probably would explain the early detection solely of IL-16 and would also suggest an unique relation between histamine and IL-16 in asthma.

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Correction

The following correction applies to the article by Corren et al. entitled "Efficacy and safety of loratadine plus pseudoephedrine in patients with seasonal allergic rhinitis and mild asthma," which appeared in volume 100, pp. 781-788, 1998, of The Journal.

In Table I, the value given for AM PEFR in the "L/P" column currently reads "295.1." It should read "395.1."