

A comparison of the airway response to segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis

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Background: Patients with allergic asthma and those with allergic rhinitis (without asthma) share many immunopathologic features but differ in the presence of lower airway symptoms in response to antigen.

Objectives: We sought to compare the airway inflammatory response to antigen in patients with atopic asthma and allergic rhinitis.

Methods: Segmental bronchoprovocation with saline or ragweed antigen was performed in 9 patients with atopic asthma and 9 patients with allergic rhinitis without asthma. The antigen dose used in segmental bronchoprovocation was 10% of the dose that caused a 20% decrease in FEV₁ in response to inhalation challenge. Bronchoalveolar lavage (BAL) was performed from the saline- and antigen-challenged segments at 5 minutes and 48 hours after challenge. BAL fluid was analyzed for cell count and differential, distribution of lymphocytes, and concentration of soluble factors (histamine, IL-5, matrix metalloproteinase 9, tissue inhibitor of metalloproteinase 1, and fibronectin). In addition, BAL cells were cultured *ex vivo*, and IL-5, IFN- γ , and IL-10 generation was measured.

Results: Antigen challenge led to similar patterns of cellular recruitment, mediator levels, and BAL cell cytokine generation in both groups; however, the dose of antigen required to promote comparable responses in the airway was significantly less in patients with asthma.

Conclusion: These data suggest that the pattern of acute airway inflammation in response to allergen does not by itself explain antigen-induced lower airway obstruction and asthma symptoms. We speculate that other factors, such as increased airway sensitivity to allergen or preexisting airway injury and remodeling, might explain why patients with asthma and rhinitis differ in their clinical and physiologic response to antigen exposure. (*J Allergy Clin Immunol* 2003;111:79-86.)

Key words: Allergy, asthma, airway inflammation, bronchoalveolar lavage, segmental bronchoprovocation, cytokines

Abbreviations used

BAL:	Bronchoalveolar lavage
FVC:	Forced vital capacity
LPR:	Late-phase response
MMP:	Matrix metalloproteinase
PNU:	Protein nitrogen units
SBP:	Segmental bronchoprovocation
TIMP:	Tissue inhibitor of metalloproteinase

There is epidemiologic and pathophysiologic evidence to suggest that atopic asthma and allergic rhinitis are linked; they often coexist, and allergic rhinitis might be a risk factor for subsequent development of asthma.¹⁻³ In both atopic asthma and allergic rhinitis, exposure to allergen promotes upper airway symptoms of rhinorrhea, sneezing, and nasal obstruction. Allergic reactions in the lung and nose involve many of the same inflammatory cells and mediators, including eosinophils, mast cells, neutrophils, basophils, and CD4⁺ T lymphocytes, along with IgE, histamine, leukotrienes, prostaglandins, and cytokines, notably IL-5.³ Despite these similarities in the response to allergens, the development of lower airway disease (ie, recurrent wheezing and bronchial obstruction) is limited to patients with asthma. It is unclear whether the magnitude of the inflammatory reaction to inhaled allergen contributes to the manifestation of the lower airway disease or if other factors, such as sensitivity of the airway, determine the expression of asthma.

Local airway antigen challenge has been established as a model to investigate the features and mechanisms of antigen-induced airway inflammation. Local administration of antigen induces an immediate allergic response characterized by mast cell-mediated histamine release, followed several hours later by eosinophilic airway inflammation. We and others have demonstrated both a decrease in airway function and a vigorous airway inflammatory response after local antigen challenge in atopic patients with or without asthma.⁴⁻⁸ Interestingly, Moore et al⁸ recently reported that patients with mild and moderate disease had a similar inflammatory response to local airway antigen challenge. It is unclear whether the response of the patients with mild asthma would be different from that of allergic patients who did not have asthma. Therefore we sought to evaluate the relationship between clinical manifestations of allergic airway diseases (rhinitis vs asthma) and the intensity (or type) of antigen-induced airway inflammation.

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Asthma, rhinitis,
other respiratory
diseases

TABLE I. Patient characteristics*

	Allergic rhinitis (n = 9)	Atopic asthma (n = 9)	P value
Mean age, y (range)	28 (21-42)	26 (20-43)	NS
Sex	7M:2F	4M:5F	
FEV ₁ (% predicted)	115 ± 12	106 ± 13	NS
Methacholine PC ₂₀ (mg/mL)	17 ± 5	2 ± 2	<.001
Antigen PD ₂₀ (PNU) [†]	971 ± 588	212 ± 310	.003
Immediate (15 min) decrease in FEV ₁ (%)	19 ± 9	27 ± 5	.03
Late (4-8 h) decrease in FEV ₁ (%) [‡]	4 ± 7	13 ± 9	.05
Ragweed dose for antigen with SBP (PNU) [†]	97 ± 59	21 ± 31	.004
Skin test response to ragweed (+ size)	3.7 ± 0.5	3.6 ± 0.5	NS
Total serum IgE (IU/mL)	2274 ± 2483	2887 ± 1890	NS

NS, Not significant.

*Data are expressed as means ± SD unless otherwise noted.

[†]1 PNU = 0.005 µg.[‡]Data are available from 6 patients with allergic rhinitis and 8 atopic asthmatic patients.

To establish what differences might exist between the allergic response in asthma versus rhinitis, we performed segmental bronchoprovocation (SBP) with antigen in the 2 groups and compared the airway response by using bronchoalveolar lavage (BAL). BAL fluid was collected at 5 minutes and 48 hours after challenge to determine whether the airway response to antigen in patients with asthma was associated with a different pattern (cells, mediators, or proteins) or intensity of response at 5 minutes (immediate) or 48 hours (late) after challenge. Such findings could shed light on the pathophysiology of lower airway obstruction and symptoms in asthma.

METHODS

Subjects

Eighteen patients with ragweed allergy, 9 with mild asthma, and 9 with allergic rhinitis without asthma were recruited for the study (Table I). Selection criteria for asthma were in accordance with the American Thoracic Society guidelines⁹ and included a history of asthma and a methacholine PC₂₀ of less than 8.0 mg/mL. Patients with allergic rhinitis had a positive skin prick test response and a history of hay fever to ragweed, no history of asthma, normal pulmonary function, and normal airway responsiveness to methacholine (PC₂₀ >8.0 mg/mL). All subjects had FEV₁ values of greater than 80% of predicted value, had a 3+ or greater skin prick test response to ragweed, were nonsmokers, were without respiratory infections within 30 days of the study, and had not received antihistamines or β-agonists within 7 days, nedocromil within 14 days, or corticosteroids or cromolyn sodium within 30 days of study enrollment. Informed consent was obtained from each subject before participation. The study was approved by the University of Wisconsin-Madison Center for Health Sciences Human Subjects Committee.

Pulmonary function testing

Spirometry¹⁰ and methacholine challenges¹¹ were performed according to American Thoracic Society guidelines. Consecutively higher concentrations of methacholine were administered through a nebulizer attached to a French-Rosenthal dosimeter¹² until the FEV₁ decreased by 20% or greater from baseline.

Selection of allergen dose for SBP

One month before bronchoscopy, a graded inhaled antigen challenge was performed in each patient to determine the antigen PD₂₀. Graded doses of ragweed antigen (GS ragweed mix; Greer Labs, Lenoir, NC) were administered as described for methacholine, except that the interval between challenge doses was increased from 5 to 10 minutes to allow for the potential development of an immediate-phase response. A cumulative dose of 1500 protein nitrogen units (PNU) was assigned to patients who did not have a 20% decrease in FEV₁ at the maximal dose of 1444 PNU (4/9 patients with allergic rhinitis). Patients were monitored for the maximum immediate (within 15 minutes) decrease in FEV₁ and late-phase response (LPR; a sustained 15% decrease in FEV₁ that lasted at least 15 minutes and occurred from 4 to 8 hours after challenge).

SBP and BAL

Spirometry was performed immediately before and 30 minutes after each bronchoscopy. Bronchoscopy and SBP were performed as previously described.⁷ Ten milliliters of saline (0.9% NaCl) was instilled into one segment (SBP with saline), and in a separate segment SBP with antigen was performed with ragweed antigen by using a dose equal to 10% of the antigen PD₂₀. The saline and antigen segments were from different lobes; an upper-lobe segment was used for the saline, whereas a middle-lobe segment (lingula) was used for the antigen challenge. BAL was performed in each segment 5 minutes (immediate) and 48 hours (late) after challenge. Spirometry measurements were obtained within 30 minutes before and again 30 minutes after each bronchoscopic procedure.

Analysis of BAL fluid

BAL fluid was kept on ice throughout processing. BAL cells were recovered from the lavage fluid by means of centrifugation at 200g for 10 minutes at 4°C. Total cell numbers were determined by means of hemacytometer, and differential cell counts were performed on cytopsin preparations stained with a modified Giemsa-based Diff-Quik stain (Baxter Scientific Products, McGraw Park, Ill).

Cell cultures

Unseparated BAL cells were cultured at 2 × 10⁶ viable cells per milliliter in the presence or absence of 10 µg/mL PHA (Sigma, St

TABLE II. Lung function 5 minutes (immediate) and 48 hours (late) after challenge*

	Immediate		Late	
	Before BAL	After BAL	Before BAL	After BAL
FEV ₁ (L)				
Rhinitis	4.5 ± 0.6	4.3 ± 0.7	4.5 ± 0.6	4.4 ± 0.7
Asthma	4.0 ± 0.7	3.9 ± 0.9	3.9 ± 0.7‡	4.0 ± 0.7
FVC (L)				
Rhinitis	5.5 ± 0.9	5.0 ± 1.0†	5.4 ± 0.7	5.0 ± 0.8†
Asthma	4.9 ± 1.1	4.5 ± 1.1†	4.7 ± 1.0‡	4.7 ± 1.0†
FEV ₁ /FVC (%)				
Rhinitis	83 ± 7	88 ± 7†	83 ± 6	88 ± 4†
Asthma	82 ± 6	87 ± 6†	83 ± 5	87 ± 5†

*Data are expressed as means ± SE.

Within-group comparisons: †before BAL with after BAL, $P < .05$; ‡immediate with late, $P < .05$; asthma versus rhinitis: no significant difference.

Louis, Mo), as previously described.¹³ Cells were cultured in triplicate for 48 hours at 37°C and 5% CO₂ in a humidified incubator.

Flow cytometric analysis

BAL cells (1×10^5 cells) or 100-μL aliquots of whole blood were stained by means of simultaneous addition of FITC- and phycoerythrin-conjugated antibodies specific for cell-surface markers (Becton Dickinson Immunocytometry Systems, San Jose, Calif), as previously described.¹³

Protein analysis

Total protein and histamine levels were measured in 1× BAL fluid by means of the Lowry assay and a radioenzymetric assay,¹⁴ respectively. To measure cytokines, BAL fluids were concentrated 20× at 4°C by using a low-protein-binding, 3-kd cut-off concentrator (Centriprep; Amicon, Beverly, Mass). A sensitive 2-step sandwich ELISA was used to measure cytokines-chemokines in BAL fluids and diluted cell-culture supernatant fluids, as previously described.¹³ Monoclonal cytokine-specific antibodies (unlabeled coating antibodies and biotinylated detection antibodies) were purchased from PharMingen (San Diego, Calif). The sensitivity for each ELISA was 3 pg/mL or less. Soluble fibronectin was measured in a similar fashion by using a purified mAb with specificity to human plasma fibronectin (clone 1601; Biotest International, Kennebunk, Md) as the coating antibody and a horseradish peroxidase-conjugated rabbit anti-human fibronectin antibody (DAKO Corp, Carpinteria, Calif) for detection. The sensitivity for the fibronectin assay was less than 12.5 ng/mL. Metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinase 1 (TIMP-1) were measured with commercially available ELISA kits (RPN 2614 and RPN 2618, respectively; Amersham Life Sciences, Arlington Heights, Ill). The MMP-9 ELISA detects free proMMP-9 and proMMP-9/TIMP complexes but not active MMP-9. The assay sensitivities were 0.6 ng/mL for MMP-9 and 1.25 ng/mL for TIMP-1. Serum concentrations of total IgE were measured by using a commercially available ELISA (AlerCHECK, Inc, Portland, Me). The assay sensitivity was 2 IU/mL.

Statistical analysis

Except where noted, data are expressed as medians with 25% and 75% interquartiles. A Wilcoxon signed-rank test (or paired *t* test for normally distributed data) was used to compare data obtained 5 minutes and 48 hours after SBP with saline or antigen within the allergic rhinitis or asthma groups. To compare data in the asthma and rhinitis groups, a Mann-Whitney rank sum test (or an unpaired

t test) was used. Correlations were tested by using Spearman rank order correlation. A *P* value of less than .05 was considered significant. Statistical analysis was performed by using the SigmaStat software package (Jandel Scientific Software, San Rafael, Calif).

RESULTS

Subject characteristics

Asthmatic subjects ($n = 9$) had mild intermittent disease with normal spirometry results (FEV₁ >80%) and 12% or greater reversibility to inhaled β-agonist, increased airway hyperresponsiveness to methacholine, or both (Table I). Subjects with allergic rhinitis ($n = 9$) had normal spirometry results and no airway hyperresponsiveness to methacholine (PC₂₀ >8 mg/mL). The ragweed PD₂₀ was significantly lower among patients with asthma. Even with a lower antigen dose, patients with asthma had a greater immediate- and late-phase decrease in FEV₁. Despite the increased airway sensitivity to ragweed, both groups had similar skin test responses to ragweed and comparable levels of total serum IgE.

Effect of airway allergen challenge on pulmonary physiology

FEV₁ did not change immediately after SBP with antigen (immediate, after BAL) or after the 48-hour (late) lavage; however, there was a modest but statistically significant post-BAL decrease in forced vital capacity (FVC) and increase in the FEV₁/FVC ratio in both groups on both days (Table II). Forty-eight hours after SBP with antigen, patients with mild asthma had a statistically significant but very slight decrease in FEV₁ and FVC compared with the values before the first BAL (Table II). This small change in lung function was not seen in patients with allergic rhinitis.

Cellular inflammation after SBP with antigen

The mean BAL fluid return volume for all segments was 75% ± 8% (range, 49%-92%). There were no significant differences among segments (within-group comparisons) or between the asthma and rhinitis groups (Table

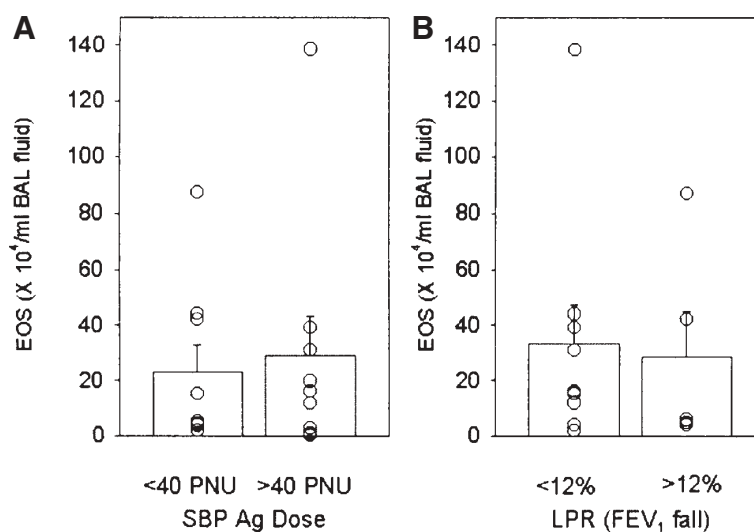


FIG 1. A, Patients were divided into 2 arbitrary groups on the basis of the median antigen dose (37.4 PNU) administered for SBP. The dose for the group that is designated less than 40 PNU ranged from 0.3 to 16.7 PNU, with a mean $\pm$ SD of 6.7 ± 7.0 PNU; the group designated greater than 40 PNU had a range of 58 to 150 PNU, with a mean $\pm$ SD of 111 ± 40 PNU. Data represent the number of eosinophils $\times 10^4/\text{mL}$ of BAL fluid obtained 48 hours after antigen with SBP. *Circles* represent data from individual subjects; *bars* represent the group mean $\pm$ SE. **B**, Patients were divided into groups on the basis of the presence or absence of an LPR (defined as an FEV₁ decrease of $\geq 12\%$ 4-8 hours after inhalation antigen challenge). The percentage decrease in the group that is designated LPR 12% or greater ranged from 14% to 30%, with a mean $\pm$ SD of $19.6\% \pm 6.3\%$; the group designated LPR 12% or less had a range of 0% to 9%, with a mean $\pm$ SD of $3.4\% \pm 4.2\%$. Data represent the number of eosinophils $\times 10^4/\text{mL}$ of BAL fluid obtained 48 hours after antigen with SBP. *Circles* represent data from individual subjects; *bars* represent the group mean $\pm$ SE. LPR data were available from 8 patients with asthma and 6 patients with rhinitis. *EOS*, Eosinophils; *Ag*, antigen.

III). In both groups total numbers of cells were significantly increased 48 hours after SBP with antigen. Forty-eight hours after challenge, the percentage of eosinophils increased significantly in all antigen-challenged segments and to a much lesser extent in the saline-challenged segments. After SBP with antigen, both total numbers and relative percentages of eosinophils tended to be greater in patients with rhinitis compared with those in asthmatic patients, but these differences were not statistically significant. Because the rhinitis group as a whole received a higher SBP antigen dose, we determined the relationship between BAL eosinophilia and antigen dose. When data from all patients were combined and individual patients were grouped according to antigen dose, there were no differences in total numbers (Fig 1, A) or relative percentages (data not shown) of BAL eosinophils. There was a small but significant increase in BAL neutrophils in both groups 48 hours after antigen or saline challenge, and the percentage of alveolar macrophages was decreased in both groups 48 hours after antigen or saline challenge.

BAL lymphocytes after SBP with antigen

In the rhinitis group there was a small but significant decrease in the percentage of CD3⁺ cells 48 hours after antigen challenge (Table III), which was not seen in the asthma group. In fact, CD3⁺ cells made up a greater pro-

portion of BAL lymphocytes in the asthma group than in the rhinitis group, both at baseline and after antigen challenge (Table III). The percentages of CD4⁺ BAL cells did not vary after antigen challenge and were not different between the asthma and rhinitis group. The percentages of CD8⁺ cells were significantly decreased after antigen challenge in both groups, and values between the 2 groups were similar. The percentages of CD4⁺CD25⁺ cells were also similar between the 2 groups and remained unchanged after challenge.

Soluble factors in BAL fluid after SBP with antigen

At baseline (saline segment, immediate), soluble factors were similar between the asthma and rhinitis groups, except for higher fibronectin levels in the asthma group (Table IV). The total protein concentration in BAL fluid significantly increased in both groups 48 hours after SBP with antigen (Table IV). This increase was greater in patients with rhinitis than in asthmatic patients. In the asthma group significantly increased levels of protein also occurred in the saline-challenged segment. Histamine levels in both groups increased significantly 5 minutes after antigen challenge (antigen, immediate vs saline, immediate) and decreased by 48 hours. IL-5, MMP-9, TIMP-1, and fibronectin levels were significantly increased 48 hours after chal-

TABLE III. BAL cells 5 minutes (immediate) and 48 hours (late) after challenge*

	Saline segment		Antigen segment	
	Immediate	Late	Immediate	Late
BAL fluid recovery (%)				
Rhinitis	76 (72-79)	74 (70-84)	78 (73-82)	76 (70-90)
Asthma	79 (73-86)	73 (63-79)	77 (68-85)	73 (72-78)
Total cells ($\times 10^4$ /mL BAL fluid)				
Rhinitis	12 (11-15)	19 (10-21)	10 (5-12)	55 (28-115) ^{†‡§}
Asthma	18 (14-21)	26 (17-49)	15 (13-24)	29 (22-49) ^{†§}
Eosinophils (%)				
Rhinitis	0 (0-0)	1 (1-2) [†]	0 (0-0)	36 (28-43) ^{†‡§}
Asthma	0 (0-1)	2 (1-3) [†]	0 (0-1)	19 (11-37) ^{†‡§}
Neutrophils (%)				
Rhinitis	1 (0-3)	5 (3-11) [†]	0 (0-1)	4 (3-7) [§]
Asthma	1 (0-1)	6 (3-15) [†]	0 (0-1)	3 (2-3) ^{†§}
Macrophages (%)				
Rhinitis	89 (81-94)	77 (75-86) [†]	90 (81-93)	46 (35-58) ^{†‡§}
Asthma	83 (79-87)	71 (62-77) [†]	83 (80-89)	60 (46-66) ^{†§}
Lymphocytes (%)				
Rhinitis	7 (4-13)	10 (8-16) [†]	7 (6-14)	14 (11-16)
Asthma	14 (11-20)	14 (10-23)	15 (9-16)	14 (11-26)
CD3 (% lymphocytes)				
Rhinitis	88 (85-91)	91 (84-94)	88 (84-90)	77 (75-86) ^{†‡}
Asthma	93 (90-95)	91 (88-92)	89 (85-93)	90 (87-92)
CD4 (% lymphocytes)				
Rhinitis	45 (40-54)	49 (44-60)	46 (42-50)	53 (43-60)
Asthma	55 (43-60)	54 (46-69)	51 (46-55)	64 (52-71)
CD8 (% lymphocytes)				
Rhinitis	40 (29-53)	32 (26-47)	33 (27-47)	18 (13-27) ^{†‡}
Asthma	33 (24-46)	26 (22-35) [†]	34 (25-45)	20 (20-29) [†]
CD25 (% of CD4 ⁺)				
Rhinitis	32 (28-33)	32 (31-33)	29 (27-33)	36 (26-42)
Asthma	36 (25-43)	36 (33-41)	27 (26-38)	38 (33-42)

*Data are expressed as median (25%-75% quartile).

Within-group comparisons: [†] $P < .05$ compared with immediate saline, [‡] $P < .05$ for late antigen versus late saline, [§] $P < .05$ for late antigen versus immediate antigen; ^{||}asthma versus rhinitis: $P < .05$.

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challenge in the antigen-challenge segment of both groups. Levels of these factors at 48 hours tended to be higher in the rhinitis group than in the asthma group, but these differences were only statistically significant for MMP-9. There was a significant positive correlation between the number of BAL eosinophils isolated at 48 hours and levels of IL-5 ($r = 0.759$, $P < .001$), TIMP-1 ($r = 0.772$, $P < .001$) and fibronectin ($r = 0.797$, $P < .001$) but not MMP-9. BAL eosinophils at 48 hours also correlated with levels of histamine at 5 minutes ($r = 0.651$, $P = .003$).

Ex vivo capacity of BAL cells to produce cytokines

When stimulated ex vivo with PHA, BAL cells generated large amounts of IL-5, IFN- γ , and IL-10 (Table V). With the exception of a greater generation of IFN- γ by BAL cells obtained from patients with rhinitis 5 minutes

after antigen challenge, there were no statistically significant differences between the asthma and rhinitis groups in absolute levels of PHA-induced cytokines. The potential of BAL cells to generate IL-5 was significantly increased 48 hours after SBP with antigen in both the asthma and rhinitis groups. In both groups IL-10 generation increased in BAL cells isolated 48 hours after antigen challenge, but this increase reached statistical significance only in the asthma group.

Relationship between LPR and antigen-induced BAL eosinophilia

Data from patients with asthma and rhinitis were combined and then divided according to the presence or absence of an LPR ($>12\%$ decrease in FEV₁). The total numbers (Fig 1, B) and the relative percentage (not shown) of BAL eosinophils were comparable in the LPR responder and nonresponder groups.

TABLE IV. BAL fluid soluble factors 5 minutes (immediate) and 48 hours (late) after SBP with antigen*

	Saline segment		Antigen segment	
	Immediate	Late	Immediate	Late
Total protein ($\mu\text{g/mL}$)				
Rhinitis	87 (58-148)	141 (67-300)	90 (56-115)	437 (293-1005)‡§
Asthma	95 (62-124)	165 (103-250)†	109 (96-153)	234 (156-320)†§
Histamine (pg/mL)				
Rhinitis	194 (114-249)	111 (87-178)	703 (255-7184)†	258 (175-401)‡§
Asthma	125 (80-177)	92 (62-102)	241 (180-723)†	160 (94-268)‡§
IL-5 (pg/mL)				
Rhinitis	0 (0-3)	0 (0-5)	0 (0-2)	50 (9-157)†‡§
Asthma	0 (0-2)	1 (0-3)	0 (0-2)	8 (2-22)†‡§
MMP-9 (pg/mL)				
Rhinitis	294 (74-971)	1547 (273-2693)	57 (24-267)	4656 (907-8813)†§
Asthma	540 (210-1230)	710 (202-1553)	90 (17-258)	1020 (442-1991)§
TIMP-1 (pg/mL)				
Rhinitis	777 (509-3146)	2047 (1190-4217)	1458 (609-2069)	7377 (5971-14,070)†‡§
Asthma	2669 (2000-3816)	2020 (910-4192)	1351 (171-2271)	5115 (2768-8798)§
Fibronectin (ng/mL)				
Rhinitis	453 (333-1106)	2068 (1599-5439)†	533 (119-1884)	20,811 (8887-28,756)†‡§
Asthma	1850 (1390-2582)	2253 (1837-15,347)	1324 (795-2259)	10,603 (4685-15,586)†§

*Data are expressed as median (25%-75% quartile) for 1 $\times$ BAL fluid.

Within-group comparisons: † $P < .05$ compared with immediate saline, ‡ $P < .05$ for late antigen versus late saline, § $P < .05$ for late antigen versus immediate antigen; ||asthma versus rhinitis, $P < .05$.

TABLE V. PHA-induced cytokine generation by BAL cells obtained 5 minutes (immediate) and 48 hours (late) after SBP with antigen*

	Saline segment		Antigen segment	
	Immediate	Late	Immediate	Late
IL-5 (pg/mL supernate)				
Rhinitis	72 (17-360)	156 (47-571)	158 (131-380)	2801 (473-3635)†‡§
Asthma	57 (30-128)	120 (83-468)†	73 (43-117)	818 (439-1549)†‡§
IFN- γ (pg/mL supernate)				
Rhinitis	3603 (1941-7285)	2332 (1213-7425)	3752 (3159-7486)	2055 (1611-5345)
Asthma	3376 (1253-7029)	2170 (1709-5795)	1144 (458-2559)	2613 (644-4434)
IL-10 (pg/mL supernate)				
Rhinitis	105 (75-135)	213 (92-350)	157 (102-525)	653 (422-952)
Asthma	138 (48-334)	233 (79-972)†	179 (35-370)	880 (235-1236)†‡§

*Data are expressed as median (25%-75% quartile).

Within-group comparisons: † $P < .05$ compared with immediate saline, ‡ $P < .05$ for late antigen versus late saline, § $P < .05$ for late antigen versus immediate antigen; ||asthma versus rhinitis, $P < .05$.

DISCUSSION

Our findings demonstrate that the airways of patients with allergic rhinitis and asthma have a similar pattern of allergic inflammation in response to antigen provocation but that asthmatic patients are more sensitive (eg, the PD₂₀ was nearly 5-fold less in asthmatic patients compared with that in patients with rhinitis). Yet when the 2 groups were given physiologically equivalent doses of antigen (ie, 10% of the individual's antigen PD₂₀), airway inflammation was comparable. The 2 groups showed similar increases in BAL cells, functional capacity of BAL cells for ex vivo generation of cytokines (IL-5, IFN- γ , and IL-10), and BAL fluid levels of histamine, IL-5, and proteins that could be associated with airway injury and repair (MMP-1, TIMP-1, and fibronectin).¹⁵ Asthmatic patients were also more

sensitive to methacholine, and increased sensitivity to a specific antigen might relate to those factors that contribute to airway responsiveness in general.

Our data corroborate and expand on recent observations of Moore et al,⁸ who performed SBP with ragweed antigen in patients with mild (mean FEV₁ 85.5% predicted) and moderate (mean FEV₁ 68.8% predicted) asthma. Twenty-four hours after challenge with an identical high dose (480 PNU) of antigen, patients with mild and moderate asthma had similar inflammatory responses, with no significant differences in BAL cell counts or BAL fluid levels of IL-1 β , IL-4, IL-5, IL-6, GM-CSF, or TNF- α . Taken together with our observations, these findings might imply that rhinitis and asthma are a continuum of disease rather than 2 separate entities, at least in regard to the allergic inflammatory response.

Our report is unique in that we compared antigen-induced airway inflammation in patients with allergic rhinitis and asthma by using an antigen dose based on the physiologic responsiveness of the airway to that antigen. Several studies comparing antigen-induced airway inflammatory response in asthma and rhinitis have been published,^{4-6,16,17} but they have differed in choice of antigen, determination of dose, timing and methods for sampling the airway, and mode of administration. Nevertheless, in most of these studies, comparable results have been obtained, suggesting that there are few or no qualitative differences in eosinophilic airway inflammation between asthma and rhinitis. One exception is a report showing that an extremely low (eg, 0.19 PNU) dose of ragweed antigen induced significant airway eosinophilia in asthmatic patients but not in patients with allergic rhinitis.¹⁷ The antigen doses used in that study might have been less than the response threshold of the patients with rhinitis, which would further support the notion that the asthmatic phenotype is determined in part by increased sensitivity to antigen rather than by the type of the inflammatory response.

In our study it is of interest that the asthma group had significantly greater sensitivity to airway antigen challenge compared with that of the rhinitis group, yet the degree of atopy, as indicated by skin test response to a single dose of ragweed and by total circulating IgE levels, was similar for the 2 groups. These data suggest that although airway sensitivity to antigen is greatly enhanced in asthma, there is not an overall increase in atopic status. Thus the primary difference between atopic patients with asthma and those with rhinitis appears to be restricted to the target organ (ie, the lower airway).

It has been reported that the ability to mount an LPR to inhaled antigen rather than the presence or absence of asthma determines the magnitude of antigen-induced airway eosinophilia.⁵ Our study did not specifically address the contribution of LPR to antigen-induced airway inflammation; however, when data from the patients with asthma and rhinitis were analyzed according to the presence or absence of an LPR, the total number of BAL eosinophils was equivalent in the LPR responders and nonresponders (Fig 1, B). Our data suggest that when a sufficient (physiologic) concentration of antigen is administered, the majority of atopic patients, regardless of disease state, have a significant airway eosinophil response. Furthermore, these findings suggest that antigen-induced LPR at 4 to 8 hours and BAL eosinophilia at 48 hours after SBP with antigen might not be linked.

In addition to airway inflammation, it is important to understand how antigen exposure affects airway remodeling. Crimi et al⁴ performed bronchial biopsies on asthmatic patients and patients with rhinitis after whole-lung antigen challenge and found no differences in the reticular basement membrane between the groups. Although our studies were limited to the airway lumen, we found that after segmental antigen challenge, BAL fluid levels of MMP-9, TIMP-1, and fibronectin (soluble factors that are linked to airway injury or repair) were similar in the

asthma and rhinitis groups. Interestingly, at baseline, fibronectin levels were higher in the asthma group than in the rhinitis group, and there was a trend toward increased levels of MMP-9 and TIMP-1. The differences in these parameters of airway injury and repair might reflect ongoing airway-remodeling events that are a key feature of asthma.

In summary, we have shown that segmental challenge of asthmatic patients with ragweed antigen provoked similar increases in inflammatory cells and soluble factors to those observed in patients with rhinitis but with a significantly lower antigen dose. Our findings suggest that the asthmatic phenotype is characterized by significantly enhanced nonspecific (methacholine) and specific (antigen) airway hyperresponsiveness. Although we did not directly address the mechanisms for this heightened responsiveness, we speculate that enhanced sensitivity to inflammatory mediators, the persistence of airway inflammation, or structural airway changes might singularly (or in concert) contribute to the development of lower airway obstruction and symptoms in response to antigen in asthmatic patients.

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