

Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways

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Background: Allergic rhinitis (AR) and asthma are characterized by means of a similar inflammatory process in which eosinophils are important effector cells. The migration of eosinophils from the blood into the tissues is dependent on adhesion molecules.

Objective: To analyze the aspects of nasobronchial cross-talk, we studied the expression of adhesion molecules in nasal and bronchial mucosa after nasal allergen provocation (NP).

Methods: Nine nonasthmatic subjects with seasonal AR and 9 healthy control subjects underwent NP out of season.

Bronchial and nasal biopsy specimens were taken before (T_0) and 24 hours after NP (T_{24}). Mucosal sections were analyzed for the presence of eosinophils, IL-5, eotaxin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, and human endothelium (CD31).

Results: At T_{24} , an influx of eosinophils was detected in nasal epithelium ($P = .01$) and lamina propria ($P < .01$), as well as in bronchial epithelium ($P = .05$) and lamina propria ($P < .05$), of the patients with AR. At T_{24} , increased expression of ICAM-1, as well as increased percentages of ICAM-1⁺, VCAM-1⁺, and E-selectin⁺ vessels, were seen in nasal and bronchial tissue of patients with AR. The number of mucosal eosinophils correlated with the local expression of ICAM-1, E-selectin, and VCAM-1 in patients with AR.

Conclusion: This study shows that NP in patients with AR results in generalized airway inflammation through upregulation of adhesion molecules. (*J Allergy Clin Immunol* 2001;107:469-76.)

Key words: Allergic rhinitis, nasal provocation, bronchial inflammation, eosinophils, adhesion molecules, nose-lung interaction

Allergic rhinitis (AR) and asthma are closely related diseases that share a common genetic background.^{1,2} Accumulating evidence underlines the importance of AR in the development³⁻⁵ and control of asthma.⁶⁻⁸ However, pathophysiologic interactions between upper and lower airways are not well described.

It has been well established that allergic mucosal inflammation in allergic asthma and rhinitis is character-

Abbreviations used

AR:	Allergic rhinitis
ICAM-1:	Intercellular adhesion molecule 1
NP:	Nasal allergen provocation
PEF:	Peak expiratory flow
PNIF:	Peak nasal inspiratory flow
VAS:	Visual analogue scale
VCAM-1:	Vascular cell adhesion molecule 1

ized by tissue eosinophilia.^{9,10} Tissue eosinophilia is the result of several sequential events. Eosinophils are released from the bone marrow under influence of serum IL-5 and eotaxin, resulting in an increase of circulating eosinophils.^{11,12} Eosinophil migration is dependent on the expression of cytokines, chemokines, and adhesion molecules.^{13,14} Upregulation of endothelial adhesion molecules through the release of IL-1 β , IL-4, and TNF- α from resident cells (eg, mast cells and T lymphocytes)¹⁵⁻¹⁷ results in firmer leukocyte-endothelial adherence and transendothelial migration of leukocytes along a chemotactic gradient. Intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin are known endothelial adhesion molecules. ICAM-1 and E-selectin mediate the attachment of all classes of leukocytes to endothelial cells, whereas VCAM-1 interacts with very late antigen 4, which is expressed on lymphocytes, monocytes, eosinophils, and basophils, but not on neutrophils.¹³ Increased expression of ICAM-1, VCAM-1, and E-selectin has been demonstrated locally after experimental nasal and bronchial allergen challenge.^{14,18}

The possibility of cross-talk between nasal and bronchial mucosa after nasal allergen provocation (NP) leading to endothelial activation has not been examined. The aim of the current study was to determine allergic mucosal inflammation and adhesion molecule expression simultaneously in the upper and lower airways after NP. Therefore we designed a study in which nasal and bronchial mucosal biopsy specimens, as well as blood samples, were taken from a group of nonasthmatic patients with AR with an isolated grass pollen allergy after NP. Eosinophils, major effector cells in allergic inflammation, IL-5⁺ cells, and eotaxin-positive cells, which are necessary for eosinophil survival and chemotaxis, were chosen as markers of mucosal allergic inflammation. Because IL-5 is vital for the differentiation of progenitor cells into mature eosinophils, we determined serum IL-5 concentrations before and after NP.

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TABLE I. Subject characteristics

Patient	Age (y)	Sex	FEV ₁ (L)	FEV ₁ (%)	IVC (L)	FEV ₁ /VC	BAR* (%)	PC ₂₀ (mg/mL)
Patients with AR								
1	22	F	3.93	108	4.72	88	103	40
2	34	M	3.20	87	3.65	88	98	40
3	26	M	4.37	95	5.67	77	104	40
4	25	M	4.51	101	4.91	92	105	40
5	22	F	3.82	112	4.06	94	100	40
6	26	F	3.28	93	4.28	77	104	38.4
7	22	M	4.26	90	5.19	82	107	19.6
8	26	F	4.72	126	5.52	86	102	20
9	27	F	3.34	102	3.80	88	105	40
Control subjects								
10	30	M	5.30	110	7.49	76	108	32.4
11	21	F	4.34	114	5.41	80	104	32.7
12	22	M	5.75	109	6.64	87	102	33.5
13	19	F	3.79	104	4.79	79	107	40
14	22	M	5.75	118	6.38	90	100	40
15	25	M	4.14	94	4.75	87	101	40
16	21	M	4.68	97	5.55	84	102	40
17	24	M	4.09	86	5.35	76	103	40
18	32	M	4.96	113	6.39	78	102	40

IVC, Inspiratory vital capacity; BAR, β -agonist response.

* β -Agonist response (terbutaline, 1000 μ g) data are presented as percentage improvement compared with initial value; a virtual value of 40 was assigned to subjects who did not reach a PC₂₀ with 38 mg/mL methacholine.

METHODS

Subject groups

Nine patients with AR (4 men and 5 women; age range, 22-34 years) and 9 nonallergic healthy control subjects (7 men and 2 women; age range, 19-32 years) were selected for the study. Subject characteristics are shown in Table I. The patients with AR had at least a 2-year history of isolated grass pollen allergy confirmed by a positive skin prick test reaction to grass pollen extract alone (Vivodiagnost; ALK Benelux BV, Groningen, the Netherlands) and not to 13 other common allergens. None of the study subjects had a clinical history of asthma. All had a normal FEV₁ and a methacholine PC₂₀ of greater than 8 mg/mL. Methacholine was administered according to a standardized tidal breathing method.¹⁹ None of the subjects smoked or used medication that could influence the results of this study. Biopsy specimens were obtained out of the grass pollen season. None of the subjects had experienced a respiratory tract infection in the 4 weeks before allergen challenge. All participants provided informed written consent, which was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands.

Experimental design

The study design is outlined in Fig 1. Bronchial and nasal biopsy specimens, as well as blood samples, were collected before (T₀) and 24 hours (T₂₄) after NP. Nasal and bronchial symptoms (visual analogue scale [VAS] scores), peak nasal inspiratory flow (PNIF), and peak expiratory flow (PEF) were recorded at baseline (T₀), at one half hour after NP (T_{1/2}), at 2-hour intervals after NP (T₂-T₁₂), and at day 2 (T₂₄ and T_{24 1/2}). PNIF was measured with a Youlten peak nasal inspiratory flowmeter (Armstrong Industries, Inc, Northbrook, Ill), and PEF was measured with a Personal Best peak expiratory flowmeter (Respironics Inc, Cedar Grove, NJ). FEV₁ was determined by using standard spirometry at T₀ and T₂₄.

Blood samples

The total number of blood eosinophils was determined by means of hemocytometry (Sysmex NE 8000). IL-5 was measured in serum

with a commercially available ELISA kit, according to the instructions of the manufacturer (Cytoscreen; BioSource International Inc, Camarillo, Calif). The sensitivity of the ELISA kit was 11.7 pg/mL.

Bronchial biopsy specimens

All bronchial biopsy specimens were taken by the same pulmonary physician (S.E.O.), according to a previously described method.²⁰ During the first bronchoscopy, mucosal biopsy specimens were taken from the carinae of the left upper and lower lobes. After 24 hours, each subject underwent a repeat bronchoscopy, during which biopsy specimens were taken from lobar segments of the right lung. The bronchial biopsy specimens were embedded in Tissue-Tek II Optimal Cutting Temperature (OCT) compound (Sakura Finetek USA Inc, Torrance, Calif), frozen, and stored at -150°C.

Nasal biopsy specimens and NP

Biopsy specimens of nasal mucosa were taken by the same investigator (G.J.B.), according to a previously described method.²⁰ Subsequently, patients and control subjects were challenged with 10,000 BU of grass pollen extract (Vivodiagnost ALK Benelux BV, Groningen, the Netherlands) by means of a pump spray delivering a fixed dose of 50 μ L into each nostril. This concentration results in a good clinical response in patients with seasonal rhinitis.²¹ The allergen was delivered after deep inspiration during breath holding. After 24 hours, a second mucosal biopsy specimen was taken from the contralateral nostril. The nasal biopsy specimens were embedded in Tissue-Tek II OCT compound, frozen, and stored at -150°C.

Monoclonal antibodies

The mAbs used in this study were anti-major basic protein antibody (IgG₁, 0.2 μ g/mL, clone No. BMK13; Sanbio, Uden, the Netherlands) for identifying eosinophils, anti-human IL-5 mAb (IgG₁, 50 μ g/mL, clone No. 5A5; a gift from Professor Jan Tavernier, University of Ghent, Ghent, Belgium), anti-eotaxin antibody (IgG₁, 10 μ g/mL, clone No. 43911.11; R&D systems, Minneapolis, Minn), anti-E-selectin antibody (IgG₁, 2 μ g/mL, clone No. ENA1,

Sanbio), anti-VCAM-1 antibody (IgG₁, 2 µg/mL, clone No. 1G11B1, Sanbio), anti-ICAM-1 antibody (IgG₁, 0.2 µg/mL, clone No. MEM-112, Sanbio), and anti-human endothelium (CD31) antibody (IgG₁, 1 µg/mL, clone No. EN4, Sanbio).

Immunohistochemical staining

Each tissue specimen was cut into serial 6-µm thick sections. The mAb stainings were developed with the supersensitive immunoalkaline phosphatase method, as previously described.²⁰ Isotype-specific control staining was done with Mouse IgG₁ antibody to *Aspergillus niger* glucose oxidase (Dako catalog No. X 0931) at the same protein concentration as the specific antibody.

Microscopic assessment of immunohistochemical staining

Biopsy specimens were coded, and 2 sections 120 µm apart were counted blind for each antibody, as has been described previously.²⁰ Cell numbers were taken to be the number of positively stained cells per square millimeter by using an Axioskop 20 microscope (Zeiss, Jena, Germany) with an eyepiece graticule at a magnification of 200×.

Expression of adhesion molecules was assessed by 2 different methods of quantification at 100× magnification. First, intensity and extent of adhesion molecule expression was determined by using a computer-assisted image analysis system (Leica, Rijswijk, the Netherlands). This method is based on densitometric differences between positively stained tissue and background after setting thresholds for color, saturation, and intensity.

Second, with the use of an eye graticule, the total lamina propria of the nasal and bronchial specimens was examined for endothelial adhesion molecules. Only stained vessels with a distinct lumen were counted, and extravascular staining was excluded, according to a method described by Lee et al.¹⁴ The percentage of endothelial adhesion molecule expression was calculated from the ratio of vessels positive for a specific adhesion molecule and the total number of vessels staining positive for CD31. The numbers of vessels stained with a specific adhesion molecule and with CD31 were obtained from consecutively cut sections.

Statistical analysis

The Wilcoxon signed-rank test for within-group analysis and the Mann-Whitney *U* test for between-group analysis were performed if there were 2 samples. In case of repeated measurements, a mixed-model ANOVA with compound symmetry was done with the SAS software package (SAS Institute Inc, Cary, NC). Data are presented as medians and ranges or mean ± SE, as indicated. Correlations were evaluated with the Spearman rank correlation test. A *P* value of less than .05 was considered significant.

RESULTS

Clinical data

At baseline, patients with AR and control subjects were comparable for clinical parameters. At the consecutive 2-hour intervals after NP, the patients with AR had increased total nasal VAS score (*P* = .0002, repeated-measurement ANOVA) and lower PNIF values (*P* = .0001) than control subjects. The shape of the nasal VAS score (Fig 2, A) and PNIF (Fig 2, B) curves in patients with AR was bimodal, with peaks at T_{1/2} and T₁₂. At T₂₄, PNIF and nasal symptom score returned to baseline values. The same bimodal pattern was seen for bronchial VAS score (Fig 2, C) and PEF (Fig 2, D) measurements in patients with AR. These findings differed significantly from those

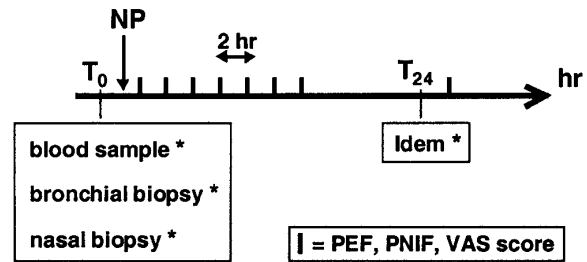


FIG 1. Study design. Bronchial and nasal biopsy specimens, as well as blood samples, were collected from patients and control subjects before (T₀) and 24 hours (T₂₄) after NP. Symptom scores (VAS), PNIF, and PEF were recorded at the beginning of each visit (T₀ and T₂₄), one half hour after NP and bronchoscopy (T_{1/2} and T_{241/2}), and at 2-hour intervals after NP (T₂-T₁₂).

of control subjects (*P* = .01 and *P* = .04, respectively). The FEV₁ did not significantly change after NP. No effect of NP could be detected on nasal VAS score, PNIF, bronchial VAS score, and PEF in control subjects.

Blood cells and mediators

At baseline, blood eosinophil numbers were comparable in patients with AR (median, 140 × 10⁶/L; range, 10-360 × 10⁶/L) and control subjects (median, 110 × 10⁶/L; range, 30-180 × 10⁶/L). At T₂₄, the number of blood eosinophils significantly increased in patients with AR (median, 300 × 10⁶/L; range, 140-550 × 10⁶/L) compared with baseline (*P* = .008) and compared with control subjects (median, 140 × 10⁶/L; range, 40-180 × 10⁶/L; *P* < .001).

At T₀ and T₂₄, the concentrations of serum IL-5 were significantly higher (*P* = .01) in patients with AR (median, 27.7 pg/mL; range, 13.0-165.1 pg/mL) than in control subjects (median, 14.6 pg/mL; range, <11.7-81 pg/mL).

Immunostaining of mucosal biopsy specimens

General description. Out of the 72 biopsy specimens, 69 reached criteria for evaluation. Two bronchial and one nasal biopsy specimen could not be evaluated. These samples were excluded from the study. In bronchial biopsy specimens the median length of evaluable basement membrane was 3.8 mm (range, 1.3-6.8 mm); in nasal biopsy specimens the median length was 4.0 mm (range, 1.2-8.1 mm). The median surface area of bronchial subepithelium was 0.38 mm² (range, 0.13-0.68 mm²), and the median surface area of nasal subepithelium was 0.42 mm² (range, 0.21-0.81 mm²). The median surface area of nasal lamina propria was 3.0 mm² (range, 1.0-6.1 mm²). Staining with isotype-specific control antibodies was negative in all cases.

Nasal specimens. At T₀, the numbers of eosinophils, IL-5⁺ cells, and eotaxin-positive cells were comparable in nasal epithelium, subepithelium, and lamina propria of patients with AR and control subjects. At T₂₄, the number of eosinophils was significantly increased in nasal epithelium (*P* = .01), subepithelium (*P* = .01), and lamina propria (*P* < .01) of patients with AR. At T₂₄, the num-

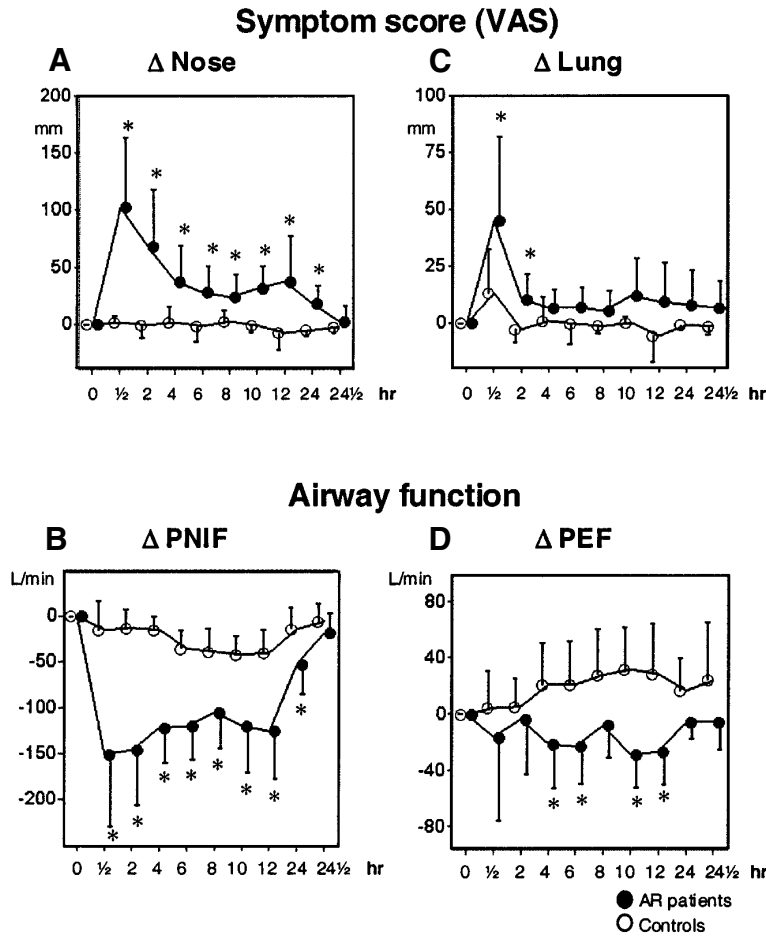


FIG 2. Symptoms were individually expressed in millimeters on a 10-cm VAS, and a composite score was obtained for nasal complaints (**A**, rhinorrhea, watery eyes, nasal itching, sneezing, and nasal blockage) and pulmonary complaints (**C**, wheezing, coughing, shortness of breath, and decreased exercise intolerance). PNIF (**B**) and PEF (**D**) were expressed in liters per minute. Baseline values were taken to be 0. Consecutive measurements were plotted as the difference from baseline (mean \pm SEM). * $P < .05$ (Mann-Whitney U test).

ber of IL-5⁺ cells was significantly higher in nasal epithelium ($P = .04$), whereas the number of eotaxin-positive cells was significantly increased in the lamina propria ($P = .05$) of the nasal mucosa.

At baseline, there were no differences in ICAM-1 (Fig 3, *A*), VCAM-1 (Fig 3, *B*), and E-selectin (Fig 3, *C*) expression in nasal mucosa between patients with AR and control subjects. At T₂₄, ICAM-1 ($P = .02$), VCAM-1 ($P = .008$), and E-selectin ($P = .02$) expression was significantly increased in the nasal lamina propria of the patients with AR compared with baseline values (Table II). However, no difference was found between patients with AR and control subjects. At T₂₄, the percentage of ICAM-1⁺ ($P = .01$; Fig 3, *E*), VCAM-1⁺ ($P = .008$; Fig 3, *F*), and E-selectin⁺ ($P = .008$; Fig 3, *G*) vessels was significantly increased in the allergic group compared with baseline (Table III) and compared with control subjects ($P = .004$, $P = .05$, and $P = .01$, respectively). No significant difference in cell numbers and adhesion molecule expression was found in the nasal mucosa of control subjects after NP. There was no difference between patients and control

subjects in the total number of vessels in the nasal mucosa at T₀ (Fig 3, *D*) and T₂₄ (Fig 3, *H*).

Bronchial specimens. At T₀, the numbers of eosinophils, IL-5⁺ cells, and eotaxin-positive cells were comparable in allergic subjects and control subjects in bronchial epithelium and subepithelium. At T₂₄, we found increased numbers of eosinophils in the bronchial epithelium ($P = .05$) and subepithelium ($P = .02$) of patients with AR. No significant changes were found in the number of IL-5⁺ and eotaxin-positive cells in bronchial mucosa before and after NP.

At baseline, there were no differences in ICAM-1 (Fig 4, *A*), VCAM-1 (Fig 4, *B*), and E-selectin (Fig 4, *C*) expression in bronchial epithelium, lamina propria, and endothelium between patients with AR and control subjects. In the bronchial lamina propria of patients with AR, ICAM-1 ($P = .05$) expression was significantly increased at T₂₄ compared with baseline values (Table II). However, no difference was found between patients with AR and control subjects. At T₂₄, the percentage of ICAM-1⁺ ($P = .01$; Fig 4, *E*), VCAM-1⁺ ($P = .04$; Fig 4, *F*), and E-

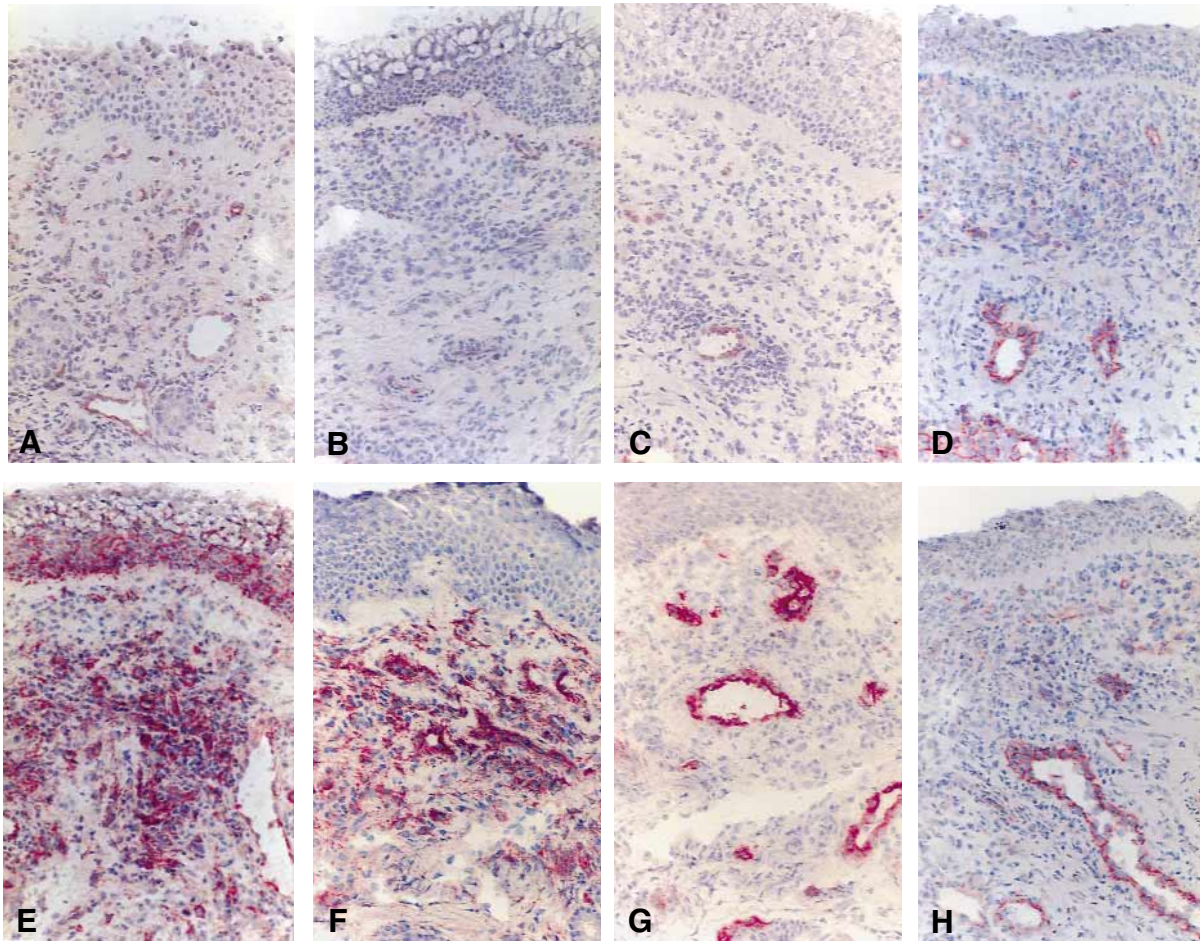


FIG 3. Nasal mucosal sections taken from patients with AR before and 24 hours after nasal provocation. Immunohistochemical staining for ICAM-1 (A and E), VCAM-1 (B and F), E-selectin (C and G), and CD31 (D and H). Counterstained with hematoxylin (original magnification, 100 \times).

selectin⁺ ($P = .02$; Fig 4, G) vessels in the bronchial mucosa was significantly increased in patients with AR compared with baseline values (Table III) and control subjects ($P = .008$, $P = .05$, and $P = .06$, respectively). No significant difference in cell numbers and adhesion molecule expression was found in the bronchial mucosa of control subjects after NP. There was no difference between patients and control subjects in the total number of vessels in the bronchial mucosa at T_0 (Fig 4, D) and T_{24} (Fig 4, H).

Correlations between inflammatory markers and symptomatology

In the bronchial subepithelium the number of eosinophils correlated with the percentage of VCAM-1⁺ vessels ($r = 0.65$, $P = .005$) and E-selectin⁺ vessels ($r = 0.58$, $P = .01$), as well as with the total bronchial symptom score ($r = 0.50$, $P = .03$).

DISCUSSION

In the current study we were able to show increased

expression of endothelial adhesion molecules and eosinophilic allergic inflammation in the nasal and bronchial mucosa of nonasthmatic patients with AR after NP. Recently, we have demonstrated that segmental bronchial provocation in patients with AR induces blood eosinophilia and mucosal inflammation characterized by increased numbers of eosinophils, IL-5⁺ cells, and eotaxin-positive cells in both upper and lower airways.²⁰ The current study further supports the hypothesis that local allergen exposure results in generalized airway inflammation.

Several studies have stressed the importance of IL-5 and eotaxin in the influx of eosinophils from the blood stream into the mucosa.^{11,12,22-24} Analysis of bronchoalveolar lavage fluid and bronchial mucosa in patients with AR and asthma has shown enhanced local IL-5 expression after segmental bronchial provocation.²⁵⁻²⁷ In the present study the expression of eotaxin and IL-5 was elevated in the nasal mucosa and blood of patients with AR. In bronchial mucosa these signaling molecules were also elevated, albeit not significantly when compared with levels found in control subjects after NP.

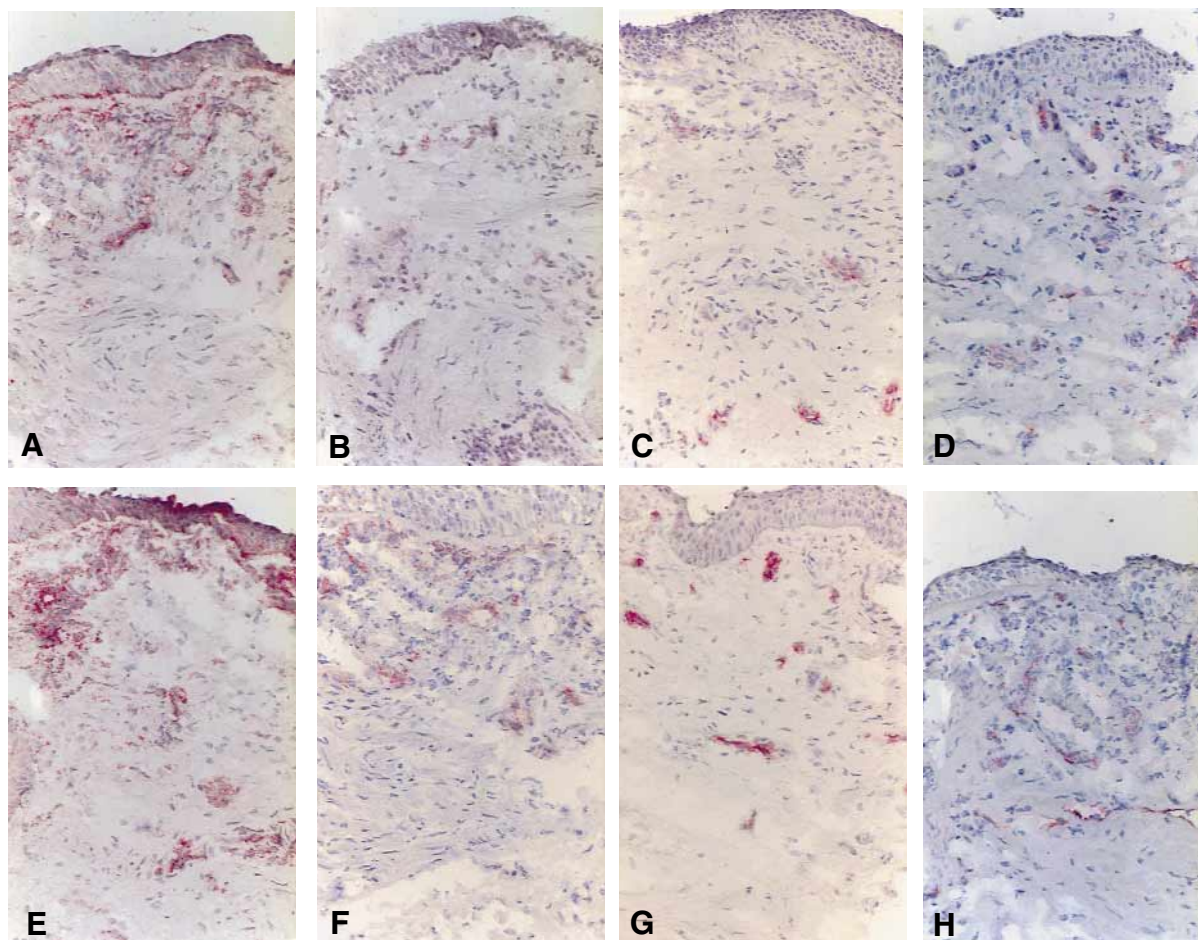


FIG 4. Bronchial mucosal sections taken from patients with AR before and 24 hours after NP. Immunohistochemical staining for ICAM-1 (A and E), VCAM-1 (B and F), E-selectin (C and G), and CD31 (D and H). Counterstained with hematoxylin (original magnification, 100 \times).

TABLE II. Image analysis results

	ICAM-1		VCAM-1		ELAM-1	
	T ₀	T ₂₄	T ₀	T ₂₄	T ₀	T ₂₄
Patients with AR						
Nose						
Epithelium	6 (1-14)	8 (1-26)	1 (0-4)	1 (0-13)		
Lamina propria	4 (1-12)	12 (1-34)*	2 (0-6)	4 (1-21)*	0 (0-1)	1 (0-2)*
Bronchus						
Epithelium	1 (0-11)	4 (1-14)	4 (0-17)	2 (0-5)		
Lamina propria	2 (0-5)	3 (1-10)*	2 (0-8)	1 (0-5)	0 (0-2)	0 (0-1)
Control subjects						
Nose						
Epithelium	12 (3-25)	10 (0-24)	1 (0-7)	0 (0-3)		
Lamina propria	7 (2-25)	6 (1-14)	2 (1-8)	4 (1-6)	0 (0-1)	0 (0-4)
Bronchus						
Epithelium	2 (0-4)	6 (0-11)	1 (0-9)	1 (0-4)		
Lamina propria	2 (0-4)	3 (2-8)	1 (0-3)	1 (0-2)	0	0

Data are expressed as medians (range). Values represent percentage of area staining positive for the particular adhesion molecule.

ELAM, Endothelial leukocyte adhesion molecule.

* $P < .05$.

TABLE III. Endothelium results

	ICAM-1/CD31		VCAM-1/CD31		ELAM-1/CD31	
	T ₀	T ₂₄	T ₀	T ₂₄	T ₀	T ₂₄
Patients with AR						
Nose						
Subepithelium	13 (0-33)	44 (19-95)*	13 (0-51)	65 (5-94)*	42 (0-60)	54 (42-82)*
Lamina propria	28 (7-58)	61 (30-94)*	28 (0-43)	67 (16-85)*	38 (17-59)	69 (48-82)*
Bronchus						
Subepithelium	50 (0-57)	82 (29-100)*	0 (0-50)	45 (0-66)*	0 (0-25)	35 (0-86)*
Control subjects						
Nose						
Subepithelium	28 (18-43)	19 (11-50)	19 (4-41)	19 (4-56)	44 (13-67)	39 (21-73)
Lamina propria	31 (16-78)	28 (12-51)	25 (15-67)	33 (10-56)	44 (18-69)	38 (13-86)
Bronchus						
Subepithelium	30 (0-50)	38 (0-75)	6 (0-48)	11 (0-69)	0 (0-14)	0 (0-62)

Data are expressed as medians (range). Values represent percentages of vessels staining positive for the particular adhesion molecule. ELAM, Endothelial leukocyte adhesion molecule.

**P* < .05.

In the process of leukocyte arrest and migration, adhesion molecules have an important role to play. Increased expression of endothelial ICAM-1, VCAM-1, and E-selectin has been demonstrated in nasal and bronchial mucosa after local allergen exposure.^{14,28,29} Others did not detect differences in adhesion molecule expression.^{30,31} These conflicting results are based on different methodologic approaches to assess adhesion molecule expression. By its nature, semi-quantitative scoring methods used in previous work^{30,31} may well be less suitable to detect small changes in adhesion molecule expression. Moreover, attention should be focused on the expression of adhesion molecules on the endothelium because this is the site where cells are recruited from the circulation. In our study we used 2 methods to quantify adhesion molecule expression: computer-assisted image analysis and microscopic evaluation of endothelium.

A significant increase was found in the ratio of ICAM-1+, VCAM-1+, and E-selectin+ vessels versus the total number of vessels in both the nose and bronchi in the AR group compared with control subjects. We used CD31 as a marker for vascular endothelium because it produced a more stringent signal in bronchial mucosa than Factor VIII (Von Willebrand) applied to nasal mucosa by Lee et al¹⁴ in a similar study. In contrast, the image analysis method showed increased expression of ICAM-1, VCAM-1, and E-selectin in both nasal and bronchial mucosa in the AR group. However, this was not significantly different from control tissue. Therefore we propose that analyzing expression of adhesion molecules by the endothelium may be more relevant with regard to eosinophil recruitment than determining the extent of adhesion molecule expression throughout the tissue.

It is very unlikely that our results are influenced by allergen spill from the nose into the lower airways. Inhalation studies with radiolabeled allergen have shown no deposition of allergen in the lungs after nasal allergen application, according to the technique we used.³² Although postnasal drainage of inflammatory mediators into the lower airways cannot be excluded, it is not like-

ly to play a role in vivo.³³ Also, bronchoscopy and taking of biopsy specimens per se do not lead to pulmonary inflammation.³⁴

Pathophysiologic mechanisms, which could explain the interaction between the nose and the lung, are neural reflex mechanisms and systemic induction of inflammatory mediators and cells. Although exposure of the nasal mucosa to cold dry air results in immediate bronchoconstriction in asthmatic patients,³⁵ no direct effect on FEV₁ could be detected after nasal allergen challenge.^{32,36,37} Nasal provocation with methacholine in asthmatic patients with rhinitis resulted in an increase in lower airway resistance that could be blocked by premedication of nasal mucosa with phenylephrine, suggesting a role for systemic mediators in the induction of lower airway resistance.³⁸ Our results are most likely to be the result of a systemic inflammatory response, which is IL-5 mediated, involving eosinophil release from the bone marrow and migration into airway mucosa.

In this study we have demonstrated that the expression of VCAM-1 and E-selectin on the bronchial microvasculature is upregulated after NP and is associated with bronchial eosinophilia. Do these findings indicate that the patients with AR in our study actually have asthma? Although significant changes in pulmonary symptom score and PEF were found in the AR subgroup, no significant effect on FEV₁ was detected 24 hours after NP. Therefore the clinical implications of single nasal provocation, with respect to the lower airways, remain disputable in patients with AR without asthma. It is, however, very well possible that repetitive allergen stimulation of the nose, inducing a continuous bombardment of the lower airways with systemic inflammatory mediators, may result in the development of asthma eventually. Moreover, in patients with AR with preexistent asthma, nasal allergen exposure could have an important contributory effect on bronchial inflammation, lung function, and pulmonary symptoms. Future studies need to address these questions.

In conclusion, we have found an allergic inflammatory response similar to asthma in the lower airways of nonasthmatic patients with AR after NP. We speculate that the absorption of inflammatory mediators at the site of allergen challenge results in release of eosinophils from the bone marrow into the blood and subsequently in their recruitment to the nasal and bronchial mucosa through upregulation of adhesion molecule expression by the endothelium.

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