

# Segmental Bronchial Provocation Induces Nasal Inflammation in Allergic Rhinitis Patients

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Allergic rhinitis and asthma often coexist and share a genetic background. Pathophysiologic connections between the nose and lungs are still not entirely understood. This study was undertaken to compare allergic inflammation and clinical findings in the upper and lower airways after segmental bronchial provocation (SBP) in nonasthmatic allergic rhinitis patients. Eight nonasthmatic, grass pollen-sensitive patients with allergic rhinitis and eight healthy controls were included. Bronchial biopsies and blood samples were taken before ( $T_0$ ) and 24 h ( $T_{24}$ ) after SBP. Nasal biopsies were obtained at  $T_0$ , 1 h after SBP ( $T_1$ ), and  $T_{24}$ . Immunohistochemical staining was performed for eosinophils (BMK13), interleukin (IL)-5, and eotaxin. The number of eosinophils increased in the challenged and unchallenged bronchial mucosa ( $p < 0.05$ ) and in the blood ( $p = 0.03$ ) of atopic subjects at  $T_{24}$ . We detected an increase of BMK13-positive and eotaxin-positive cells in the nasal lamina propria and enhanced expression of IL-5 in the nasal epithelium of atopic subjects only at  $T_{24}$  ( $p < 0.05$ ). SBP induced nasal and bronchial symptoms as well as reductions in pulmonary and nasal function in the allergic group. No significant changes could be observed in healthy controls. The study shows that SBP in nonasthmatic allergic rhinitis patients results in peripheral blood eosinophilia, and that SBP can induce allergic inflammation in the nose.

Epidemiologic (1), pathophysiologic (2, 3), and clinical studies (4, 5) strongly suggest a link between rhinitis and asthma. Asthma and rhinitis, which are considered to be manifestations of the atopic syndrome, often coexist and share a common genetic background. Although several studies have shown that asthma and rhinitis are characterized by a similar inflammatory process (6–9), pathophysiologic interactions between upper and lower airways are not entirely understood. It is clear that the condition of the upper airways definitely influences the lower airways. In allergic rhinitis patients without bronchial hyperreactivity (BHR), signs of allergic inflammation of the lower airways have been found in induced sputum, bronchoalveolar lavage fluid (BALF), and bronchial biopsy specimens (10–13). The nasal-bronchial reflex, an altered breathing pattern, pulmonary aspiration of nasal contents, and increased levels of inflammatory factors in the blood are possible mechanisms for lower airway dysfunction among patients with rhinitis (14). To shed more light on the role of systemic induction in the allergic inflammatory response, we designed a study in which blood samples and nasal and bronchial mucosal biopsy specimens were taken from a group of nonasthmatic allergic rhinitis patients with an isolated grass pollen allergy after segmental bronchial provocation (SBP) at a time other than the grass pollen season. The aim of the study was to compare allergic mucosal inflammation and clinical findings in the upper

and lower airways. Eosinophils, major effector cells in allergic inflammation, interleukin (IL)-5-positive cells and eotaxin-positive cells (necessary for eosinophil survival and chemotaxis), were chosen as markers of mucosal allergic inflammation.

## METHODS

### Subject Groups

Eight allergic rhinitis patients (two men and six women, age range 21 to 31 yr) and eight nonallergic healthy controls (four men and four women, age range 18 to 29 yr) were selected for the study. Subject characteristics are shown in Table 1. The rhinitis patients had a history of isolated grass pollen allergy for at least 2 yr, confirmed by a positive skin-prick test reaction to grass pollen extract alone (Vivodiagnost; ALK Benelux BV, Groningen, the Netherlands), and not to a panel of 13 other common allergens. The control subjects had no symptoms or signs of rhinitis and had negative skin-prick tests. None of the allergic rhinitis patients or controls had a clinical history of asthma. All had a normal FEV<sub>1</sub> and provocative concentration of methacholine causing a 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub> methacholine) > 8 mg/ml. Methacholine was administered according to a standardized tidal breathing method (15). The response to methacholine was measured as change in FEV<sub>1</sub>, expressed as a percent of the initial value. None of the subjects smoked or used any medication known to influence the results of the study. Biopsy specimens were obtained between February and April 1998, before the grass pollen season. None of the patients or control subjects had an infection of the respiratory tract or any nasal complaints during the 4 wk preceding the allergen challenges. All participants gave informed consent to the study, which was approved by the medical ethics committee of the Erasmus Medical Center Rotterdam.

### Experimental Design

The study design is outlined in Table 2. Baseline nasal and bronchial biopsy specimens were collected from patients and controls before SBP ( $T_0$ ). Nasal biopsy specimens were obtained 1 h ( $T_1$ ) and 24 h ( $T_{24}$ ) after SBP. Bronchoscopy and biopsy were repeated 24 h after SBP ( $T_{24}$ ). Signs and symptoms of the patients and controls were recorded at the beginning of each visit (at  $T_0$  and  $T_{24}$ ) on a 10-cm visual analogue scale (VAS). Symptoms were divided into nasal complaints (rhinorrhea, watery eyes, nasal itching, sneezing, and nasal blockage) and pulmonary complaints (wheezing, coughing, shortness of breath, and decreased exercise tolerance). Upper and lower airways obstruction was measured through peak nasal inspiratory flow (PNIF) and FEV<sub>1</sub>. FEV<sub>1</sub> was determined at  $T_0$ ,  $T_1$ , and  $T_{24}$ . PNIF was measured with a Youtlen peak nasal inspiratory flow meter (Armstrong Industries, Inc., Northbrook, IL) at  $T_0$  and  $T_{24}$ . Blood samples were taken at  $T_0$  and  $T_{24}$ . Blood eosinophils were counted by hemocytometry.

### Bronchial Biopsies and Segmental Allergen Bronchoprovocation

All bronchial biopsy specimens were taken by the same pulmonary physician (S.E.O.). After intramuscular premedication of subjects with atropine (0.5 mg), oropharyngeal anesthesia was accomplished with topical 1% xylocaine spray. The vocal cords, trachea, and bronchial tree were then anesthetized with oxybuprocaine. A fiberoptic bronchoscope was introduced into the airway via the oral route, and mucosal biopsy specimens were taken from the carina of the left up-

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TABLE 1  
SUBJECT CHARACTERISTICS AT BASELINE\*

Patient	Age (yr)	Sex	FEV <sub>1</sub> (L)	FEV <sub>1</sub> (%)	IVC (L)	FEV <sub>1</sub> /VC	BAR* (%)	PC <sub>20</sub> (mg/ml)
Allergic rhinitis								
1	31	F	4.00	108	4.99	80	102	40
2	21	M	4.02	85	5.15	79	107	11.8
3	25	F	4.74	126	5.62	84	102	23.6
4	25	M	4.35	94	5.44	80	104	40
5	27	F	3.71	114	4.06	91	100	40
6	23	F	3.91	113	4.54	86	104	40
7	23	F	4.00	122	4.65	86	105	40
8	21	F	4.06	111	4.70	86	100	40
Controls								
9	23	F	2.96	82	3.43	86	110	40
10	29	M	5.59	115	7.42	76	108	32.4
11	24	M	4.14	94	4.72	88	101	40
12	28	F	2.52	80	2.67	95	102	40
13	25	F	4.21	112	4.72	89	101	40
14	21	M	5.99	100	6.98	86	102	40
15	20	M	4.81	114	5.44	88	102	9
16	18	F	3.83	111	5.12	76	107	40

Definition of abbreviations: BAR =  $\beta$ -agonist response; IVC = inspiratory vital capacity; PC<sub>20</sub> = concentration of methacholine causing a 20% decrease in FEV<sub>1</sub>.

\* BAR (terbutaline, 1,000  $\mu$ g) data are presented as percentage improvement compared to initial value; a virtual value of 40 was assigned to subjects who did not reach a PC<sub>20</sub> with 38 mg/ml methacholine.

per and lower lobe. Subsequently, SBP was accomplished with a method described previously (16). The tip of the bronchoscope (BF, Type P20 D; Olympus Tokyo, Japan), was wedged in the anterior segment of the right upper lobe (RUL), and 10 ml of 0.9% sterile saline was instilled as a control challenge. After the control challenge, the bronchoscope was wedged in a segmental bronchus of the right middle lobe (RML), and allergen challenge was achieved by instilling 100 BU of grass pollen extract (Vivodiagnost) made up in 5 ml of sterile saline. The challenge site was observed for 5 min. In the absence of local bronchoconstriction, a further 400 BU of allergen in 5 ml of saline was administered, after which the bronchial segment was observed for 5 more minutes and the bronchoscope then quickly removed. After 24 h, each subject underwent a repeat bronchoscopy, during which biopsy specimens were taken from lobar segments of the unchallenged left side, the saline-challenged RUL and the allergen-challenged RML. The biopsy specimens were embedded in Tissue-Tek II optimal cutting temperature (OCT) compound (Sakura Finetek USA Inc., Torrance, CA), frozen, and stored at  $-150^{\circ}$  C.

### Nasal Biopsies

All biopsy specimens of nasal mucosa were taken by the same investigator (G.J.B.) according to the study design shown in Table 2. First, local anesthesia was induced by placing a cotton-wool carrier with 50 to 100 mg of cocaine and 3 drops of epinephrine (1:1,000) under the inferior turbinate, without touching the biopsy site. Second, mucosal biopsy samples were obtained from the lower edge of the inferior turbinate, about 2 cm posterior to the edge, by using a Gerritsma forceps with a cup diameter of 2.5 mm. The nasal biopsy specimens were

TABLE 2  
STUDY DESIGN

Time Point	VAS Score	FEV <sub>1</sub>	PNIF	Bronchial Biopsy	Nasal Biopsy	Blood Samples
T <sub>0</sub> Baseline	X	X	X	X	X	X
T <sub>1</sub> 1 h after SBP*		X			X	
T <sub>24</sub> 24 h after SBP	X	X	X	X	X	X

Definition of abbreviations: PNIF = peak nasal inspiratory flow; SBP = segmental bronchial provocation; VAS = visual analogue scale for scoring nasal and pulmonary symptoms.

embedded in Tissue-Tek II OCT compound, frozen, and stored at  $-150^{\circ}$  C. (17).

### Monoclonal Antibodies

The monoclonal antibodies (mAbs) used in the study were BMK-13 (IgG<sub>1</sub>, 0.2  $\mu$ g/ml; Sanbio, Uden, The Netherlands) for total eosinophils, an antibody to human (IL)-5 (IgG<sub>1</sub>, 50  $\mu$ g/ml; clone 5A5, a gift from Prof. Jan Tavernier of the University of Ghent, Ghent, Belgium), and an anti-eotaxin antibody (IgG<sub>1</sub>, 10  $\mu$ g/ml; clone 43911.11; R&D Systems, Minneapolis, MN).

### Immunohistochemical Staining

Staining for eosinophils with BMK13 was done with a modified alkaline phosphatase (AP) method (18). (IL)-5 and eotaxin staining were done as follows: briefly, each tissue specimen was cut into serial, 6- $\mu$ m-thick sections on a Reichert-Jung 2800e Frigocut cryostat (Leica, Wetzlar, Germany) and transferred onto poly-L-lysine-coated slides (Sigma Chemical Co., St. Louis, MO), dried, and stored at  $-70^{\circ}$  C. The slides were stained within 3 mo. They were heated to room temperature and subsequently dried and fixed in acetone for 10 min at room temperature. The slides were then rinsed in phosphate-buffered saline (PBS; pH 7.8) and placed in a semiautomatic stainer (Sequenza; Shandon, Sewickley, PA). Sections were incubated with 10% normal goat serum (CLB, Amsterdam, The Netherlands) for 10 min and were subsequently incubated for 60 min with the appropriate mAb (diluted in 10% normal human serum with 1% bovine serum albumin in PBS). They were then rinsed with PBS for 5 min, incubated with biotin-labeled goat-antimouse (Biogenex, San Ramon, CA), rinsed with PBS, incubated with AP-conjugated goat-antibiotin (Sigma) for 30 min, rinsed once more with PBS for 5 min, rinsed with Tris buffer (0.2 mol/L, pH 8.5) for 5 min, and incubated for 30 min with new fuchsin (Chroma, Kӧngen, Germany) substrate (containing levamisole to block endogenous AP enzyme activity). The sections were then rinsed in distilled water, counterstained with Gill's hematoxylin, and mounted in glycerin gelatin. Control staining was done with an irrelevant mAb of the same subclass as the specific antibody.

### Microscopic Evaluation

Biopsy specimens were coded, and two sections 120  $\mu$ m apart were counted in a blinded fashion for each antibody. Bronchial sections were divided into epithelium and subepithelium (an area 100  $\mu$ m deep in the lamina propria, along the length of the epithelial basement membrane) and were counted as previously described (19). Nasal biopsy sections were divided into epithelium, subepithelium, and lamina propria (total subepithelial mucosa). Positively stained epithelial and subepithelial cells were counted along the basement membrane, which had to be undamaged for a length of at least 1 mm before being accepted for evaluation. For lamina propria, a minimum area of 1 mm<sup>2</sup> was required for analysis. Cell numbers were determined as the number of positively stained cells per mm<sup>2</sup>, using an Axioskop 20 microscope (Zeiss, Jena, Germany) with an eyepiece graticule at a magnification of  $\times 200$ .

### Statistical Analysis

Statistical analysis was done with Friedman's and Wilcoxon's test for intragroup analysis and the Mann-Whitney U test for intergroup analysis. Data are presented as median  $\pm$  range. Correlations were evaluated with Spearman's rank correlation test. A value of  $p < 0.05$  was considered significant.

## RESULTS

### Clinical Data

The allergic rhinitis patients reported significantly more pulmonary symptoms after SBP than did the controls (Table 3), expressed by an increased total bronchial VAS score at T<sub>24</sub> ( $p = 0.03$ ). A significant decrease in FEV<sub>1</sub>, of 9% (range:  $-20$  to  $+3\%$ ), was measured at T<sub>1</sub> in allergic patients ( $p = 0.03$ ), whereas in controls, FEV<sub>1</sub> barely changed (median:  $-1\%$ ; range:  $-4$  to  $+4\%$ ). At T<sub>24</sub>, FEV<sub>1</sub> was still 9% (range:  $-11$  to  $0\%$ ) lower than its baseline value in allergic patients, and only

TABLE 3  
CLINICAL DATA FOR ALLERGIC RHINITIS PATIENTS AND CONTROLS BEFORE (T<sub>0</sub>) AND 24 h (T<sub>24</sub>) AFTER SBP SEGMENTAL BRONCHIAL PROVOCATION

Patient	FEV <sub>1</sub> (L)		PNIF (L)		Lung Symptoms		Nose Symptoms	
	T <sub>0</sub>	T <sub>24</sub>	T <sub>0</sub>	T <sub>24</sub>	T <sub>0</sub>	T <sub>24</sub>	T <sub>0</sub>	T <sub>24</sub>
Allergic rhinitis								
1	4.00	3.82	235	190	6	37	7	56
2	4.02	4.05	265	250	26	68	52	128
3	4.74	4.34	200	180	0	123	0	35
4	4.35	4.21	270	260	36	16	35	35
5	3.71	3.35	230	220	24	31	46	54
6	3.91	3.94	220	215	13	34	0	47
7	4.00	3.77	250	230	10	27	36	49
8	4.06	3.64	220	220	8	50	12	28
Controls								
9	2.96	2.97	120	115	na	na	na	na
10	5.59	5.13	290	185	28	15	36	53
11	4.14	4.10	160	240	4	9	7	6
12	2.52	2.46	230	240	31	68	49	203
13	4.21	4.08	235	205	7	6	19	63
14	5.99	4.69	300	265	8	30	11	43
15	4.81	5.68	180	195	21	24	56	53
16	3.83	3.62	230	210	3	10	10	17

Definition of abbreviations: na = not available; PNIF = peak nasal inspiratory flow; VAS = visual analogue scale.

Symptoms were individually expressed in mm on a 10-cm visual analogue scale, and a composite score was obtained for nasal complaints (rhinorrhea, watery eyes, nasal itching, sneezing, and nasal discharge) and pulmonary complaints (wheezing, coughing, shortness of breath, and decreased exercise intolerance).

\*  $p < 0.05$ .

2% (range: -9 to 0%) below its baseline value in healthy controls. Also, the nasal VAS score was significantly increased ( $p = 0.02$ ) and PNIF was reduced ( $p = 0.02$ ) at T<sub>24</sub>, as compared with their baseline values in the allergic group. No effect on either nasal VAS score or on PNIF could be detected in control subjects.

#### Blood Eosinophils

No significant difference in baseline blood eosinophil count was observed in allergic patients (median:  $175 \times 10^6/L$ ; range:  $70$  to  $440 \times 10^6/L$ ) as compared with healthy controls (median:  $95 \times 10^6/L$ ; range:  $40$  to  $190 \times 10^6/L$ ). Twenty-four hours after SBP, total blood eosinophil counts were significantly elevated over baseline in allergic patients ( $p = 0.02$ ) and as compared with those of controls ( $p = 0.001$ ; Figure 1).

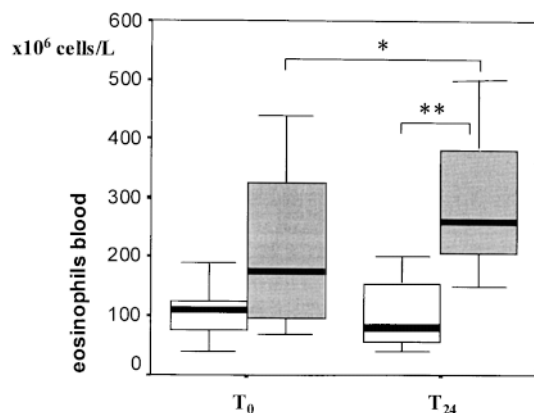


Figure 1. Number of peripheral blood eosinophils before (T<sub>0</sub>) and 24 h (T<sub>24</sub>) after SBP. Grey boxes indicate allergic patients, open boxes indicate controls. Data are presented as median  $\pm$  range. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

#### Immunostaining

**General description.** Three nasal mucosa specimens and four bronchial biopsy specimens were collected per patient. Of the 112 biopsy specimens, 106 met the criteria for evaluation. Neither bronchial epithelium (median length of evaluable basement membrane: 3.6 mm; range: 0 to 7.8 mm) nor subepithelium (median: 3.4 mm; range: 0 to 7.8 mm) could be evaluated in five samples. Nasal epithelium (median: 5.2 mm; range: 1.1 to 12.5 mm), subepithelium (median: 5.4 mm; range: 1.1 to 12.5 mm), and lamina propria (median:  $4.63 \text{ mm}^2$ ; range: 0.88 to  $8.31 \text{ mm}^2$ ) could not be evaluated in one case. These samples were excluded from the study. However, for all time points, a minimal number of seven subjects per subgroup could be included.

**Bronchial specimens.** In bronchial epithelium, baseline (BMK13-positive) BMK13<sup>+</sup> cell numbers were equal in allergic subjects and controls (Figure 2A). In the subepithelium, BMK13<sup>+</sup> cell numbers were higher in atopic than in control subjects ( $p < 0.01$ ), but absolute cell numbers were very low at baseline (Figure 2B). At T<sub>24</sub>, we found increased numbers of BMK13<sup>+</sup> cells in the bronchial epithelium and subepithelium of allergic rhinitis patients (Figure 3), in the saline-challenged bronchial segment (epithelium,  $p = 0.02$ ; subepithelium  $p = 0.03$ ) as well as in the allergen-challenged bronchial segment (epithelium,  $p = 0.01$ ; subepithelium,  $p = 0.02$ ). In the unchal-

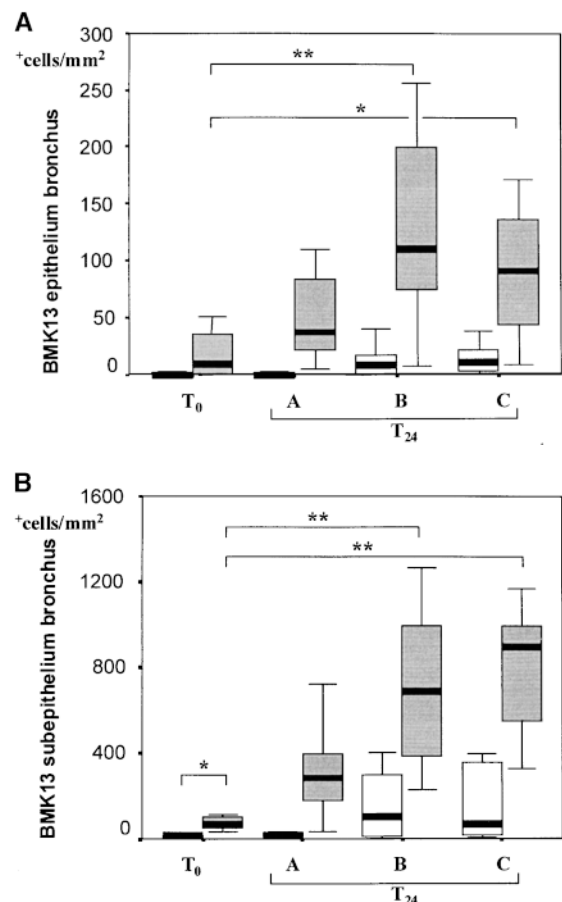
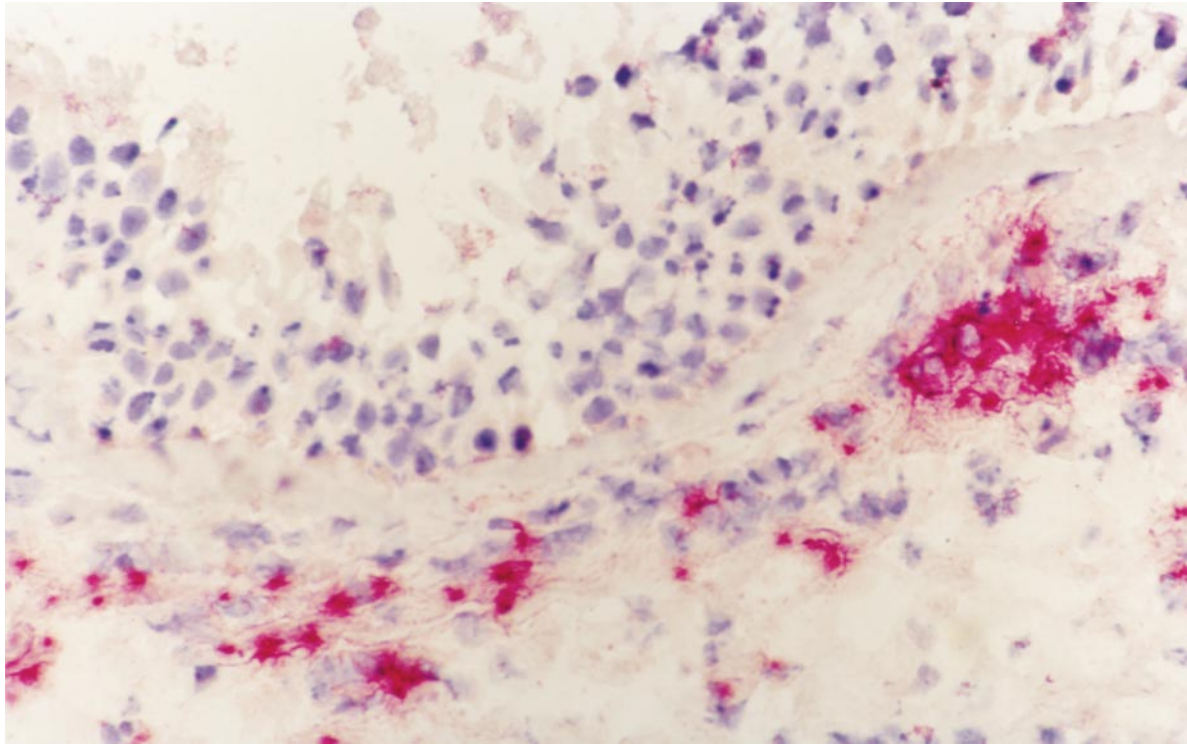


Figure 2. Number of BMK13<sup>+</sup> cells in bronchial epithelium (A) and subepithelial layer (B) before (T<sub>0</sub>) and 24 hours (T<sub>24</sub>) after SBP. A = unchallenged left lung; B = allergen challenged right middle lobe; C = saline challenged right upper lobe. Grey boxes indicate allergic patients, open boxes indicate controls. Data are presented as median  $\pm$  range. \*  $p < 0.05$ , \*\*  $p < 0.01$ .



**Figure 3.** BMK13 immunoreactivity of a section of bronchial biopsy taken from an allergen-challenged segment of an allergic rhinitis patient 24 h after SBP. Counterstained with hematoxylin. Original magnification:  $\times 400$ .

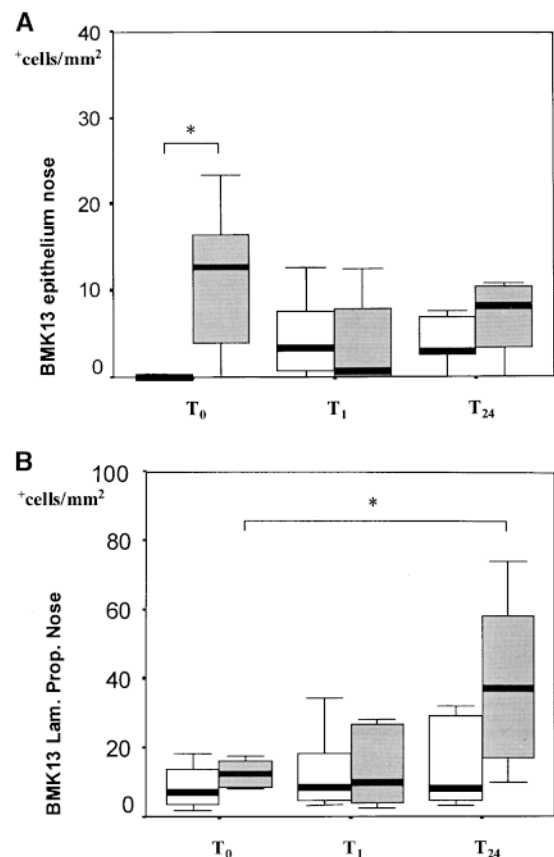
lenged segment, BMK13<sup>+</sup> cell numbers were not significantly affected by SBP ( $p = 0.07$  for both epithelium and subepithelium). At T<sub>24</sub> we found a significant difference between allergic patients and controls in the number of eosinophils in the allergen-challenged segment (epithelium,  $p = 0.003$ ; subepithelium,  $p = 0.002$ ), saline challenged segment (epithelium,  $p = 0.01$ ; subepithelium,  $p = 0.03$ ), and even in the unchallenged segment (epithelium,  $p = 0.001$ ; subepithelium,  $p = 0.001$ ). In none of the tissue areas of the control groups, were BMK13<sup>+</sup> cell numbers significantly altered at T<sub>24</sub>.

After SBP, IL-5-positive cell numbers were markedly increased only in the allergen-challenged bronchial subepithelium in allergic rhinitis subjects ( $p = 0.07$ ). No significant differences were found in the other investigated segments.

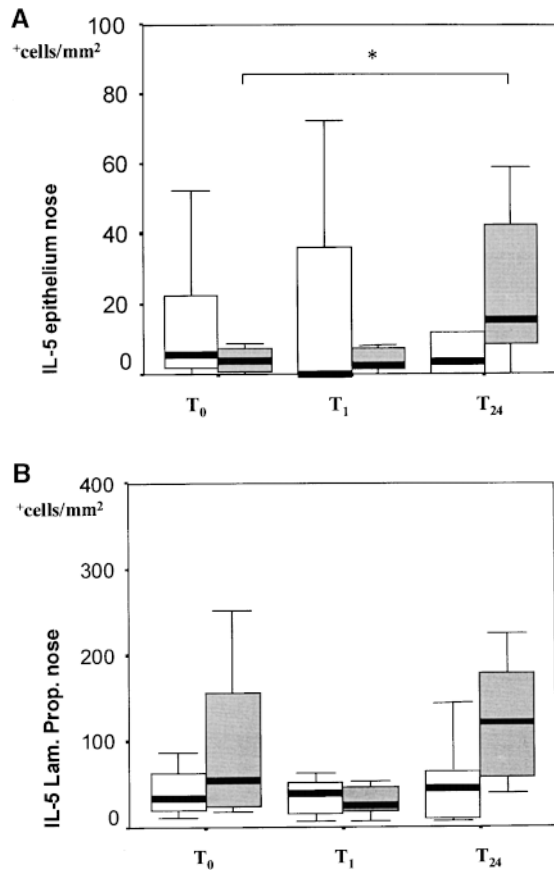
No significant changes were found in the number of eotaxin-positive cells in bronchial mucosa from before to after SBP.

**Nasal specimens.** Although at T<sub>0</sub>, BMK13<sup>+</sup> cell numbers in the nasal epithelium (Figure 4A) and subepithelium were slightly higher in the allergic group than in the controls, no differences were found at T<sub>1</sub> and T<sub>24</sub> as compared with baseline or between the two groups. BMK13<sup>+</sup> cell numbers were not significantly different in the lamina propria of the allergic and control groups at T<sub>0</sub> (Figure 4B). At T<sub>24</sub>, however, the number of BMK13<sup>+</sup> cells in the nasal lamina propria was significantly greater in allergic patients than at baseline ( $p = 0.04$ ). A significant increase in the number of IL-5-positive cells was found in the nasal epithelium at T<sub>24</sub> ( $p = 0.02$ ; Figures 5A and 6). The numbers of IL-5-positive cells in the nasal subepithelium and lamina propria were increased (Figure 5B), but this did not reach significance.

In the nasal epithelium, the eotaxin-positive cell number was significantly increased at T<sub>24</sub> as compared with that at T<sub>1</sub> ( $p = 0.05$ ) in allergic patients, but not in controls. As compared with baseline, eotaxin-positive cells were significantly



**Figure 4.** Number of BMK13<sup>+</sup> cells in nasal epithelium (A) and lamina propria (B) before (T<sub>0</sub>) and 1 h (T<sub>1</sub>) and 24 h (T<sub>24</sub>) after SBP. Grey boxes indicate allergic patients, open boxes indicate controls. Data are presented as median  $\pm$  range. \* $p < 0.05$ .



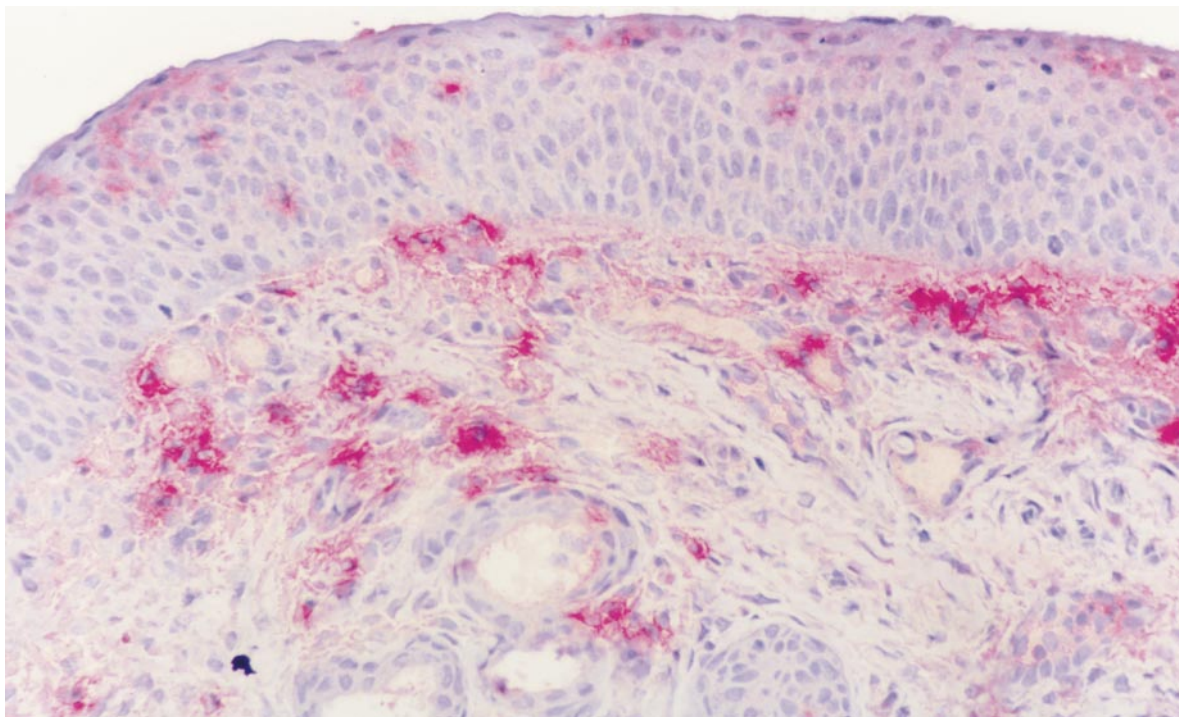
**Figure 5.** Number of IL-5-positive cells in nasal epithelium (A) and lamina propria (B) before (T<sub>0</sub>) and 1 h (T<sub>1</sub>) and 24 h (T<sub>24</sub>) after SBP. Grey boxes indicate allergic patients, open boxes indicate controls. Data are presented as median ± range. \*p < 0.05.

increased in the subepithelium ( $p = 0.01$ ) and lamina propria ( $p = 0.03$ ) of the nasal mucosa at T<sub>24</sub> in allergic patients, but not in controls. However, differences between allergic patients and controls did not reach statistical significance.

**Comparison of nasal and bronchial inflammatory cell numbers.** At baseline, the numbers of eosinophils, IL-5-positive cells, and eotaxin-positive cells in rhinitis patients and controls were low and not statistically different in bronchial and nasal biopsy specimens. The reason that only epithelial and subepithelial layers were compared is because of the difference in size between nasal and bronchial biopsy specimens. In atopic patients, the eosinophil number at T<sub>24</sub> was higher in bronchial mucosa (RML) than in nasal mucosa for both epithelium ( $p = 0.02$ ) and subepithelium ( $p = 0.01$ ). In controls, no differences were found. In allergic patients, the number of IL-5-positive cells at T<sub>24</sub>, however, was significantly greater in the nasal subepithelial layer than in the bronchial subepithelium ( $p = 0.01$ ), but this was not found in controls. In both atopic subjects and controls, the number of eotaxin-positive cells at T<sub>24</sub> was greater in the epithelium and subepithelium of the nasal mucosa than in the bronchial mucosa, but the difference did not reach statistical significance.

**Correlations between inflammatory markers, airway function and symptomatology.** In the allergic subgroup, the number of bronchial eosinophils (RML) correlated with the number of blood eosinophils (epithelium Spearman's  $r = 0.56$ ,  $p = 0.03$ ; subepithelium:  $r = 0.49$ ,  $p = 0.05$ ). No correlation could be found between nasal inflammatory cell numbers, PNIF, and nasal symptom score.

A correlation was found between the increase in bronchial eosinophils (subepithelium, RML) and the total nose symptom score in atopic subjects ( $r = 0.57$ ,  $p = 0.02$ ), which was not found in the controls. In the allergic subgroup, total nose symptom score and total lung symptom score were highly correlated ( $r = 0.57$ ,  $p = 0.02$ ). No correlation was found between symptom scores and airway function (FEV<sub>1</sub> and PNIF).



**Figure 6.** IL-5 immunohistochemical staining of a section of a nasal biopsy taken from an allergic rhinitis patient 24 h after SBP. Counterstained with hematoxylin. Original magnification:  $\times 200$ .

## DISCUSSION

In this study, we were able to demonstrate allergic inflammation, characterized by eosinophil infiltration, in the bronchi of subjects with nonasthmatic allergic rhinitis at 24 h after SBP. This occurred not only in allergen-challenged, but to a lesser extent also in saline-challenged and control segments. These data suggest a more generalized effect on the lower airways after local allergen deposition, which is also reflected by a sustained decrease in FEV<sub>1</sub> and increase in pulmonary symptoms after SBP in allergic subjects. The increased nasal obstruction and symptom score at 24 h after SBP in allergic rhinitis patients indicate that the upper respiratory tract is also involved in the allergic response. Peripheral blood eosinophilia and increased eosinophil numbers deep in the nasal mucosa after SBP further support the idea that a more extended systemic inflammatory reaction takes place in these nonasthmatic patients. Although other authors have also reported activation and mobilization of blood eosinophils in nonasthmatic allergic subjects after SBP (12, 20), we have found no published reports of signs of allergic inflammation in a remote and "upstream" organ after local allergen challenge. Generalized pulmonary inflammation cannot be explained by bronchoscopic and biopsy findings, *per se* (21). To minimize intraluminal spread of antigen from the allergen-challenged bronchial segment to other segments, we held the bronchoscope in the wedged position for 5 min, as has been suggested by other authors (11). It is also very unlikely that allergen spilled into the nose after SBP, since we performed bronchoscopy by the oral route, and it did not lead to excessive coughing. Other mechanisms that are more likely to explain the interaction between the nose and the lung are neural reflex mechanisms and systemic induction of inflammatory mediators and cells. Our results are most suggestive of a general systemic activation of eosinophils, which leads to migration of these cells into both the upper and lower respiratory mucosa. The detection of increased expression of the proeosinophilic cytokines (IL)-5 and granulocyte-macrophage colony-stimulating factor in the serum of allergic rhinitis patients after nasal allergen provocation supports our hypothesis (22). Nasal provocation with methacholine in asthmatic patients with rhinitis resulted in an increase in lower airway resistance that could be blocked by premedication of the nasal mucosa with phenylephrine, also suggesting a role for systemic absorption in the induction of lower airway resistance (23). The contribution of a bronchonasal reflex mechanism to nasal allergic inflammation after SBP is currently under investigation.

Several studies have demonstrated the role of IL-5 and eotaxin in the influx of eosinophils into the mucosa (24–26). Previous studies involving allergic rhinitis and asthma patients have shown a significant increase in IL-5 concentrations in BALF after SBP (11, 27, 28). We could also demonstrate an increased expression of both IL-5 and eotaxin in the bronchial mucosa of allergic patients 24 h after SBP. The increase did not reach statistical significance, but this could have been due to the relatively late time point of sampling and the brief, transient presence of these cytokines in the bronchial tissue. A significant increase in both IL-5-positive and eotaxin-positive cells, coinciding with the influx of eosinophils into the nasal lamina propria, was detected in the nasal mucosa at 24 h after SBP, illustrating a delay in activation of the inflammatory pathway in the nasal mucosa as compared with the bronchial mucosa.

Several investigators have shown that eosinophilic inflammation of the airways is correlated with the severity of asthma (6, 29). In our study, we also found a strong correlation of clinical data (FEV<sub>1</sub> and VAS score) with inflammatory parameters (eosinophils and IL-5 expression) in allergic lower air-

ways, but were unable to demonstrate any correlation between clinical parameters and inflammatory cell numbers in the nose. Some authors (30, 31) reported a correlation between the number of eosinophils or mediators released by eosinophils and symptoms after nasal provocation. Usually, no correlation was found (18, 32, 33).

It is not clear why allergic rhinitis patients without BHR do not have asthma in the natural situation. Our results have led us to hypothesize that allergic rhinitis patients do have asthma, but that the dose of allergen required to initiate an allergic response in the bronchi is probably higher in allergic rhinitic than in clinical asthma patients. Although the type and degree of inhalant allergy are known to play important roles in the mechanism underlying nonspecific BHR (34), other factors, such as duration and severity of exposure, could also affect the clinical manifestation of the allergic response.

In conclusion, we have found an allergic inflammatory response, similar to asthma, in the lower airways of rhinitis patients without BHR after SBP. Interestingly, the allergic response was not restricted to the allergen-challenged bronchial segment, but was more widespread: eosinophilia also occurred in other parts of the lung, and was detectable in peripheral blood. Moreover, we found increased inflammatory cell numbers in the nasal mucosa after SBP, together with signs and symptoms of allergic rhinitis, indicating that the nose is also involved in this systemic effect. We also observed a clear difference in the time course and the degree of clinical and immunopathologic findings in the nose and bronchi of allergic subjects after local allergen challenge.

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