Segmental Bronchoprovocation in Allergic Rhinitis Patients Affects Mast Cell and Basophil Numbers in Nasal and Bronchial Mucosa

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Mast cells and basophils are cells that play an important role in the initiation and control of allergic inflammation in asthma and rhinitis. This study was undertaken to determine the presence and dynamics of mast cells and basophils in the nasal and bronchial mucosa of allergic rhinitis patients after segmental bronchial provocation (SBP). Eight nonasthmatic, grass pollen-allergic rhinitis patients and eight healthy controls were included. Bronchial and nasal biopsies, as well as blood samples, were taken before (T_0) and 24 h (T_{24}) after SBP. Immunohistochemical staining was performed for mast cells (tryptase and chymase; phenotypes MC_T, MC_{TC}, MC_C) and basophils (BB1). In the bronchial mucosa, the number of $BB1^+$ cells increased significantly ($p < 0.05$) in allergic rhinitis patients after SBP. In the nasal mucosa, the numbers of MC_C and MC_{TC} cells decreased significantly, whereas the numbers of [$BB1^+$] cells increased significantly in allergic rhinitis patients after SBP ($p < 0.05$). In blood, the number of basophils decreased ($p < 0.05$) and the level of interleukin (IL)-5 increased ($p < 0.05$) in atopic patients after SBP. No significant changes could be observed in healthy controls. This study shows that SBP in nonasthmatic allergic rhinitis patients reduces numbers of mast cells in the nose as a result of enhanced degranulation. At the same time, there is evidence for an influx of basophils from the blood into the nasal and bronchial mucosae.

Keywords: bronchoprovocation; asthma; rhinitis; biopsy; inflammation

Asthma and rhinitis, which are considered manifestations of the atopic syndrome, often coexist (1, 2) and share a common genetic background (3). Although several studies have demonstrated that asthma and rhinitis are characterized by a similar inflammatory process (4–7), pathophysiologic interactions between upper and lower airways are not entirely understood. It is clear that the condition of the upper airways 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 (8–10).

Mast cells and basophils are metachromatically staining cells that are believed to play an important role in the initiation and control of upper and lower respiratory allergy (11). Both cell types express high-affinity receptors for IgE (FcεRI). Binding of IgE and subsequent crosslinking of FcεRI lead to degranulation of mast cells and release of histamine and proteases, among other effects, characteristic of the early phase of the allergic immune response (EAR). Mast cells are also sources of interleukin (IL)-4, IL-5, IL-6, and tumor necrosis factor (TNF)-α (12). Through the release of mediators, mast cells can clearly orchestrate the infiltration of leukocytes into sites of mast cell activation (13). Although basophils comprise a minor component of the inflammatory cell influx, they are capable of producing more IL-4 and IL-13 per cell than any other cell type (14, 15). Therefore, basophils are considered important modulators of the allergic immune response. Moreover, their total number and relative contribution increase during the late phase of the allergic response (LAR), and correlate with the severity of disease (16). The pattern of mediator release, particularly the lack of a secondary increase in prostaglandin D_2 and the corticosteroid sensitivity of the LAR (17), indicates that the basophil and not the mast cell is the main source of histamine in the LAR. An increase in the number of basophil-like cells in the nasal mucosa has been reported after nasal allergen provocation (18, 19). In a previous article, we demonstrated that eosinophils, IL-5^+, and eotaxin$^+$ cells are increased in the nasal mucosa of AR patients at 24 h after segmental bronchial provocation (SBP). This indicates that a simultaneous LAR may take place in other organs than those exposed to the allergen (20). Here we report on the contribution of metachromatic cells to the inflammatory process in both the challenged lung and in the nose, with special emphasis on early allergic events. Bronchial and nasal mucosal biopsy specimens and peripheral blood samples were taken from a group of nonasthmatic rhinitis patients with an isolated grass pollen allergy after SBP at a time other than the grass pollen season. The presence of mast cells was established after immunohistochemical double staining for tryptase and chymase (phenotypes MC_T, MC_{TC}, MC_C). The presence of basophils was assessed with a novel monoclonal antibody, BB1, which recognizes a unique granule constituent of basophils (21).

METHODS

Subject Groups

Eight nonasthmatic 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 included in 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, as 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_1 and a provocative concentration of methacholine causing a 20% decrease in FEV_1 (PC_{20} methacholine) > 8 mg/ml. Methacholine was administered according to a standardized tidal breathing method (22). 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 challenge. All partici-

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TABLE 1. SUBJECT CHARACTERISTICS AT BASELINE

Patient	Age (*yr*)	Sex	FEV_1 (*L*)	FEV_1 (%)	IVC (*L*)	FEV_1/VC	BAR* (%)	PC_{20} (*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	4.7	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 (terbutaline 1,000 μg, 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_{20} with 38 mg/ml methacholilne; IVC = inspiratory vital capacity.

pants 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 just before SBP (T_0). Nasal biopsy specimens were obtained at 1 h (T_1) and 24 h (T_{24}) after SBP. Collection of bronchial biopsy specimens was repeated 24 h after SBP (T_{24}). Signs and symptoms were scored 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 and ocular 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 airway obstruction were measured through peak nasal inspiratory flow (PNIF) and FEV_1. FEV_1 was determined at T_0, T_1, and T_{24}. PNIF was measured with a Youlten 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}. The basophil percentage of total white blood cells was determined by hemocytometric differential cell counting (Sysmex NE 8000; Sysmex Corporation, Kobe, Japan). IL-5 was measured in serum with a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the instructions of the manufacturer (Cytoscreen; BioSource International Inc., Camarillo, CA). The minimal detectable dose of hIL-5 with this assay is < 4 pg/ml, and no cross-reactivity was found with other cytokines.

Bronchial Biopsies and Segmental Allergen Bronchoprovocation

All bronchial biopsy specimens were taken by the same pulmonary physician (S.E.O.). After intramuscular premedication with atropine (0.5 mg), oropharyngeal anesthesia was accomplished with topical xylocaine spray 1%. 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 upper and lower lobes. Subsequently, SBP was accomplished with a method described previously (20). The tip of the bronchoscope (Model BF, type P20 D; Olympus Optical, 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. Following 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 challenged 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 a further 5 min. The bronchoscope was 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 bronchial biopsy specimens were embedded in Tissue-Tek II Optimal Cutting Temperature (OCT) compound (Sakura Finetek USA, Inc., Torrance, CA), frozen, and stored at −150° C.

TABLE 2. STUDY DESIGN

	Time Point	VAS Score	FEV_1	PNIF	Bronchial Biopsy	Nasal Biopsy	Blood Samples
T_0	Baseline	x	x	x	x	x	x
T_1	1 h after SBP*		x			x	
T_{24}	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.

Nasal Biopsies

All biopsy specimens of nasal mucosa were taken by the same investigator (G.J.B.) according to the study design as 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. Nasal biopsy specimens were embedded in Tissue-Tek II OCT compound, frozen, and stored at −150° C (23).

Monoclonal Antibodies

Monoclonal antibodies (mAbs) used in this study were anti-IgE (IgG_1; 0.2 μg/ml; clone HM25M; CLB, Amsterdam, The Netherlands), mouse antibody to human chymase (IgG_1; 1 μg/ml; clone B7; Chemicon, Brunschwig, The Netherlands), mouse antibody to human tryptase (IgG_1;

TABLE 3. CLINICAL DATA FOR ALLERGIC RHINITIS PATIENTS AND CONTROLS BEFORE (T_0) AND 24 h (T_{24}) AFTER SEGMENTAL BRONCHIAL PROVOCATION

	FEV_1 (*L*)		PNIF (*L*)		Local Symptoms		Nose Symptoms	
Patient	T_0	T_{24}	T_0	T_{24}	T_0	T_{24}	T_0	T_{24}
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.

Symptoms were individually expressed in millimeters 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 exercise intolerance).

0.7 μg/ml; clone G3; Chemicon), and antibody to basophilic granules (IgG_{2a}, < 1 μg/ml; clone BB1; Immunopharmacology Group, Southampton General Hospital, Southampton, UK). The mAb BB1 recognizes a unique granule constituent of basophils (21).

Double Staining for Tryptase and Chymase

Staining was done according to a modified method as previously described (24). Briefly, each tissue specimen was cut into serial 6-μm-thick sections on a Reichert–Jung 2800e Frigocut cryostat (Leica, Wetzlaar, Germany) and transferred onto poly-L-lysine–coated slides (Sigma Chemical Co., St. Louis, MO), dried, and stored at −80° C. Before staining, slides were heated to room temperature (RT) and subsequently dried and fixed in acetone for 10 min at RT. Slides were then rinsed in phosphate-buffered saline (PBS; pH 7.8) and placed in a semiautomatic stainer (Sequenza; Shandon, Sewickley, PA). Endogenous peroxidase was blocked with 0.1% sodium azide and 0.01% hydrogen peroxide in PBS for 30 min. Sections were then rinsed in PBS for 10 min, incubated with 10% normal goat serum (CLB) and 10% normal rabbit serum (CLB). Subsequently, slides were incubated with mouse antibody to human tryptase for 60 min at RT. Sections were rinsed with PBS for 5 min, incubated with peroxidase (PO)-conjugated rabbit antimouse (1:200; Sigma) immunoglobulin, rinsed with PBS, and incubated with PO-conjugated mouse anti-PO antibody (1:200; Sigma) for 30 min. Slides were incubated with 10% normal mouse serum (CLB) for 10 min and were then incubated with biotinylated mouse antibody to human chymase (B7) for 60 min. They were rinsed once more with PBS for 5 min and incubated with alkaline phosphatase (AP)-conjugated goat antibiotin antibody (1:50; Sigma) for 30 min. Next, slides were rinsed with PBS for 5 min and with Tris buffer (0.1 M, pH 8.5) for 5 min, and were incubated for 30 min in Fast Blue substrate containing levamisole to block endogenous AP enzyme activity. Sections were then rinsed with sodium acetate (0.1 M, pH 4.6) for 5 min and incubated with aminoethylcarbazole (AEC; 0.05% in sodium acetate 0.1 M, pH 4.6, and 0.01% peroxide) substrate for 30 min. Following this, the slides were rinsed with distilled water and mounted in glycerin gelatin. Control staining was done with an irrelevant mAb of the same subclass and at the same protein concentration as the specific antibody.

Single Staining and Tyramide Signal Amplification

The mAb stains were developed with the supersensitive immuno-AP (ss-AP) method as previously described (24). The basophil staining method differed from the staining method described earlier in the inclusion of a tyramide signal amplification (TSA) (NEN Inc., Boston, MA) step to enhance the BB1 signal. Briefly, tissue fixation was done as previously described, followed by blocking of endogenous avidin and biotin with the Vector Blocking Kit, (Vector Laboratories, Burlingame, CA) according to the specifications of the manufacturer. Subsequently, slides were rinsed in PBS (5 min) and incubated with BB1 antibody for 1 h. After rinsing in PBS, endogenous peroxidase was blocked with azide (0.2%), peroxidase (0.02%), and methanol (50%) in PBS. TSA was done by subsequent incubation with streptavidin PBS/bovine serum albumin (BSA) solution (1:100) (NEN Inc.) for 10 min, rinsing with PBS, and incubation with AP-conjugated goat antibiotin antibody (1:50, Sigma) for 30 min. This was followed by incubation in new fuchsin (Chroma, Kongen, Germany) substrate (containing levamisole to block endogenous AP enzyme activity) for a maximum of 30 min. 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 and at the same protein concentration as the specific antibody.

Microscopic Evaluation

Biopsy specimens were coded and stained cells in two sections 120 μm apart were counted under blind conditions for each antibody. Bronchial sections were divided into epithelium and subepithelium (an area 100 μm deep into the lamina propria, along the length of the epithelial basement membrane), and stained cells were counted as previously described (20). 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 (BM), 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^2 was required for analysis. Cell numbers in the subepithelial mucosa were determined as the number of immunostained cells per mm^2, and those in the epithelium were determined as cells per mm of BM, using an Axioskop 20 microscope (Zeiss, Jena, Germany) with an eyepiece graticule at a magnification of ×200.

Statistical Analysis

Data were first analyzed with Friedman's test for several related samples. In cases of significance, further analysis was done with Wilcoxon's test for intragroup analysis and the Mann–Whitney U test for intergroup analysis. Data are presented as median ± range. Correlations were evaluated with Spearman's rank correlation test. A value of $p < 0.05$ was considered significant.

RESULTS

Clinical Data

All control subjects and allergic rhinitis patients received a total dose of 500 BU grass pollen solution without exhibiting any macroscopic bronchoconstriction. An overview of the clinical data is given in Table 3. A significant decrease in FEV_1 of 9% (range: −20% to +3%) was measured at T_1 in the allergic rhinitis group ($p = 0.03$), whereas in controls, FEV_1 barely changed (median: −1%; range: −4% to +4%). At T_{24}, FEV_1 was still 9% (range: −11% to 0%) lower than its baseline value in allergic patients, but only 2% (range: −9% to 0%) below its baseline value in healthy controls. Although the overall increase in nasal and pulmonary symptoms was greater in the allergic subgroup at T_{24}, no significant differences were found in total nasal and pulmonary VAS scores for allergic rhinitis patients versus controls.

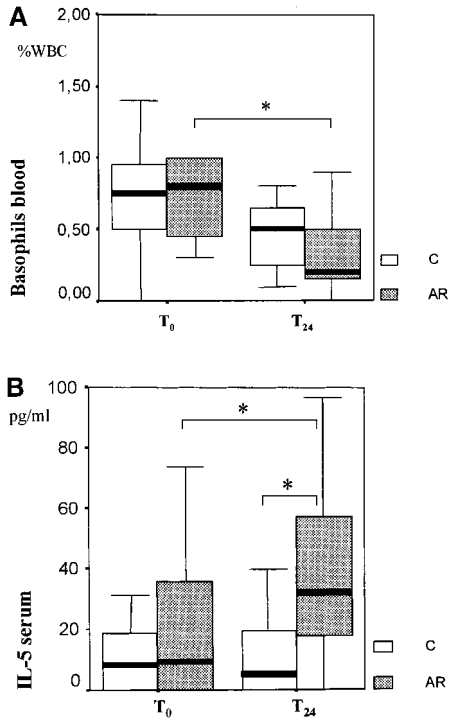

Figure 1. (*A*) Percentage of peripheral blood basophils in white blood cell (WBC) count before (T_0) and 24 h (T_{24}) after SBP. AR = allergic rhinitis patients; C = control subjects. Data are presented as median ± range. *$p < 0.05$. (*B*) Concentration of hIL-5 (pg/ml) in serum before (T_0) and 24 h (T_{24}) after SBP. AR = allergic rhinitis patients; C = control subjects. Data are presented as median ± range. *$p < 0.05$.

Peripheral Blood Parameters

No significant difference in baseline blood basophil percentage was observed in allergic patients (median: 0.8%; range: 0.3% to 2.0%) as compared with healthy controls (median: 0.75%; range: 0% to 1.4%). At T_{24}, the percentage of blood basophils was significantly decreased in allergic patients as compared with baseline ($p = 0.02$; Figure 1a). This was accompanied by a smaller reduction in the control group ($p = 0.2$). No significant difference was found between allergic rhinitis patients and controls at T_{24}.

At T_0, the concentration of IL-5 in the serum of allergic rhinitis patients (median: 9.6 pg/ml; range: 0 to 73.6 pg/ml) was comparable to that of controls (median: 8.3 pg/ml; range: 0 to 31.3 pg/ml). At T_{24}, however, the median concentration of IL-5 in allergic rhinitis patients was increased by threefold over the baseline concentration ($p = 0.02$; Figure 1b). The serum concentration of IL-5 in the control group did not change after SBP, and was significantly lower than the serum concentrations of IL-5 in the allergic rhinitis group ($p = 0.05$).

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. In bronchial biopsy specimens, the median length of evaluable basement membrane was 3.7 mm (range: 1.1 to 7.8 mm). The median surface area of subepithelium was 0.37 mm^2 (range: 0.11 to 0.78 mm^2). Bronchial epithelium and subepithelium could not be evaluated in five samples. In nasal biopsy specimens, the median length of evaluable basement membrane was 5.2 mm (range: 1.1 to 12.5 mm), the median surface area of nasal subepithelium was 0.54 mm^2 (range: 0.11 to 1.25 mm^2), and the median surface area of lamina propria was 4.69 mm^2 (range: 1.88 to 8.31 mm^2). The specified areas could not be evaluated in one case. A minimal number of seven subjects per subgroup could be included for every time point.

Bronchial specimens. At T_0, the numbers of IgE^+, $BB1^+$, $tryptase^+$, and $chymase^+$ cells (phenotypes MC_T, MC_{TC}, and MC_C) in bronchial epithelium and subepithelium were low and not significantly different in the allergic rhinitis patients and controls (Table 4). At neither T_0 nor T_{24} could changes in IgE^+, MC_T, MC_{TC}, MC_C, and $BB1^+$ cells be detected in control subjects in any of the tissues studied. In bronchial epithelium of allergic rhinitis patients, only a few mast cells and $BB1^+$ cells (Figure 2) could be detected before and after SBP. At T_{24}, the epithelial IgE^+ cell number tended to be higher in the unchallenged ($p = 0.05$) and saline challenged ($p = 0.1$) segments, and was significantly higher in the allergen challenged ($p = 0.04$) segment of allergic rhinitis patients than of controls. At T_{24}, the numbers of IgE^+ cells and mast cells in the subepithelium of allergic rhinitis patients were not significantly affected. The number of $BB1^+$ cells in the bronchial subepithelium (Figure 3) of allergic rhinitis patients, however, increased significantly in the allergen challenged segment ($p = 0.02$), saline control segment ($p = 0.02$), and even in the unchallenged segment ($p = 0.08$). At T_{24}, the number of $BB1^+$ cells in the bronchial subepithelium was significantly increased in the allergic rhinitis subjects as compared with the controls for the allergen challenged segment ($p = 0.002$) and saline challenged segment ($p = 0.01$), but not for the unchallenged segment ($p = 0.6$).

Nasal specimens. As in the bronchial mucosa, only few $chymase^+$, $tryptase^+$ (Figure 4), or $BB1^+$ cells were seen in the nasal epithelium, of any subject either before or after SBP (Table 5). At T_0, IgE^+ cells could be detected in the nasal epithelium, and the numbers of such cells were higher in the allergic rhinitis patients than in controls ($p = 0.06$). At T_0, the numbers of IgE^+ cells and MC_{TC}, MC_T, and $BB1^+$ cells in the nasal lamina propria showed no significant differences in the allergic rhinitis patients and control subjects. The number of MC_C cells, however, was higher in the rhinitis patients than in the controls at T_0 ($p = 0.03$).

At T_1, no significant changes in cell numbers were found for mast cells, IgE^+ cells, or $BB1^+$ cells in nasal epithelium and lamina propria of the allergic rhinitis patients as compared

TABLE 4. CELL NUMBERS IN BRONCHIAL SPECIMENS BEFORE (T_0) AND 24 h (T_{24}) AFTER SEGMENTAL BRONCHIAL PROVOCATION

				T_{24}		
Variable	Group	Layer	T_0	UC	AC	SC
IgE	AR	Epithelium	0 (0–22)	14 (0–48)	2 (0–29)	3 (0–58)
		Subepithelium	21 (0–442)	41 (21–264)	76 (0–128)	138 (32–943)
	C	Epithelium	0 (0–3)	0 (0–2)	0	0 (0–21)
		Subepithelium	15 (0–35)	4 (0–133)	10 (0–245)	10 (0–120)
BB1	AR	Epithelium	0	0 (0–1)	0	0
		Subepithelium	0 (0–4)	9 (0–144)	88 (22–223)*	25 (5–350)*
	C	Epithelium	0	0	0	0
		Subepithelium	0 (0–4)	5 (0–16)	5 (0–88)	2 (0–33)
MC_C	AR	Epithelium	0	0 (0–3)	0	0
		Subepithelium	2 (0–28)	0 (0–43)	0 (0–21)	4 (0–143)
	C	Epithelium	0	0	0 (0–8)	0 (0–3)
		Subepithelium	0 (0–8)	0 (0–3)	0 (0–2)	2 (0–5)
MC_{TC}	AR	Epithelium	0 (0–33)	0 (0–3)	0 (0–5)	0
		Subepithelium	36 (0–141)	42 (0–126)	31 (4–131)	33 (0–81)
	C	Epithelium	0 (0–3)	0 (0–3)	0 (0–3)	0 (0–8)
		Subepithelium	14 (0–79)	8 (0–184)	15 (0–89)	27 (0–265)
MC_T	AR	Epithelium	1 (0–10)	8 (0–55)	0 (0–22)	0 (0–24)
		Subepithelium	111 (0–622)	161 (28–351)	139 (25–323)	74 (0–326)
	C	Epithelium	0 (0–19)	0 (0–13)	8 (0–31)	9 (0–36)
		Subepithelium	127 (8–203)	56 (10–140)	151 (0–414)	110 (0–300)

Definition of abbreviations: AC= allergen challenged right middle lobe; AR = allergic rhinitis patients; C = control subjects; SC = saline challenged right upper lobe; UC = unchallenged left lung.

Data are presented as median ± range.

*$p < 0.05$ (Wilcoxon's signed ranks test).

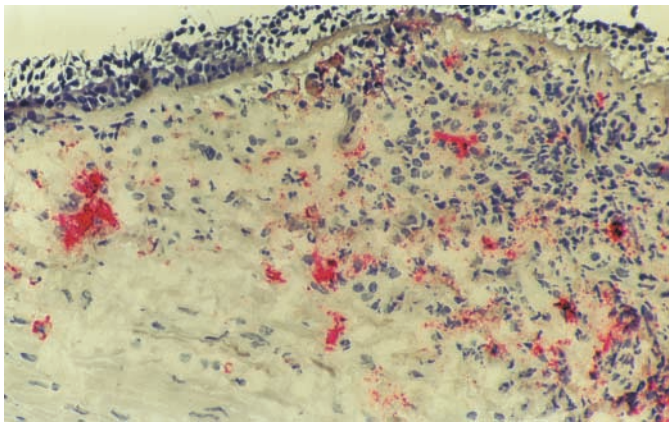

Figure 2. Bronchial biopsy specimens of a challenged allergic subject stained immunohistochemically for $BB1^+$ cells and counterstained with hematoxylin. Original mangnification ×200.

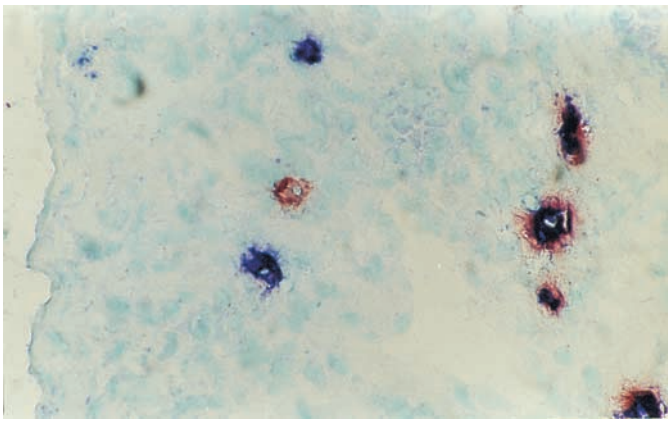

Figure 4. Nasal biopsy sections of an allergic subject showing immunoreactivity for mast cells at T_0. Counterstained with methyl green. Original magnification: ×400.

with baseline numbers. At T_{24}, however, the numbers of MC_C ($p = 0.02$) and MC_{TC} ($p = 0.04$) cells in the nasal lamina propria of the allergic rhinitis patients were significantly decreased (Figures 5a and 5b). In contrast, the numbers of MC_T and IgE^+ cells remained stable, whereas that of $BB1^+$ cells was significantly increased ($p = 0.01$; Figure 5c). At T_{24}, the $BB1^+$ cell number in the nasal subepithelium ($p = 0.01$) and lamina propria ($p = 0.02$) was significantly higher in allergic rhinitis patients than in controls. No significant increases from baseline were seen for IgE^+, mast cell, or $BB1^+$ cell numbers in healthy controls at either T_1 or T_{24}.

Comparison of Cell Counts in Upper and Lower Airways

At T_0, IgE^+ cell numbers were significantly higher in nasal epithelium (Table 5) than in bronchial epithelium (Table 4) in the allergic rhinitis patients ($p = 0.02$). In the controls, IgE^+ cells were predominantly present in nasal epithelium as compared with bronchial epithelium ($p = 0.02$), but cell counts were lower than in the allergic rhinitis subjects. In the subepithelium there was no difference between the number of IgE^+ cells in either the nose or the bronchi of the allergic rhinitis patients and control subjects. At T_0, $BB1^+$ cells could hardly be detected in either nasal or bronchial mucosa of the two study groups. At T_{24}, however, the influx of $BB1^+$ cells was much higher in the bronchial subepithelium than in the nasal subepithelium ($p = 0.04$) of the allergic rhinitis patients, whereas no difference was found in the control subjects. There was a clear difference in the relative distribution of $chymase^+$ and $tryptase^+$ cells between nasal and bronchial mucosa. In the bronchi, MC_T was the predominant cell type (74% in atopic subjects and 89% in controls), followed by MC_{TC} (23% in atopic subjects versus 10% in controls) with almost no MC_C cells (3% in atopic subjects versus 1% in controls). In contrast, MC_{TC} cells were the most abundant cell type in the nose (79% in atopic subjects and 89% in controls), followed by MC_C cells (19% in atopic subjects and 5% in controls), and with very few MC_T cells (3% in both groups). At T_{24}, the percentage of MC_T cells increased significantly in the nasal subepithelium ($p = 0.04$) and to a lesser extent also in the bronchial subepithelium ($p = 0.2$) of allergic rhinitis patients, at the expense of MC_{TC} and MC_C cells.

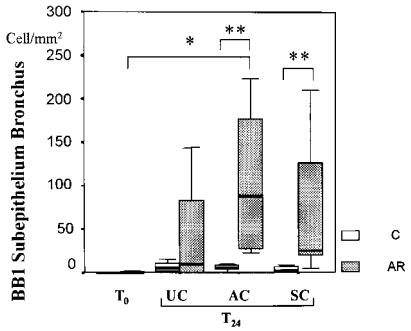

Figure 3. Number of $BB1^+$ cells in subepithelial layer before (T_0) and 24 h (T_{24}) after SBP. AC = allergen challenged right middle lobe; AR = allergic rhinitis patients; C = control subjects; SC = saline challenged right upper lobe; UC = unchallenged left lung. Data are presented as median ± range. *$p < 0.05$, **$p < 0.01$.

DISCUSSION

In this study, we were able to demonstrate changes in nasal mast cell and basophil numbers in nonasthmatic allergic rhinitis patients at 24 h after SBP. Furthermore, we found an influx of basophils into the bronchi of these nonasthmatic allergic rhinitis patients at 24 h after SBP. This occurred not only in challenged but also, albeit to a lesser extent, in saline challenged and unchallenged segments. In addition to these inflammation-related effects, we noticed a sustained decrease in FEV_1 after SBP in subjects with allergic rhinitis. In a previous study, we demonstrated that SBP in allergic rhinitis patients induced eosinophilia in the blood and mucosal inflammation characterized by increased numbers of eosinophils, and of $IL\text{-}5^+$ and $eotaxin^+$ cells in both upper and lower airways (18). These data suggest a more generalized effect of local allergen deposition.

Granule secretion by mucosal mast cells, and the influx of basophils from blood into tissue, have been associated with disease activity in the upper and lower airways of patients with allergic rhinitis and asthma (16, 18, 25–27). Since these two cell types are important for initiation and modulation of the allergic reaction (11, 14, 15), we studied the dynamics of mast cells and basophils in the nasal mucosa after allergen provocation in a distant and "downstream" organ.

In the nasal mucosa, the numbers of $chymase^+$ (MC_C) and $tryptase^+/chymase^+$ (MC_{TC}) mast cells decreased significantly, whereas the numbers of $BB1^+$ cells increased significantly in allergic rhinitis patients at 24 h after SBP. In contrast to the

TABLE 5. CELL NUMBERS IN NASAL SPECIMENS BEFORE (T_0) AND 1 h (T_1) AND 24 h (T_{24}) AFTER SEGMENTAL BRONCHIAL PROVOCATION

Variable	Group	Layer	T_0	T_1	T_{24}
IgE	AR	Epithelium	34 (0–140)	13 (0–215)	6 (0–42)
		Subepithelium	106 (9–1,211)	157 (19–794)	39 (0–282)
		Lamina propria	74 (6–255)	71 (14–192)	46 (5–88)
	C	Epithelium	4 (0–20)	1 (0–11)	1 (0–24)
		Subepithelium	32 (10–55)	29 (3–168)	16 (0–34)
		Lamina propria	14 (6–40)	12 (8–34)	10 (2–28)
BB1	AR	Epithelium	0	0 (0–1)	0
		Subepithelium	1 (0–2)	0 (0–1)	4 (1–12)*
		Lamina propria	1 (0–5)	2 (0–3)	7 (3–14)*
	C	Epithelium	0	0 (0–1)	0 (0–1)
		Subepithelium	1 (0–1)	1 (0–1)	1 (0–9)
		Lamina propria	1 (0–5)	1 (0–4)	2 (0–10)
MC_C	AR	Epithelium	0 (0–16)	0 (0–6)	0
		Subepithelium	29 (8–86)	22 (11–45)	15 (0–179)
		Lamina propria	18 (5–48)	11 (3–21)	3 (1–25)*
	C	Epithelium	0	0	0
		Subepithelium	9 (0–55)	6 (2–59)	5 (1–19)
		Lamina propria	4 (1–27)	5 (1–47)	3 (0–15)
MC_{TC}	AR	Epithelium	0 (0–14)	1 (0–27)	0 (0–5)
		Subepithelium	235 (54–694)	449 (189–555)	118 (0–451)
		Lamina propria	92 (22–178)	114 (77–137)	42 (0–111)*
	C	Epithelium	0 (0–15)	0	0
		Subepithelium	228 (46–540)	223 (80–431)	179 (0–324)
		Lamina propria	105 (47–185)	95 (56–127)	89 (0–172)
MC_T	AR	Epithelium	0 (0–7)	4 (0–9)	0 (0–15)
		Subepithelium	5 (0–40)	11 (0–123)	9 (0–26)
		Lamina propria	2 (0–15)	2 (0–11)	2 (0–6)
	C	Epithelium	0	0	0 (0–3)
		Subepithelium	6 (0–23)	3 (0–78)	3 (0–45)
		Lamina propria	2 (1–6)	4 (0–11)	1 (0–13)

Definition of abbreviations: AR = allergic rhinitis patients; C = control subjects.
Data are presented as median ± range.
* $p < 0.05$ (Wilcoxon's signed ranks test).

changes found in the nose, the number of mast cells in the bronchial subepithelium, which are predominantly tryptase$^+$ (24, 28, 29), did not change significantly after SBP. A possible explanation for the relatively stable number of tryptase$^+$ cells in the nasal and bronchial mucosae could be the influx of tryptase$^+$ basophils from the peripheral blood into the bronchial mucosa (30).

Discrepancies in mast cell counts in different studies, probably caused by different methods for detecting of mast cells, have led to various opinions about mast cell dynamics. Transmucosal migration of mast cells into the epithelium has been suggested as a possible mechanism for an increase in epithelial mast cells after allergen exposure (5, 31). Degranulation has been proposed and demonstrated by other authors, and could also contribute to changes in mast cell numbers in the nasal and bronchial mucosae (10, 26, 32, 33). Immunohistochemical analysis of mast cells is hampered by the lack of a universal mast cell membrane-associated marker. In our study we found no indication of a redistribution of mast cells in nasal and bronchial mucosae at either an early (T_1) or a late (T_{24}) time point. Therefore, degranulation is a more likely mechanism for the sudden decrease in MC_C and MC_{TC} cells in the nasal lamina propria of allergic rhinitis patients after SBP, whereas the number of IgE$^+$ cells remains stable. Degranulation of mast cells in the nose after SBP occurred at a relatively late time point as compared with that found in experiments after nasal allergen challenge. KleinJan and coworkers demonstrated an increase in the number of basophils and a reduction in mast cell numbers in nasal mucosa of allergic rhinitis subjects within 1 h after nasal allergen provocation (28). In our study, no changes in the number of nasal mast cells or basophils were seen at 1 h after SBP. At T_{24}, however, more than 50% of MC_{TC} cells in the nasal lamina propria of allergic rhinitis patients (as compared with only 10% in controls) had lost their granule contents, which is in accordance with findings during natural exposure (26). Lozewicz and Wagenmann have shown that unilateral nasal allergen challenge results in an immediate reduction in the number of stainable mast cells (34) and in a delayed increase in basophil numbers and histamine production in the contralateral unstimulated nostril (35). Lozewicz and Wagenmann speculated that a neural reflex mechanism was most likely to contribute to mast cell degranulation in the unchallenged nostril. The delayed effect on nasal mucosal mast cells that we see in our model, however, is more suggestive of a systemic allergic response.

In allergic rhinitis patients, we found a decreased number of basophils in the peripheral blood at 24 h after SBP. The decrease in circulating basophils at this time point could have been the result of active migration and redistribution of these cells from the blood to the affected tissue (16). Moreover, we detected increased expression of IL-5 in the serum of allergic rhinitis patients after SBP, which suggests that a systemic allergic response is triggered. Cytokines such as IL-5, but also IL-1, IL-3, granulocyte–macrophage colony-stimulating factor (GM-CSF), stem-cell factor, nerve growth factor, histamine-releasing factor, and interferon-γ are capable of priming basophils (36). Both IL-3 and GM-CSF were undetectable in serum samples in our study (data not shown). Comparable serum IL-5 results have been demonstrated in patients with allergic asthma after bronchial allergen provocation (37) and natural exposure to allergen (38). Furthermore, nasal provocation with methacholine in asthmatic patients with rhinitis resulted in an

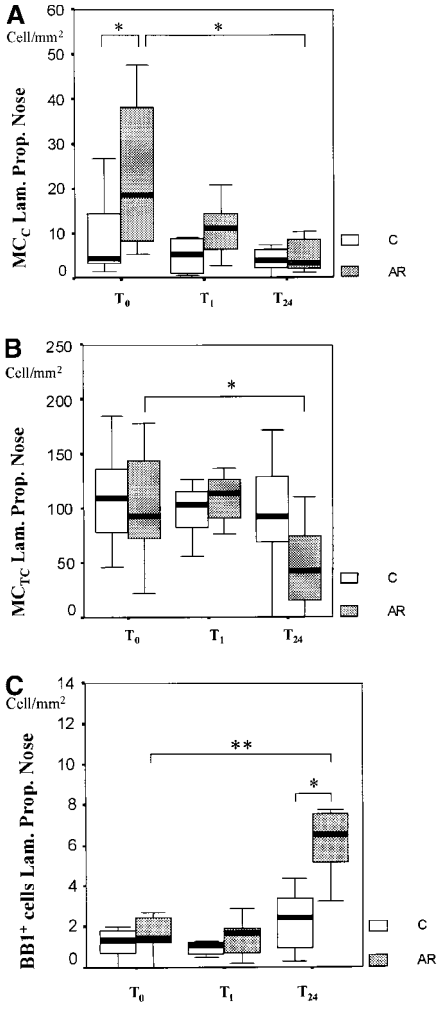

Figure 5. Number of MC_C (*A*), MC_{TC} (*B*), and $BB1^+$ (*C*) cells in the nasal lamina propria before (T_0) and at 1 h (T_1) and 24 h (T_{24}) after SBP. AR = allergic rhinitis patients; C = control subjects. Data are presented as median ± range. *$p < 0.05$, **$p < 0.01$.

increase in lower airway resistance that could be blocked by premedication of the nasal mucosa with phenylephrine (39). This is also suggestive of a systemic effect of inflammatory mediators in the induction of lower airway resistance. Another possible interaction mechanism could be systemic allergen absorption, as was suggested by Kontou-Karakitsos and colleagues in the early 1970s (40). However, the allergen we used, grass pollen, has a much higher molecular weight than the antigen fragments used in the peanut extract in their study. Therefore, we find it less plausible that grass pollen particles cross the mucosal barrier and merge in the blood.

It is highly unlikely that the methodologic approach used in our study would contribute to a systemic allergic response. Bronchoscopy and biopsy *per se* do not result in generalized bronchial inflammation (41). To minimize the intraluminal spread of antigen from the allergen-challenged segment to other segments, the bronchoscope in our study was held in the wedged position. It is also very unlikely that allergen spilled into the nose after SBP, since bronchoscopy was performed via the oral route and did not lead to excessive coughing. This is supported by a small study that addressed the issue of cross-contamination of the nose and lungs with a radioactive tracer (42). In this pilot study, a radionuclide was placed bronchoscopically into the bronchial tree in four patients, and was still clearly visible in the same position after 24 h, suggesting that contamination of the nose through intraluminal spread is unlikely to take place (42).

On the basis of our results, we hypothesize that allergic rhinitis patients without bronchial hyperresponsiveness can express "asthma-like" symptoms and inflammation. However, the dose of allergen required to initiate an allergic response in the bronchi is probably higher in allergic rhinitis patients than in patients with clinical asthma. Apparently, such a dose is not encountered during natural exposure to allergen. Moreover, other factors, such as the type of inhalant allergy and the duration and severity of exposure, are important in this context (43). We also found that SBP influences the number of stainable mast cells and basophils in the upper airways of allergic rhinitis patients. We speculate that enhanced production of inflammatory mediators at the site of allergen provocation leads to a more generalized effect on metachromatic cells throughout the respiratory system and the blood.

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