

Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin

Alison J. Macfarlane, MRCP,^a Onn M. Kon, MRCP,^a Susan J. Smith, PhD,^a Kyriaki Zeibecoglou, MD,^a L. Nasreen Khan, MRCP,^a Luis T. Barata, MD,^a Alan R. McEuen, PhD,^b Mark G. Buckley, PhD,^b Andrew F. Walls, PhD,^b Qiu Meng, MD,^a Marc Humbert, MD,^c Neil C. Barnes, FRCP,^d Douglas S. Robinson, MD,^a Sun Ying, MD,^a and A. Barry Kay, MD^a *London and Southampton, United Kingdom, and Clamart, France*

Background: Previous studies used indirect methods to identify basophils in the bronchi in asthma, and the numbers were not compared with eosinophils and mast cells. Furthermore, differences in basophil numbers between atopic and nonatopic asthma at baseline and between late-phase skin and asthmatic reactions have not been previously documented.

Objective: The basophil granule-specific mAb BB1 was used to identify basophils in (1) bronchial biopsy specimens from atopic asthmatic subjects and nonatopic asthmatic subjects and control subjects, (2) biopsy specimens from atopic asthmatic subjects before and after inhalational allergen challenge, and (3) late-phase skin reactions. Basophil numbers were compared with EG2⁺ eosinophils and tryptase⁺ mast cells.

Methods: Cells were enumerated in bronchial and skin biopsy specimens by means of immunohistochemistry with the alkaline phosphatase-antialkaline phosphatase method.

Results: There were elevated numbers of basophils in baseline biopsy specimens in atopic asthmatic subjects compared with atopic control subjects or normal control subjects, although eosinophils and mast cells were 10-fold higher. There was an intermediate number of basophils in nonatopic asthmatic subjects. Basophils increased after allergen inhalation, but again basophils were less than 10% of eosinophils. In contrast, basophils in cutaneous late-phase reactions were approximately 40% of infiltrating eosinophils. The peak of basophil accumulation was at 24 hours, whereas maximal eosinophil infiltration occurred at 6 hours. One third of cutaneous basophils had morphologic appearances suggestive of degranulation.

Conclusion: Numerous basophils infiltrated cutaneous late-phase reactions in atopic subjects. However, this cell was not prominent in bronchial biopsy specimens of asthmatic sub-

jects, either at baseline or after allergen challenge. (*J Allergy Clin Immunol* 1999;99:107.)

Key words: Basophil, eosinophil, mast cell, skin, bronchial provocation

Basophils express the high-affinity IgE receptor FcεRI together with membrane-bound surface IgE.¹ IgE-dependent and IgE-independent activation of basophils results in the release of a range of potent mediators, including histamine, leukotrienes, proteoglycans, and a variety of cytokines, including IL-4 and IL-13.²⁻⁷ Basophils and eosinophils arise from similar CD34⁺ precursor cells of myeloid origin and circulate within the blood stream.⁸ Although basophils are not usually detected within healthy human tissues, this cell type has been identified in a variety of inflammatory tissue reactions by using a range of indirect techniques.⁹⁻¹³

It is difficult to distinguish mast cells from basophils in tissues because both cell types have metachromatic granules and are FcεRI⁺. Earlier attempts to distinguish these cell types were based on morphology, mediator profiles, and/or markers in flow cytometry, which were recognized by basophils rather than mast cells.¹⁴⁻¹⁷ Attempts were also made to enumerate metachromatic cells with multilobular nuclei, tryptase⁺/metachromatic cells or IgE⁺/tryptase⁻ cells (ie, putative basophils) within the airways^{18,19} of symptomatic asthmatic subjects and in the lungs of asthmatic subjects dying of fatal asthma.²⁰ Increased metachromatic cells with the nuclear morphology of basophils and increases in mediators suggestive of basophil activation have been demonstrated in sputum^{21,22} and in bronchoalveolar lavage fluid after the late asthmatic reaction.²³ However, the lack of a sensitive and specific marker for basophils has hampered confident identification of basophils within the tissues.

A murine mAb, 2D7, directed against granule determinants and of a molecular size of approximately 72 and 76 kd was described in 1995.²⁴ No immunostaining was observed in normal human lung, skin, or bowel tissue.²⁵ 2D7⁺ cells were identified at the sites of allergen-induced cutaneous late-phase reactions (LPRs), with peak infiltration occurring at 6 hours after challenge.²⁵

Because, as stated, previous attempts to identify

From ^aAllergy and Clinical Immunology, Imperial College School of Medicine, National Heart and Lung Institute, London; ^bImmunopharmacology Group, Southampton General Hospital, Southampton; ^cService de Pneumologie et de Réanimation Respiratoire, Hôpital Antoine Bécclère, Clamart; ^dRespiratory Medicine, The London Chest Hospital, London.

Supported by a grant from the National Asthma Campaign (UK) and funding from Zeneca Pharmaceuticals, UK.

Received for publication May 25, 1999; revised July 29, 1999; accepted for publication Aug 30, 1999.

Reprint requests: A. Barry Kay, MD, Professor and Head, Allergy and Clinical Immunology, Imperial College School of Medicine, National Heart and Lung Institute, Dovehouse St, London SW3 6LY, UK.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/102686

Abbreviations used

AA:	Atopic asthmatic subject
AC:	Atopic control subject
APAAP:	Alkaline phosphatase-antialkaline phosphatase
EAR:	Early asthmatic reaction
IHC:	Immunohistochemistry
LAR:	Late asthmatic reaction
LPR:	Late-phase reaction
NAA:	Nonatopic asthmatic subject
NC:	Normal control subjects
MBP:	Major basic protein
MFI:	Mean fluorescence intensity
SMF:	Specific mean fluorescence

basophils in tissue from asthmatic subjects used indirect methods, the aim of the present study was to re-evaluate this issue by using BB1, another recently available basophil-specific mAb.²⁶ We have compared basophils, eosinophils, and mast cell numbers in bronchial biopsy specimens from atopic and nonatopic asthma at baseline and after allergen challenge. We have also compared late asthmatic and skin reactions to determine whether there are differences between tissues in terms of allergen-induced basophilia.

METHODS

Subjects were recruited from the Allergy Clinic, Royal Brompton Hospital, and the London Chest Hospital. The ethics committee at each center approved the study, and all patients gave written consent. All patients were nonsmokers, with no other intercurrent illness. Asthmatic patients were stable at the time of recruitment with an FEV₁ greater than 70% of predicted value at each clinic visit. No patient had been treated with inhaled, nasal, or oral corticosteroids for at least 3 months before entry into the study. Patients were asked to refrain from use of all medication, including short-acting β_2 -agonists for at least 8 hours before each visit.

Asthma was defined as reversible airway obstruction, with a documented decrease in FEV₁ greater than 20% from baseline in response to allergen (atopic asthmatic subjects) or histamine (nonatopic asthmatic subjects). Nonatopic asthmatic subjects demonstrated a histamine PC₂₀ of less than 2 mg/mL, whereas nonasthmatic volunteers demonstrated a histamine PC₂₀ of greater than 16 mg/mL. Atopic and nonatopic status was described as previously.²⁷

For the bronchoscopy studies, patients were classified as atopic asthmatic subjects (AAs), nonatopic asthmatic subjects (NAAs), atopic nonasthmatic control subjects (ACs), or nonatopic, nonasthmatic normal control subjects (NCs). These subjects were carefully documented, as described in previous studies.²⁷⁻³⁰

Bronchial biopsy studies

There were 2 asthmatic study populations. The first (baseline study) consisted of 24 AAs (9 male and 15 female patients) with a mean age of 28 ± 1.4 years and a mean FEV₁ of $83.3\% \pm 6.7\%$ of predicted value, 9 NAAs (5 male and 4 female patients) with a mean age of 54 ± 2.3 years and a mean FEV₁ of $79.1\% \pm 6.3\%$ of predicted value, 8 ACs (4 male and 4 female patients) with a mean age of 23.8 ± 1 years and a mean FEV₁ of $104.8\% \pm 2.8\%$ of predicted value, and 10 NCs (6 male and 4 female patients) with a mean age of 24.2 ± 0.5 years and a mean FEV₁ of $106.7\% \pm 4.0\%$ of predicted value. Baseline bronchial biopsy specimens were obtained from

each patient. The second population of asthmatic subjects (inhalational allergen challenge study) consisted of 11 AAs (3 male and 8 female patients) with a mean age of 30.6 ± 1.5 years and FEV₁ of $94.2\% \pm 2.2\%$ of predicted value. They were selected on the basis of a demonstrated late asthmatic reaction (LAR) with decrease in FEV₁ of at least 15% occurring 2 to 8 hours after inhalational allergen challenge. These patients were identified and recruited in a double-blind, placebo-controlled intervention study for which they represent the placebo arm.³¹ A minimum of 3 weeks after their screening allergen challenge, these patients attended for a baseline bronchoscopy performed in accordance with established guidelines.³² They received a dose of placebo medication, and then, 24 hours later, the inhalational allergen challenge was repeated. FEV₁ was monitored for 8 hours after challenge, and 24 hours later, bronchoscopy was again performed.

Inhalational allergen challenge

Inhalational allergen challenge was performed by using a standardized protocol based on that described by Chai et al³³ by using a breath-activated dosimeter (Mefar). AAs inhaled a dose of allergen known from a screening, incremental allergen challenge to cause a decrease in FEV₁ of at least 15% from baseline during the late asthmatic response.

Cutaneous LPRs

Late-phase skin reactions were performed on 10 atopic subjects (4 female and 6 male subjects) with a mean age of 31.8 ± 2.4 years, and the sites underwent biopsy at time intervals, as previously described.^{28,34}

Immunohistochemistry

Bronchial and skin biopsy specimens were prepared, as previously described.^{27,35} The alkaline phosphatase-antialkaline phosphatase (APAAP) method was used to enumerate cells binding to mAbs against human activated eosinophils (EG2; Pharmacia, Milton Keynes, UK) and mast cells (anti-tryptase; Dako, High Wycombe, UK).^{27,35} Basophils were detected by using the murine mAb BB1. The APAAP technique was performed as previously described.^{27,35} Optimal concentrations of all antibodies used were determined in pilot experiments. Briefly, the sections were incubated with the mAbs against phenotypic marker for 30 minutes, washed in PBS, and incubated with rabbit anti-mouse immunoglobulin (Dako) for 30 minutes. After washing in PBS, the sections were incubated with soluble complexes of APAAP (Dako) for a further 30 minutes and developed with Fast Red (Sigma Chemicals Co, Poole, UK) as chromogen for signal visualization. For immunostaining of BB1⁺ basophils, the slides were pretreated with 0.1% saponin (Sigma)-PBS for 30 minutes, incubated overnight with BB1 (1:10 in 0.1% saponin-PBS), and then processed as described above by using 0.1% saponin-PBS as the buffer for all stages.

Positive cells stained red after development with Fast Red. Omission or substitution of the primary antibody with isotype-matched murine antibodies were included as negative controls.

Quantification of immunostaining

Two sections per biopsy specimen were counted for each cellular marker in a blinded fashion by using an Olympus microscope equipped with an eyepiece graticule (Olympus Corp, Lake Success, NY). For bronchial biopsy specimens, the area immediately below the intact basement membrane was counted to a depth of 220 μ m (one graticule at $\times 400$ magnification). Results are expressed as immunopositive cells per millimeter of basement membrane. The epithelial cell layer was not intact on all bronchial biopsy specimens, and therefore no counts were made on the epithelial side of the base-

TABLE I. Intracellular staining of permeabilized, peripheral blood leukocytes with BB1 and BMK13 (anti-MBP) and phycoerythrin-labeled F(ab')₂ goat anti-mouse IgG (heavy- and light-chain specific)

	Peripheral blood leukocytes				
	Basophils	Eosinophils	Neutrophils	Lymphocytes	Monocytes
MBP	10.6 (2.3-23.1)	20.9 (8.89-57.82)	ND	<0.1 (0.0-0.1)	<0.1 (0.0-0.2)
BB1	48.6 (18.7-116.3)	0.6 (0.0-3.1)	<0.1 (0.0-0.2)	<0.1 (0.0-0.1)	<0.1 (0.0-0.1)

Results are expressed as median values and ranges (n = 8) of the specific mean fluorescence.

ment membrane. Skin biopsy specimens were counted for the whole area of the biopsy. Results are therefore expressed as immunopositive cells per square millimeter of skin biopsy specimen. In addition, the pattern of granules around BB1⁺ cells was counted either as discrete intact basophils or as dispersed degranulated basophils.

Flow cytometry

The specificity of BB1 for basophil granules was further confirmed by comparing permeabilized basophils, eosinophils, neutrophils, lymphocytes, and monocytes. We also wished to determine whether all basophils stained with BB1 and whether there were differences between atopic and nonatopic subjects. Mononuclear cells (including basophils) were isolated from the peripheral blood of both atopic (n = 7) and nonatopic subjects (n = 4). The cells were prepared by a combination of dextran sedimentation and density-gradient centrifugation on Histopaque (Sigma Aldrich, Poole, UK). Eosinophils were further purified to greater than 97% purity by using CD16⁺ selection by MACS (Miltenyi Biotec Ltd, Bisley, UK). The cells were fixed, permeabilized with saponin, and stained as previously described.³⁶ Briefly, 100 μ L of PBMCs (20×10^6 mL⁻¹) or eosinophils (5×10^6 mL⁻¹) were incubated for 30 minutes at room temperature with either 5 μ L of BB1, 10 μ L of BMK-13 (directed against major basic protein [MBP]),³⁷ or 10 μ L of 100 μ g mL⁻¹ of either mouse IgG1 or IgG2a isotype control antibodies and subsequently stained as previously described.

Cells were analyzed by using a Coulter Epics Elite flow cytometer (Beckman Coulter, High Wycombe, UK). Basophils (1000 events) and eosinophils (5000 events) were stained with BB1 and BMK13 and compared with the IgG2a and IgG1 isotype-matched antibodies, respectively. The voltage used to detect fluorescence of phycoerythrin was altered according to the cell type being acquired, and therefore the mean fluorescence intensity (MFI) of the basophils and the eosinophils were equal (MFI of 5). Results are expressed as specific mean fluorescence (SMF), which was calculated by subtracting the MFI of the respective isotype control antibody from the MFI of the test antibody.

We have previously identified the high staining Fc ϵ RI α cell population in mononuclear cell preparations as basophils.³⁸ The lymphocytes and monocytes were identified by their characteristic forward and side-scatter profiles, which were confirmed by staining with antibodies against CD3 and CD14, respectively. The eosinophils were analyzed as purified populations and confirmed by using an antibody against CD9. The antibody BMK13 was used as a positive control for both eosinophil and basophil staining because it has previously been demonstrated that basophils, as well as eosinophils, contain MBP.³⁹

Statistical analysis

Statistical analyses were performed with the aid of a commercially available statistical package (Minitab for Windows, Minitab Release 9.2; Minitab Inc, State College, Pa). Nonparametric statistics were used throughout the study. All immunohistochemistry and flow cytometry data are expressed as medians and interquartile ranges. The Mann-Whitney *U* test was used for intergroup compar-

isons. The 2-tailed Wilcoxon signed-rank test was used to analyze changes in inflammatory cell numbers in response to allergen challenge. Correlation coefficients were calculated by using the Spearman rank order method. A *P* value of less than .05 was accepted as statistically significant.

RESULTS

Flow cytometry

Analysis of staining on permeabilized peripheral blood leukocytes by flow cytometry confirmed that BB1 was specific for basophils. There was negligible SMFs with eosinophils, neutrophils, lymphocytes, and monocytes (n = 8) stained with BB1 (Table I). In contrast, the total basophil population was BB1⁺ because there was a significant shift in the fluorescence of the whole population of basophils (Fig 1). There was no significant difference in the intensity or profile of staining of BB1⁺ basophils from atopic subjects (median SMF, 48.6; range, 18.7-116.3) compared with basophils from normal nonatopic subjects (median SMF of BB1, 51.2; range, 22.9-144.9; n = 7).

Baseline bronchial biopsy specimens

For all groups studied, the numbers of basophils in bronchial biopsy specimens was approximately 10-fold less than eosinophils and mast cells (Fig 2). The numbers of BB1⁺ basophils in bronchial biopsy specimens of AAs were significantly elevated compared with either ACs (*P* = .0061) or NCs (*P* = .0037; Fig 2). In contrast, the median basophil count in the NAA bronchial biopsy specimens was not significantly different from any of the other groups. The mean numbers of BB1⁺ cells in all asthmatic subjects (ie, AAs and NAAs) was significantly elevated compared with those of ACs and NCs (*P* = .003).

EG2⁺ eosinophils were significantly elevated in AAs compared with NCs (*P* = .02) and in NAAs compared with NCs (*P* = .002). In ACs eosinophil numbers were intermediate among NCs, AAs, and NAAs (Fig 2). There were no significant differences in the numbers of tryptase⁺ cells between any of the 4 groups studied (Fig 2). There were no correlations between BB1⁺ cells and EG2⁺ or tryptase⁺ cells.

Preallergen and postallergen challenge bronchial biopsy specimens

The allergen-induced LAR was associated with an increase in BB1⁺ basophils (*P* = .007) and EG2⁺ eosinophils (*P* = .009; Fig 3) in bronchial biopsy specimens taken at 24 hours after challenge. This followed a

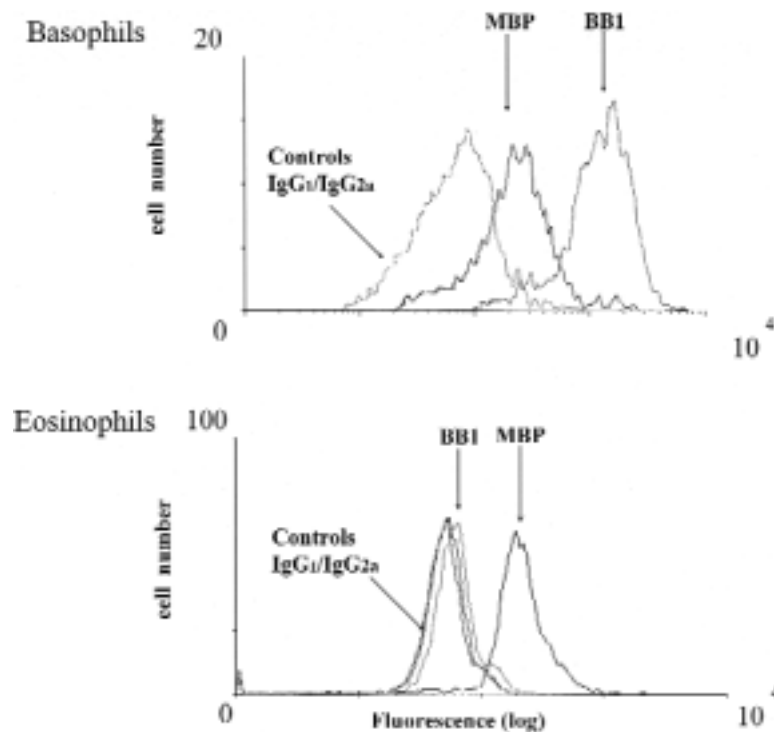


FIG 1. Representative flow cytometry profiles of peripheral blood basophils and purified peripheral blood eosinophils showing BB1 and MBP binding compared with respective IgG2a and IgG1 isotypic controls.

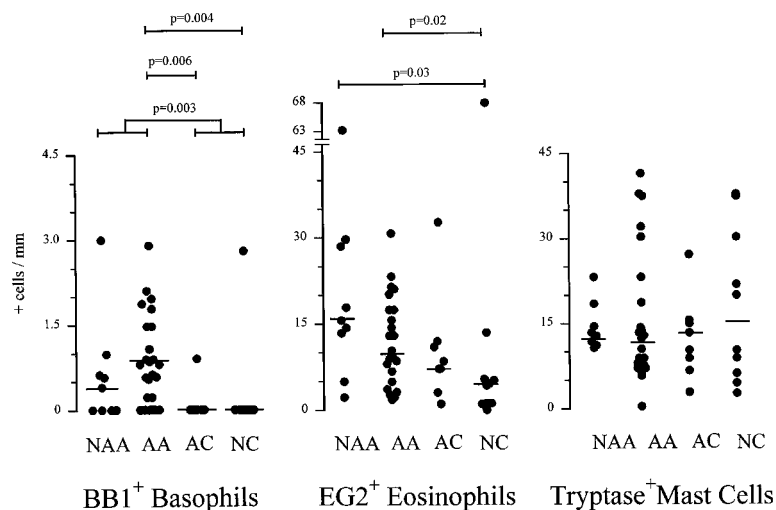


FIG 2. BB1⁺, EG2⁺, and tryptase⁺ cells in baseline bronchial biopsy specimens from NAAs, AAs, ACs, and NCs. Results expressed as immunoreactive cells per millimeter of intact basement membrane. Individual and median values are shown. Statistics were performed by using the Mann-Whitney *U* test. Note the difference in vertical scales.

median decrease in FEV₁ of 18% (11%-37%) during the early asthmatic reaction and 19% (17%-47%) during the LAR after allergen inhalation. Eosinophil numbers were approximately 10-fold higher than basophil numbers at baseline and after challenge. There was no association in individual patients between the increase in BB1⁺ cells and the increase in EG2⁺ cells. Some of the basophils at

24 hours after challenge in biopsy specimens, but not at baseline, were degranulated and represented 12% of the total basophil infiltrate.

Cutaneous LPR biopsy specimens

All subjects exhibited a cutaneous LPR at the site of allergen injection, which was maximal at 24 hours (Fig

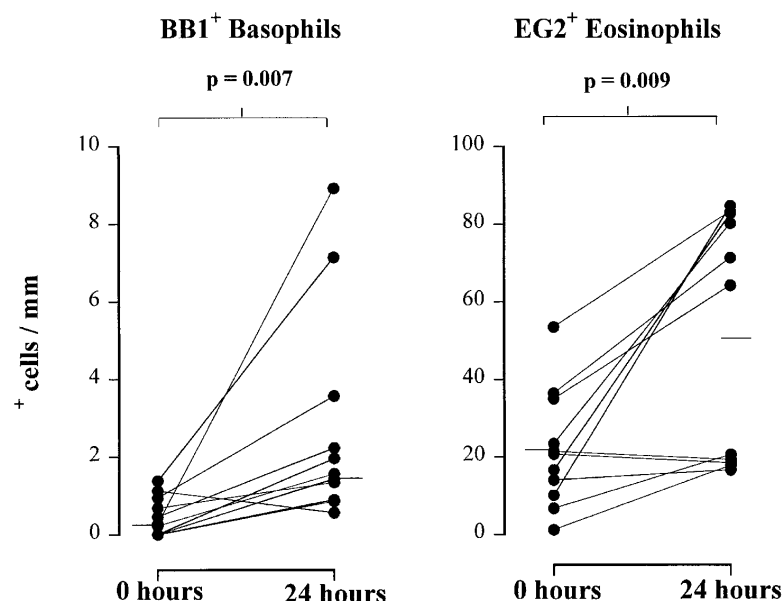


FIG 3. BB1⁺ and EG2⁺ cells in bronchial biopsy specimens before and 24 hours after inhalational allergen challenge. Cell counts are expressed as in Fig 2. Statistics were performed by using the Wilcoxon signed-rank test. Note the difference in vertical scales.

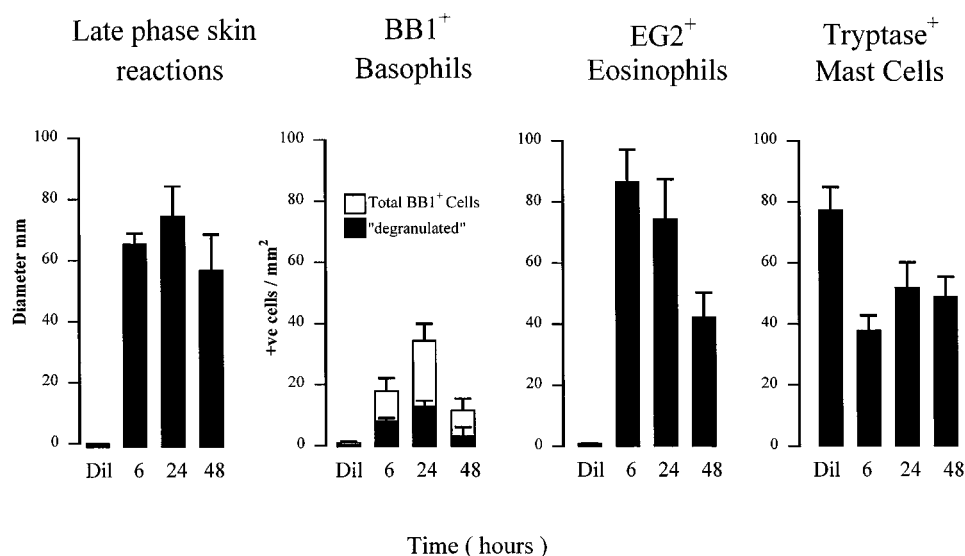


FIG 4. Time course study of the magnitude of the late-phase skin reaction and the numbers of infiltrating BB1⁺ (total and degranulated), EG2⁺, and tryptase⁺ cells. Results represent immunoreactive cells per square millimeter of skin biopsy specimen, with mean values ($\pm$ SEM) shown. Dil, Diluent.

4). No changes were observed at the sites of diluent challenge. The cutaneous LPR was associated with an increase in BB1⁺ basophils, which was maximal at 24 hours after challenge and occurred later than the peak infiltration of EG2⁺ eosinophils ($P < .01$ at all time points compared with diluent challenge for both markers; Fig 4). Tryptase⁺ mast cell numbers were significantly reduced at 6 hours after challenge (Fig 4) and remained significantly lower than in diluent biopsy specimens at all time points examined ($P < .01$ at all time points com-

pared with diluent). In general, basophil numbers were about 40% of eosinophil numbers. There was no correlation between eosinophil and basophil numbers or between BB1⁺ and tryptase⁺ cell numbers at any time point. About one third of the BB1⁺ basophils at the 6-, 24-, and 48-hour time points had an appearance consistent with degranulation (ie, a dispersed pattern of granules; Fig 4). At all time points, basophils were evenly distributed throughout the dermis and often clustered around blood vessels.

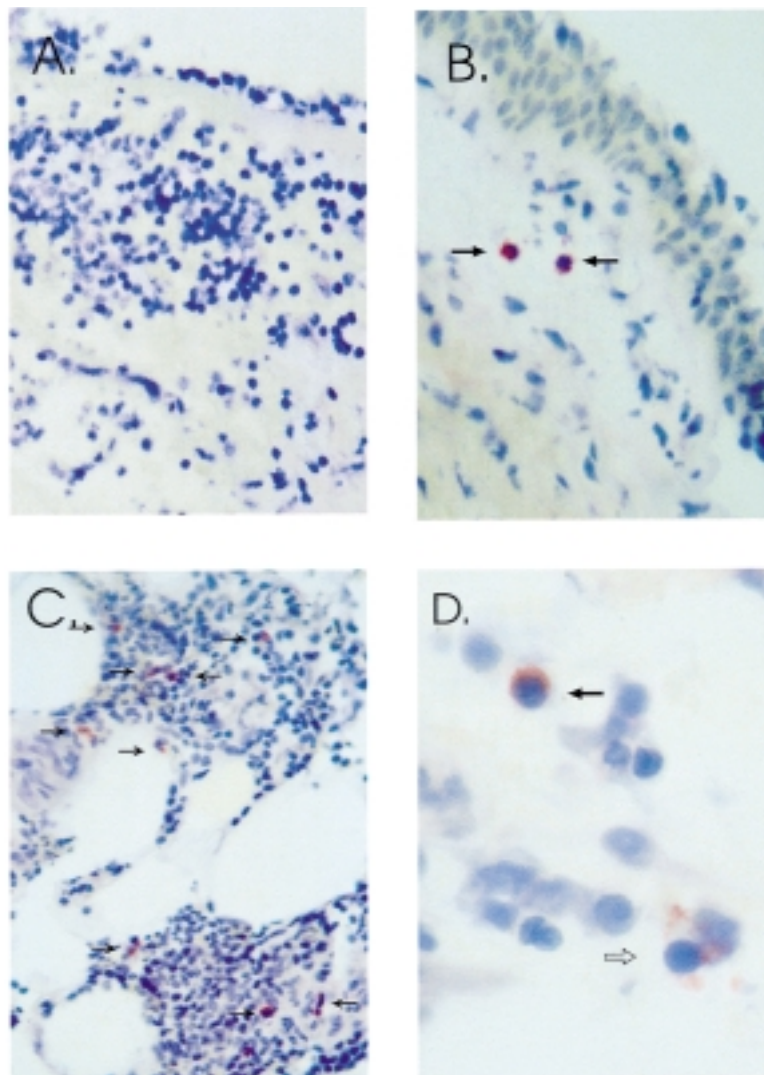


FIG 5. Immunohistochemistry of BB1⁺ cells in bronchial and skin biopsy specimens. **A**, Negative staining with an irrelevant antibody (magnification $\times 200$); **B**, BB1⁺ cells in bronchial biopsy specimens 24 hours after inhalational allergen challenge (magnification $\times 400$); **C**, BB1⁺ cells in skin biopsy specimen (magnification $\times 200$); and **D**, BB1⁺ cells in skin biopsy specimen (magnification $\times 1000$) taken 24 hours after cutaneous allergen challenge (compact, intact granule pattern [filled arrow] and dispersed degranulated pattern [open arrow]).

Examples of immunostaining are shown in Fig 5, including negative and BB1 staining in bronchial biopsy specimens (Fig 5, A and B) and BB1 staining in the cutaneous LPR (Fig 5, C and D). BB1⁺ basophils with intact and dispersed granule patterns of staining are shown in Fig 5, D.

DISCUSSION

The novel findings in this study are (1) the identification of basophils in bronchial biopsy specimens from atopic and nonatopic asthmatic subjects by using a basophil-specific mAb; (2) the observations that there were approximately 10 times fewer basophils in the biopsy specimens from late-phase asthmatic, as compared

with late-phase skin, reactions; (3) that the numbers of eosinophils and mast cells were 10 times higher than basophils in asthma biopsy specimens; and (4) the numbers of basophils in cutaneous LPRs were approximately 40% of mast cells and infiltrating eosinophils. One third of basophils in cutaneous LPRs had morphologic appearances suggestive of degranulation.

We also further confirmed the specificity of BB1 by using flow cytometric analysis of permeabilized peripheral blood leukocytes. This revealed that the antigen bound by BB1 is expressed by basophils alone (Table I) and that this antibody stains the total basophil population (Fig 1). The intensity and binding pattern of this antibody was not related to atopic status, indicating that the basophil population expresses the BB1 antigen in a uni-

form fashion. Thus the low numbers of BB1⁺ basophils detected in baseline skin and bronchial biopsy specimens from NCs and ACs are unlikely to be due to poor expression of the BB1 antigen but to the very low numbers of these cells in normal tissues.

Using immunohistochemistry, we have confirmed that BB1⁺ basophils were undetectable within the normal, nonasthmatic bronchial biopsy specimens from either ACs or NCs, but that small, statistically significant numbers of basophils were detected in baseline bronchial biopsy specimens from AAs (Fig 2). In addition, inhalational allergen challenge induced an increase in BB1⁺ cell numbers in the bronchial biopsy specimens of sensitized AAs at 24 hours after challenge (Fig 3). However, BB1⁺ basophils were less than 10% of EG2⁺ eosinophils in the bronchial biopsy specimens of AAs both at baseline and after allergen challenge. In contrast, although BB1⁺ basophils were undetectable within the skin at baseline, the cutaneous LPR was associated with an increase in basophils detectable at 6 hours and peaking at 24 hours after allergen challenge (Fig 4). In the skin the number of basophils was approximately 40% of the number of infiltrating eosinophils. Morphologically, up to 45% of basophils infiltrating the cutaneous LPR appeared to have degranulated compared with only 12% of basophils in the LAR. The interpretation of this finding is uncertain at present. BB1⁺ cells were readily detectable in cutaneous and bronchial biopsy specimens in both their intact and degranulated states (Fig 5) because there was little background staining. Individual cells showed some variation in intensity of staining, which may have been the result of either different levels of sectioning of cells or different stages of degranulation. We did not observe diffusion staining, as described by Irani et al.²⁵ In our study degranulated cells had BB1⁺ material clearly located around the cells with some BB1⁺ granules remaining within the cell. Even when the entire granule contents appeared to have been released, BB1⁺ antigen remained around the circumference of the cell. We cannot exclude the possibility that some basophils may have degranulated entirely and that the antigen could have been destroyed by local enzyme digestion or other processes. Thus we may have failed to detect some BB1⁺ degranulated cells. The granule constituent identified by BB1 is unknown. The exact nature of its structure and function are currently under investigation. Nevertheless, the basophil specificity of BB1 is clearly established, and this mAb serves as a useful tool to monitor basophil activation in inflammatory reactions.

Bronchial biopsy studies from atopic and nonatopic asthmatic subjects suggest that the intrinsic variant of the disease is not a distinct pathologic entity.²⁸ Thus in AAs and NAAs, there were comparable increases in eosinophils,⁴⁰ activated lymphocytes, and FcεRI⁺ cells compared with ACs and NCs.⁴¹ Similar findings were observed for IL-3, IL-4, IL-5,^{28,34} IL-13,²⁹ RANTES, and monocyte chemotactic protein-3.²⁹ Given these findings, one might expect the accumulation not only of eosinophils but also of basophils within the bronchial

mucosa in both AAs and NAAs. However, although EG2⁺ cells were increased in both AAs and NAAs compared with NCs, BB1⁺ cells were only increased in the biopsy specimens from AAs, but not NAAs, compared with either ACs or NCs. There are several possible explanations for this. First, our sample size (n = 9) may have been too small to have sufficient power to detect a small increase in BB1⁺ cells in the NAAs. However, a similar number of patients were used in previous studies comparing the bronchial immunopathology of NAAs with AAs, ACs, and NCs.^{28,30,34} Second, immunohistochemistry may underestimate the numbers of basophils present within the bronchial mucosa because of lower antigen expression, although our flow cytometry data show that, at least in peripheral blood, all of the basophil population can be detected by using BB1, and basophils from atopic and nonatopic individuals stain with an equal intensity. Third, there may be a genuine difference between AAs and NAAs, with eosinophil and basophil recruitment being controlled by separate factors that are expressed differently in AAs and NAAs.

Our study of the LPRs suggests that basophils may play a more prominent role in pathogenesis in the skin than in the lung because higher numbers of basophils appear to accumulate cutaneous LPRs. It is possible that during recruitment, basophils may become activated, with resultant reduced sensitivity to BB1. McEuen et al²⁶ showed in vitro release of the BB1 antigen after IgE-dependent and IgE-independent mediated degranulation of basophils. However, we were able to detect a sizeable increase in BB1⁺ cells within the cutaneous LPR involving both intact and degranulated basophils and have counted both intact and degranulated basophils within the lung.

The explanation as to why there were so few basophils in the bronchi of asthmatic subjects at baseline or even after allergen challenge is unclear. Basophils might perhaps only remain for a short period of time in the tissues and then move further to be localized in bronchoalveolar lavage fluid¹⁷ or in sputum.^{21,22}

Irani et al²⁵ demonstrated a peak of 2D7⁺ basophil infiltration at 6 hours that remained elevated at 24 hours after cutaneous allergen challenge. We have shown that BB1⁺ basophils were also elevated at 6 hours, although in our study they were maximal at 24 hours, with eosinophils being present in greater numbers at 6 hours. In general basophil numbers were approximately 40% of eosinophils in the cutaneous LPR. Activation and degranulation of basophils by means of FcεRI by using anti-FcεRIα mAbs has been demonstrated to be associated with reduced staining with 2D7. Thus local activation of incoming basophils may make it difficult to detect basophils later in the development of LPR.

The pattern of tryptase⁺-staining mast cells detected in the cutaneous LPR emphasizes the need for specific cellular markers for basophils because tryptase⁺ mast cell numbers at the control skin sites were higher in all patients than at any time point after allergen challenge. This suggests that allergen-induced degranulation of mast cells with release of tryptase from the mast cells resulted in

a loss of tryptase⁺ immunoreactivity. Thus IgE⁺/tryptase⁻ cells could be erroneously identified as basophils rather than degranulated tryptase⁻ mast cells.²⁰ Because the median reduction in tryptase⁺ cells was approximately 40 cells/mm² compared with the increase in BB1⁺ cells of 35 cells/mm², this may also explain why previous studies have detected a more substantial increase in basophils in bronchial biopsy specimens and lavage fluid after inhalational allergen challenge. Increased levels of IgE⁺/tryptase⁻ cells detected within the lungs in patients after fatal asthma attacks²⁰ may also be a result of loss of mast cell tryptase staining.

In summary, using a specific mAb (BB1), we detected small but significantly raised numbers of basophils in the bronchial mucosa of asthmatic subjects compared with nonasthmatic subjects. Basophil numbers were significantly higher in AAs than either ACs or NCs but were small compared with eosinophil and mast cell numbers. Although basophils accumulated in both the bronchi in the LAR and the skin in the cutaneous LPR, they represented less than 10% of infiltrating eosinophils within the bronchial reaction but at least 30% in the skin. Basophils may therefore play a more important role in the pathogenesis of the cutaneous LPR than the LAR. Eosinophil and basophil recruitment appear to be controlled independently. The mechanisms contributing to the selective accumulation of basophils within the skin remain to be established.

We thank Dr Philip W. Askenase, Yale University, for his critical review of the manuscript.

REFERENCES

1. Froese A. Structure and function of the receptor for IgE. *Crit Rev Immunol* 1980;1:79-132.
2. Ochensberger B, Rihs S, Brunner T, Dahinden CA. IgE-independent interleukin-4 expression and induction of a late-phase of leukotriene C₄ formation in human blood basophils. *Blood* 1995;86:4039-49.
3. MacGlashan D Jr, White JM, Huang SK, Ono SJ, Schroeder JT, Lichtenstein LM. Secretion of IL-4 from human basophils. The relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994;152:3006-16.
4. Sarmiento EU, Espiritu BR, Gleich GJ, Thomas LL. IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor potentiate basophil mediator release stimulated by eosinophil granule major basic protein. *J Immunol* 1995;155:2211-21.
5. Ochensberger B, Daepf GC, Rihs S, Dahinden CA. Human blood basophils produce interleukin-13 in response to IgE-receptor-dependent and -independent activation. *Blood* 1996;88:3028-37.
6. Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, et al. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur J Immunol* 1996;26:2493-8.
7. Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. *J Immunol* 1996;156:4833-8.
8. Denburg JA, Woolley M, Leber B, Linden M, O'Byrne P. Basophil and eosinophil differentiation in allergic reactions. *J Allergy Clin Immunol* 1994;94:1135-41.
9. Dvorak HF, Mihm MC. Basophilic leukocytes in allergic contact dermatitis. *J Exp Med* 1972;135:235-54.
10. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. *J Allergy Clin Immunol* 1988;81:580-9.
11. Zeiger RS, Heller S. Development of nasal basophilic cells and nasal eosinophils from age 4 months through 4 years in children of atopic parents. *J Allergy Clin Immunol* 1993;91:723-34.
12. Charlesworth EN, Hood AF, Soter NA, Kagey Sobotka A, Norman PS, Lichtenstein LM. Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. *J Clin Invest* 1989;83:1519-26.
13. Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. *J Clin Invest* 1976;58:408-20.
14. Naclerio RM, Proud D, Togias AG, Adkinson NF, Meyers DA, Kagey-Sobotka A, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
15. Sedgwick JB, Calhoun WJ, Gleich GJ, Kita H, Abrams JS, Schwartz LB, et al. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. *Am Rev Respir Dis* 1991;144:1274-81.
16. Iliopoulos O, Baroody FM, Naclerio RM, Bochner BS, Kagey Sobotka A, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992;148:2223-8.
17. Guo C-B, Liu MC, Galli SJ, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Identification of IgE-bearing cells in the late-phase response to antigen in the lung as basophils. *Am J Respir Cell Mol Biol* 1994;10:384-90.
18. Maruyama N, Tamura G, Aizawa T, Ohru T, Shimura S, Shirato K, et al. Accumulation of basophils and their chemotactic activity in the airways during natural airway narrowing in asthmatic individuals. *Am J Respir Crit Care Med* 1994;150:1086-93.
19. Koshino T, Arai Y, Miyamoto Y, Sano Y, Itami M, Teshima S, et al. Airway basophil and mast cell density in patients with bronchial asthma: relationship to bronchial hyperresponsiveness. *J Asthma* 1996;33:89-95.
20. Koshino T, Teshima S, Fukushima N, Takaishi T, Hirai K, Miyamoto Y, et al. Identification of basophils by immunohistochemistry in the airways of post-mortem cases of fatal asthma. *Clin Exp Allergy* 1993;23:919-25.
21. Foresi A, Leone C, Pelucchi A, Mastropasqua B, Chetta A, Dippolito R, et al. Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997;100:58-64.
22. Kimura I, Tanizaki Y, Saito K, Takahashi K, Ueda N, Sato S. Appearance of basophils in sputum of patients with bronchial asthma. *Clin Allergy* 1975;5:95-8.
23. Liu MC, Hubbard WC, Proud D, Stealey BA, Galli SJ, Kagey Sobotka A, et al. Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Cellular, mediator, and permeability changes. *Am Rev Respir Dis* 1991;144:51-8.
24. Kepley CL, Craig SS, Schwartz LB. Identification and partial characterization of a unique marker for human basophils. *J Immunol* 1995;154:6548-55.
25. Irani AM, Huang C, Xia HZ, Kepley C, Nafie A, Fouda ED, et al. Immunohistochemical detection of human basophils in late-phase skin reactions. *J Allergy Clin Immunol* 1998;101:354-62.
26. McEuen AR, Buckley MG, Compton SJ, Walls AF. Development and characterisation of a monoclonal antibody specific for human basophils and the identification of a unique secretory product of basophil activation. *Lab Invest* 1999;79:27-38.
27. Humbert M, Grant JA, Taborda Barata L, Durham SR, Pfister R, Menz G, et al. High-affinity IgE receptor (Fc epsilon RI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. *Am J Respir Crit Care Med* 1996;153:1931-7.
28. Humbert M, Durham SR, Ying S, Kimmitt P, Barkans J, Assoufi B, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against "intrinsic" asthma being a distinct immunopathologic entity. *Am J Respir Crit Care Med* 1996;154:1497-504.
29. Humbert M, Ying S, Corrigan C, Menz G, Barkans J, Pfister R, et al. Bronchial mucosal expression of the genes encoding chemokines RANTES and MCP-3 in symptomatic atopic and nonatopic asthmatics: relationship to the eosinophil-active cytokines interleukin (IL)-5, granulocyte macrophage-colony-stimulating factor, and IL-3. *Am J Respir Cell Mol Biol* 1997;16:1-8.
30. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J Allergy Clin Immunol* 1997;99:657-65.
31. Khan LN, Ying S, Macfarlane AJ, Barnes NC, Kay AB. The effect of cyclosporin A (CsA) on allergen-induced infiltration of EG2+ eosinophils, BB1+ basophils and eotaxin+ cells in bronchial biopsies

- from mild atopic asthmatics [abstract]. *J Allergy Clin Immunol* 1999;103:178.
32. Anonymous. Investigative use of bronchoscopy, lavage and bronchial biopsies in asthma and other airways diseases. *J Invest Allergol Clin Immunol* 1991;1:271-7.
33. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323-7.
34. Ying S, Meng Q, Barata LT, Robinson DS, Durham SR, Kay AB. Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages, and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* 1997;158:5050-7.
35. Ying S, Robinson DS, Meng Q, Barata LT, McEuen AR, Buckley MG, et al. C-C chemokines in allergen-induced late-phase cutaneous responses in atopic subjects: Association of eotaxin with early 6-hour eosinophils, and eotaxin-2 and MCP-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (MCP-3 and RANTES). *J Immunol*. 1999;163:3976-84.
36. Kon OM, Sihra BS, Till SJ, Kay AB, Grant JA. Unstimulated basophils in atopic and non-atopic subjects express intracellular interleukin-4: detection by flow cytometry. *Allergy* 1998;53:891-6.
37. Moqbel R, Barkans J, Bradley BL, Durham SR, Kay AB. Application of monoclonal antibodies against major basic protein (BMK-13) and eosinophil cationic protein (EG1 and EG2) for quantifying eosinophils in bronchial biopsies from atopic asthma. *Clin Exp Allergy* 1992;22:265-73.
38. Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997;99:699-706.
39. Ackerman SJ, Kephart GM, Habermann TM, Greipp PR, Gleich GJ. Localization of eosinophil granule major basic protein in human basophils. *J Exp Med* 1983;158:946-61.
40. Bentley AM, Durham SR, Kay AB. Comparison of the immunopathology of extrinsic, intrinsic and occupational asthma. *J Invest Allergol Clin Immunol* 1994;4:222-32.
41. Humbert M, Menz G, Ying S, Corrigan CJ, Robinson DS, Durham SR, et al. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today*. 1999;20:528-33.