Human Basophil/Mast Cell Releasability

V. Functional Comparisons of Cells Obtained from Peripheral Blood, Lung Parenchyma, and Bronchoalveolar Lavage in Asthmatics[1-3]

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SUMMARY Releasability of human basophils and mast cells is an important parameter in allergic disorders. We compared IgE- and non-IgE-mediated releasability of human peripheral blood basophils with that of mast cells obtained from lung parenchyma (isolated by mechanical or enzymatic dissociation) and from bronchoalveolar lavage of normal and asthmatic donors. In a first study, the response to anti-IgE, Staph A, Con A, f-met peptide, and Ca^{2+} ionophore A23187 of basophils obtained from 52 donors was compared with that of mast cells isolated enzymatically (PMC_E) or mechanically (PMC_M) from lung parenchyma obtained during surgery. The histamine content of basophils (1.1 ± 0.1 pg/cell) was significantly lower than that of PMC_E (4.1 ± 0.3 pg/cell; $p < 0.001$) and PMC_M (3.7 ± 0.3; $p < 0.001$). The maximal percent anti-IgE-induced histamine secretion in basophils (41.3 ± 3.6) was higher than in PMC_E (17.5 ± 1.8) and in PMC_M (13.8 ± 1.5). Similarly, the response to Staph A and Con A was higher in basophils (29 ± 3.9 and 31.6 ± 4.9, respectively) than in PMC_E (3.5 ± 0.6 and 3.3 ± 0.8, respectively) and PMC_M (5.1 ± 1.3 and 8.8 ± 2.2, respectively). A positive correlation between the maximal percent of histamine release induced by anti-IgE and Staph A was found in basophils ($r_s = 0.61$; $p < 0.001$), whereas there was a negative correlation between the reactivity of PMC_E ($r_s = -0.67$; $p < 0.001$) and PMC_M ($r_s = -0.40$; $p < 0.001$) to anti-IgE and their reactivity to Staph A. The maximal percent f-met-peptide-induced histamine release in basophils (34.9 ± 3.1) was higher than in PMC_E (3.4 ± 0.7) and in PMC_M (5.4 ± 1.4). The reactivity of PMC_M to compound A23187 (43.9 ± 3.5) was lower than that of basophils (72.1 ± 2.7) and PMC_E (71.4 ± 2). There was no correlation between the maximal percent histamine release induced by all the stimuli in basophils, PMC_E, and PMC_M and the serum concentration of IgE. In a second study, the response to anti-IgE, f-met peptide, and compound A23187 of human basophils obtained from 17 normal donors and 19 asthmatics undergoing bronchoalveolar lavage was compared with that of mast-basophiloid cells (BMC) in the BAL. The histamine level in BAL of asthmatics (1,628 ± 632 pg/ml) was higher than that in control subjects (371 ± 65 pg/ml). The histamine content of BMC (2.5 ± 0.3 pg/cell) was higher than that of peripheral blood basophils (1.3 ± 0.1 pg/cell). The maximal percent anti-IgE-induced histamine release in BMC from asthmatics (21.9 ± 4.8) was higher than that in normal donors (7.1 ± 3.8). The maximal percent anti-IgE-, f-met-peptide-, and A23187-induced histamine release in basophils was significantly higher than in BMC of both control subjects and asthmatics.

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Introduction

There is compelling evidence that mast cells and basophils are the primary effector cells in the pathogenesis of allergic (1–3) and probably of other immune disorders (4). Tissue mast cells and circulating basophils are present throughout the respiratory tract of humans, with mast cells located in the bronchoalveolar lavage (BAL), alveoli wall, and bronchi (5–7). In patients with asthma, the number of basophils or mast cells in the bronchoalveolar lumen is increased (5, 8). Furthermore, mast cells and basophils synthesize a variety of chemical mediators that are released *in vivo* during allergic reactions (9, 10); in humans, the administration of these chemical mediators induces some of the symptoms of allergic reactions (11, 12).

Until recently, differences between basophils and mast cells were ignored, and it was even suggested that the two cells have a common cellular progenitor (13). However, there is now firm evidence that basophils and mast cells are distinct cells (14). Furthermore, there are differences between mast cells from different tissues (15, 16), in addition to quantitative and qualitative differences between mast cells isolated from the same tissue (17, 18).

It is now possible to isolate mast cells from human lung parenchyma (17, 19) and to obtain mast cells by BAL (20, 21), and therefore to study the *in vitro* release of chemical mediators.

The term "releasability" implies that biochemical events in basophils or mast cells, not only the surface density of IgE molecules, determine the capacity of these cells to release mediators in response to activating stimuli (22). In the basophil system, IgE- and non-IgE-mediated releasability is influenced by genetic and, presumably, by as yet undefined environmental factors (23). IgE-mediated releasability is also influenced by an age-dependent factor and is correlated in a complex fashion to serum IgE levels and to the cell donor's age (24). Until now no detailed studies have been performed on the relationship in a single subject between *in vitro* responsiveness of basophils and mast cells isolated from lung parenchyma or BAL.

The present study was designed to provide systematic information on IgE- and non-IgE-mediated releasability of basophils versus that of pulmonary mast cells. Two series of experiments were performed: in the first group of 52 donors, responsiveness of basophils to a variety of immunologic and nonimmunologic stimuli was compared with that of mast cells obtained from human lung parenchyma by enzymatic or mechanical dispersion. In the second group of 36 donors, basophil releasability was compared with that of mast cells obtained from BAL of normal donors and of asthmatics.

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Methods

Reagents

The following were purchased: RPMI 1640 with 25 mM HEPES buffer and 5.0 mM glutamine (MA Bioproducts, Walkersville, MD); fetal calf serum (FCS) (Flow Lab, McLean, VA); PIPES, chymopapain, diamine oxidase, histamine dihydrochloride, and elastase type I (Sigma Chemical Co., St. Louis, MO); collagenase (Worthington, Freehold, NJ); *N*-formyl-methionyl-leucyl-phenylalanine (f-met-peptide), the Ca^{2+} ionophore A23187, deoxyribonuclease, and pronase (Calbiochem-Behring Co., La Jolla, CA); protein A from *Staphylococcus aureus* Cowan I, concanavalin A, Percoll, and Dextran 70 (Pharmacia Fine Chemicals, Uppsala, Sweden); [^{3}H]S-adenosyl-L-methionine ([^{3}H]SAM) (78 Ci/mM) (New England Nuclear Co., Boston, MA). Rabbit antihuman IgE-Fc antibody was a generous gift from Drs. Teruko and Kimishige Ishizaka (Johns Hopkins University School of Medicine, Baltimore, MD).

Buffers

The PIPES buffer (P) used in these experiments was made up of 25 mM PIPES, 110 mM NaCl, and 5 mM KCl at pH 7.4; buffer PCG was P with the addition of 1.0 mM $CaCl_2$ and 1 g/L glucose (25). Tyrode's buffer contains (g/L): NaCl, 8.0; KCl, 0.2; NaH_2PO_4, 0.05; $CaCl_2 \times 2H_2O$, 0.26; $MgCl_2 \times 6H_2O$, 0.25; and glucose, 1.0; pH was titrated to 7.4 with sodium bicarbonate (26).

Subjects

The use of human volunteers was approved by the Committee of Clinical Investigations of the University of Naples, Second School of Medicine, and informed consent was always obtained. Two series of experiments were performed. In the first series, 52 patients (47 male and five female) 14 to 71 yr of age (mean, 56.6 ± 1.5 yr), undergoing thoracic surgery mainly for lung cancer, were studied. General anesthesia in these patients was performed using the following drugs: droperidol plus fentanyl and atropine (premedication); droperidol plus fentanyl, thiopental, succinylcholine, and pancuronium (anesthesia). All patients except six (four male and two female) were cigarette smokers. By personal history, four patients were affected by allergic rhinitis. In the second group of experiments, 19 asthmatics (nine male and 10 female) 13 to 64 yr of age (mean, 35.2 ± 3.3 yr) and 17 nonasthmatics (ten male and seven female) 18 to 64 yr of age (mean, 40.9 ± 3.4 yr) undergoing diagnostic bronchoscopy were studied. Asthmatic subjects were characterized on the basis of personal positive history and skin tests to common allergens (*Dermatophagoides pteronyssinus, Dermatophagoides farinae, Parietaria officinalis*, grass, and tree pollens). Control subjects were normal donors and subjects undergoing diagnostic bronchoscopy and subsequently found negative for pulmonary disease. All control subjects were negative for skin tests to common allergens. In all subjects, after informed consent was obtained, approximately 50 ml of blood were drawn 2 h before the surgical or diagnostic procedure. Two milliliters of blood were saved for serum IgE determinations, and the rest was drawn into a mixture of 0.008 M EDTA, 1.1% Dextran 70, and glucose.

Preparation of Peripheral Blood Leukocytes (Containing Basophils)

Leukocytes were isolated from blood samples by dextran sedimentation as previously described (25). Cells were allowed to sediment for 90 min at 22° C. The leukocyte-rich upper layer was drawn off, pelleted, and washed as previously described (26). All cell preparations were > 95% viable as assessed from the ability to exclude erythrosin B.

Isolation of Human Lung Parenchymal Cells by Enzymatic Dispersion

These cells were prepared as described elsewhere (17, 26). These preparations (1 to 10% mast cells) contain more than 95% viable cells and are referred to in this paper as parenchymal lung mast cells enzymatically dispersed (PMC_E).

Isolation of Human Lung Parenchymal Cells by Mechanical Dispersion

These cells were prepared by a modification of the technique described by Church and coworkers (19). The modified technique does not require an enzymatic treatment of human lung tissue. In brief, peripheral rims of grossly normal human lung tissue were obtained from thoracotomy specimens from patients undergoing surgery for lung cancer. Tissue was carefully dissected free of tumor, pleura, large airways, and blood vessels. Approximately 10 g of lung parenchyma were cut into strips about 5 mm wide and then carefully minced into pieces about 1 mm^3 with scissors. The chopped tissue was filtered over Nytex cloth (Tetko, Elmsford, NY) with a 150-μm pore size and washed at 22° C with 30 ml Tyrode's buffer. The cells filtered through the Nytex cloth were pelleted (200 × *g* at 22° C for 8 min), and washed twice in Tyrode's buffer. Between the two washes the cells were filtered through Nytex cloth with a 75-μm pore size to remove large aggregates that occasionally formed. These preparations, containing more than 95% viable cells and about 1% mast cells, are referred to in this report as parenchymal lung mast cells mechanically dispersed (PMC_M).

Bronchoalveolar Lavage

Thirty-six patients were studied at the Department of Pulmonary Medicine of the University of Naples, Second School of Medicine, and at the Division of Pulmonary Medicine of the Cardarelli Hospital, Naples, Italy, after informed consent was obtained. These studies were performed in accordance with the guidelines of the National Institutes of Health (Bethesda, MD) on the investigative use of bronchoalveolar lavage (27), and conformed to the principles embodied in the International Declaration of Helsinki on the protection of human subjects.

Bronchoalveolar lavage was performed at least 1 wk after subjects had received corticosteroids, antihistamines, beta-adrenergic drugs, theophylline, or antibiotics. The subjects were in a stable clinical state with no evidence of bronchospasm (forced expiratory volume > 70%) within a week of bronchoalveolar lavage. All patients were treated intramuscularly with atropine (0.5 mg) and diazepam (5 mg) 30 min before testing. The fiberoptic bronchoscope (BF type 10; Olympus Corp. of America, New Hyde Park, NY) was passed pernasally. Bronchoalveolar lavage was performed in the medial segment of the right middle lobe using three 50-ml aliquots of buffered (pH, 7.4) isotonic saline prewarmed to 37° C. Fluid was recovered under minimal (approximately 5 cm H_2O) negative pressure into 50-ml polyethylene tubes at room temperature and filtered through two layers of sterile gauze. Lavage fluid was rapidly (within 15 min) transferred in our laboratory, centrifuged (1,000 × *g* at 4° C for 8 min), and stored (−70° C) for the analysis of histamine content. Cell pellets were washed twice and used for histamine release assays.

Histamine Release Assay

In the experiments with peripheral blood leukocytes (containing basophils) or with PMC_E, PMC_M, and mast-basophiloid cells (BMC), 0.4 ml of the cell suspension was placed in polyethylene tubes 12 × 75 mm (Sarstadt Inc., Princeton, NJ) and warmed to 37° C; 0.2 ml of each prewarmed stimulus for release was added, and incubation was continued at 37° C for 45 min. At the end of the incubation, the reaction was stopped by centrifugation (1,000 × *g* at 22° C for 2 min), and the cell-free supernatants were stored for subsequent assay of histamine. All values are based on means of duplicate determinations, which differed from each other by less than 10%. For calculation of histamine release as a percentage of total cellular histamine, "spontaneous" release of histamine from basophils or mast cells was subtracted from both numerator and denominator. In the experiments performed with basophils, PMC_E, and PMC_M, histamine was assayed by a sensitive, automated fluorometric method (28). The histamine content in BAL fluid and in the supernatants of BMC was assayed by a specific and sensitive radioenzymatic method (29) that utilizes histamine *N*-methyltransferase purified from rat kidney tissue. Incubations were performed in borosilicate tubes 12 × 75 mm, and all samples were assayed in duplicate. The radioenzymatic assay of histamine was performed as described previously (29). In brief, 25 μl of BAL fluid or histamine standards (0, 30, 50, 100, 200, 400, 800, 1,000, and 2,000 pg histamine/ml) were incubated with 10 μl of histamine-*N*-methyltransferase, 5 μl of [^{3}H]SAM in 0.1 M phosphate buffer (pH, 7.4), and 20 μl of phosphate buffer for 60 min at 5° C. The reaction was stopped by the addition of 75 μl 2.5 M

sium borate (pH, 11). Four milliliters of ene-isopentyl alcohol (3:1) were added, e tubes were vortexed and centrifuged to acilitate phase separation, and 3.8 ml of the organic phase were transferred to a second tube containing 250 µl of 1.0 M potassium phosphate (pH, 7.1). The tubes were vortexed and centrifuged, the organic phase was then removed by aspiration, and 150 µl of the aqueous phase were transferred to a scintillation vial for liquid scintillation spectrometry (Model 2660; Packard Instrument Co., Downers Grove, IL). The specificity of this assay for histamine was determined by degradation of histamine in BAL by incubation (37° C for 60 min) in the presence of appropriate concentrations of diamine oxidase, and by adding exogenous histamine to BAL and comparing the results to a standard curve run in phosphate-buffered saline. The assay is accurate from 50 to 2,000 pg/ml. The intra-assay variance is less than 10% and the interassay variance between samples stored at −70° C for 2 to 3 months is approximately 10%. The anti-IgE, Staph A, Con A, f-met peptide, and A23187 used throughout the study were taken from a single stock preparation that had been stored at −20° C.

IgE Measurement

Serum samples were stored at −70° C from collection until analysis. Serum IgE was determined using a previously described radioimmunoassay (Phadebas IgE PRIST kit; Pharmacia) (23). Because total IgE values follow a log-normal distribution, all analyses were done on a log-transformed scale (Log IgE) when serum IgE level was examined as a continuous variable.

Statistical Analysis

The results were expressed as the mean ± SEM. The data were analyzed using Student's *t* test. The rank correlation was calculated using Spearman's rank coefficient (r_s) (30). All the correlations and significance analyses were performed using paired tests.

Results

Comparison of Histamine Content of Basophils, PMC_E, and PMC_M

Basophils and lung parenchymal mast cells obtained by two different techniques (PMC_E and PMC_M) from 52 donors of a rather narrow range of ages were compared with respect to the total histamine content/cell (table 1) and reactivity to a variety of immunologic and nonimmunologic stimuli. The histamine content in basophils (1.1 ± 0.1 pg/cell) was significantly lower than in PMC_E (4.1 ± 0.3 pg/cell; $p < 0.001$) and PMC_M (3.7 ± 0.3 pg/cell; $p < 0.001$). There was no correlation between the histamine content of basophils versus PMC_E (r_s = 0.10; NS) and PMC_M (r_s = 0.00; NS). No correlation was found between the histamine content of PMC_E and that of PMC_M (r_s = 0.22; NS).

TABLE 1

AGE, HISTAMINE CONTENT OF BASOPHILS, PMC_E AND PMC_M, AND SERUM IgE LEVELS*

Age (yr)	Histamine Content (pg/basophil)	Histamine Content (pg/PMC_E)	Histamine Content (pg/PMC_M)	Serum IgE (IU/ml)
56.6 ± 1.5 (14–71)	1.1 ± 0.1† (0.3–2.3)	4.1 ± 0.3 (1.4–7.8)	3.7 ± 0.3 (1.1–7.4)	218.2 ± 46.5 (2–1,157)

* Values are the means ± SEM. Numbers in parentheses represent the range.
† $p < 0.001$ when compared with the histamine content in PMC_E and PMC_M.

Comparison of Basophil and PMC_E and PMC_M Releasability

The "spontaneous" releasability of basophils was significantly lower than that of PMC_E and PMC_M ($p < 0.001$) (table 2). We next examined the releasability of basophils and lung parenchymal mast cells exposed to a range of concentrations (10^{-2} to 5 µg/ml) of anti-IgE previously shown to be optimal for histamine release (17). Therefore, an entire dose-response curve was obtained with basophils, PMC_E, and PMC_M for each individual donor; table 2 shows that the maximal percent anti-IgE-induced histamine secretion from basophils was significantly higher than that from PMC_E and PMC_M ($p < 0.001$). The increased IgE-mediated releasability of basophils compared with that of parenchymal mast cells is illustrated in figure 1A. The percent histamine release was significantly higher in basophils than in parenchymal mast cells at all concentrations of anti-IgE. Furthermore, the optimal concentration of anti-IgE for release in basophils was 3×10^{-1} µg/ml, whereas in mast cells it was at least 10 times higher.

Protein A of *Staphylococcus aureus* Cowan I induces histamine release from human basophils (31) through the interaction of the alternative site with the Fab portion of a percentage of IgE on the membrane surface (32). Basophils and parenchymal mast cells were challenged with a wide range of concentrations of Staph A (10^{-2} to 100 µg/ml) previously shown to be optimal for histamine release (32). The maximal percent Staph-A-induced histamine release in basophils was higher ($p < 0.001$) than in PMC_E and PMC_M (table 2). The percent histamine release in basophils was significantly higher than in lung mast cells at all concentrations of Staph A (figure 1B). In fact, PMC_E and PMC_M were essentially unresponsive to Staph A.

Con A activates human basophils to release histamine, presumably by binding to carbohydrate groups on the IgE molecule located on the basophil membrane (33). With Con A used at a range of concentrations (3×10^{-1} to 10 µg/ml) previously shown to be optimal for histamine release (33), the maximal percent of Con-A-induced histamine secretion from basophils was significantly ($p < 0.001$) higher than from both PMC_E and PMC_M (table 2), and figure 1C shows that there was a significant increase in the percent of histamine release at all concentrations of Con A from basophils versus PMC_E and PMC_M. In fact, PMC_E and PMC_M were almost unresponsive to Con A.

To study non-IgE-mediated releasability we employed f-met peptide, which activates a specific cell surface receptor independent of the IgE receptor (25). It can

TABLE 2

"SPONTANEOUS" AND MAXIMAL PERCENT HISTAMINE RELEASE INDUCED BY DIFFERENT STIMULI*

	"Spontaneous" Histamine Release	Anti-IgE	Staph A	Con A	f-met Peptide	A23187
Basophils	6.6 ± 1.0† (0–35.3)	41.3 ± 3.6† (1–93)	29 ± 3.9† (0–100)	31.6 ± 4.9† (0–77)	34.9 ± 3.1† (1–90)	72.1 ± 2.7 (27–100)
PMC_E	17.4 ± 1.2 (2.6–49.1)	17.5 ± 1.8 (0–56)	3.5 ± 0.6 (0–20)	3.3 ± 0.8 (0–16.5)	3.4 ± 0.7 (0–20)	71.4 ± 2.0 (32–98)
PMC_M	22 ± 1.7§ (9.3–51.8)	13.8 ± 1.5 (0–41.5)	5.1 ± 1.3 (0–28.5)	8.8 ± 2.2 (0–57.5)	5.4 ± 1.4§ (0–27.5)	43.9 ± 3.5‡ (10.5–96.5)

* Values are the means ± SEM. Numbers in parentheses represent the range.
† $p < 0.001$ when compared with the corresponding values of PMC_E and PMC_M.
‡ $p < 0.001$ when compared with the corresponding values of basophils and PMC_E.
§ $p < 0.05$ when compared with the corresponding values of PMC_E.

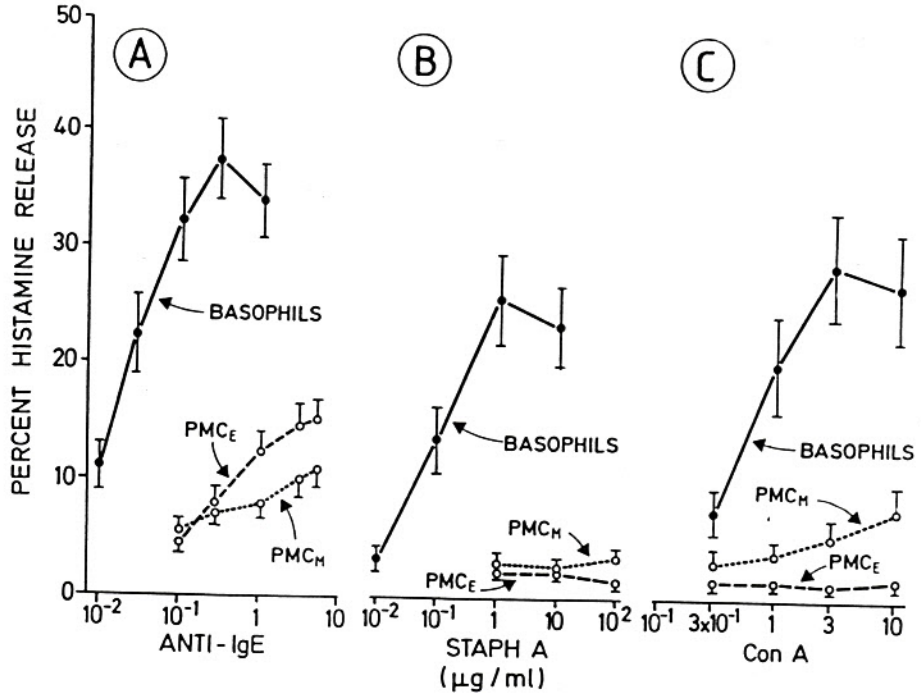

Fig. 1. Histamine release as a function of anti-IgE (A), Staph A (B), and Con A (C) concentration from peripheral blood basophils or mast cells isolated enzymatically (PMC$_E$) or mechanically (PMC$_M$) from lung parenchyma of 52 individual donors. Values are expressed as mean ± SEM.

be seen in figure 2A that the percent of histamine release induced by all concentrations (10^{-7} to 10^{-5} M) of f-met peptide from basophils was significantly higher than from PMC$_E$ and PMC$_M$. In addition, the maximal percent f-met-peptide–induced histamine secretion from basophils was significantly higher ($p < 0.001$) than that obtained from PMC$_E$ and PMC$_M$ (table 2). In fact, parenchymal lung mast cells were almost unresponsive to f-met peptide.

With the Ca^{2+} ionophore A23187 used at a range of concentrations (3×10^{-2} to 1 μg/ml) previously shown to be optimal for activation of human basophil and mast cells (26, 34), the maximal percent of histamine secretion from PMC$_M$ was significantly lower ($p < 0.001$) than that from PMC$_E$ and basophils (table 2). It can be seen in figure 2B that the decreased response of mechanically dispersed lung mast cells occurred at the three higher concentrations of A23187.

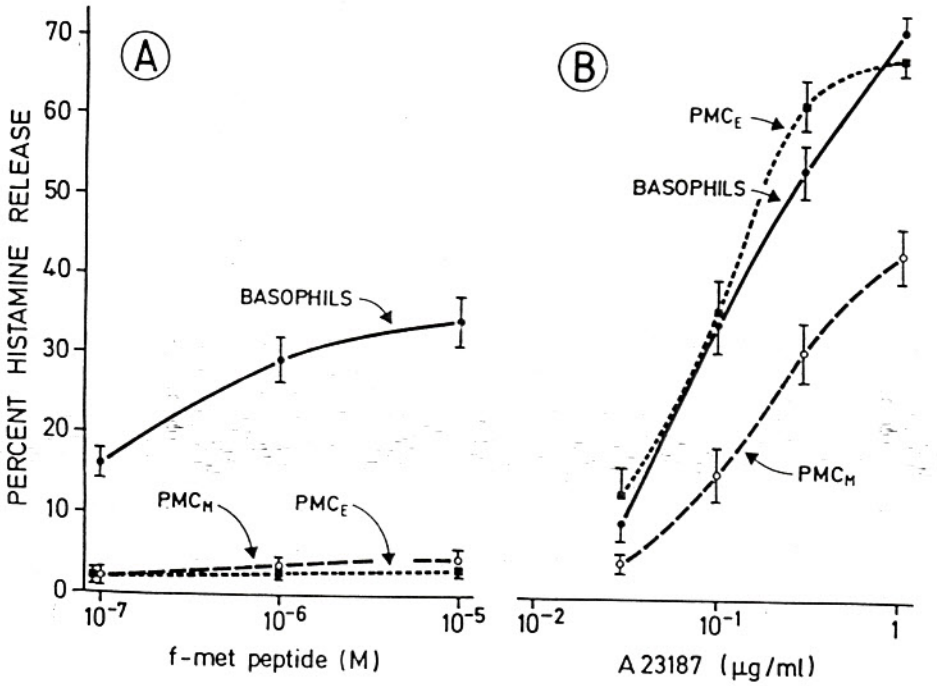

Fig. 2. Histamine release as a function of f-met peptide (A) and A23187 (B) concentration from peripheral blood basophils or mast cells isolated enzymatically (PMC$_E$) or mechanically (PMC$_M$) from lung parenchyma of 52 individual donors. Values are expressed as mean ± SEM.

We then examined the correlation between the maximal percent of histamine release with the five releasing agents (anti-IgE, Staph A, Con A, f-met peptide and A23187) in basophils, PMC$_E$, and PMC$_M$. We first examined the correlation between the maximal percent of histamine release induced by IgE-mediated stimuli (anti-IgE, Staph A, and Con A) in the basophil system. There was a positive correlation between the reactivity of the cells to release with anti-IgE and their response to Staph A ($r_s = 0.62$; $p < 0.001$) and Con A ($r_s = 0.83$; $p < 0.001$). There was also a significant correlation between the maximal percent histamine release caused by Staph A and Con A ($r_s = 0.92$; $p < 0.001$). In contrast, there was a negative correlation between the reactivity of PMC$_E$ to anti-IgE and their response to Staph A ($r_s = -0.67$; $p < 0.001$) and Con A ($r_s = -0.46$; $p < 0.05$). However, a significant correlation between the maximal percent histamine release induced by Staph A and Con A ($r_s = 0.90$; $p < 0.001$) was found in PMC$_E$. In PMC$_M$ a negative correlation ($r_s = -0.40$; $p < 0.01$) between the maximal percent of histamine release induced by anti-IgE and Staph A and a positive correlation ($r_s = 0.38$; $p < 0.05$) between the maximal percent histamine release induced by anti-IgE and Con A were found. A significant correlation between the maximal percent histamine release by Staph A and Con A ($r_s = 0.70$; $p < 0.001$) was found also in PMC$_M$.

There was a positive correlation between the reactivity of basophils to release with anti-IgE and their response to f-met peptide ($r_s = 0.33$; $p < 0.05$) and to compound A23187 ($r_s = 0.51$; $p < 0.001$). In basophils there was also a significant correlation between the maximal percent histamine release caused by f-met peptide and A23187 ($r_s = 0.38$; $p < 0.05$). In PMC$_E$, a negative correlation was found between the response to anti-IgE versus f-met peptide ($r_s = -0.66$; $p < 0.001$), whereas there was a positive correlation between the release caused by anti-IgE versus A23187 ($r_s = 0.41$; $p < 0.01$). In PMC$_M$ a positive correlation between the reactivity of cells to anti-IgE

A23187 ($r_s = 0.56$; $p < 0.001$) and response to f-met peptide versus 3187 ($r_s = 0.51$; $p < 0.001$) was found. hese correlations are summarized in table 3.

We next examined the correlation between the maximal percent histamine release induced by anti-IgE from basophils, PMC_E, and PMC_M. We also examined the correlation between the reactivity of basophils, PMC_E, and PMC_M to Staph A and Con A. There was no correlation between the maximal percent histamine release caused by anti-IgE from basophils and both PMC_E ($r_s = 0.01$; NS) and PMC_M ($r_s = 0.18$; NS). However, there was a significant correlation ($r_s = 0.36$; $p < 0.05$) between the maximal percent histamine release caused by anti-IgE from PMC_E and PMC_M. Similar results were obtained with Staph A and Con A (table 4).

We also examined the correlation between the maximal percent histamine release caused by non-IgE–mediated stimuli (f-met peptide and compound A23187). The results are summarized in table 4. There was a significant correlation between the maximal percent histamine release caused by f-met peptide from basophils and PMC_M ($r_s = 0.33$; $p < 0.05$) and PMC_E and PMC_M ($r_s = 0.68$; $p < 0.001$). No significant correlations were found with respect to the release caused by compound A23187 from basophils, PMC_E and PMC_M.

Comparison of Basophil and BMC Releasability in Patients with Bronchial Asthma

Mast-cell-like cells and histamine have been found in BAL fluid obtained from humans (6, 8, 20, 21, 35). We measured the histamine levels in BAL fluids of 19 asthmatics and 17 nonasthmatics, and we compared basophil versus BMC releasability in both groups of donors. The histamine concentration in BAL of asthmatics (1,628 ± 632 pg/ml) was significantly greater than in nonasthmatics (371 ± 65 pg/ml; $p < 0.05$) (figure 3).

The histamine content in basophils of control subjects (1.3 ± 0.2 pg/cell) and asthmatics (1.4 ± 0.2 pg/cell) was significantly lower than in BMC of the corresponding groups (2.6 ± 0.3 pg/cell and 2.4 ± 0.4 pg/cell; $p < 0.001$). The serum IgE level did not differ in the two groups examined (table 5). This can be attributed to the small sample of patients studied or to the fact that all the asthmatics studied can be considered to have mild asthma.

We next examined the "spontaneous" release of histamine from basophils and BMC during a 45-min culture in standard buffer (table 6). The "spontaneous" releasability of BMC was significantly higher than that of basophils in both groups ($p < 0.05$).

We then studied histamine release from human basophils and BMC obtained from patients with asthma and from control subjects. These cells were challenged with various concentrations of anti-IgE, f-met peptide, and A23187 to obtain an entire dose-response curve for each individual donor; table 6 shows that the maximal percent anti-IgE-, f-met-peptide-, and A23187-induced histamine release from basophils of asthmatics did not differ from that of control subjects. In contrast, the maximal percent anti-IgE-, f-met peptide-, and A23187-induced histamine release from BMC of both control subjects and asthmatics was significantly lower than that of basophils of both groups of donors ($p < 0.001$). There was no correlation between the maximal percent histamine release induced by anti-IgE from basophils and BMC in control subjects ($r_s = 0.29$; NS) and in asthmatics ($r_s = 0.38$; NS). In addition, the maximal percent of anti-IgE-induced histamine release from BMC of asthmatics was significantly higher than in control subjects ($p < 0.05$). It can be seen in figure 4 that the increased IgE-mediated releasability of BMC of asthmatics compared with that of control subjects is present at all concentrations of anti-IgE.

TABLE 3

CORRELATIONS BETWEEN THE MAXIMAL PERCENT HISTAMINE RELEASE INDUCED BY DIFFERENT STIMULI FROM PERIPHERAL BLOOD BASOPHILS, PMC_E, AND PMC_M

	Basophils (r_s)	PMC_E (r_s)	PMC_M (r_s)
Anti-IgE versus Staph A	0.62*	−0.67*	−0.40†
Anti-IgE versus Con A	0.83*	−0.46†	0.38‡
Staph A versus Con A	0.92*	0.90*	0.70*
Anti-IgE versus f-met peptide	0.32‡	−0.66*	0.25
Anti-IgE versus A23187	0.51*	0.41†	0.56*
f-met peptide versus A23187	0.38‡	0.13	0.51*

* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

Discussion

In this study, we have compared basophils and lung mast cells obtained from different anatomic sites (parenchyma and alveoli) and with different techniques (enzymatic and mechanical dispersion of lung parenchyma). Furthermore, we compared basophils with mast cells obtained from individual subjects of an ample population of normal and asthmatic donors.

The first parameter in which we found heterogeneity between basophils, parenchymal mast cells, and BMC was the histamine content. Human lung parenchymal mast cells, mechanically and enzymatically dispersed, contain approximately 4 pg histamine/cell, whereas peripheral blood basophils and BMC contain approximately 1 and 2.5 pg, respectively. The histamine content of lung parenchymal mast cells that we found agrees with a previous report (17), and our results suggest that the enzymatic treatment presumably does not alter the mediator content of these cells. However, it should be mentioned that the lack of correlation between the histamine content of PMC_E and PMC_M is compatible with the hypothesis that the different isolation pro-

TABLE 4

CORRELATION BETWEEN THE MAXIMAL PERCENT HISTAMINE RELEASE INDUCED BY DIFFERENT RELEASING AGENTS IN BASOPHILS, PMC_E, AND PMC_M

Correlation between	Anti-IgE (r_s)	Staph A (r_s)	Con A (r_s)	f-met Peptide (r_s)	A23187 (r_s)
Basophils and PMC_E	0.01	−0.09	−0.21	0.07	0.24
Basophils and PMC_M	0.18	0.29	0.14	0.33‡	0.13
PMC_E and PMC_M	0.36‡	0.69*	0.55†	0.68*	0.05

* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

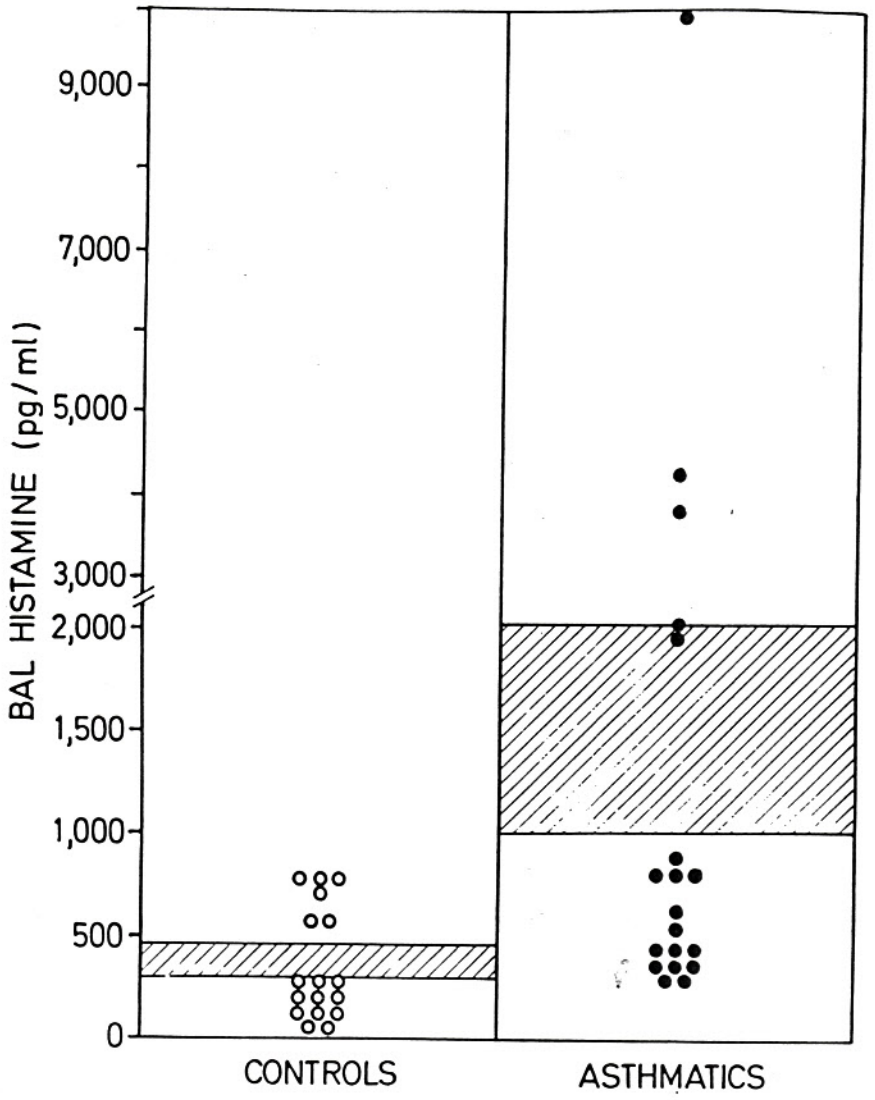

Fig. 3. BAL histamine levels in 17 normal donors (*open circles*) and 19 patients with asthma (*closed circles*). Each symbol represents the mean of duplicate determinations. The shaded area represents the mean ± SEM.

cedure leads to distinct populations of parenchymal mast cells.

The "spontaneous" releasability of basophils is significantly lower than that of PMC_E and PMC_M. In addition, marked differences were found with respect to the response to IgE-mediated stimuli between basophils and PMC_E and PMC_M from individual subjects. We confirm the observation that the reactivity to anti-IgE of parenchymal mast cells is lower than that of basophils (17). Interestingly, the response to anti-IgE of PMC_M did not differ from that of PMC_E. This is an indication that the enzymatic procedure used to isolate PMC_E presumably does not alter the IgE molecule or the biochemical pathways underlying IgE-mediated release of histamine. However, we were surprised to find that Staph A and Con A, which induce histamine release from human basophils presumably by interacting with different portions of the IgE molecule on the basophil membrane (32,33), caused little or no release from both PMC_E and PMC_M. In addition, the excellent positive correlation between the histamine release caused by anti-IgE, Staph A, and Con A from basophils was not always found in parenchymal mast cells. These results indicate that diffe ent IgE-mediated stimuli might selectiv ly activate lung parenchymal mast cell This observation raises several possib ties. For example, it might be that a cri cal portion of IgE (interacting with Sta A or Con A) is shed on human lung p renchymal mast cells. Alternatively, different degree of glycosylation of Ig present on the mast cell membrane mig explain, at least in part, the lack of r sponse to Con A. Furthermore, the po sibility exists that an IgE receptor on lu mast cells different from that of bas phils binds a portion of IgE that do not interact with Staph A and Con A It is known that Staph A binds throug the alternative site with the Fab portio of a small percentage (approximate 15%) of human polyclonal IgE (36, 37

It is unlikely that the lack of respon of lung parenchymal mast cells to Co A and Staph A is due to trivial alter tions of the membrane and cytoplasm apparatus caused by the enzymatic trea ment required to isolate PMC_E. In fac similar results were obtained wi PMC_M, which were not exposed to e zymatic treatment during the isolatio procedure we used.

An interesting difference betwe PMC_E and PMC_M was found with r spect to the response to the Ca^{2+} ion phore A23187. The reason why PMC released less histamine in response compound A23187 is not clear, but aga it is possible that the isolation procedu yields a subpopulation of lung parench mal mast cells less responsive to this co pound. This hypothesis is compatib with the observation that there is no co relation between PMC_E and PMC_M the maximal percent histamine release i duced by compound A23187.

In a previous study conducted wi basophils from a wide age group, w had no correlation between the maxim percent histamine release induced b anti-IgE, f-met peptide, and compoun A23187 (24). In contrast, in young d

TABLE 5

AGE, HISTAMINE CONTENT OF BASOPHILS AND BMC, HISTAMINE LEVEL IN BAL, AND SERUM IgE LEVELS IN CONTROL SUBJECTS AND ASTHMATIC PATIENTS*

	Age (yr)	Histamine Content (pg/basophil)	(pg/BMC)	(pg/ml BAL)	Serum IgE (IU/ml)
Control subjects	40.9 ± 3.4 (18–64)	1.3 ± 0.2† (0.7–3.0)	2.6 ± 0.3 (0.4–5.1)	371 ± 65 (120–2,100)	251 ± 65 (14–1,000)
Asthmatics	35.2 ± 3.3 (13–64)	1.4 ± 0.2† (0.4–3.7)	2.4 ± 0.4 (0.6–7.7)	1,628 ± 632 (150–12,000)	277 ± 50 (55–672)

* Values are the means ± SEM. Numbers in parentheses represent the range.
† $p < 0.001$ when compared with the corresponding values of BMC.

..., we found a significant correlation ...ween the maximal percent histamine ...lease induced by the f-met peptide and A23187 both in control subjects and in patients with atopic dermatitis (38). In the present study performed in adults of a narrow range of ages, we found several correlations between the maximal percent histamine release induced by IgE- and non-IgE-mediated stimuli from basophils, PMC_E, and PMC_M. In particular, we confirmed the significant correlation between the maximal percent histamine release induced by anti-IgE and A23187 (19). This finding is yet another indication that the parameter of basophil and mast cell releasability must be defined with respect to the age of cell donors (24).

Furthermore, with the exception of a weak correlation between the maximal percent histamine release caused by f-met peptide, no correlations were found between basophils and both PMC_E and PMC_M. In contrast, with the exception of compound A23187, significant correlations were found between PMC_E and PMC_M with respect to all other releasing agents.

Lastly, although our experimental procedures included extensive washing of both the lung parenchyma and the isolated mast cells, we cannot exclude that drugs used in premedication and for anesthesia could at least in part be responsible for the differences found in mast cell and basophil releasability.

The second part of this study was devoted to the comparison of basophil and BMC releasability in asthmatics and in control subjects. Although the histamine levels in BAL fluid of both control subjects and asthmatics found in this study are slightly higher than those reported by others (20, 21), we have confirmed the observation by Casale and coworkers (20) that the BAL histamine level of asthmatics is increased compared with that in control subjects. We have also demonstrated that BMC IgE-mediated releasability in asthmatics is increased, reinforcing the hypothesis that asthmatics have ongoing BMC degranulation that might contribute *in situ* to the etiology of airway hyperresponsiveness.

In this small group of asthmatics, we did not detect any significant alterations of basophil releasability and serum IgE level compared with those in control subjects. Previous studies have shown that basophil releasability is altered in patients with such allergic disorders as atopic dermatitis (34) and chronic urticaria (39). Therefore, as asthmatics do not appear to show alterations of IgE-mediated releasability of basophils (40), it appears that the evaluation of releasability of effector cells that would come into immediate contact with inhaled antigens (e.g., BMC) is a more sensitive way to distinguish asthmatic from normal donors than is the determination of serum IgE level or basophil releasability.

The importance of altered BMC releasability in asthmatics is indirectly supported by the observation that BMC isolated from normal donors are almost insensitive to IgE-mediated stimuli. This indicates that asthmatics have an intrinsic alteration of BMC releasability, which is presumably responsible for the increased histamine level in BAL fluid that has been related to the etiology of airway hyperresponsiveness (20).

There is now compelling evidence that functional mast cell heterogeneity exists in rodents (15, 41) and in humans (16, 42). Our results extend previous observations by showing functional differences between basophils and mast cells isolated from different anatomic sites of human lung. Furthermore, differences in terms of histamine content and mast cell releasability have been documented between lung parenchymal mast cells and BMC. Differences have been found even between PMC_E and PMC_M, despite their many similarities. It is of interest that heterogeneity of mast cells in lung paren-

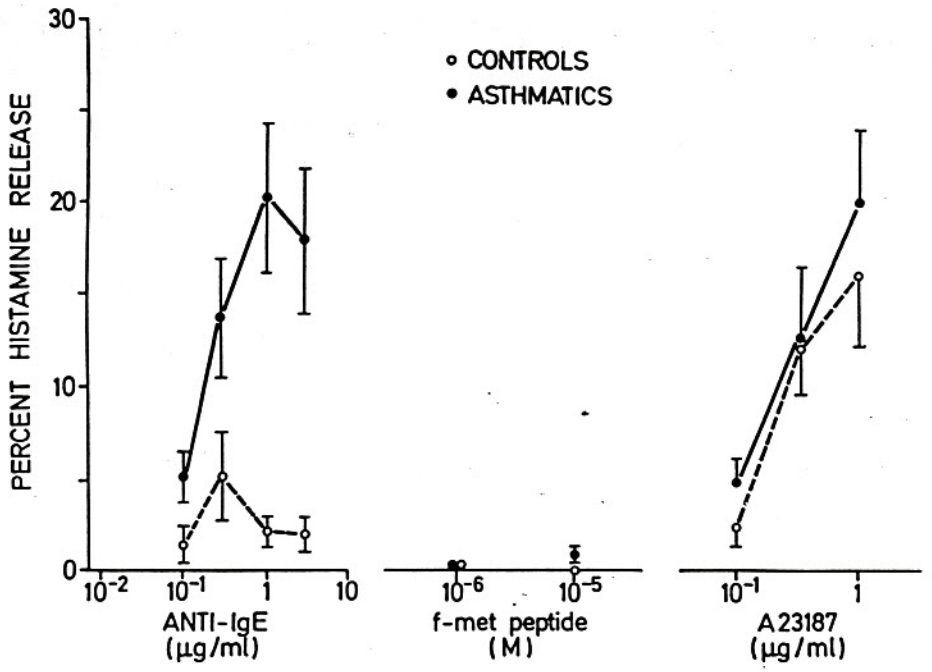

Fig. 4. Histamine release as a function of anti-IgE, f-met peptide, and A23187 concentration in BMC from 17 normal donors (*open circles*) and 19 patients with asthma (*closed circles*). Values are expressed as mean ± SEM.

TABLE 6

"SPONTANEOUS" AND MAXIMAL PERCENT HISTAMINE RELEASE FROM BASOPHILS AND BMC IN CONTROL SUBJECTS AND ASTHMATIC PATIENTS*

	"Spontaneous" Histamine Release	Anti-IgE	f-met Peptide	A23187
Control subjects				
Basophils	9 ± 1.7 (1–21)	36.8 ± 6.9 (2–100)	38.1 ± 5.5 (8–93)	77.5 ± 3.3 (52–100)
BMC	13.2 ± 3.5† (0–57.5)	7.1 ± 3.8† (0–66)	0.2 ± 0.1† (0–1)	19 ± 5.3† (0–66)
Asthmatics				
Basophils	12.6 ± 1.7 (2–33.5)	44.4 ± 6.1 (0–87)	33 ± 4.5 (0–82)	66.3 ± 6.5 (0–100)
BMC	19.8 ± 2.0† (0–34.5)	21.9 ± 4.8†‡ (0–63)	0.8 ± 0.4† (0–4)	20.6 ± 4† (0–56)

* Values are the means ± SEM. Numbers in parentheses represent the range.
† Significantly different ($p < 0.001$) from the corresponding group of basophils.
‡ Significantly different ($p < 0.05$) from the corresponding group of control BMC.

chyma and alveoli has been already documented by immunohistochemical techniques (18).

In conclusion, the results of this study performed on a large population of donors indicate that IgE- and non-IgE-mediated releasability of basophils differs from that of mast cells isolated from different anatomic sites of human lung. Furthermore, releasability of mast cells isolated from lung parenchyma differs from that of cells obtained from BAL. We have also found that patients with bronchial asthma have increased BMC releasability and histamine levels in BAL fluid. The interrelationships among mast cell degranulation, chemical mediators, and the pathogenesis of bronchial asthma are certainly complex and largely unknown. However, the observations arising from this study could contribute to the understanding of the pathophysiology of airway hyperreactivity in bronchial asthma.

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