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Selectively Enhanced Sensitivity of Bronchoalveolar Lavage Fluid (BALF) Mast Cells to IgE Dependent Stimulation in Mild Asthmatics

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SUMMARY The aim of this study is to investigate the histamine-releasing ability of mast cells from asthmatic bronchoalveolar lavage fluid (BALF). Following the measurement of the forced expiratory volume at the first second (FEV1), 29 mild asthmatics were included in the study and were subjected to fibreoptic bronchoscopy. The cells recovered from the BALF were challenged with anti-IgE, calcium ionophore A23187 (CI) or adenosine, and the released histamine was measured with an enzyme-linked chromogenic assay. Enzymatically dispersed mast cells from human lung or colon tissues were employed as control groups. The results showed that mast cells from BALF were at least 100 fold more sensitive to anti-IgE than those from lung or colon tissues. However, there was little difference between mast cells from BALF, lung or colon tissues in response to CI. Adenosine failed to stimulate histamine release from BALF mast cells. In conclusion, asthmatic BALF mast cells are much more sensitive to IgE-dependent stimulation than the non-IgE-dependent ones, indicating that mast cells may play a role in the pathogenesis of asthma.

The mast cell has long been considered as one of the important cells in asthma, in which it plays a primary effector cell role. Through releasing its pro-inflammatory mediators such as histamine, tryptase, PGD2, and certain cytokines it is able to induce an early asthmatic response, and is also able to accumulate and activate eosinophils and neutrophils, the key cells for late asthmatic responses in the airways.[1] Evidence that certain anti-asthmatic drugs, such as salmeterol and salbutamol, possess mast cell stabilizing activity[2-4] may further suggest the critical role of mast cells in the pathogenesis of allergic asthma.

Over the last two decades, the bronchoalveolar lavage technique has been broadly applied in the investigation of the pathogenesis of asthma. People now firmly believe that asthma is a chronic inflammatory disease of the airways.[5] The typical pathological changes in the airways of asthmatics include activation and increased numbers of mast cells,[6-7] eosinophils,[8] neutrophils[9-10] and lymphocytes[11] in the BALFs of asthmatics, together with airway hyperresponsiveness.[12-13] The close association of increased airway obstruction in asthmatics with elevated levels of histamine and tryptase[14-15] in their BALFs further indicates that mast cell activation could be a critical step in the pathogenesis of asthma.

However, information on the functional properties of mast cells from BALF is relatively limited although initial work on this topic was published 16 years ago.[16] Since learning the functional properties of mast cells of asthmatic airways is crucial for understanding the patho-

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genesis of asthma, this study investigated the histamine-releasing ability of mast cells from asthmatic BALF in response to various stimuli.

MATERIAL AND METHODS

Materials

The following compounds were purchased from Sigma Chemical Co. (Poole, Dorset, UK): Adenosine (9-β-D-ribofuranosyladenine), collagenase (type I), hyaluronidase (type I), BSA (fraction V), penicillin and streptomycin, minimal essential medium (MEM) containing 25 mM HEPES, calcium ionophore A23187 (CI), *o*-phthaldialdehyde (OPD). Goat anti-human IgE (inactivated) and a histamine enzyme immunoassay kit were purchased from Serotec (Kidlington, Oxford, UK).

Subjects

The study included 29 (age between 22-55 years old, 15 males and 14 females) mild asthmatics (according to the criteria published in GINA 2002) who had asthma for less than two years, had no asthma attack for the last two weeks and whose chest was quite clear with FEV_1 > 80% predicted (Table 1). All subjects gave their informed consent for BAL.

Preparation of BAL cells and challenge

BALF was obtained with an Olympus BF fibreoptic bronchoscope and was filtered through a sterile nylon gauze. Following washing with MEM (containing 2% fetal calf serum [FCS]) twice, cells were resuspended in complete Hanks' balanced salt solution (HBSS) with 1.8 mM $CaCl_2$ and 0.5 mM $MgCl_2$. After taking 10 µl out for cell counting, cells were added to tubes containing either anti-IgE, CI, adenosine or buffer alone, 100 µl per tube, and incubated at 37°C for 20 minutes. The reactions were stopped by placing the tubes on ice and adding 100 µl cold incomplete HBSS (HBSS without $CaCl_2$ and $MgCl_2$). Soon after the tubes were centrifuged at 750 x *g* at 4°C for 7 minutes, the cell supernatants were collected into Eppendorf tubes and stored at -20°C. Approximately 8.5×10^5 (median value, range $6.8\text{-}9.7 \times 10^5$) mast cells were recovered from each subject.

Preparation of dispersed cells from tissues

Macroscopically normal lung and colonic tissues were obtained from patients at lobectomy for lung cancer or colectomy for colon cancer. The procedure for cell

Table 1 The values of FEV_1 and the numbers of mast cells recovered from BALF of the mild asthmatic subjects

Subject	FEV_1 (%)	BALF recovered (ml)	Mast cell recovered ($\times 10^5$)
1	93.0	55	8.2
2	81.1	71	8.6
3	93.3	52	7.6
4	85.7	76	8.1
5	96.8	62	7.4
6	81.3	68	9.1
7	96.5	72	8.8
8	89.1	63	9.3
9	97.7	59	7.8
10	89.6	75	8.9
11	98.3	69	9.5
12	81.5	81	6.8
13	81.6	77	7.5
14	99.1	73	8.5
15	82.4	78	8.8
16	93.8	65	9.2
17	91	64	9.4
18	94.2	80	8.7
19	118.0	59	9.5
20	104.1	74	7.4
21	85.4	65	6.8
22	89.3	68	8.4
23	88.3	57	9.1
24	102.2	75	8.3
25	97.4	64	8.5
26	92.8	77	7.6
27	88.5	58	8.5
28	83.3	79	9.7
29	90.2	67	8.8

BALF = bronchoalveolar lavage fluid; FEV_1 = forced expiratory volume at the first second.

dispersion was similar to that described previously with human tonsil tissues.[17] Briefly, the tissue was chopped finely with scissors into fragments of 0.5-2.0 mm^3, and incubated with 1.5 mg/ml collagenase and 0.75 mg/ml hyaluronidase in MEM containing 2% FCS (1 g lung/10 ml buffer) for 70 minutes at 37°C. Dispersed cells were separated from undigested tissue by filtration through nylon gauze (pore size 100 µm diameter), and were maintained in MEM (containing 10% FCS, 200 U/ml penicillin, 200 µg/ml streptomycin) on a roller overnight at room temperature. Mast cell numbers were determined by light microscopy after staining by Kimura stain. The percentage of lung mast cells with this procedure ranged from 2.3 to 4.5%, and those of colonic cells was 3.2 to 5.3%.

Prior to the challenge, the cells were washed with incomplete HBSS (500 x *g*, 10 minutes, 25°C), and then resuspended in complete HBSS. Aliquots of 100 µl containing 4-6 x 10^3 mast cells were added to a 50 µl aliquot of anti-IgE or CI and incubated for 20 to 60 minutes at 37°C. The reaction was terminated by the addition of 150 µl ice cold HBSS and immediate centrifugation of the tubes (500 x g, 10 minutes, 4°C). All experiments were performed in duplicates. The suspension was boiled for 6 minutes for the measurement of total histamine concentration. The supernatants were stored at -20°C until the histamine concentrations were determined.

Histamine measurement

An enzyme-linked histamine chromogenic assay was employed to detect the histamine released from BALF mast cells. The assay was performed according to the procedures supplied by the manufacturer. In brief, 100 µl of histamine standards, controls or samples were mixed with 50 µl acylation solution in a 96-well plate. Following 60 minutes incubation at room temperature, 50 µl of the acylated standards, controls or samples were pipetted onto an antibody-coated plate and mixed with 200 µl of enzymatic conjugate at 4°C for 18 hours. The reactions were visualized by the addition of a substrate supplied by the manufacturer and the plate was read at 410 nm on a plate reader.

A glass fiber-based fluorometric assay was used to measure histamine in the supernatants of dispersed mast cells and was performed as described previously.[17] The procedure is performed in a glass-fiber matrix (Lundbeck Diagnostics, Copenhagen, Denmark) that selectively binds histamine. The histamine is detected by adding *o*-phthaldialdehyde (OPD) to the glass fibre plate and reading the results on a spe trophotofluorometer (Perkin-Elmer LS 2, Denmark). The histamine release was expressed as a percentage of total cellular histamine levels, and corrected for the spontaneous release measured in tubes in which

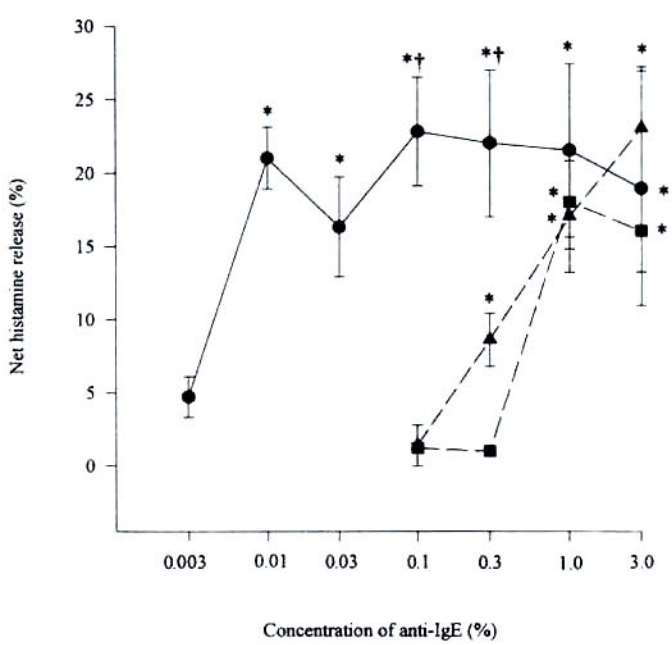

Fig. 1 The effects of anti-IgE on the histamine release from the mast cells from BALF, lung or colon tissues. The values shown are mean ± SEM for 6 to 9 separate experiments performed in duplicates. The BALF (●), lung (■) or colon cells (▲) were incubated with various concentrations of anti-IgE for 20 minutes at 37°C, respectively. *$p < 0.05$ compared with spontaneous histamine release (Student's t test). †$p < 0.05$ compared with the histamine release from lung and colon mast cells induced by corresponding dose of anti-IgE (Student's t test).

cells had been incubated with the HBSS diluent alone.

Statistics

All statistical analyses were performed using SPSS (Version 10.0). Data are shown as the mean ± SEM for the number of experiments(n) indicated and the Student's t test was applied to evaluate two paired independent samples. In all analyses $p < 0.05$ was taken as significant.

RESULTS

The mast cells from BALF were at least 100 times more sensitive to anti-IgE stimulation than mast cells dispersed from lung or colon tissues. As little as 0.01% of anti-IgE was able to induce some 20% histamine release from BALF mast cells, whereas to achieve a similar degree of release of histamine from lung or colonic mast cells, at least 1% of anti-IgE was required, which was a 100 fold more than the quantity needed for provoking BALF mast cells. However, when the concentrations of anti-IgE increased from 0.01% to as high as 3% the quantity of histamine released from BALF mast cells did not show any significant change (Fig. 1).

In contrast, BALF mast cells had no significantly different reaction from lung or colon mast cells in response to CI stimulation. The maximum net histamine release was approximately 40%, 45% and 40% for BALF, lung and colon mast cells, respectively, induced by 1 μM (for BALF and colon mast cells) or 10 μM CI (for lung mast cells) (Fig. 2). Adenosine virtually failed to stimulate histamine release from BALF mast cells at concentrations up to 100 μM. Nevertheless, as much as 1,000 μM adenosine was able to induce some 10% histamine release from BALF mast cells (Fig. 3).

DISCUSSION

A selectively enhanced sensitivity of BALF mast cells to IgE dependent stimulation in asthmatics was demonstrated in the current study, indicating that mast cells from asthmatic patients are more easily activated by allergens than those from non-asthmatics. This could represent one of the mechanisms why asthma occurs to some people, but not to others when two groups of people live under similar conditions. Serving as an antigen, as little as 0.01% anti-IgE (approximately 100 ng/ml) was able to induce some 20% release of histamine from BALF mast cells of asthmatics. This concentration of antigens should be easily achieved during the pollen seasons or a situation of close contact. The 100 fold increased sensitivity of asthmatic BALF mast cells to anti-IgE observed in the current study is in concordant to previous reports, [18-19] in which also described a trend that asthmatic BALF mast cells were more sensitive to IgE-dependent stimulation.

Asthmatic BALF mast cells did not show any significant difference from dispersed lung or colonic

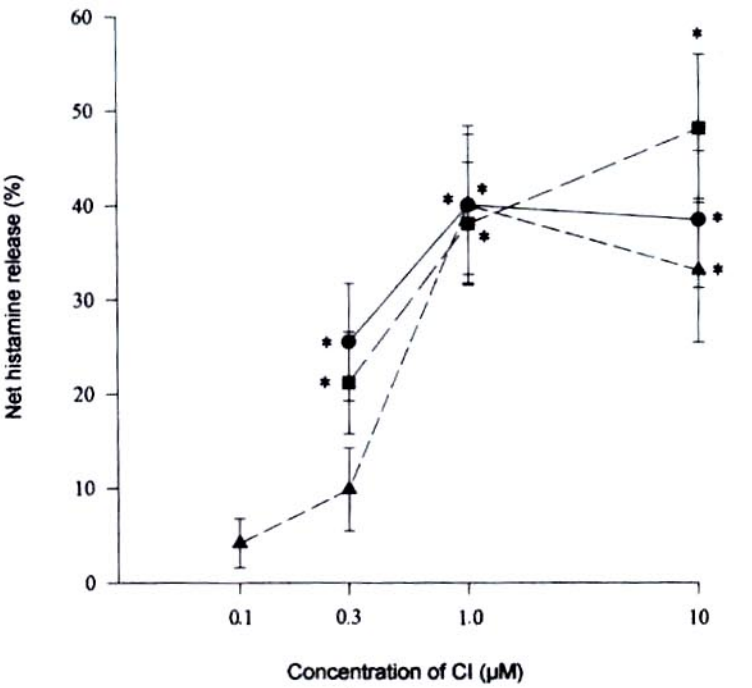

Fig. 2 The effects of calcium ionophore A23187 (CI) on the histamine release from the mast cells from BALF, lung or colon tissues. The values shown are mean ± SEM for 6 to 9 separate experiments performed in duplicates. The BALF (●), lung (■) or colon cells (▲) were incubated with various concentrations of CI for 20 minutes at 37°C, respectively. *$p < 0.05$ compared with spontaneous histamine release (Student's t test).

mast cells in response to CI stimulation, indicating that the response of asthmatic BALF mast cells to anti-IgE was selective. Since mast cell degranulation is a key event in the pathogenesis of asthma, the selectively enhanced responsiveness of asthmatic BALF mast cells to anti-IgE could be an important finding in asthma research. Our finding suggests that a reduction of the influence of IgE on mast cells including mast cell stabilization could be a valuable approach for the development of effective anti-asthmatic drugs. While the extent of histamine release from asthmatic BALF mast cells induced by CI in the current study was similar to that reported previously,[20] adenosine showed little effect on asthmatic BALF mast cells. This was quite different from a previous report suggesting adenosine was a relatively potent stimulus of BALF mast cells.[21] The reason for this could be that BALF mast cells from normal subjects responded to adenosine, but the cells from asthmatics did not.

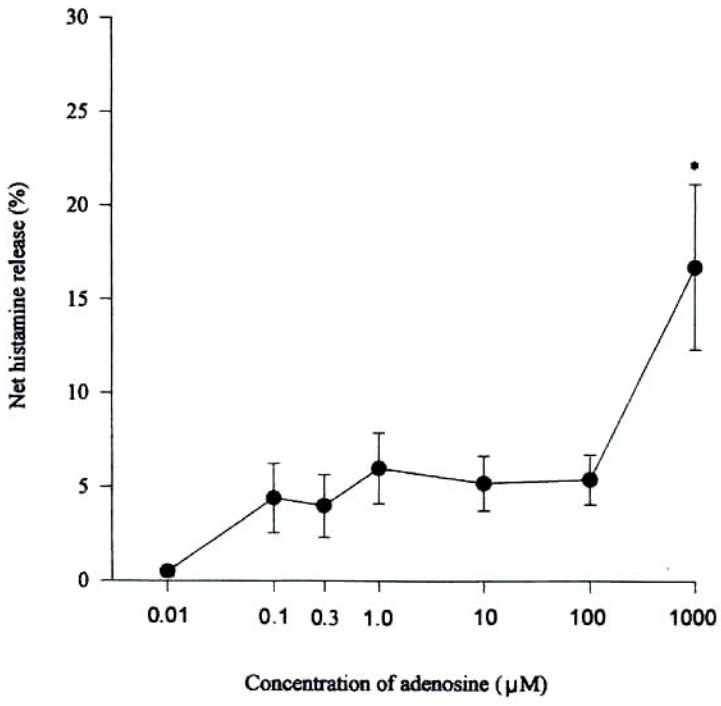

Fig. 3 The effects of adenosine on the histamine release from the BALF mast cells. The BALF cells were incubated with various concentrations of adenosine for 20 min at 37°C. The values shown are mean ± SEM for 7 to 8 separate experiments performed in duplicates. $^{*}p < 0.05$ compared with spontaneous histamine release (Student's t test).

In conclusion, the selective enhancement of responsiveness of asthmatic BALF mast cells to IgE-dependent stimulation is of importance in understanding the pathogenesis of asthma, and could explain at least in part the reason why asthma occurs in one population, but not in others.

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REFERENCES

1. Church MK, Bradding P, Walls AF, Okayama Y. Human mast cells and basophils. In: Kay AB, ed. Allergy and Allergic Diseases. Oxford, Blackwell, 1997; pp 149-70.
2. Okayama Y, Church MK. Comparison of the modulatory effect of ketotifen, sodium cromoglycate, procaterol and salbutamol in human skin, lung and tonsil mast cells. Int Arch Allergy Appl Immunol 1992; 97: 216-22.
3. Butchers PR, Vardey CJ, Johnson M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. Br J Pharmacol 1991; 104: 672-6.
4. Naclerio RM, Kagey-Sobotka A, Lichtenstein LM, Freidhoff L, Proud D. Terfenadine, an H1 antihistamine, inhibits histamine release *in vivo* in the human. Am Rev Respir Dis 1990; 142: 167-71.
5. Holgate ST. Inflammatory and structural changes in the airways of patients with asthma. Respir Med 2000; 94 Suppl D: S3-6.
6. Kassel O, de Blay F, Duvernelle C, Oligart C, Israel-Biet D, Krieger P, Moreau L, Muller C, Pauli G, Frossard N. Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose. Clin Exp Allergy 2001; 31: 1432-40.
7. Olsson N, Rak S, Nilsson G. Demonstration of mast cell chemotactic activity in bronchoalveolar lavage fluid collected from asthmatic patients before and during pollen season. J Allergy Clin Immunol 2000; 105: 455-61.
8. Brown JR, Kleimberg J, Marini M, Sun G, Bellini A, Mattoli S. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. Clin Exp Immunol 1998; 114: 137-46.
9. Nocker RE, Out TA, Weller FR, Mul EP, Jansen HM, van der Zee JS. Influx of neutrophils into the airway lumen at

4 h after segmental allergen challenge in asthma. Int Arch Allergy Immunol 1999; 119: 45-53.
10. Le Bourgeois M, Goncalves M, Le Clainche L, Benoist MR, Fournet JC, Scheinmann P, de Blic J. Bronchoalveolar cells in children < 3 years old with severe recurrent wheezing. Chest 2002; 122: 791-7.
11. Wahlstrom J, Dahlen B, Ihre E, Wigzell H, Grunewald J, Eklund A. Selective CD8+ T cells accumulate in the lungs of patients with allergic asthma after allergen bronchoprovocation. Clin Exp Immunol 1998; 112: 1-9.
12. Boulay ME, Boulet LP. Influence of natural exposure to pollens and domestic animals on airway responsiveness and inflammation in sensitized non-asthmatic subjects. Int Arch Allergy Immunol 2002; 128: 336-43.
13. Sterk PJ. Airway hyperresponsiveness: using bronchial challenge tests in research and management of asthma. J Aerosol Med 2002; 15: 123-9.
14. Wenzel SE, Fowler III AA, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. Am Rev Respir Dis 1988; 137: 1002-8.
15. Arjour NN, Calhoun WJ, Schwartz LB, Busse WW. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatic are associated with increased airway obstruction. Am Rev Respir Dis 1991; 144: 83-7.
16. Leung KB, Flint KC, Brostoff J, Hudspith BN, Johnson NM, Pearce FL. Some properties of mast cells obtained by human bronchoalveolar lavage. Agents Actions 1986; 18: 110-2.
17. He S, Gaça MDA, Walls AF. A role for tryptase in the activation of human mast cells: modulation of histamine release by tryptase and inhibitors of tryptase. J Pharmacol Exp Ther 1998; 286: 289-97.
18. Pearce FL, Flint KC, Leung KB, Hudspith BN, Seager K, Hammond MD, Brostoff J, Geraint-James D, Johnson NM. Some studies on human pulmonary mast cells obtained by bronchoalveolar lavage and by enzymic dissociation of whole lung tissue. Int Arch Allergy A ppl Immunol 1987; 82: 507-12.
19. Leung KB, Flint KC, Hudspith BN, Brostoff J, Johnson NM, Seager K, Hammond MD, Pearce FL. Some further properties of human pulmonary mast cells recovered by bronchoalveolar lavage and enzymic dispersion of lung tissue. Agents Actions 1987; 20: 213-5.
20. Heaney LG, Cross LJ, Stanford CF, Ennis M. Substance P induces histamine release from human pulmonary mast cells. Clin Exp Allergy 1995; 25: 179-86.
21. Forsythe P, McGarvey LP, Heaney LG, MacMahon J, Ennis M. Adenosine induces histamine release from human bronchoalveolar lavage mast cells. Clin Sci 1999; 96:349-55.