

Histamine release from bronchoalveolar lavage cells from asthmatic subjects after allergen challenge and relationship to the late asthmatic response

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Summary

Background Metachromatic cells obtained from asthmatic subjects demonstrate increased spontaneous and stimulated histamine release *in vitro*. Their ability to synthesize and store proinflammatory cytokines has focused renewed interest on their role in asthma.

Objective The late asthmatic response provides a useful model of clinical asthma. The aim of the study was to examine metachromatic cell derived mediators and histamine releasability *in vitro* after *in vivo* allergen exposure in atopic subjects with and without asthma and relate them to the type of physiological response observed.

Methods Bronchoalveolar lavage (BAL) cells were obtained 4 h after challenge from asthmatics exhibiting a single early response (EAR, $n = 5$), a dual response (LAR, $n = 7$), unchallenged (basal, $n = 5$), atopic non-asthmatic (ANA, $n = 6$) and non-atopic non-asthmatics (normal, $n = 5$). BAL histamine and tryptase concentrations and *in vitro* histamine release (HR) after stimulation with anti-IgE, allergen, A23187, concanavalin A and substance P were compared.

Results Metachromatic cell numbers were lower in normal controls compared with all asthmatic groups and in LAR compared with EAR. Metachromatic cell derived mediators were higher in asthmatic compared with normal subjects. Spontaneous HR in LAR ($20.5 \pm 5.0\%$) was lower than EAR ($29.5 \pm 3.9\%$) and ANA ($30.2 \pm 1.4\%$) ($P < 0.05$). No differences were seen in stimulated HR between EAR and LAR. HR in ANA stimulated with anti-IgE was greater than LAR ($P < 0.05$). HR in ANA stimulated with anti-IgE was greater than LAR ($P < 0.05$). After stimulation with ionophore A23187 ($1 \mu\text{M}$), release was greater in LAR compared with basal ($P < 0.05$) and no different at $5 \mu\text{M}$. All subject groups responded to substance P (SP) but was significantly more in the asthmatic subjects compared to normal controls ($P < 0.05$). Allergen challenge did not modify the response of asthmatic subjects to SP.

Conclusion Functional differences in metachromatic cell reactivity are present in atopic subjects 4 h after *in vivo* allergen exposure which relate to the physiological response observed after this time and suggest that there is ongoing metachromatic cell degranulation subjects who subsequently develop LAR.

Keywords: asthma, basophil, bronchoalveolar lavage, bronchoscopy, histamine, histamine liberation, late asthmatic response, mast cell

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Introduction

Bronchoalveolar lavage (BAL) has demonstrated increased numbers of mast cells in both atopic [1,2] and non-atopic

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asthma [3] and mast cell numbers have been shown to correlate with bronchial hyperresponsiveness in both adults [1] and children [4]. Functionally these cells are activated with both increased spontaneous histamine release *in vitro* [1,5] and increased concentrations of mast cell derived mediators *in vivo*, which correlate with bronchial hyperresponsiveness [6,7]. Morphological and ultrastructural evidence from endobronchial biopsies has demonstrated mast cell degranulation *in vivo* [8,9] which is greatest in asthmatic subjects [10].

Allergen challenge of human subjects with asthma has been used to study inflammatory mechanisms in asthma. After inhalation allergen challenge in asthmatic subjects, an early asthmatic response (EAR) occurs, which is characterized by the development of airflow obstruction, is maximal at 15–30 min and resolves within 2–3 h [11]. Approximately 60% of asthmatic subjects go on to develop a late phase of airflow obstruction of varying severity (LAR), which begins at 3–4 h and is maximal between 6 and 12 h [11]. This late asthmatic reaction has many features which are typical of clinical asthma and can be used as a laboratory model of the disease [11].

After allergen challenge, recruitment to the airways of eosinophils and their immunological activation with release of toxic granule products appears to be crucial to the development of LAR. In comparative studies of LAR and EAR, Aalbers *et al.* and Rossi *et al.* have demonstrated eosinophilia 3 and 4 h respectively after allergen exposure [12,13]. This would suggest that the immunological mechanisms involved in eosinophilic recruitment are established in the first few hours after allergen exposure. Thus, examination of airway events 4 h after allergen exposure allows study of the dynamic processes related to the development of LAR. This is also a convenient timepoint to study asthmatics who subsequently develop a late asthmatic response as significant airflow obstruction is not present and bronchoscopy can be performed with few difficulties.

After allergen exposure, basophils are also recruited to the airway and have been demonstrated in BAL 24 h post challenge [14] and in nasal lavage fluid 4–8 h post challenge [15]. Both metachromatic cells (mast cell and basophil) store many proinflammatory mediators including cytokines and renewed interest has been shown in these cells as central to the inflammatory events which take place after allergen exposure. Little work has been done on metachromatic cell reactivity after allergen challenge and it is unclear if challenge modulates cellular secretion prior to the onset of LAR. This study examined *in vitro* histamine releasability and the relation of this reactivity to concentrations of metachromatic cell derived mediators and metachromatic cell numbers in BAL fluid 4 h after allergen challenge in allergic asthmatic subjects and its relationship to the development of LAR.

Subjects and methods

All subjects ($n = 25$, 18–37 years, Table 1) were recruited on a voluntary basis and gave voluntary informed written consent to take part in the study, which was approved by the Faculty of Medicine Research Ethical Committee, The Queen's University of Belfast. Asthma was defined according to the American Thoracic Society as a history of intermittent wheeze, cough or chest tightness on allergen exposure or exercise [16]. Subjects were excluded if there was any history of smoking, diabetes, current pregnancy, cardiac or renal disease or any chest disease other than asthma. Asthmatic subjects had current asthma defined as symptoms within the past 12 months and atopy was defined as positive skin test to one or more of four common environmental allergens (house dust mite, *Dermaphagoides pteronyssinus* (HDM), mixed grass pollen, cat, and dog, [Biodiagnostics]). All atopic subjects were sensitive to HDM with a positive skin test and HDM specific IgE in the serum measured by radioallergosorbent test (RAST) (Pharmacia Ltd, Sweden). In atopic subjects sensitive to grasses, all studies were performed outside the grass pollen season (May–July). Asthmatic subjects on theophylline, inhaled steroids or cromoglycate or who had taken oral steroids in the preceding 4 weeks were also excluded to ensure recruitment of asthmatic subjects with mild clinical disease and no recent exacerbations. Prior to attending for each study day, asthmatic subjects were advised to omit inhaled β_2 -agonists for the preceding 12 h and to avoid tea or coffee on the morning of attendance. Baseline spirometry was measured and if greater than 70% predicted for age, sex and height, the subject proceeded to inhalation challenge.

Inhalation challenges

Methacholine (Sigma-Aldrich Company Ltd, UK) challenge was performed using the tidal breathing method of Cockcroft *et al.* [17]. Allergen challenge was performed in similar fashion with inhalation of increasing doses (500 U/mL, 1000 U/mL, 5000 U/mL HDM allergen solution) until FEV₁ fell by at least 20% from baseline value, or the maximum concentration of HDM had been inhaled. FEV₁ was measured 10, 15 and 20 min after each inhalation challenge and the maximum fall in FEV₁ was recorded. Atopic subjects attended on two days at least 7 days apart for allergen and placebo inhalation. Challenges were performed single blind at 9.00 am on each day. Inhalation challenge began with either HDM or placebo and spirometry was recorded every 15 min for 1 h and then every 30 min until 8 h post challenge. The early response was defined as the maximal fall in FEV₁ occurring in the first 30 min after allergen challenge. A delayed or late asthmatic response was considered to have occurred if the FEV₁ fell by greater

Table 1. Age, sex, atopic status, baseline spirometric indices and subject group (LAR, late asthmatic response; EAR, early asthmatic response only; ANA, atopic non-asthmatic; normal, non-atopic control; basal, unchallenged asthmatic; three subjects were studied on two separate occasions). †Radioallergosorbent test (RAST) is given as 0–6 (0 = negative, 6 = strongly positive). *Concentration of methacholine (PC₂₀) causing a 20% fall in FEV₁. ‡The calculated values (see text) for the EAR and LAR after allergen challenge are given

Patient no	Allergen response	Age (years)	Sex	IgE U/L	†RAST HDM	*PC ₂₀ (mg/mL)	FEV ₁ (I) %	FVC (I) (%)	‡EAR (%)	LAR (%)
1	LAR	36	m	27	3	0.28	3.05 (91)	5.15 (126)	30	18
2	LAR	28	f	266	5	0.2	2.38 (74)	4.34 (108)	37	22
3	LAR	22	m	121	4	0.18	3.24 (78)	5.86 (119)	21	24
4	LAR	23	m	159	5	5.72	6.59 (155)	8.15 (160)	50	22
5	LAR	23	m	291	4	12.9	4.29 (103)	6.0 (121)	48	19
6	LAR	37	f	153	2	0.9	2.48 (87)	3.73 (104)	38	23
7	LAR	18	f	297	6	3.17	2.73 (96)	3.67 (114)	41	23
8	EAR	21	m	152	3	0.8	4.79 (108)	6.59 (128)	21	8
9	EAR	21	f	1556	6	0.45	3.41 (99)	4.53 (99)	52	9
10	EAR	19	m	163	4	8.4	5.3 (128)	5.8 (117)	23	1.9
11	EAR	22	m	802	5	3.9	4.54 (103)	5.23 (99)	21	7
12	EAR	26	m	161	4	3	4.06 (104)	5.78 (124)	41	3
13	ANA	21	m	222	2	21.8	5.35 (113)	6.28 (113)	6.5	4.1
14	ANA	29	f	174	2	64	4.69 (151)	6.26 (162)	0	0
15	ANA	22	m	137	3	>128	4.86 (106)	5.59 (102)	7	1
16	ANA	26	m	14	2	46	3.91 (96)	5.15 (105)	0	0
17	ANA	21	m	54	3	6.4	5.62 (125)	6.96 (129)	8	0
18	ANA	21	m	274	2	32	4.71 (107)	6.12 (118)	0	0
19	normal	21	m	12	0	44.2	5.34 (113)	7.46 (131)	0	
20	normal	22	m	14	0	64	4.02 (92)	5.05 (97)	0	
21	normal	22	m	57	0	16	3.89 (96)	5.46 (115)	0	
22	normal	21	m	6	0	128	5.47 (121)	6.44 (119)	0	
23	normal	21	m	7	0	55	4.93 (114)	5.81 (114)	0	
24	basal	19	m	1256	6	0.5	3.95 (91)	5.25 (102)	unchallenged	
25	basal	25	m	15	3	7.8	4.67 (107)	6.9 (132)	unchallenged	
8	basal	21	m	152	3	1.0	4.08 (92)	6.18 (120)	unchallenged	
12	basal	26	m	161	4	3.4	3.82 (96)	4.3 (94)	unchallenged	
7	basal	18	f	297	6	2.9	2.62 (96)	5.7 (115)	unchallenged	

than 15% in the 3–8 h period after challenge when corrected for the maximal fall in FEV₁ in the same time period after placebo challenge on a separate day [17]. After assessment the subject groups were non-challenged asthma (basal, $n = 5$), late asthmatic response (LAR, $n = 7$), early asthmatic response (EAR, $n = 5$), atopic non-asthmatic control (ANA, $n = 6$) and non-atopic non-asthmatic control (normal, $n = 5$).

Bronchoscopy and BAL

On a subsequent day, subjects attended for bronchoscopy. Challenged asthmatics, atopic non-asthmatics and normal subjects underwent inhalation challenge with HDM (PD ≥ 20 allergen) at 08.30 h. All bronchoscopies were performed at 12.30 h using a standardized technique. The bronchoscope (Olympus IT20D) was inserted under direct

vision via the nasal or oral route and local anaesthesia (1% lignocaine administered as required to suppress coughing. The bronchoscope was wedged in the medial or lateral segment of the right middle lobe and BAL performed (3 $\times$ 60 mL aliquots of sterile isotonic phosphate buffered saline, pre-warmed to 37°C). The fluid was aspirated and the return samples pooled and placed immediately on ice.

Processing of lavage fluid

The total cell count was measured in a single threshold Coulter counter as previously described [18]. Cytospin preparations were made using the glass coverslip method of Laviolette *et al.* [19]. Two coverslip preparations were stained in acidic Toluidine Blue (BDH Ltd, UK, 0.5% w/v in 0.5 M HCL, 30 min) following fixation in Carnoy's fluid

[2.5 h]). Between 2000 and 5000 cells were counted per slide and the metachromatic cell count expressed as a percentage of total cells counted.

Mediator assays

Tryptase (Pharmacia Ltd, Sweden) and histamine (IBL Laboratories, Germany) were measured in lavage fluid using commercially available kits. The histamine assay was modified according to manufacturer's instructions for lavage fluid and the resultant range of the assay was 25–8000 ng/L.

Functional studies

The BAL fluid was centrifuged (200 *g*, 10 min, 4°C), washed twice in Tyrode's buffer and resuspended in a volume appropriate for the experiment. The cells were prewarmed (37°C, 5 min) and challenged by a 20 min incubation with the secretagogues anti-IgE (Dako, 18.75, 37.5, 75 ng/mL), HDM allergen (Biodiagnostics, 2000 IU/mL), concanavalin A (Sigma, 5, 10 µg/mL), substance P (SP) (Bachem, 25, 50 µM) and A23187 (Calbiochem, 1, 5 µM). Anti-IgE was chosen because it will activate metachromatic cells with surface bound IgE independent of atopic status whereas activation by house dust mite will demonstrate specific activation in sensitized subjects. Concanavalin A is thought to act via sugar receptors on the IgE molecule and thus is another immunological stimulus.

Neuropeptide activation of airway metachromatic cells may be important in asthma and we have previously demonstrated SP activates BAL cells with high spontaneous histamine release *in vitro* and speculated that BAL cells from asthmatic subjects would also be activated by this peptide [27]. A23187 does not activate cells via a receptor dependent mechanism but rather transports calcium directly into the cells. Thus, the choice of stimulants examines both immunological and non-immunological activation of airway metachromatic cells and allows examination of any alteration in this reactivity after allergen challenge and the relationship to the type of asthmatic response. The reactions were stopped by the addition of ice cold Tyrode's buffer and the cells and supernatants separated by centrifugation. Histamine was measured in the cell pellets and the supernatants using an automated fluorometric assay [20]. Histamine release was expressed as a percentage of the total cellular histamine content. When the results are described as corrected, the spontaneous release of histamine (i.e. that occurring in the absence of any stimulus) has been subtracted.

Statistical analysis

Results are presented graphically as the group mean ($\pm$ standard error of the mean) to facilitate comparison

with other data presented in this field. Non-parametric statistical methods are used throughout. The Kruskal–Wallis analysis of variance was used to examine for significant inter-group differences and if significant the Mann–Whitney *U*-test was used for between group comparison. Correlation coefficients are calculated using the Spearman's rank method. A *P*-value of less than 0.05 was considered statistically significant.

Results

Metachromatic cell numbers and metachromatic cell derived mediators

There was no difference in total cell numbers between groups (data not shown). Metachromatic cell numbers (Fig. 1) were significantly lower in normal controls compared to all asthmatic groups. Metachromatic cell numbers were significantly lower in the LAR group compared with EAR. No significant differences were found in the histamine or tryptase concentrations between the individual asthmatic groups though concentrations tended to be higher in LAR (Fig. 2). Histamine and tryptase concentrations were lower in both control groups compared to basal asthmatics. There was a significant correlation between histamine and tryptase concentrations in the lavage fluid ($n = 28$, $r = 0.53$, $P < 0.01$ data not shown).

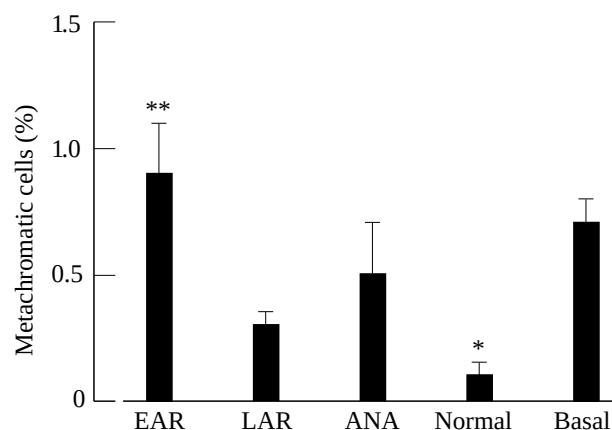


Fig. 1. Metachromatic cell (%) in lavage fluid in subject groups (EAR = subjects demonstrating an early asthmatic response only, LAR = subjects demonstrating a late asthmatic response, ANA = atopic non-asthmatic subjects, Normal = non-atopic non-asthmatic subjects, Basal = unchallenged asthmatic subjects). Statistical comparison was made using the Mann Whitney *U* test. *denotes $p < 0.01$ compared to all asthmatic groups, **denotes $P < 0.01$ compared to LAR.

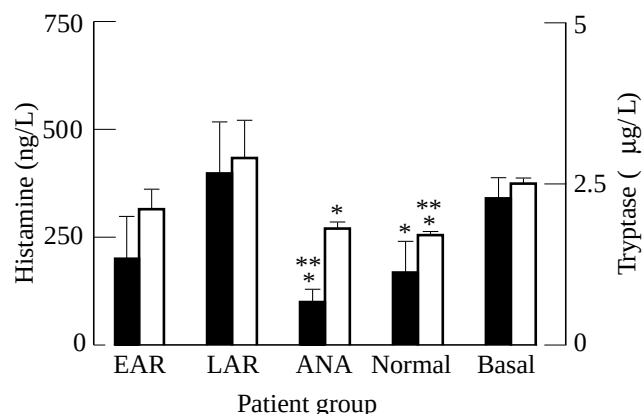


Fig. 2. Histamine (closed columns, ng/L) and tryptase (open columns $\mu\text{g/L}$) concentrations in lavage fluid in subject groups (EAR = subjects demonstrating an early asthmatic response only, LAR = subjects demonstrating a late asthmatic response, ANA = atopic non-asthmatic subjects, Normal = non-atopic non-asthmatic subjects, Basal = unchallenged asthmatic subjects). Statistical comparison was made using the Mann Whitney U test. *denotes $p < 0.05$ compared to basal asthmatic subjects and **denotes $P < 0.01$ compared to LAR.

Functional studies

Spontaneous histamine release The spontaneous histamine release was lowest in the normal control group (Fig. 3). Allergen challenge reduced the spontaneous histamine

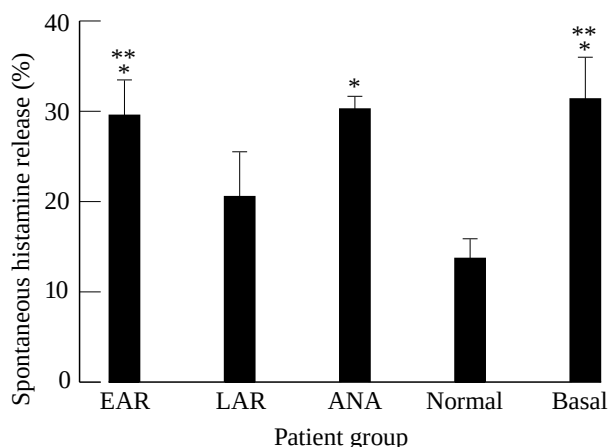


Fig. 3. Spontaneous histamine release (%) from bronchoalveolar lavage cells in subject groups (EAR = subjects demonstrating an early asthmatic response only, LAR = subjects demonstrating a late asthmatic response, ANA = atopic non-asthmatic subjects, Normal = non-atopic non-asthmatic subjects, Basal = unchallenged asthmatic subjects). Statistical comparison was made using the Mann Whitney U test. *denotes $p < 0.05$ compared to normal and **denotes $p < 0.05$ compared to LAR.

release in a group of subjects who went on to develop LAR compared to all other atopic groups and it did not differ significantly from that in the non-atopic control group. In atopic subjects challenged with allergen, there was a significant inverse correlation between spontaneous histamine release and both histamine and tryptase in the lavage fluid (Fig. 4).

Stimulated histamine release

There was significant inter-subject variation in the response to anti-IgE (Fig. 5), with cells from control subjects and non-asthmatic atopics tending to release the most histamine. The responses of cells from challenged asthmatics did not differ significantly from those of basal asthmatics. The type of asthmatic reaction (early or late) did not influence the response to anti-IgE. Allergen

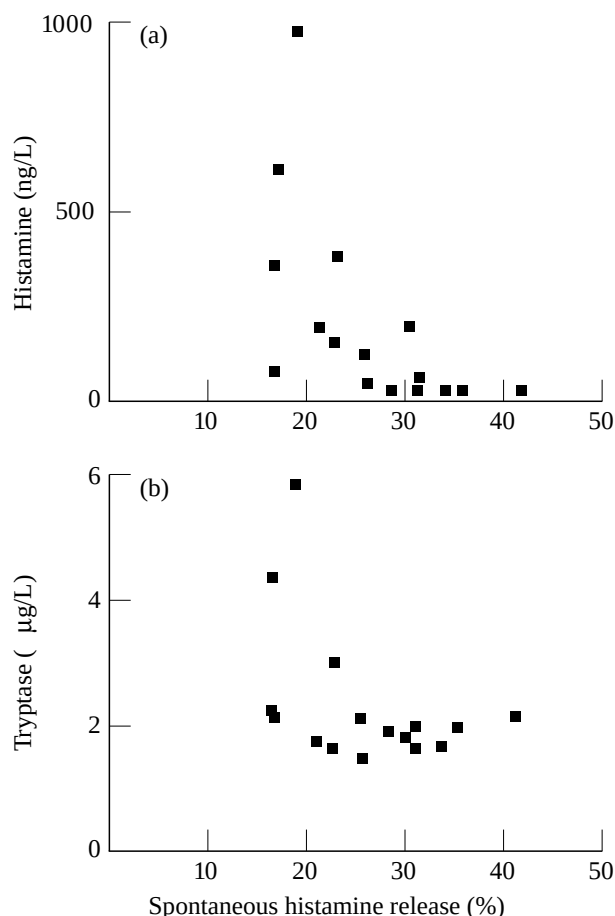


Fig. 4. Correlation between spontaneous histamine release (%) from bronchoalveolar lavage cells and (a) histamine (ng/L) ($r = -0.76$, $P < 0.01$) and (b) tryptase ($\mu\text{g/L}$) ($r = -0.52$, $P < 0.05$) in atopic subjects after allergen challenge ($n = 23$). Correlation is calculated using the Spearman's rank method.

challenge elicited histamine release from all atopic groups but not from non-atopic controls (Fig. 5). There were no significant differences between challenged and non-challenged subjects. Concanavalin A induced histamine release was similar in all groups (Fig. 6). Cells from basal asthmatics released the least histamine on challenge with the calcium ionophore A23187 (Fig. 6). Allergen challenge tended to increase this response

although there was no difference between subjects who exhibited EAR or LAR. Although all groups responded to substance P, the histamine release was lowest in the control subjects and greatest in the non-asthmatic atopics (Fig. 7). Allergen challenge did not modify the response of asthmatic subjects to substance P.

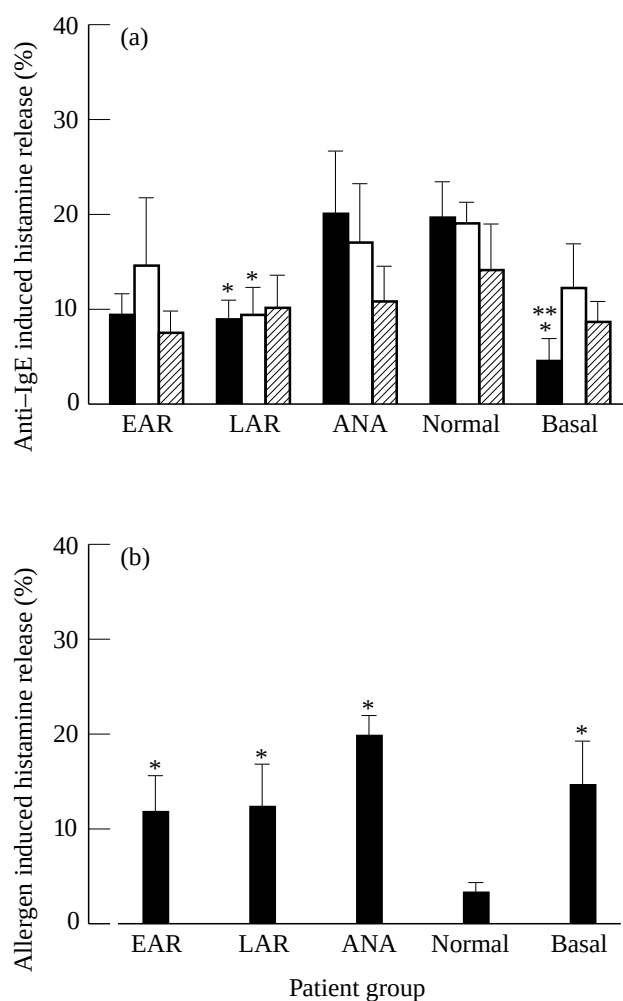


Fig. 5. Anti-IgE (■ 18.75 ng/mL, □ 37.5 ng/mL, ▨ 75 ng/mL) and allergen (HDM) (2000 IU/mL) induced histamine release (%) from bronchoalveolar lavage cells in subject groups (EAR, subjects demonstrating an early asthmatic response only; LAR, subjects demonstrating a late asthmatic response; ANA, atopic non-asthmatic subjects; normal, non-atopic non-asthmatic subjects; basal, unchallenged asthmatic subjects). Mean histamine release is corrected for spontaneous release. Statistical comparison was made using the Mann-Whitney *U*-test. * $P < 0.05$ compared with normal and ** $P < 0.05$ compared with ANA at the same concentration.

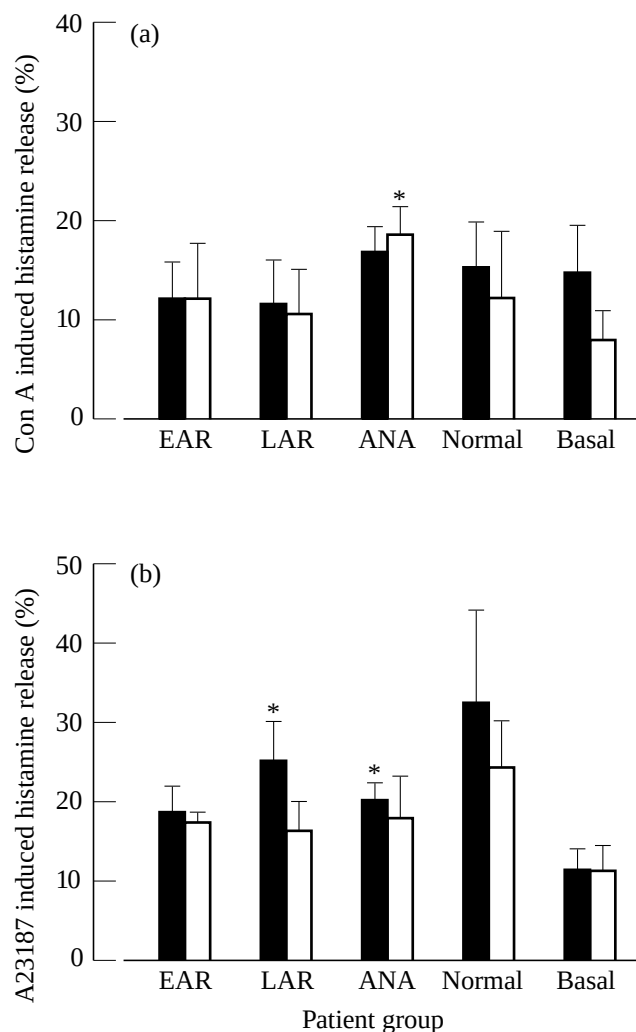


Fig. 6. Concanavalin A (■ 5 µg/mL, □ 10 µg/mL) and calcium ionophore (A23187) (■ 1 µM, □ 5 µM) induced histamine release (%) from bronchoalveolar lavage cells in subject groups (EAR = subjects demonstrating an early asthmatic response only, LAR, subjects demonstrating a late asthmatic response; ANA, atopic non-asthmatic subjects; Normal, non-atopic non-asthmatic subjects; Basal, unchallenged asthmatic subjects). Mean histamine release is corrected for spontaneous release. Statistical comparison was made using the Mann-Whitney *U*-test. * $P < 0.05$ compared with basal at the same concentration.

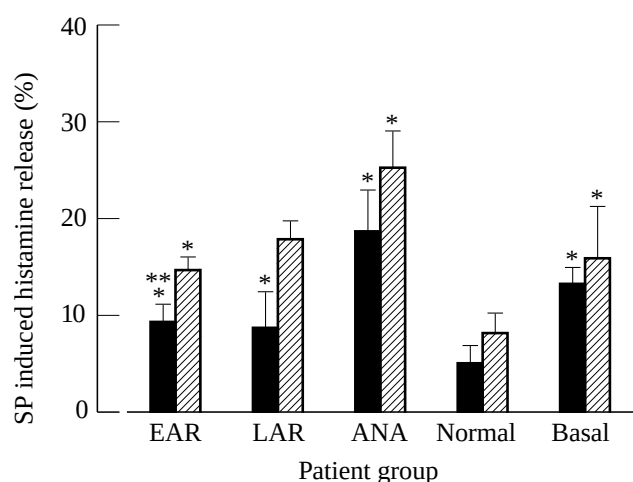


Fig. 7. Substance P (■ 25 µM, ▨ 50 µM) induced histamine release (%) from bronchoalveolar lavage cells in subject groups (EAR, subjects demonstrating an early asthmatic response only; LAR, subjects demonstrating a late asthmatic response; ANA, atopic non-asthmatic subjects; normal, non-atopic non-asthmatic subjects; Basal, unchallenged asthmatic subjects). Mean histamine release is corrected for spontaneous release. Statistical comparison was made using the Mann–Whitney *U*-test. **P* < 0.05 compared with normal and ***P* < 0.05 compared with ANA at the same concentration.

Discussion

This study examined *in vitro* metachromatic cell reactivity after allergen challenge in atopic individuals and found some differences which related to the type of physiological response observed. The late asthmatic response represents a useful model which has many features consistent with clinical asthma [11]. After allergen exposure, much evidence has pointed to the early asthmatic reaction being mast cell dependent however the contribution of the mast cell to the late response is less clearly established.

We refer to the cellular source of histamine *in vitro* as metachromatic cells as both mast cells and basophils could contribute to the histamine release observed. Basophils have been demonstrated in BAL after allergen exposure at 24 h [14] and in nasal lavage fluid 4–8 h after exposure [15]. Our study was performed 4 h after allergen exposure and we feel that the histamine releasing cells are predominantly mast cells. The correlation between lavage tryptase and histamine does suggest that they are derived from the same cellular source, namely the mast cell. Differential immunohistochemical staining with anti-tryptase and anti-histamine antibodies or *in vitro* releasability with f-met leu phe may have helped to clarify this issue but would still not totally exclude some contribution from basophils. The cells examined on cytopsin were morphologically mast cells but again

difficulty exists in definitively distinguishing mast cells from basophils using metachromatic stains.

Metachromatic cell numbers were significantly higher in all asthmatic groups compared with normal controls. This has been reported in a number of lavage studies and suggests recruitment of metachromatic cells to the bronchial mucosa in asthma [1–3]. The concentrations of histamine and tryptase are similar to those previously reported [5,6] and were higher in the baseline asthmatic group compared with normal and atopic non-asthmatic controls suggesting more basal metachromatic cell degranulation in the asthmatic group. Within the asthmatic groups no significant differences were observed though both histamine and tryptase tended to be higher in the LAR group. This trend was associated with significantly fewer countable metachromatic cells in the lavage fluid presumably because the LAR subjects have more degranulated metachromatic cells and thus are more difficult to stain and count. A previous study demonstrated the ratio of degranulated to granulated mast cells was greater in subjects who develop a LAR both at baseline and 4 h after allergen challenge [21]. In another study, Diaz *et al.* reported histamine was elevated at 6 h post allergen challenge in five of seven subjects who demonstrated a late response [22]. These studies are in keeping with fewer intact mast cells and higher mast cell granule products in subjects who develop LAR.

Spontaneous histamine release was significantly higher in asthmatic subjects at baseline compared with non-atopic controls as previously reported [5,23,24]. This increased reactivity is supported by the significantly higher concentrations of both metachromatic cell derived mediators in the lavage fluid of asthmatics at baseline compared to both control groups.

After allergen challenge, spontaneous release is not altered in subjects demonstrating EAR only and indeed is similar to spontaneous release in atopic non-asthmatic subjects, who demonstrated no airway response after allergen challenge. Further, no difference was observed in metachromatic cell derived mediators in these groups. This would suggest that in sensitised subjects 4 h after allergen challenge, baseline metachromatic cell degranulation *in vivo* is similar in asthmatic subjects who have completed an intact immediate response and those who do not demonstrate any immediate response. In the LAR group however, spontaneous release is significantly less than both other sensitive challenged groups and the basal asthmatics and indeed is not significantly different from normals. The LAR group tended to have higher metachromatic cell derived mediators in BAL fluid and in atopic subjects a significant inverse correlation is observed between spontaneous histamine release *in vitro* and both histamine and tryptase *in vivo*. Histamine is cleared rapidly from the target organ, thus the histamine measured in lavage fluid probably

represents histamine released at the time of lavage. This would suggest that after allergen challenge in atopic subjects the lower *in vitro* histamine release reflects cells, which *in vivo* are actively degranulating. In contrast, the low spontaneous histamine release seen in the non atopic control subjects is associated with low concentrations of metachromatic cell derived mediators and in this situation low spontaneous histamine release reflects cells with little ongoing degranulation *in vivo* and little stimulation by allergen. In LAR subjects therefore, there appears to be a greater degree of metachromatic cell degranulation *in vivo* 4 h after allergen challenge. The significance of this ongoing metachromatic cell degranulation in LAR subjects and its overall contribution to the development of the resultant airflow obstruction is still unclear. Interestingly, subjects with steroid-dependent intractable asthma as well as having more severe disease, also have lower histamine and LTC₄ release *in vitro* when compared with non steroid-dependent asthmatics [25] suggesting that an analogous situation may exist in severe clinical disease.

The dose range of anti-IgE used *in vitro*, has previously been shown to correspond to the ascending part of the dose response curve for lavage mast cells for normal and asthmatic subjects [23]. In the normal group, histamine release was not different from atopic controls, while it is higher than the asthmatic groups. This can be explained in a number of ways. Firstly, metachromatic cells from the normal group may be fully granulated when retrieved *in vivo* and consequently demonstrate a greater *in vitro* response with anti-IgE stimulation. This situation would also be true to a lesser extent for the atopic controls and this is supported by the fact that metachromatic cell mediators *in vivo* are higher than normals and lower than asthmatics [7]. Further, after endobronchial allergen challenge both tryptase and histamine concentrations in BAL fluid rise in atopic subjects without asthma [26]. Thus, histamine release in the atopic non-asthmatic group after anti-IgE stimulation, although tending to be less, was not significantly different from normals. When challenged asthmatics are sub-divided into subjects exhibiting EAR and LAR, LAR subjects demonstrate significantly less histamine release after anti-IgE stimulation, which again suggests that cells from LAR subjects are more degranulated *in vivo*. This is supported by the higher concentrations of metachromatic cell mediators in the lavage fluid and the lower spontaneous release mentioned above.

Stimulation with concanavalin A did not demonstrate any of the trends seen with the other immunological stimuli. Of interest after stimulation with A23187, LAR subjects had the highest histamine release of all atopic groups at both concentrations suggesting that the differences discussed above are true and do not relate to cells that have merely released all their mediators after *in vivo* allergen exposure.

We have previously demonstrated that SP can stimulate BAL metachromatic cells and that this release was associated with high spontaneous histamine release [27]. We speculated that since high spontaneous histamine release is observed in asthmatic subjects, lavage metachromatic cells from this group would respond to SP. This study confirms that SP does stimulate metachromatic cells from asthmatic subjects in a dose dependent manner and supports the use of our *in vitro* model to examine functional characteristics of metachromatic cell reactivity. Lung parenchymal mast cells do not respond to substance P [28,29] and this is further evidence for functional heterogeneity between pulmonary metachromatic cells. Lavage metachromatic cells may be more relevant to airways diseases such as asthma being located close to the mucosal surface and ideally placed to interact with inhaled agents.

In summary, this study demonstrates functional differences in metachromatic cell reactivity in atopic subjects 4 h after *in vivo* allergen exposure which relate to the physiological response observed after this time. It suggests that there is ongoing metachromatic cell degranulation 4 h after allergen exposure in subjects who subsequently develop LAR. In subjects with EAR only, after the initial phase of metachromatic cell degranulation and subsequent mediator release, metachromatic cells return to the basal state with similar spontaneous and stimulated histamine release. In view of the numerous pro-inflammatory mediators which are both stored and synthesized by metachromatic cells after allergen exposure, this ongoing mediator release may contribute to the development of LAR.

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