

Short communication

The allergen specificity of the late asthmatic reaction

Background: Allergen inhalation challenge in asthma may induce both early (EAR) and late (LAR) asthmatic reactions. The EAR is IgE and mast cell dependent. The mechanism of the LAR is less well defined and we have hypothesized may be allergen dependent. The aim of this study was to investigate the allergen specificity of the LAR to allergen inhalation in asthma.

Methods: In a randomized, double-blind, crossover design six asthmatic volunteers with dual sensitization to house dust mite (HDM) allergen and grass pollen (GP) allergen underwent inhalation allergen challenge with these separate allergens on two occasions separated by 14 days. Lung function changes were followed for 8-h postchallenge. Bronchial reactivity (histamine PC₂₀) and airway inflammation, assessed by induced sputum differential cell count, were measured 24-h pre and postallergen challenge. The allergen inhalation challenges were matched to achieve the same magnitude of EAR.

Results: Despite comparable group mean EAR percent falls in FEV₁ (25.8% following GP and 28.0% following HDM ($P = 0.917$), the LAR was statistically greater on the HDM challenge day (13.0% vs 22.8% [$P = 0.046$]) and was associated with a significant airway eosinophil recruitment (mean (SD) of 5.4 (4.8)% to 22.1 (18.2)% ($P = 0.028$) that was not evident on the GP allergen challenge day.

Conclusions: These findings identify the allergen specificity of the LAR and indicate that factors independent of IgE contribute to the LAR. Such findings have relevance both to the understanding of the allergen-induced airway responses in asthma and the need for homogeneity in inhaled-allergen challenge studies in asthma.

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Inhaled allergen challenge in sensitized asthmatics induces an early asthmatic reaction (EAR) as a result of IgE-mediated mast cell activation and subsequent bronchial smooth muscle cell constriction (1). This is succeeded by a late asthmatic reaction (LAR) 3–8 h later in some individuals, that is characterized by further airway obstruction, airway inflammation and an increase in airways hyperresponsiveness (2). The late reaction is thus considered a better predictor of clinical asthma than the immediate reaction. The mechanisms underlying the LAR are less clear than the early response but are considered likely to be generated, at least in part, by T cell activation (2–4). The ability of different allergens to induce a LAR may differ; seasonal asthma during the hay fever season is not as common as, and differs from, house dust mite-related perennial asthma, in that it responds well to antihistamine therapy (5), inferring that this form of asthma may be more dependent on immediate type responses.

To evaluate of the allergen specificity of the LAR, we examined the presence and magnitude of the LAR in asthmatics sensitized to both house dust mite and grass pollen allergens, by examining the airway bronchoconstrictor response, the change in airway reactivity and induced airway inflammation after separate inhalation challenges with these allergens, matched to achieve the same magnitude EAR.

Methods

Subjects

Six volunteers (three male) with mild asthma were recruited. All were nonsmokers and had documented physician diagnosed asthma, positive skin prick tests to grass pollen and house dust mite, resting FEV₁ > 75% of predicted and a PC₂₀ to histamine of ≤ 10 mg/ml at screening. Subject baseline characteristics are shown at Table 1. Subjects were excluded if they had experi-

Table 1. Subject baseline characteristics

Subject	Sex	Age	PC ₂₀	% pred FEV ₁	% pred FVC	SPT HDM	SPT Grass
1	M	34	2.39	98.0	107.0	7	6
2	F	35	9.1	85.2	87.2	8	4
3	M	46	0.5	75.4	79.4	7.5	7
4	F	29	0.97	94.0	106.9	12	11.5
5	F	23	8.62	92.8	91.3	5	5
6	M	33	0.55	97.0	99.6	3.5	5.5

PC₂₀, provoking concentration of histamine resulting in 20% reduction in FEV₁. SPT, skin prick test wheal diameter in mm; HDM, house dust mite; M, male; F, female; % pred FEV₁, percentage of predicted forced expiratory volume in 1 s; FVC, forced vital capacity.

enced a respiratory infection within 4 weeks of the study, an exacerbation requiring oral steroids within the preceding three months or deteriorating asthma requiring an increased dose of inhaled steroids within six months. None of the subjects were prescribed long acting β -agonists, theophyllines, leukotriene receptor antagonists or H₁ receptor antagonists. The Local Research Ethics Committee approved the study and all subjects provided written informed consent.

Study design

The study was a randomized, double-blind, crossover design. Subjects attended for two study phases, each 14 days apart. Each phase consisted of attendances on three consecutive days; days 1 and 3 involved assessment of lung function followed by histamine challenge and sputum induction. On day 2, an inhalation allergen challenge was performed. All subjects attended during the months of February and March, outside the grass pollen season in the UK.

Bronchial challenge tests

Airways reactivity to histamine was measured by inhalation of doubling doses of histamine (0.03–16 mg/ml), with the provocative concentration of histamine causing a 20% decrease in FEV₁ (PC₂₀) calculated by linear interpolation. Inhaled allergen challenges were performed as previously described (6). Briefly, allergen extracts of either house dust mite (HDM) (*Dermatophagoides pteronyssinus*) or mixed grasses (Aquagen SQ 100000 SQ-U/ml; ALK, Horsholm, Denmark) were diluted to give concentrations from 250 to 32 000 SQ-U/ml. Following inhalation of 0.9% sodium chloride from a Spira dosimeter (Spira, Hammeelina, Finland), allergen challenge was performed if FEV₁ fell by <10%. After initial inhalation of allergen, a fourfold increment was administered at 20-min intervals providing the FEV₁ had not fallen by more than 10% from baseline. If the fall in FEV₁ was between 10% and 15% a twofold increment was administered. The challenge was terminated when a fall of FEV₁ of $\geq 20\%$ from the baseline was achieved. FEV₁ was recorded at 10-min intervals up to 60 min then every 30 min to 8 h. The allergen-induced EAR was defined as occurring between 0 and 120 min and the LAR as between 150 and 480 min after challenge.

Sputum induction

After recovery from histamine challenge and inhalation of salbutamol (200 μ g), subjects inhaled nebulized 4.5% hypertonic saline for up to four periods of 5 min to induce sputum expectoration (7). Sputum samples were stored on ice and processed within 2 h.

Briefly, an equal weight of 0.01 M dithiothreitol and a four times the volume of phosphate buffered saline (PBS) was added to the sample; liquefaction, filtration and centrifugation followed (8). After removal of the supernatant, the cell pellet was resuspended in 1 ml of PBS and total cell counts and viability determined. Cytospins were performed and after drying, stained with Rapi-Diff 2 (Raymond Lamb Ltd, London, UK) to enable a differential cell count on 500 nonsquamous cells.

Statistical analysis

Statistical tests were performed using SPSS 17.0 for Mac except for area under the curve which was performed using PRISM 3.0 for Windows. *P*-values less than 0.05 were considered significant.

Results

All six subjects developed an early asthmatic response with a significant fall in FEV₁ to both allergens. As shown Fig. 1 the group mean maximum (standard deviation) percentage fall in FEV₁ during the early asthmatic reaction was similar with each allergen challenge; 25.8 (2.2)% following grass and 28.0 (9.7)% following HDM (*P* = 0.917). The area under the curve (AUC) calculated for the change in FEV₁ during the EAR was also similar, 1479.7 (406.6) for grass and 1433.8 (677.3) for HDM (*P* = 0.753). The LAR was significantly less severe following grass challenge compared to HDM whether examined by maximal fall in FEV₁, or by AUC. The LAR mean (SD) maximum percentage fall in FEV₁ following grass challenge was 13.0 (7.1)% and following HDM was 22.8 (8.9)% (*P* = 0.046) whilst AUC was 1861.3 (864.4) and 4077.7 (2452.0) (*P* = 0.043), respectively.

Prior to inhaled allergen challenge, there was no significant difference in the cellular composition of sputum samples on the two challenge days (Table 2). There was no significant change 24 h after the grass pollen challenge (Table 2), whereas HDM inhalation

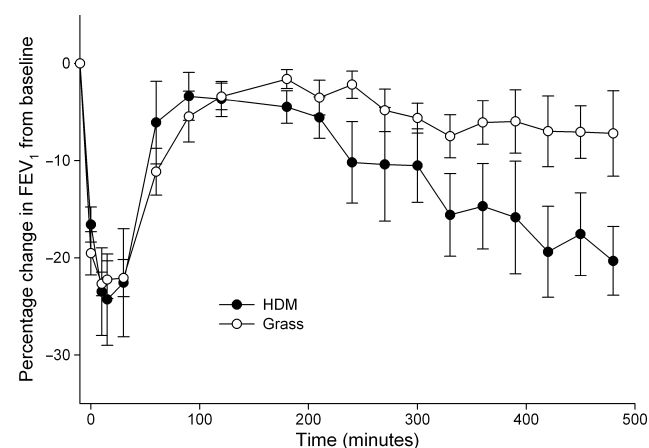


Figure 1. Percentage change in FEV₁ from baseline over time following inhaled challenge with house dust mite (HDM) or grass allergen. Values are means, bars indicate SEM.

Table 2. Differential cell percentages in sputum before and after inhaled allergen challenge with grass or house dust mite

	Grass			HDM		
	Pre	Post	<i>P</i>	Pre	Post	<i>P</i>
Eosinophils	6.7 (7.3)	11.0 (12.7)	0.600	5.4 (4.8)	22.1 (18.2)	0.028
Macrophages	38.3 (20.8)	33.0 (23.3)	0.463	37.9 (6.6)	24.1 (13.1)	0.046
Neutrophils	43.9 (20.6)	50.4 (26.6)	0.600	50.1 (9.3)	50.5 (20.6)	0.686
Lymphocytes	0.6 (0.8)	0.2 (0.2)	0.138	0.6 (0.6)	0.5 (0.5)	1.00
Epithelial cells	3.6 (3.1)	2.3 (2.9)	0.138	5.0 (6.5)	2.4 (3.2)	0.043

Values are mean percentages with standard deviations in parentheses. HDM: house dust mite. No significant difference between grass and HDM before allergen challenge. *P*-values calculated using Wilcoxon signed rank test.

resulted in an increase in the proportion of eosinophils 24-h postchallenge from a mean (SD) of 5.4 (4.8)% to 22.1 (18.2)% ($P = 0.028$).

There was no challenge-induced change in bronchial reactivity with either grass pollen or HDM allergen ($P = 0.753$), with respective geometric mean PC₂₀ histamine values of 3.5 mg/ml and 4.6 mg/ml prechallenge and 3.1 and 3.1 mg/ml 24-h postchallenge.

Discussion

In asthmatic patients allergic to house dust mite and grass pollen we have demonstrated that bronchial provocation with these allergens matched for similar early asthmatic reactions results in late asthmatic reactions of different severity and nature. In contrast to grass pollen inhalation, house dust mite allergen inhalation was associated with a significantly greater late reaction and airway eosinophil recruitment. Neither challenge was associated with an increase in airway reactivity thus dissociating allergen-induced airway eosinophilic infiltration and induced bronchial reactivity changes. This suggests that these arise from separate processes and is consistent with the previous identification that an anti IL-5 monoclonal antibody that inhibited allergen-induced eosinophil responses had no impact on allergen-induced bronchial reactivity changes (9). These findings also divorce the mechanisms of the early reaction as being the sole determinant of the allergen-induced late bronchoconstrictor response and induced airway eosinophil recruitment, as the grass pollen and house dust mite challenges were matched to achieve the same early reaction. This suggests that house dust mite allergen, in addition to promoting IgE-dependent mast cell degranulation, has actions extra to that conferred by grass pollen allergen and that these additional actions have relevance to the late bronchoconstrictor response and airway eosinophil recruitment.

Potentially, as the study was undertaken out of the pollen season in February and March, it is possible that continuous allergen exposure is needed to recruit allergen-specific T cells and that the difference between the

grass pollen and HDM late reaction is not allergen specific but reflects the timing of the challenge and, as such, a difference would not have been evident if the study was undertaken during a period of seasonal pollen exposure. This cannot be excluded although studies with cat allergen peptide fragments suggest that this may not be the explanation. These cat allergen peptide fragments, which do not promote IgE-dependent reactions, can induce isolated late reactions following inhalation in certain asthmatics. As these fragments are presented to T cells it has been concluded that this late airway response is T cell dependent. These reactions are, however, independent of airway eosinophil recruitment and prominently associated with alterations in airway hyperreactivity, separate from that which has been demonstrated in this study with naturally arising whole allergen (3). This suggests that these allergen-related differences may have other explanations potentially independent of the T cell activation properties of these allergens.

We have previously demonstrated that HDM but not GP allergen can directly activate epithelial cells in culture (10), an action possibly related to the greater serine and cysteine protease activity of the HDM allergens. Epithelial activation is an important aspect of the airway inflammation and will promote chemokine-directed luminal cell recruitment. It has also recently been demonstrated in rodent studies that HDM antigen triggers airway epithelial cells via toll-like receptor (TLR) 4, and that TLR4 negative mice fail to develop salient features of allergic inflammation (such as eosinophilic infiltration and goblet cell hyperplasia) (11). It is possible that the differential response to HDM and GP allergens is due to differential activation of TLR4 on lung structural cells, though the above findings have yet to be corroborated in humans. Additionally, HDM allergens but not those from grass pollens have chitin-like activity. Chitin in house dust mite may act through alternatively activated macrophages to mediate eosinophil recruitment via leukotriene B₄ activation (12). The biological relevance of these additional nonIgE dependent actions remains to be further elucidated but the identification in murine studies that aspergillus allergen, which also has potent protease activity and contains chitin, upregulates a distinct array of airway genes in comparison to ovalbumin, which is more comparable to grass pollen allergen, suggests that their biological relevance is worth pursuing (13).

These findings not only have relevance to the understanding of the allergen-induced airway responses in asthma but also have relevance to inhaled-allergen challenge studies in asthma. This study indicates that it is important that such experimental series are performed using single allergens, rather than a variety in different individuals, as has occurred previously (14), as distinct allergens may mechanistically contribute differently to the late bronchoconstriction, airway inflammation and airway reactivity changes characterizing the late asthmatic reaction.

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