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ORIGINAL ARTICLE

Effect of inhaled dust mite allergen on regional particle deposition and mucociliary clearance in allergic asthmatics

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Summary

Background Acute exacerbations in allergic asthmatics may lead to impaired ability to clear mucus from the airways, a key factor in asthma morbidity.

Objective The purpose of this study was to determine the effect of inhaled house dust mite challenge on the regional deposition of inhaled particles and mucociliary clearance (MCC) in allergic asthmatics.

Methods We used gamma scintigraphy (inhalation of ^{99m}Tc -sulphur colloid particles) to measure the regional particle deposition and MCC in allergic asthmatics ($n = 12$) 4 h following an inhaled dust mite allergen challenge (*Dermatophagoides farinae* extract; PD_{max} = fall in forced expiratory volume in 1 s of 10%) for comparison with baseline non-challenge measures.

Results In responders ($n = 9$ PD_{max} dose), lung function returned to pre-challenge values by 3 h but was significantly decreased at 6 and 24 h in three of the responders (i.e. late-phase response) and induced sputum eosinophils were increased at 24 h post-challenge ($P < 0.05$). Responders showed enhanced bronchial airway deposition of inhaled particles ($P < 0.05$) and slowed clearance from the central lung zone ($P < 0.01$) at 4 h post-challenge compared with the baseline (no allergen challenge) that was predicted by the PD_{max} allergen concentration ($r = -0.70$, $P < 0.05$). The decline in lung function at 24 h post-challenge correlated with reduced MCC from the central lung zone ($r = -0.78$, $P < 0.02$) and PD_{max}. Non-responders ($n = 3$) showed no change in lung function, regional deposition or MCC post-challenge vs. baseline.

Conclusions and Clinical Relevance These data suggest that regional deposition and clearance of inhaled particles may be sensitive for detecting mild airway obstruction associated with early- and late-phase allergen-induced effects on mucus secretions. The study was listed on clinicaltrials.gov (NCT00448851).

Keywords airway deposition, dust mite allergen, mucus, particle inhalation
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Introduction

Acute exacerbation of asthma is a leading cause of morbidity and mortality associated with this disease. While change in spirometry is the most validated physiological end-point for defining acute asthma exacerbation, other physiological changes play a role in this disease. Severe exacerbations of asthma are associated with the inability to improve lung function with β-agonists and mucus accumulation in the airways, impeding airflow. Mucus accumulation likely results from the hypersecretion of mucus and the failure of the mucociliary apparatus to effectively clear this mucus and airway debris. Asthmatics are thought to have impaired ability to clear mucus from their airways [1], especially during acute exacerbations [2]. The mechanisms that account for the acute impairment of mucociliary clearance (MCC) are poorly understood and few experimental models have been developed to study the relationship between inflammation and MCC in humans.

One model for the investigation of asthma exacerbation is an inhaled allergen challenge. Allergen challenge in

allergic asthmatics often induces both an immediate-phase response that occurs within minutes and then rapidly resolves, and a late-phase decrease in lung function that occurs 2–8 h later and is associated with increased airway inflammation [3, 4]. Ragweed antigen challenge has been shown to decrease tracheal mucus velocity in asthmatic patients immediately following challenge [5] and in allergic animals up to 2 days post-challenge [6, 7]. However, there have been few studies to assess mucociliary function from the whole lung in asthmatics following an allergen challenge.

The relationship between airway obstruction, inflammation, and MCC is poorly understood in allergic asthmatics as these end-points have not been assessed simultaneously in the same study. In the current study, we used an inhalational challenge using house dust mite (HDM, *Dermatophagoides farinae*) allergen as a model of acute exacerbation. We assessed regional lung deposition and clearance of inhaled, radiolabelled particles by gamma scintigraphy 4 h after challenge and the inflammatory cell content of airway sputum recovered by hypertonic saline induction 24 h after challenge.

Whole-lung MCC measurements are highly dependent on the regional particle deposition pattern in the lung [8] that, in turn, might vary with the airway changes induced by allergen challenge. Thus, while a confounder for MCC comparisons, these changes in deposition heterogeneity may also be a very sensitive indicator of mild, heterogeneous bronchoconstriction. Previous scintigraphy studies have shown both an increase in central airway deposition [9] and increased 'patchiness' of particle deposition [10] associated with induced bronchoconstriction.

To confirm that an individual was responsive to allergen, we assessed the change in forced expiratory volume in 1 s (FEV_1) from baseline measurements (obtained immediately before challenge). Our primary hypotheses were that in allergen responders, regional particle deposition would be more heterogenous and MCC depressed as part of a late-phase reaction to allergen challenge compared with the baseline measures made during an earlier study visit. We also hypothesized that increases in eosinophils would correlate with changes in MCC and particle deposition as well as changes in spirometry. This report summarizes the development of a model of allergen-induced asthma exacerbation that assesses MCC, particle deposition and airway inflammation.

Methods

Subjects

Twelve (6 M/6 F) mild allergic, non-smoking asthmatics, ages 20–39, with skin sensitivity to HDM and normal baseline lung function (FEV_1 %pred >80, FEV_1/FVC ratio >0.70) (without the use of bronchodilating medications for 12 h) were studied. Subjects had to have a history of episodic wheezing, chest tightness or shortness of breath consistent with asthma or physician-diagnosed asthma. All subjects took only albuterol as needed and none were on chronic asthma therapy (i.e. no daily LABA, inhaled steroids, etc.). Pre- and post-bronchodilator spirometry and a standard graded dose methacholine bronchoprovocation challenge test [11, 12] to determine non-specific bronchial reactivity was performed on each subject during a screening visit. A provocative methacholine concentration of 10 mg/mL or less producing a 20% decline in FEV_1 (PC_{20} methacholine) was required for subject inclusion. Informed written consent was obtained from all subjects before their participation in the study, which was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill. The study was listed on clinicaltrials.gov (NCT00448851).

Study design

Figure 1 illustrates the timeline of measurements on the baseline and challenge study days. During the subject's baseline visit, we measured the MCC of inhaled, radiolabelled particles by gamma scintigraphy [13, 14]. The subject returned the next day for a follow-up gamma camera scan (24 h retention) and an induced sputum sample was collected [14, 15]. At least 2 days after the baseline visit, the subject returned for their allergen challenge study visit. At 4 h post-allergen challenge, we measured MCC, and then the following day, the subject returned for the 24-h retention scan, spirometry and induced sputum procedure. Subjects were monitored overnight in the CTRC inpatient unit from the time of the challenge until the 24-h follow-up scan. Comparisons were made with MCC obtained from the same subject during the baseline visit, and sputum samples were analysed and compared at 24 h post-challenge vs. the baseline sample.

Inhaled allergen challenge

Each subject inhaled sequential doses of inhaled HDM extract (*D. farinae*; Greer®, Lenoir, NC, USA) delivered as five inhalations from a Devilbiss 646 nebulizer (Sunrise Medical, Somerset, PA, USA) (mass median aerodynamic diameter of 5 μm, GSD = 2.0 [16]) at concentrations of zero (saline control), 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32, 64, 125, 250, 500, 1000 and 2000 allergen units (AU)/mL (Fig. 2). For each of the breaths of inhaled HDM at each dose, subjects were instructed to perform a full rapid inhalation to total lung capacity with a 5-s breath hold. FEV_1 was measured before and 10 min after each aerosol inhalation. The FEV_1 following saline challenge and before the first dose of antigen was considered the baseline value. If the FEV_1 declined by less than 10% of the baseline after a

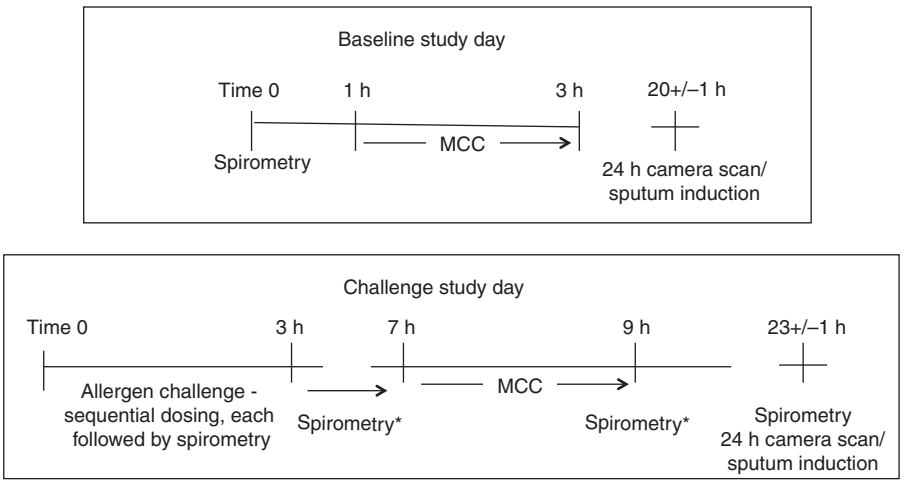


Fig. 1. Schematic of the study design showing the order and timing of procedures for baseline and allergen challenge study days. *Post-challenge spirometry on the challenge day was performed at 10 min, 20 min, 30 min, 1 h, 90 min, 2 h, 3 h and 6 h post end of challenge. MCC, mucociliary clearance.

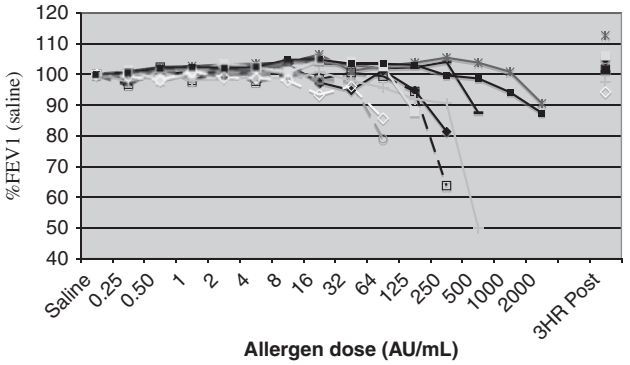


Fig. 2. Forced expiratory volume in 1 s (FEV_1) as % of the initial saline dose as a function of allergen dose in the nine patients who showed at least a 10% decline associated with the challenge. Each symbol represents data from one patient. Those patients who showed a late-phase response, i.e. a $>10\%$ decline in FEV_1 from pre-challenge at 6 and 24 h post-challenge, are indicated with dashed lines.

given concentration of allergen had been inhaled, the next higher concentration was given. If the decline in FEV_1 was between 10% and 15%, spirometry was repeated each 5 min for 15 min or until a clear nadir in the decline had been reached. If the nadir after 15 min was a decline in FEV_1 of less than 10%, the next higher concentration was administered, and if it was a decline of 10–15%, the challenge was stopped. Once the challenge was stopped, the FEV_1 was repeated every 10 min until 30 min, each 30 min until 2 h, at 3 and 6 h and again the next day. It was not measured at 4 and 5 h post-challenge in order to avoid forced exhalation manoeuvres during and immediately before the MCC scanning. A few responders received albuterol by MDI during the first 30 min following the challenge. Non-responders as well as those with only small drops in FEV_1 or milder symptoms were not given albuterol at the end of the challenge.

Regional particle deposition and mucociliary clearance

The procedure we used for measuring MCC in humans has been described in detail previously [14, 15]. A xenon (^{133}Xe) equilibrium lung scan was recorded for each subject on their baseline visit to allow the creation of suitable regions of interest (ROIs) for determining regional lung deposition and MCC. For each measure of MCC, the subject inhaled an aerosol (mass median aerodynamic diameter of 5 μm, GSD = 2.0) of sulphur colloid labelled with ^{99m}Tc (^{99m}Tc-SC) (40 μCi) (CIS-US Inc., Bedford, MA, USA) from a Devilbiss 646 nebulizer. While breathing the radiolabelled aerosol, the subject matched his/her tidal flow and breathing rate at 500 mL/s and 30/min, respectively, by following a visual flow signal while breathing in time to a metronome. Immediately following inhalation of radioaerosol (duration of less than 2 min), an initial deposition scan was recorded (sum of two 2-min images) and then continuous 2-min images were recorded for a period of 2 h to monitor the clearance of particles from the lung as the subject remained seated in front of the gamma camera. The subject returned the following day after the radiolabelled aerosol exposure to obtain a 30-min scan of 24-h lung activity/retention.

Only the right lung was used to analyse both regional deposition and MCC because of the potential overlap of the stomach and lung activity on the left side. To assess central (C) vs. peripheral (P) deposition (Fig. 3), two outline ROI were created over the right ^{133}Xe lung image: (1) a rectangular region around the entire right lung and (2) a central (C) ROI, with dimensions equal to half the whole lung ROI's width and one-half its height. The C region was positioned on the medial boundary of the lung, centred by height, 25% of the area of the whole lung ROI. The peripheral region (P) is the area lying between the central and the whole lung outline. These regions were displayed

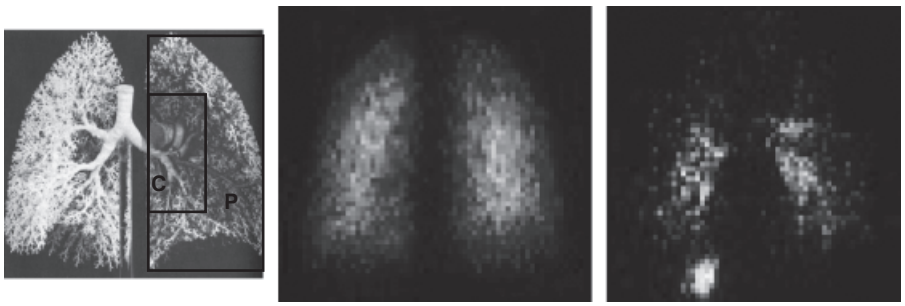


Fig. 3. Central (C) vs. peripheral (P) deposition and clearance. Schematic (left) illustrating C and P regions based on the outline of the xenon equilibrium image (centre). A deposition image from the same individual is shown on the right.

over the initial aerosol scans to determine the initial counts in each region. We then calculated the ratio of central to peripheral counts, (C/P)Tc, and normalized this ratio by dividing by the central-to-peripheral ratio for the ^{133}Xe scan, (C/P)Xe;

$$(\mathrm{C/P})\mathrm{Tc}/(\mathrm{C/P})\mathrm{Xe} = \mathrm{C/P}.$$

This normalization was carried out to account for the difference in the relative lung areas and thickness between the central and the peripheral regions. C/P provides an index of relative deposition between the two regions. A C/P of 1.0 reflects equal deposition in each region. However, because the central region outlines both bronchial airways and lung parenchyma surrounding these airways, a C/P of near unity reflects primarily deposition in the pulmonary airspaces distal to the anatomic dead space. Increases in C/P to values greater than unity reflect an increase in central vs. peripheral deposition primarily as a result of increased bronchial deposition.

Another measure of regional deposition heterogeneity is the skew of the histogram distribution (counts/pixel vs. #pixels) [17] within the right whole lung ROI, increasing with increased frequency of 'hot spots' in the lung. These hot spots are presumed to be due to increased deposition within bronchial airways throughout the lung so that skew is independent of the specific region within the lung (e.g. central vs. peripheral). To determine skew, frequency distribution histograms were constructed from the right lung deposition images, with the number of pixels with a given count value (expressed as a fraction of total pixels) on the y-axis and the count values on the x-axis (Fig. 4). These histograms were analysed for skew (a measure of histogram symmetry, the third moment about the mean of the histogram) [16]. The heterogeneity of deposition increases with increasing skew (i.e. more pixels with high counts/pixel).

The whole lung ROI bordering the right lung was used to determine, by computer analysis, the whole lung retention (decay and background corrected) as a fraction of the initial counts in the right lung, over the 2-h clearance period at 10-min intervals (two 2-min images summed for each 10-min time-point, e.g. images 1 and 2 for initial time 0 and images 6 and 7 for time 10 min). Similarly, the 24-h retention (R_{24}) was calculated. For these experiments, we assumed that R_{24} primarily represented the fraction of aerosol initially deposited in the alveolar region [18–21], or conversely, that 24-h clearance represented the deposition in the bronchial airways that could be cleared by ciliary action [18]. To determine tracheobronchial (TB) retention (R_t) vs. time for the initial 2-h period of observation, R_{24} was subtracted from the retention measurements during the initial 2-h clearance period and re-normalized (divided) by $(1-R_{24})$, i.e.

$$\mathrm{TB}\,R_\mathrm{t} = (R_\mathrm{t} - R_{24})/(1 - R_{24}),$$

where t is the time between 0 and 2 h.

Finally, both central (C) and peripheral (P) TB retention vs. time were also determined from the respective regions described above to allow comparisons of MCC from a region with a preponderance of large, bronchial airways (C) to a region lacking in such airways (P). For each retention vs. time data set (e.g. mean data shown in Fig. 5), the average retention over the 2-h period of observation (Ave120 Ret) was computed (i.e. average of the 10-min retention values from 10 to 120 min).

Induced sputum, cell count assessments

This procedure has been described in detail previously [15]. The numbers and percent of airway eosinophils and neutrophils in sputum were assessed and compared for baseline vs. allergen challenge study visits.

Statistical methods

Comparisons between baseline and post-challenge measurements were analysed using non-parametric statistics for paired samples (Wilcoxon signed-rank test, Stata for MacIntosh). The significance of the relationships between individual variables was tested using Spearman's correlation (Stata for MacIntosh). An overall significance level of $P \leqslant 0.05$ was considered to be significant. All values are expressed as the mean (±standard deviation). Comparison of TB retention vs. time between baseline and allergen

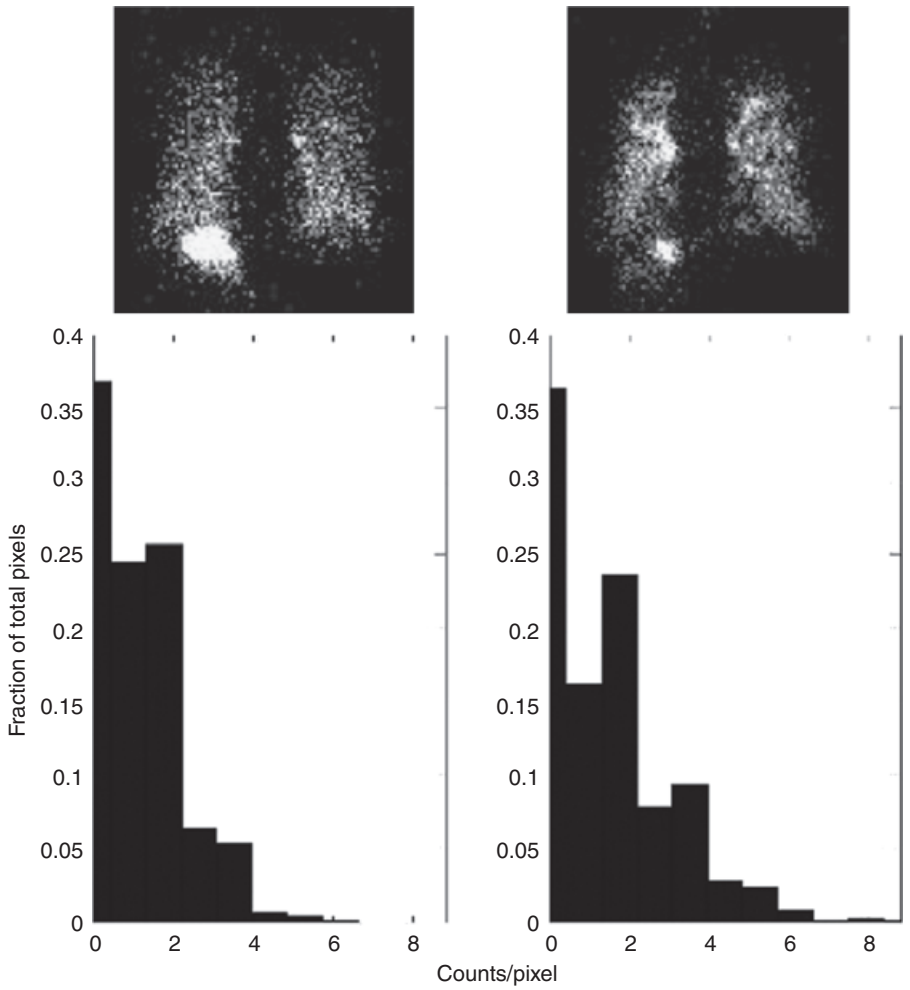


Fig. 4. Baseline vs. post-challenge (C/P = 1.08 vs. 1.72 and skew = 1.27 vs. 1.56, respectively).

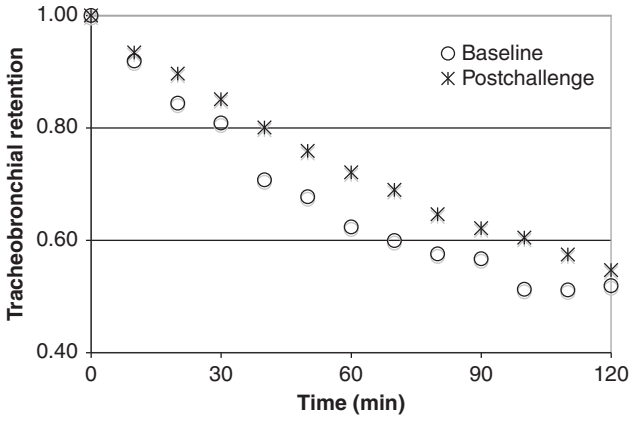


Fig. 5. Whole lung tracheobronchial retention vs. time for responders, baseline vs. 4 h post-challenge.

study days was made by mixed model analysis (SAS) for retention as a function of visit (i.e. baseline vs. allergen), time, time-squared and their interactions. The Ave120 Ret (described above) for post-challenge retention vs. time data was used to compare the relationships between MCC and other post-challenge parameters.

Results

Characterization of volunteers with allergic asthma

The mean FEV_1 (expressed as % of predicted value) was 97±12% before and 105±13 post the use of albuterol (bronchodilator). All subjects were responsive to methacholine, with the PD_{20} for methacholine ranging from 1.25 to 10 mg/mL. All subjects also demonstrated skin test reactivity to *D. farinae*.

Response to inhaled allergen

Of the 12 patients studied, nine (5M/4F) responded to an inhaled allergen challenge with $\geqslant$10% reduction in FEV_1 (individual responses depicted in Fig. 2). The mean (SD) PD_{max} for all responders was 639 (788) AU/mL. In all cases, FEV_1 and FEF25–75 returned to pre-challenge

Table 1. Mean (SD) changes in spirometry associated with allergen challenge (all values are as % saline pre-challenge) for responders [late-phase (LPR) and non-late-phase (nLPR)] and non-responders

	Mean allergen response	3 h post-challenge	6 h post-challenge	24 h post-challenge
FEV_1				
Responders ($n = 9$)	79 (14)	103 (5)	94 (11)*	92 (8)#
nLPR ($n = 6$)	82 (15)	104 (5)	100 (5)	97 (2)
LPR ($n = 3$)	76 (11)	100 (5)	82 (10)	82 (3)
Non-responders ($n = 3$)	–	104 (3)	106 (3)	102 (2)
FEF25–75				
Responders	68 (21)	104 (11)	86 (22)#	80 (14)#
nLPR	74 (25)	108 (11)	100 (10)	89 (4)
LPR	58 (4)	96 (6)	60 (11)	62 (4)
Non-responders	–	106 (6)	112 (13)	104 (7)

*$P < 0.02$,

#$P < 0.01$ compared with 3 h post-challenge.

FEV1, forced expiratory volume in 1 s; LPR, late-phase responder.

Table 2. Mean (SD) regional deposition and clearance indices of inhaled aerosol at 4 h post-challenge (PC) compared with the baseline (Base) for responders [late-phase (LPR) and non-late-phase (nLPR)] and non-responders

	Skew		C/P		24 h clear (%)		Central TB Ave120Ret	
	Base	PC	Base	PC	Base	PC	Base	PC
Responders ($n = 9$)	2.00 (0.45)	2.53* (0.78)	1.85 (0.43)	2.20 (0.61)	52 (16)	66* (12)	0.69 (0.17)	0.79 (0.22)
nLPR ($n = 6$)	2.17 (0.32)	2.64 (0.68)	2.17 (0.32)	2.32 (0.72)	59 (12)	66 (14)	0.71 (0.17)	0.74 (0.20)
LPR ($n = 3$)	1.67 (0.54)	2.31 (1.10)	1.50 (0.36)	1.97 (0.28)	38 (12)	66 (10)	0.67 (0.22)	0.88 (0.25)
Non-responders ($n = 3$)	1.75 (0.60)	1.65 (0.65)	2.01 (0.82)	2.11 (1.13)	43 (6)	41 (13)	0.77 (0.20)	0.69 (0.25)

*$P < 0.02$ compared with the baseline.

values by 3 h post-challenge (Table 1). In responders, lung function significantly declined by 6 and 24 h post-challenge relative to the pre-challenge values (Table 1). This was primarily determined by a late-phase response (LPR), $\geqslant 10\%$ reduction in FEV_1 6 h post-challenge, occurring in three of the responders (Table 1). The non-late-phase responders (nLPR) all had returned to pre-challenge spirometry by 30 min post-challenge and remained normal throughout the 24-h testing period. Among the three late-phase responders (LPR), the early response had all resolved (i.e. returned to pre-challenge FEV_1) by 2 h (specifically 30, 90 and 120 min) before all declining again at 6 h post-challenge. The mean (SD) PD_{max} for nLPR and LPR was 896 (868) and 126 (107) AU/mL, respectively. Three of the responders (one LPR and two nLPR) received albuterol [a total of four puffs from a MDI (90 μg/actuation)] during the first 30 min post-challenge. Two additional late-phase responders received albuterol by a nebulizer [0.083% (2.5 mg/3 mL) solution] at approximately 7 h post end of challenge. One of the two late responders required repeated doses of two albuterol puffs via MDI at 10, 20 and 24 h post end of the challenge. The other individual required two puffs of albuterol at 10 and 24 h post-challenge as well as albuterol via a nebulizer at 20 h post-challenge. Both of these late-phase responders were treated with prednisone taper at discharge on the post-challenge follow-up day. The PD_{max} for allergen significantly predicted the reduction in FEV_1 at 24 h post-challenge ($R = -0.75$, $P = 0.02$). Within all subjects, there was no significant correlation between HDM-specific and non-specific (methacholine) airway reactivity ($R = 0.13$ for correlation of PD_{max} of allergen concentration vs. PC_{20} of methacholine concentration).

Regional particle deposition after allergen challenge

Table 2 summarizes the changes in regional particle deposition between the baseline and the 4 h post-allergen challenge. Among the responders, skew of the deposition distribution was significantly increased ($P = 0.02$) after the allergen challenge compared with the baseline. There was also a trend for C/P to be increased among responders ($P = 0.07$). Figure 4 shows an example of deposition images from one responder, baseline study day vs. 4 h post-challenge, and the corresponding number vs. counts/pixel histogram for the whole right lung for each after normalizing to a common median count. Both skew of the distribution and C/P were increased in this subject for post-challenge vs. baseline (values given in legend). Finally, within the responders, the percent of deposited particles cleared through 24 h was significantly increased post-challenge compared with that at baseline ($P < 0.02$).

Again, the percent of particles cleared through 24 h is a measure of regional deposition (not clearance rate in these subjects) and therefore reflects a greater initial deposition of particles in airways vs. alveoli for post-challenge vs. baseline [18]. Finally, using multivariate analysis, skew and 24 h clear were both modelled as a function of the presence of an LPR, study day and their interaction. As with the paired analysis, both skew and 24-h clear were found to be a significant function of study day. Neither was significantly predicted by the presence of LPR or its interaction with study day. There was a trend, however, for 24-h clear to be predicted by an interaction of study day and presence of LPR ($P = 0.10$), i.e. the late-phase responders tending to have the largest difference in 24-h clear between study days (Table 2).

Mucociliary clearance as reflected by particle retention in responders

Figure 5 illustrates the mean whole lung TB retention vs. time for baseline vs. post-allergen challenge for the responders, suggesting a trend ($P = 0.07$) towards a reduction in whole lung MCC (increased retention) following the antigen challenge compared with the baseline. There was a significant ($P = 0.01$) slowing of central airway clearance post-challenge compared with baseline in responders depicted in Fig. 6a (mean Central TB Ave120Ret increased from 0.69 to 0.79 for baseline vs. allergen challenge, respectively). On the other hand, there was no difference in retention vs. time from the P region between the two study days. The PD_{max} of allergen predicted the slowing of TB MCC in the C region between baseline and challenge, $r = -0.70$, $P < 0.05$). This reduction in MCC from the C region also significantly correlated with the post-challenge 24 h FEV_1 (as % of saline control pre-challenge) ($r = -0.78$, $P < 0.02$, respectively). In fact, when we categorized the central TB MCC by LPR vs. nLPR (Fig. 6b), it was clear that the slowing of MCC post-challenge was determined primarily by those patients with an LPR. When the difference in retention between study days at each time-point (10–120 min) (Fig. 6a) was modelled as a function of both time and the presence of an LPR, we found that the presence of an LPR was a significant predictor of retention difference, interacting with time ($P = 0.03$). Finally, non-responders showed no change in MCC from the whole lung central airways (Fig. 6c) associated with allergen challenge.

Inflammatory response to allergen challenge

Table 3 gives the differential cell counts from induced sputum samples collected at baseline and 24 h post-allergen challenge. Only 10 of the 12 subjects (seven of the responders and the three non-responders) were able to provide sufficient samples for cells counts on both days

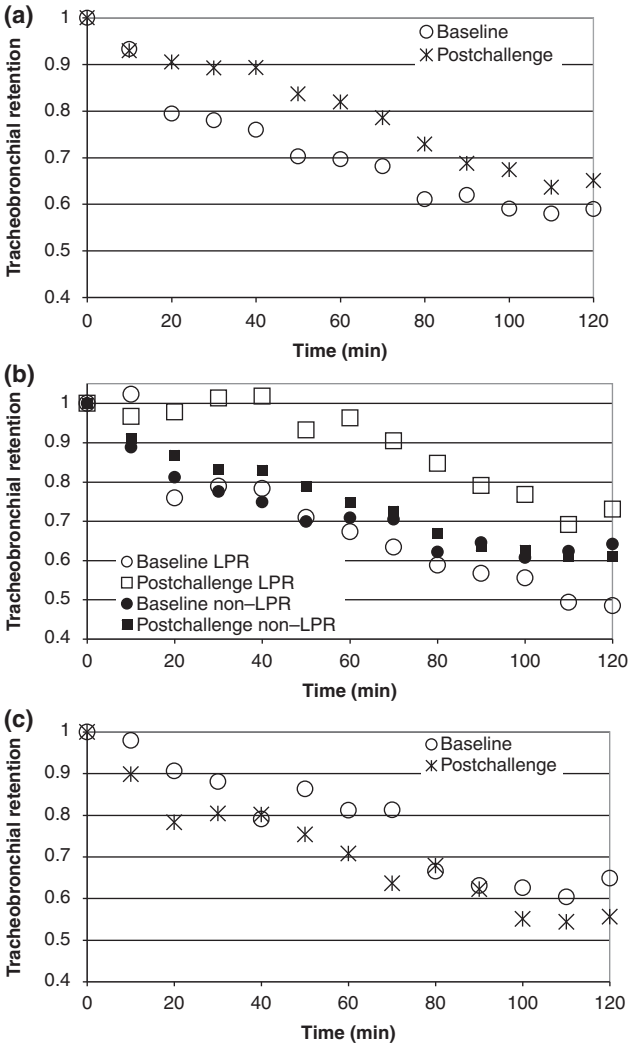


Fig. 6. (a). Central (C) lung TB retention vs. time (for the C region of Fig. 2) for responders, baseline vs. 4 h post-challenge. (b) Central (C) lung TB retention vs. time (bottom) for LPR and nLPR at baseline vs. 4 h post-challenge. (c) Central (C) lung TB retention vs. time for non-responders, baseline vs. 4 h post-challenge. LPR, late-phase response.

for comparison. Eosinophils (EOS) were significantly increased in all subjects 24 h following post-allergen challenge. The two subjects with the highest eosinophil concentration (cells/mg) at 24 h post-challenge were both LPRs. Accordingly, within the responders, the concentration of eosinophils (cells/mg) post-challenge was significantly correlated with the FEV_1 at 24 h post-challenge relative to the saline control before challenge ($r = -0.93$, $P < 0.005$), suggesting that resolution of the inflammatory response to allergen exposure was not yet normalized by 24 h post-challenge. Within the responders, there was also a trend towards eosinophil concentrations post-challenge to correlate with the reduction in TB clearance from the C region ($r = 0.64$, $P = 0.12$, $n = 7$) but it was not statistically significant.

Table 3. Mean (SD) granulocyte cell counts from induced sputum (% and cells/mg in sample)

	EOS (% cells/mg)		PMN (% cells/mg)	
	Base	PC	Base	PC
Responders ($n = 7$)	1.3 (2.7)	14.6 (11)*	28.7 (13.8)	35.1 (12.3)
	12 (18)	136 (169)*	307 (331)	334 (461)
nLPR ($n = 5$)	1.5 (3.2)	12.1 (0.68)	25.5 (13.8)	30.7 (11.3)
	10 (22)	57 (83)	166 (82)	129 (105)
LPR ($n = 2$)	0.9 (0.1)	20.9 (1.10)	37.6 (10.1)	46 (7.6)
	15 (6)	335 (182)	497 (469)	845 (706)
Non-responders ($n = 3$)	0.8 (0.8)	8.6 (11.3)	56 (32)	30 (7.7)
	5 (7)	13 (15)	286 (397)	134 (150)

*$P < 0.05$ compared with the baseline.

Discussion

In this study, we used an inhaled *D. farinae* allergen challenge to induce model exacerbations in mild allergic asthmatic volunteers. In addition to the traditional characterization of response by changes in spirometry after challenge, we used inhalation of ^{99m}Tc-labelled sulphur colloid (^{99m}Tc-SC) particles and gamma scintigraphy to assess the particle deposition pattern and both whole and regional lung MCC. We also collected sputum 24 h after challenge and examined changes in inflammatory cells to explore the relationships between inflammatory cells and spirometric, particle deposition and MCC end-points. Of the 12 volunteers who participated in this study, nine were spirometric responders and three were non-responders to the inhalational allergen challenge. Furthermore, the spirometric function of all responders had returned to normal 3 h after the end of the allergen challenge procedure. Of the nine responders, however, three showed an additional LPR, i.e. a decline of $> 10\%$ in FEV_1 from the pre-challenge values at 6 and 24 h post-challenge. These data provided us with an opportunity to determine whether MCC and particle deposition, as measured by the ratio of particles deposited in the central vs. peripheral airways (C/P ratio) and skew (or lack of homogeneity of particle distribution) at 4 h post-allergen challenge, were sensitive indicators of allergen responsiveness, either the initial or the late phase, compared with traditional spirometric measures.

One of the most intriguing observations was that both skew, a measure of regional particle deposition, and fraction of particles cleared through 24 h were significantly increased in responders, with a trend towards an increased C/P ratio as well. Skew increases with increased heterogeneity of particle deposition in the lung that may occur with increased frequency of localized deposition areas, known as 'hot spots' [16]. This increased patchiness of deposition may result from non-homogeneous mucus accumulation or local bronchial smooth muscle constriction. The percent of particles cleared through 24 h is an estimate of the percent of total particles depositing in bronchial airways. As such, the increase in 24 h clear also indicated enhanced airway deposition post-allergen challenge. Inhalation of ^{99m}Tc-SC occurred 4 h after challenge while spirometry had already been normalized 3 h after challenge (either with or without albuterol). Furthermore, the presence of an LPR among the early responders was not a significant predictor of changes in the deposition pattern (skew). We suggest that these changes in airway deposition reflect residual effects on airway surface biology associated with the immediate response to allergen. Such effects may include increased mucus secretion, plasma exudate and intermittent presence of allergen-induced airway oedema.

We also examined the effect of allergen challenge on MCC as reflected in the retention of ^{99m}Tc-SC particles, and found a significant decrease in MCC in the central region of the right lung in responders (Fig. 6a). On the other hand, there was no change in MCC between baseline and post-challenge for the three patients who had no spirometric response to allergen challenge (Fig. 6c). While the effects on MCC were observed an hour after spirometry had returned to normal, the depression in MCC inversely correlated with the maximal dose of allergen required to induce a response in the nine responders. Furthermore, it was clear that the presence of an LPR was a significant predictor of the observed slowing in central airways MCC (Fig. 6b). We think that the most likely mechanism associated with both MCC inhibition from the central airways and enhanced 'hot spot' particle deposition post-allergen challenge is the increased release of mucins from airway epithelial cells [22, 23], regardless of the induction pathway, that are not readily or easily cleared from the airway surface. Many of the inflammatory mediators implicated in the pathophysiology of asthma have been shown to affect mucus secretion. Markedly up-regulated production of MUC5AC together with stimulated secretion may contribute to airflow obstruction in asthma [22, 23]. Furthermore, an imbalance in mucin release without appropriate hydration may contribute to MCC defects associated with airways disease [24].

We examined sputum eosinophilia in sputum 24 h after challenge and 18 h after the completion of the MCC studies. Consistent with others, we found a profound increase in eosinophils associated with allergen challenge in these asthmatics [25–27]. We further observed a trend towards a relationship between eosinophils and MCC ($R = 0.64$), but this did not reach statistical significance ($P = 0.12$). Eosinophilia also strongly correlated with FEV_1 at 24 h after challenge in responders, as well as with the PD_{max} of allergen required to induce a response. Three of the responders received albuterol during the first 30 min post-challenge to ease associated chest tightness and bronchoconstriction. As a β-adrenergic agonist, albuterol

has been shown to be effective at stimulating MCC in healthy and asthmatic subjects, although its effectiveness is diminished in airways disease [28]. Thus, it might be expected that the albuterol may alleviate the effects on MCC and regional deposition associated with allergen challenge. However, discerning the effect of albuterol treatment in concert with the effects of LPR on our measures would require larger numbers of subjects than those studied here.

We selected the 4–6 h post-allergen time-points as the time to conduct scintigraphic measurements of airway function because this is the period during which LPRs have been reported in other experimental procedures [25, 29]. This limited, however, our examination of earlier time-points. Future studies should examine earlier time-points (immediately after challenge) that extend to 24 h to encompass the time frame in which a clinical response to allergen occurs. Another limitation to using the combination of scintigraphy, spirometry and sputum induction is that it is necessary to perform sputum induction after other measures, as hypertonic saline and cough clearance, two components of sputum induction, may disrupt other measures. Nonetheless, using this combination of measures, we were able to show that allergen induces not only changes in lung function, but changes in particle deposition pattern and slowing of MCC. Furthermore, we have been able to associate post-challenge changes in lung function with inflammatory responses.

In developing models of disease, it is important that the model actually reflect the events that occur in naturally occurring disease. Messina et al. [2] have shown that in severe cases of asthma exacerbations requiring hospitalization, MCC is nearly static with no discernible clearance of radiolabelled particles over a 2-h period of observation. Using an inhalation challenge model that used ragweed extract, Mezey et al. [5] showed impairment in mucus transport in asymptomatic ragweed-sensitive asthmatics both immediately and 1 h after challenge. Allergen challenge in animal models of allergic airways disease also shows decreased TMV for several days [6, 7, 30] with no clear linkage to the time course of bronchoconstriction in the animals. Thus, the observations presented in this report are consistent with earlier animal and human studies as well as those seen in naturally occurring disease. Others have also shown that inflammation modifies MCC. Compared with non-asthmatics, MCC may actually be enhanced in mild asthmatics with normal lung function [14, 31]. However, with further progression of airway inflammation and obstruction in these patients, MCC may be depressed relative to normal [19, 20, 23].

In conclusion, we used an inhaled HDM allergen challenge to produce a model of asthma exacerbation in mild allergic asthmatics. We found decreases in MCC and changes in the deposition pattern of inhaled particles in asthmatics who responded to an inhalational challenge. There were also changes in inflammation, most notably an association between eosinophils and lung function (FEV_1) response to allergen. We propose that IgE-mediated responses to allergen modify MCC. Finally, we anticipate that agents interfering with IgE-mediated processes (e.g. omalizumab) or mucus clearance (e.g. hypertonic saline) may improve or even promote airway clearance during acute exacerbations of disease.

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