Small airways response to naturalistic cat allergen exposure in subjects with asthma

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Background: It is currently unclear whether the small airways (diameter <2 μm) contribute significantly to late asthmatic reactions to inhaled allergen.
Objectives: We sought to determine whether naturalistic exposure to cat allergen induced late responses in the small airways as measured by pulmonary function testing and high-resolution computed tomography (HRCT) of the chest performed at end-expiration.
Methods: In a group of 10 subjects with cat-induced asthma, physiologic studies (spirometry and lung volumes, including closing volume) and HRCT were performed before and 6 and 23 hours after a cat room challenge that caused a 20% or greater acute fall in FEV_1.
Results: There was no significant decline in FEV_1 at 6 or 23 hours after cat exposure. Forced expiratory flow at 25% to 75% of forced vital capacity was significantly decreased at 6 hours after the challenge and returned to normal by 23 hours. HRCT image analysis as well as closing volume demonstrated increased air trapping from baseline at both 6 and 23 hours after the challenge. In addition, image analysis demonstrated a significant increase in small airways hyperresponsiveness to methacholine at 23 hours after the challenge. No significant mean changes were noted in lung volumes at either 6 or 23 hours or in PC_{20} FEV_1 at 23 hours postchallenge.
Conclusion: These findings demonstrate that naturalistic exposure to cat allergen results in significant small airways obstruction and hyperresponsiveness persisting for at least 23 hours, at which time these changes cannot be detected by conventional physiologic measures.
Clinical implications: Physiologically silent distal lung inflammation persists after an antigenic challenge.
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Key words: *Asthma, bronchial hyperreactivity, Fel d 1 protein, image analysis, computer-assisted, tomography, x-ray computed*

Distal airways inflammation, well documented in both symptomatic and asymptomatic patients with asthma, is emerging as an important component in the pathophysiology of asthma.[1,2] Histologic and immunologic data show significant increases in distal lung inflammation and remodeling as evidenced by infiltration of T cells and eosinophils as well as thickening of the submucosa, adventita, and smooth muscle in the small airways.[3-7]

Pulmonary allergen challenge has frequently been employed as a model for studying allergic responses in the lower airways. It is recognized that administration of allergen by nebulizer results in an early-phase reaction followed by a late-phase reaction in 20% to 70% of subjects.[8,9] Although the cellular events associated with late-phase responses have been well characterized, it remains unclear whether the small airways (mean airway diameter <2 μm) play a significant role in late-phase responses.[10,11] This lack of understanding relates, in part, to the difficulty in studying this area of the lung, long recognized as being clinically silent, using either physiologic tests or bronchoscopy.[1,2] Conventional physiologic measures of the small airways, including forced expiratory flow at 25% to 75% of forced vital capacity (FEF_{25-75}), residual volume (RV), and closing volume (CV), are limited by their test-retest variability and lack of specificity in reflecting small airways function.[12,13] Histopathologic studies of the distal airways using transbronchial biopsies are informative but are invasive and pose potential risks to the subject. We have previously shown that quantitative image analysis of thoracic high-resolution computed tomography (HRCT), performed at RV both before and after a methacholine challenge test (MCT), demonstrates small airways obstruction and/or regional airways hyperresponsiveness that cannot be detected by conventional physiologic studies.[14,15] In the current study, therefore, we sought to investigate the effects of cat room challenge (CRC) on small airways function using quantitative image analysis of HRCT of the

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Abbreviations used

BDP-CFC: Beclomethasone–dipropionate chlorofluorocarbon
BDP-HFA: Beclomethasone dipropionate hydrofluoroalkane
CRC: Cat room challenge
CV: Closing volume
CV/VC%: Closing volume as a percent of the vital capacity
FEF_{25-75}: Forced expiratory flow at 25% to 75% of forced vital capacity
FRC: Functional residual capacity
FVC: Forced vital capacity
HRCT: High-resolution computed tomography
HU: Hounsfield unit
LAC: Lung attenuation curve
MCT: Methacholine challenge test
RV: Residual volume
TLC: Total lung capacity

chest, in addition to physiologic measures of the small airways.

METHODS

Ten subjects with a clinical diagnosis of cat-induced asthma were studied. Inclusion and exclusion criteria are listed in Table I.

Subjects were informed of the risks and benefits of the study and signed an informed consent approved by the Western Institutional Review Board.

The total radiation dose was 9 millisievert (mSv). This exposure just exceeds the dose given with 1 conventional diagnostic computed tomography (CT) scan and is equivalent to about 3 years background radiation, which in Los Angeles is 3 mSv/y.[16,17]

Study design

Eligible subjects underwent baseline studies, including pulmonary function tests, CV, and HRCT of the chest, at 0800 and 1500 hours on day 1, one day before the CRC. In addition, at the 0800-hour time point on day 1, immediately following the baseline HRCT, a methacholine inhalation challenge was performed, and the HRCT was repeated immediately thereafter. At approximately 0900 hours on day 2, subjects underwent a CRC. At 1500 hours on day 2, six hours postchallenge, pulmonary function tests and HRCT were repeated. At 0800 hours on day 3, twenty-three hours postchallenge, pulmonary function tests and HRCT were repeated once more, with the HRCT performed both before and after inhaled methacholine. The study timeline is outlined in Fig 1.

Pulmonary function and methacholine inhalation challenge testing

Spirometry, lung volumes, and CVs were performed according to published guidelines.[18-20] CV, expressed as percentage of the vital capacity, was determined in triplicate; identification of the inflection point was performed visually by 2 independent readers (masked as to the subject or the phase of the study); and consensus was obtained.

Methacholine challenge test was performed in accordance with American Thoracic Society guidelines using Provocholine, 100 mg/vial (Metapharm, Coral Springs, Fla) diluted to concentrations of 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16 mg/mL with normal saline.[21] The 5-breath dosimeter technique was used. Subsequent MCTs during the HRCT studies were performed in the same manner, except that the Provocholine dose was initiated at a 2-fold concentration less than the PC_{20} determined from the screening MCT. Whenever HRCT was performed before and after a MCT, the screening PC_{20} dose was used. If a PC_{20} was not achieved at this repeated dose, the MCT was continued until a PC_{20} or a concentration of 8 mg/mL was reached. The subject was rescanned after the final dose had been reached.

TABLE I. Inclusion and exclusion criteria

Inclusion	Exclusion
Age 18-65 y	Use of inhaled corticosteroids in the previous 1 mo
≥5-mm positive skin prick test to cat allergen (Standardized Cat Allergen Extract, 10,000 antigen units/mL; ALK, Milford, Conn)	Parenteral or oral corticosteroids within the previous 3 mo
	Leukotriene modifiers within 1 mo
	Cromolyn sodium within 2 wk
	Theophylline within 7 d
	Smoking within the past year or had a greater than 5 pack-year history of smoking
$FEV_1 \geq 70\%$ predicted[33]	
$PC_{20} \leq 8$ mg/mL	Respiratory infection in the previous 3 wk
	Significant medical problems other than asthma
	Pregnant or lactating women

CRC

The CRC protocol has been described previously.[22] Briefly, subjects entered a 50-m^3 room that contained 2 neutered cats (1 male and 1 female). The room was furnished with wall-to-wall carpeting, a futon covered with a blanket, 2 metal chairs, and a litter box. During the CRC, the cats were placed into 2 separate wire cages 60 cm × 60 cm × 90 cm. Spirometry was performed outside the room immediately before and every 10 minutes during the CRC until the subject's FEV_1 declined by 20% or greater from the pre-CRC baseline value. After this drop in FEV_1, the subjects were removed from the room, and FEV_1 was remeasured every 10 minutes until it recovered to within 10% of the baseline value. Two puffs of albuterol (2 × 90 μg) were administered if the FEV_1 fell ≥ 50% below baseline or the subject requested the medication.

Fel d 1 measurements

Fel d 1 was sampled during every challenge using a portable air filtration device. The filtration pump (Gillian Instrument Corp, Wayne, NJ) was calibrated to a flow rate of 4 L/min. Antigen was collected on a 25-mm fiber glass filter (Millipore Corp, Bedford, Mass) and later measured with a 2-site antibody ELISA. A Sierra Series 260 Marple Cascade Impactor (Andersen Samplers Inc, Atlanta, Ga) was used to determine the size distribution of the particles.

Functional imaging

Subjects were scanned on a GE 9800 HiSpeed Advantage scanner (General Electric, Milwaukee, Wis) under spirometric control in the supine position. After training, under the guidance of experienced pulmonary function technologists, subjects performed forced expiratory maneuvers to RV according to American Thoracic Society standards to determine their best effort. Subjects were required to reproduce their forced vital capacity (FVC) to within 10% of their best effort before each imaging sequence. Subjects remained attached to the spirometer during CT scanning to ensure a suspended breath hold at RV. HRCT studies were performed immediately before and after MCT. A standardized image acquisition technique was used for all sequences using 120 kilovolt peak (kVp), 300 milliamperes (mA), with nominal slice collimation of 1 mm and a table increment

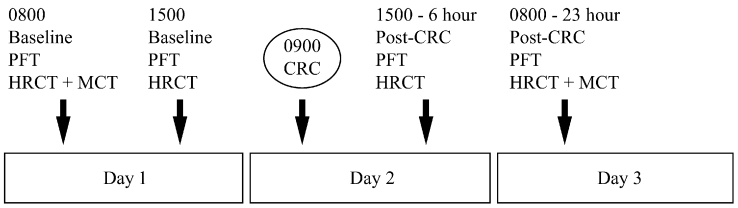

FIG 1. Study timeline. *PFT,* Pulmonary function test, including CV.

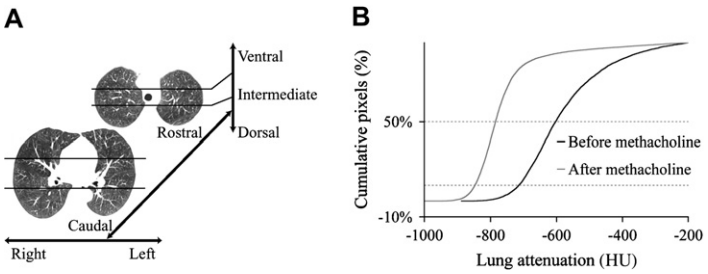

FIG 2. HRCT data analysis. **A,** The acquisition of 12 regions of interest consisting of right rostral, right caudal, left rostral, and left caudal, each subdivided into ventral (nondependent), intermediate, and dorsal (dependent) sections. A lung attenuation curve is then derived for each regions of interest. **B,** A representative lung attenuation curve. Reproduced with permission from Zeidler MR, Goldin J, Kleerup EC, Truong D, Simmons M, Kim HJ, et al. Montelukast improves regional air-trapping due to small airways obstruction in asthma. Eur Respir J 2006;27:307-15.[15]

of 3 mm/s. Images were acquired in single-volume sets of 6 to 10 seconds through the 2 regions of interest (rostral and caudal lung zones) commencing 3 cm above and below the carina, respectively. Images were reconstructed every 1 mm with a high spatial frequency algorithm using the narrowest field of view that included the rib margins at the widest diameter of the chest at a matrix of 512 × 512. After the procedure, the images were transferred to the Thoracic Imaging Laboratory image analysis work station.

HRCT data analysis

Anatomically registered 1-mm slices, which were obtained before and after methacholine, were selected for the rostral (upper) and caudal (lower) lung zones. After image quality checks, automated segmentation was performed on each of the acquired slices as previously described.[14] Twelve nonoverlapping regions of interest were segmented from the lung portion of the matched slices. The 12 regions of interest consist of right rostral, right caudal, left rostral, and left caudal regions, each subdivided into ventral (nondependent), intermediate, and dorsal (dependent) sections. Lung attenuation curves (LACs) representing the cumulative frequency distribution of lung attenuation (Hounsfield units [HU]) by pixel were derived for each of the regions of interest, and the median and 10th percentile attenuation were determined (Fig 2). Six and 23 hours after the CRC, all premethacholine regions of interest were analyzed for a leftward shift of the LAC from the baseline LAC. A shift of the LAC to the left (as measured by a more negative median or 10th percentile) represents lower lung attenuation, more air trapping, and, by inference, reduced small airways patency.[14]

After methacholine the post-pre methacholine shift was calculated for each region of interest. This post-pre methacholine shift pre-CRC was then compared with the post-pre methacholine shift after the CRC to determine the allergen-induced change in small airways hyperresponsiveness to methacholine on HRCT.

Data analysis

Primary and secondary outcomes. The primary outcome variables were (1) the change in the degree of regional air trapping calculated from the premethacholine HRCT LAC at 6 and 23 hours after antigen challenge compared with the pre-CRC baseline LAC at the corresponding hours during the preceding day, and (2) the change in the response to methacholine (ie, in the degree of leftward post-pre methacholine shift of the LAC, indicating regional hyperresponsiveness) at 23 hours after CRC compared with the pre-CRC baseline post-pre methacholine shift.

Secondary outcomes included changes in spirometry, plethysmography, and CVs at 6 and 23 hours and in methacholine PC_{20} FEV_1 at 23 hours after CRC.

HRCT analysis. A generalized least squares random-effects model was used to consider the correlations among the regions of interest at each of the subject's visits. We applied the generalized least squares random-effects model to test for changes before and after a naturalistic cat antigen challenge in both the 10th percentile and median Hounsfield units of the LAC. The following images were evaluated per subject both before and after CRC: (1) scans at 1500 hours (performed both before and 6 hours after CRC—no methacholine administered), (2) premethacholine scans at 0800 hours (performed before and 23 hours after a CRC), and (3) differences between post-pre methacholine 0800-hour scans (performed both before and 23 hours after a CRC). For each scan, 11 indicator variables (regions of interest) were identified. The right upper lobe posterior regions of interest were identified as the baseline reference regions of interest

TABLE II. Subject characteristics

	Mean ± SD
Age (y)	31.6 ± 7.4
Sex	70% Female
Duration of asthma (y)	20.5 ± 9.2
Fel d 1 mean wheal diameter (mm)	10.9 ± 3.0
FEV_1 (L)	3.27 ± 0.89
FEV_1 (% predicted)[26]	84% ± 13%
PC_{20} (mg/mL)*	0.45 ± 1.94

*Geometric mean.

TABLE III. Change in spirometric and plethysmographic measures at baseline (before CRC) and 6 and 23 hours after CRC

Variable	Baseline* (mean ± SD)	% Change from baseline post-CRC (mean ± SD)	Hours post-CRC	*P* value
FEV_1 1500 (% predicted)†	84 ± 13	−10 ± 14	6	.052
FEV_1 0800 (% predicted)	85 ± 13	−5 ± 11	23	.115
FVC 1500 (% predicted)	94 ± 12	−4 ± 12	6	.286
FVC 0800 (% predicted)	94 ± 10	−3 ± 4	23	.078
FEF_{25-75} 1500 (% predicted)	66 ± 22	−23 ± 17	6	.004
FEF_{25-75} 0800 (% predicted)	68 ± 20	−5 ± 21	23	.221
TLC 1500 (% predicted)‡	99 ± 15	−2 ± 4	6	.244
TLC 0800 (% predicted)	98 ± 15	0 ± 5	23	.909
FRC 1500 (% predicted)	91 ± 22	0 ± 27	6	.782
FRC 0800 (% predicted)	92 ± 21	−1 ± 9	23	.856
RV 1500 (% predicted)	101 ± 26	19 ± 34	6	.346
RV 0800 (% predicted)	95 ± 23	7 ± 15	23	.092
PC_{20} (mg/mL)	0.6 ± 2.7	−0.2 ± 0.8§	23	.441
CV/VC (%) 1500‖	3.3 ± 4.2	4.5 ± 3.1	6	.005
CV/VC (%) 0800	2.6 ± 3.2	3.3 ± 3.3	23	.024

*Baseline studies were performed at 0800 and 1500 the day before the CRC to correspond with the post-CRC studies.
†Hankinson et al[33] used for spirometry.
‡Goldman and Becklake[34] used for plethysmography.
§Doubling dose.
‖Closing volume as a percentage of vital capacity.[35]

against which the other 11 regions of interest were compared. The covariates of mean change after the CRC for each of the 11 regions of interest were calculated referenced to the baseline constant, and intraclass correlations were then estimated. Analyses were performed using STATA (STATA Corp 8.0, Statistics Data Analysis, College Station, Tex).

Pulmonary function test analysis. Pulmonary function data were evaluated using a paired Student *t* test.

RESULTS

Subject characteristics are described in Table II. No significant diurnal variation in the 0800 and 1500 pre-CRC spirometry (day 1) was observed (Table III). All 10 subjects had an immediate decline in FEV_1 of at least 20% during the CRC (mean decrease, 30% ± 11%), with the mean time to a 20% or greater decrease 25 ± 12 minutes (range, 10-50 minutes). Four subjects required the use of a β_2-agonist for significant symptoms after the CRC, whereas the remaining 6 subjects had a spontaneous improvement of their FEV_1 to within 10% of their baseline after they were removed from the cat room.

Table III shows the mean (±SD) percent spirometric and plethysmographic changes at 6 and 23 hours after the CRC. Fig 3 illustrates the time course of changes in FEV_1 and FEF_{25-75} beginning from 0800 on day 1 through the CRC on day 2 and ending at 0800 on day 3. One subject had late-phase responses 6 hours after CRC (defined as a 20% or greater decrease in FEV_1 compared with the baseline value measured at 1500 on day 1). At 23 hours after the CRC, 1 subject had a persistently reduced FEV_1 late-phase response. The mean reductions in FEV_1 at 6 and 23 hours after CRC were –10.4% ± −14.2% ($P = .052$) and 4.9% ± 11% ($P = .115$), respectively.

The mean reductions in FEF_{25-75} at 6 and 23 hours after CRC were –23.0% ± 17.1% ($P = .004$) and –5.0% ± 21% ($P = .221$), respectively. Two subjects had an FEF_{25-75} decline ≥23% at 6 hours, and 3 subjects exhibited this degree of decline at 23 hours.

The CV as a percent of the vital capacity (CV/VC%) changes at 6 and 23 hours were 4.5 ± 3.1 ($P = .005$) and 2.6 ± 3.2 ($P = .024$). There were no significant changes in FVC, TLC, FRC, or RV at 6 or 23 hours or in methacholine PC_{20} at 23 hours after the CRC.

The changes in lung attenuation (10th percentile and median) calculated from the LAC derived from the HRCT scans before and after CRC are shown in Table IV. Cat room exposure resulted in a significant shift in both the 10th percentile and the median of all of the LACs at 6 and 23 hours (Table IV and Figs 4 and 5) after the allergen challenge, indicating the development of a late-phase response manifested as significant regional air trapping that, although most prominent after 6 hours, persisted for at least 23 hours. We found 0.26 to 0.69 intraclass correlations across the 12 regions of interest in both the 0800 and the 1500 premethacholine scans of individual subjects, indicating distinct heterogeneity in regional lung attenuation within the lungs of these subjects with mild asthma. At 23 hours post-CRC (day 3), the postmethacholine-premethacholine shift (decrease) in lung attenuation was significantly greater than that measured at 0800 hours on day 1 (the day before the CRC), indicating the development of increased small airways hyperresponsiveness as a late-phase response to the CRC.

Fel d 1 concentrations measured in the room over the course of the study were 117 ± 79.5 ng/m^3; 20% of the Fel d 1 particles were less than 6 μm in size, and 7% were less than 3.2 μm.

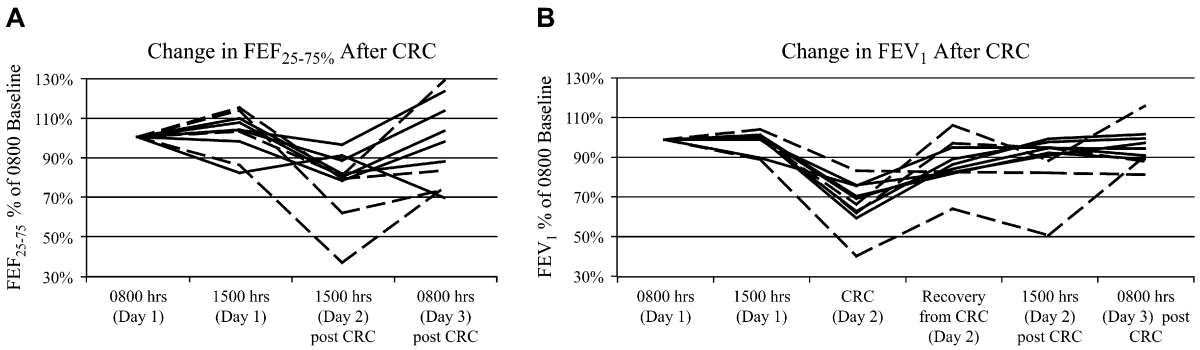

FIG 3. Time course of changes in FEF_{25-75} (**A**) and FEV_1 (**B**) at baseline, CRC, and recovery.

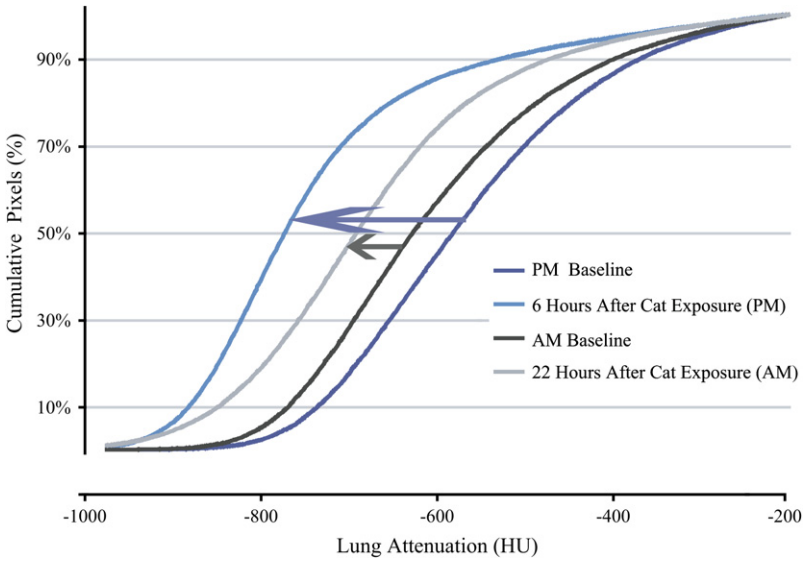

FIG 4. Representative lung attenuation curves at baseline and 6 hours and 23 hours after CRC.

TABLE IV. Changes in lung attenuation 6 and 23 hours, and changes in airways hyperreactivity to methacholine 23 hours after CRC

	6 Hours after CRC		23 Hours after CRC		23 Hours after CRC−postmethacholine-premethacholine	
Assessment	**Shift in HU (mean ± SE)**	***P* value**	**Shift in HU (mean ± SE)**	***P* value**	**Shift in HU (mean ± SE)**	***P* value**
10th Percentile	−45.6 ± 5.1	<.001	−10.4 ± 4.2	<.014	−12.8 ± 4.4	.004
Median	−54.0 ± 6.8	<.001	−15.8 ± 5.0	.002	−16.8 ± 5.4	.002
	Intraclass Constant Correlation		**Intraclass Constant Correlation**		**Intraclass Constant Correlation**	
10th Percentile	0.68		0.69		0.26	
Median	0.57		0.67		0.31	

DISCUSSION

In cat-sensitive subjects with mild asthma, we have demonstrated evidence of worsening small airways obstruction at 6 and 23 hours after exposure to natural cat allergen, as measured by both HRCT scanning of the lungs and CV. Although there was physiologic evidence of airways obstruction at 6 hours as detected by a borderline significant decline in FEV_1 and a significant decline in FEF_{25-75}, this was undetectable at 23 hours. In addition, at 23 hours postallergen challenge, the small airways were hyperresponsive to inhaled methacholine when assessed using HRCT but not FEV_1. We chose changes in lung attenuation on HRCT after allergen as our primary outcome because previous studies suggest that this technique is more sensitive for detecting small airways

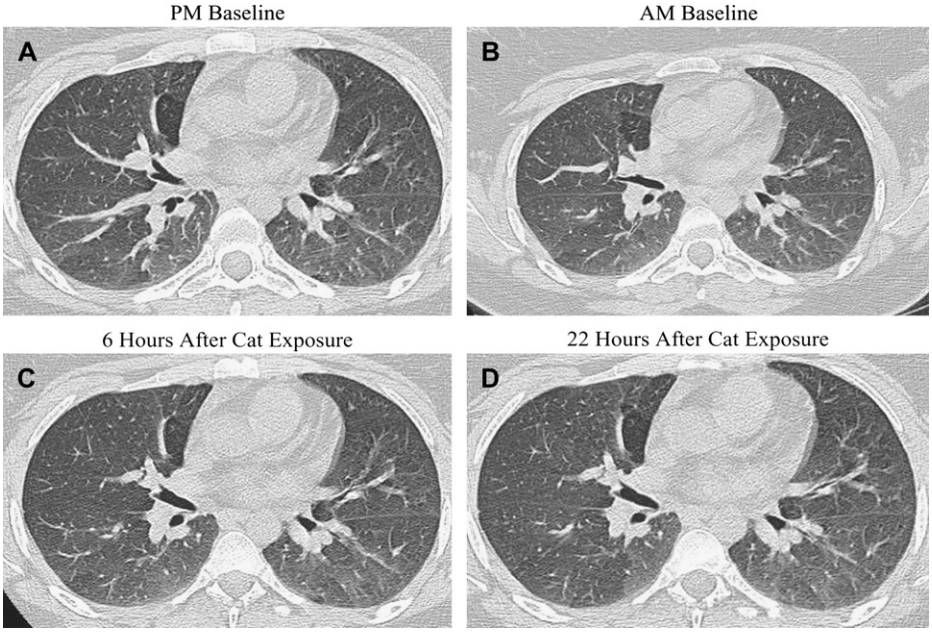

FIG 5. Representative images at PM baseline (**A**), AM baseline (**B**), and 6 (**C**) and 23 hours (**D**) after CRC.

obstruction, as well as changes therein, than most existing physiologic tests. For example, in a pediatric asthma population, Pifferi et al[23] showed the presence of qualitatively assessed low attenuation areas on HRCT as well as an RV >150% predicted after inhaled salmeterol/fluticasone therapy even after normalization of FEV_1 and FEF_{25-75}. Furthermore, our group demonstrated that use of an extrafine inhaled corticosteroid for 4 weeks (beclomethasone dipropionate hydrofluoroalkane [BDP-HFA]) resulted in improved small airways obstruction in subjects with asthma measured by shifts in lung attenuation on HRCT but not by changes in FEV_1 or FEF_{25-75}.[24] In contrast, no significant changes in lung attenuation shifts were noted with beclomethasone–dipropionate chlorofluorocarbon (BDP-CFC), a standard coarse particle inhaled corticosteroid, which does not penetrate the distal lung, although significant and equivalent increases in FEV_1 during the 4-week treatment period were noted in both groups. In both the BDP-HFA and BDP-CFC groups, there were no significant changes in the diameters of the larger airways on HRCT (airways > 2 mm in diameter), suggesting that the observed changes in lung attenuation were a result of improvement in small airways patency. In the latter study, we also observed a decrease in small airways hyperresponsiveness to methacholine documented by HRCT in the BDP-HFA group but not the BDP-CFC group. Similar results were obtained in subjects with asthma after treatment with an oral leukotriene antagonist, montelukast, which has access to the small airways indirectly via the circulation.[15] In this study, a significant decrease in air trapping after montelukast was noted on HRCT analysis but not by changes in FEV_1, FEF_{25-75}, lung volumes, or CVs compared with placebo. Collectively, results of these previous studies suggest that tests with a high degree of sensitivity, such as quantitative image analysis of HRCT scans, are most likely to detect small airways changes.

The unique sensitivity of HRCT in detecting small airways changes may be a result, in part, of the heterogeneous nature of airways disease in subjects with asthma. This heterogeneity is evidenced by the intraclass correlations (0.26-0.69) of lung attenuation between regions of interest noted within the lungs of individual subjects. For this reason, physiologic measures, which are global measures of lung function, may be less likely to detect subtle changes in the small airways.

Accumulating data show that small airways inflammation as measured by CV is a significant component of asthma in general and is linked to recurrent exacerbations, even in the presence of a normal FEV_1.[25] Exposure to small particulate antigens, which can penetrate the distal lung, is a common daily occurrence in both indoor and outdoor settings. Exposure to fine particulate indoor pollutants is becoming more important as both adults and children spend more time indoors. A significant portion of indoor suspended particulate matter is composed of particles less than 2.5 μm in diameter.[26] Many of these particles consist of soot (carbon aggregates) and are effective allergen carriers. Relevant to these observations are studies of cat antigen in homes, which demonstrate that up to 25% of Fel d 1 is present in a size that can penetrate the distal airways, and that these small particles can stay airborne much longer than the larger particles.[27-29] In one study of cat allergens in homes, 23% of airborne Fel d 1 was found to be less than 4.7 μm in diameter,[28] whereas in another, 25% of particles were less than 2.5 μm in diameter.[27]

Our finding of a late-phase FEV_1 response to the CRC in only 10% of subjects (1/10 subjects) is consistent with the results of previous studies of naturalistic exposure to cat allergen in cat-sensitive subjects with asthma. Diaz-

Sanchez et al[30] failed to show a late-phase response in any of 10 subjects with asthma monitored with FEV_1 as long as 8 hours after a CRC, whereas Corren et al,[22] using peak expiratory flow rate, showed a late-phase response in only 10% of subjects. With nebulized antigen, on the other hand, a late-phase response has been seen 5% to 50% of the time in cat-sensitive subjects with asthma.[3,4,7,31,32] The measurement of FEV_1 at only 6 and 23 hours after the initial CRC may have missed additional late-phase responses that might have occurred between 6 and 12 hours.

The results from the current study demonstrate the presence of significant small airways involvement in late asthmatic responses to naturalistic cat allergen exposure with persistence for up to 23 hours after the exposure. These findings have clinical implications regarding possible persistence of distal lung inflammation and subsequent structural changes with repeated natural exposure to cat allergen. Because evidence of inflammation and remodeling of small airways has been noted in patients with mild asthma, the small airways are a rational target for anti-inflammatory therapy delivered as extrafine particle aerosols or in oral form to access the distal lung.[3,4,7,31,32] In future studies of such targeted therapies, assessment of inhibition of late asthmatic responses to naturalistic allergen exposure using sensitive tools such as HRCT quantitative image analysis could serve as a useful model for evaluating therapeutic efficacy.

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