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ORIGINAL ARTICLE

New data analysis in a population study raises the hypothesis that particle size contributes to the pro-asthmatic potential of small pet animal allergens

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ABSTRACT

Background: The size of inhaled particles influences where they deposit and theoretically should be important for the development of airway inflammation and responsiveness. Our aim was to assess if sensitization to smaller-sized aeroallergens relates to higher prevalence of treated asthma, increased airway responsiveness, and airway and systemic inflammation.

Methods: Molecular-based IgE antibody determination was done in 467 subjects. Sensitized subjects were grouped based on the particle size of the aeroallergen: (1) Large particles only (mainly pollen); (2) Medium-sized particles (sensitized to mainly mite and mold and possibly to large particles); and (3) Small particles (sensitized to pet allergens and possibly to medium- and/or large-sized particles). Airway responsiveness to methacholine, exhaled nitric oxide (F_ENO), and serum eosinophil cationic protein (S-ECP) were measured. Asthma and rhinitis were questionnaire-assessed.

Results: Subjects sensitized to small particles had higher prevalence of treated asthma (35% versus 10%, $P < 0.001$), higher F_ENO₅₀ (32 versus 17 ppb, $P < 0.001$), higher S-ECP (10 versus 7.5 ng/mL, $P = 0.04$), and increased bronchial responsiveness (dose-response slope, 5.6 versus 7.5, $P < 0.001$) compared with non-atopics. This was consistent after adjusting for potential confounders. Sensitization to only large or to medium and possibly also large aeroallergen particles was not related to any of these outcomes after adjustments.

Conclusions: Sensitization to smaller particles was associated with a higher prevalence of asthma under treatment, higher airway responsiveness, and airway and systemic inflammation. Mapping of IgE sensitization to small particles might help to detect subjects having increased airway and systemic inflammation and bronchial responsiveness, indicating increased risk of developing asthma.

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Introduction

With the advance of multiplex, molecular-based technique it is possible to obtain information on profiles of IgE sensitization to more than 60 individual aeroallergens in one assay (1). The profile of IgE sensitization is important as IgE sensitization against perennial aeroallergens is related to asthma, airway inflammation, and hyperresponsiveness stronger than seasonal aeroallergens. This association of allergic sensitization patterns with airway inflammation (assessed by the fraction of nitric oxide in exhaled air, F_ENO) can be found both in a general population and asthma subjects (2).

Inflammation is a key feature in asthma (3), and anti-inflammatory treatment in the form of inhaled corticosteroids is currently the most effective treatment for subjects with persistent atopic asthma. Recent studies highlight even the importance of systemic eosinophil inflammation for asthma outcomes such as asthma attacks or asthma-related

emergency room visits (4). We have recently reported (2) an independent relation of IgE sensitization to aeroallergens (furry animals and mold) with systemic eosinophil inflammation (blood eosinophil counts) in patients with asthma. Serum eosinophilic cationic protein (S-ECP) is another marker of systemic eosinophil inflammation in asthma and allergic diseases (5). To our knowledge only one previous study used the information from the multiplex IgE determination (animal-derived lipocalin, kallikrein, and secretoglobulin) in order to relate to systemic eosinophil inflammation (blood eosinophils) in asthma (6).

The dysfunction of the small airways (those with internal diameters of 2 mm or less) is associated with worse control of asthma and higher airway responsiveness (6–8). Particle size is the most important aerosol characteristic that determines the site of airway deposition of inhaled drugs (9), and several other studies (10–12) demonstrated the deposition patterns of

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inhaled particles in relation to particle size and the importance of particle size of inhaled aeroallergens.

The alveolar region deposits mostly particles varying from 1 to 4 μm (12–14), although even a proportion of 4% to 15% of large particles may deposit in the alveoli (15). A majority of larger particles tend to deposit in the trachea and oropharyngeal tract (12–14), and inhaled pollen particles with a diameter of 10 to 70 μm (16) localize mainly in the tracheobronchial tree. Moreover, mathematical modeling of pulmonary NO dynamics can estimate fractional concentration of NO in the gas phase of the alveolar or acinar region ($C_A\text{NO}$). $C_A\text{NO}$ has been investigated as a potential biomarker of small airways inflammation (17), but the impact of IgE sensitization to allergen particles of small size on alveolar NO has been poorly studied (18).

The aim of the present study was to examine the hypothesis that the size of inhaled particles influences their deposition area and therefore is also important for the development of airway inflammation and responsiveness. In particular, we aimed to investigate if there is a difference regarding levels of airways and systemic inflammation, airway responsiveness, and prevalence of asthma under treatment with inhaled corticosteroids, in relation to IgE sensitization to small compared to medium and large allergen particles.

Methods

Population

This study is a retrospective analysis of previous data based on subjects who participated in the European Community Respiratory Health Survey (ECRHS) II, which is the 10-year follow-up of ECRHS I. The design of ECRHS I and II has been published in detail (19). In the Uppsala study center, 679 subjects were reinvestigated in ECRHS II. This study included 467 people who were examined with component-resolved IgE antibody determination, based on microarray techniques (ImmunoCap ISAC; Phadia/Thermo Fisher Scientific, Uppsala, Sweden), of whom 96 fulfilled criteria for current asthma. The characteristics of the 212 subjects who were not examined with ISAC are presented in Table E1 (available online). Those that were lost in the follow-up were more often females, younger, with lower prevalence of rhinitis and asthma. Lung function test results (forced expiratory volume in 1 s (FEV_1) absolute levels and $\text{FEV}_1\%$ predicted) were available for 411 subjects. Eosinophil cationic protein (ECP) was measured in blood after our recent work (2), where we correlated allergic sensitization patterns with systemic eosinophil inflammation (blood eosinophil counts) in patients with asthma. Therefore we wanted to analyze this correlation as well in a population-based material, and serum samples were available for measuring ECP. Moreover, in the present study we also analyzed nitric oxide (NO) flow-independent parameters, such as tissue concentration of NO of the airway wall ($C_{\text{aw}}\text{NO}$), airway transfer factor for NO ($D_{\text{aw}}\text{NO}$), and F_ENO (20). These were available for 231 subjects. Methacholine challenge test results were available from 295 subjects, and ECP measurements for 404 subjects (Figure 1). All these measurements were conducted randomly throughout the whole year both outside and during pollen and mold season. In the

previously published results from the same material we grouped allergens as perennial/pollen/food allergens and had as outcomes asthma, bronchial responsiveness, and F_ENO (1). No data were available on ECP, and no data on NO flow-independent parameters were used in our previous publication (1).

IgE sensitization

The presence of IgE antibodies was examined using microarray chip technology (ImmunoCAP ISAC 103; Phadia/Thermo Fisher Scientific, Uppsala, Sweden) (21,22). The ImmunoCAP ISAC 103 has allergen components from 43 allergen sources and yields results as ISAC Standardized Units (ISU) for specific IgE, a semi-quantitative estimate of specific IgE antibody titers. Subjects were considered non-IgE-sensitized if the signal was non-measurable or very low (<0.3 ISU) for all allergen components. Previous air sampling studies (23–26) have examined the particle size of various aeroallergens and have shown that approximately 20%–30% of airborne animal allergens are present on small particles of 1–5 μm diameter, in contrast to mite and cockroach allergens, which are carried on larger particles of 10–40 μm diameter (23,25). Airborne pollen allergen can vary because of the different atmospheric conditions (24), but particles between 20 and 60 μm in diameter are often and can be carried in the wind causing nasal and ocular symptoms (allergic rhinoconjunctivitis) (27).

Detailed data on our subjects with regard to the different allergen components have been published elsewhere (1), but for the clarity of this paper they have been presented here in a somewhat modified way (Table E2, available online). Subjects sensitized to aeroallergens were divided into three groups depending on the particle size of the allergen: (1) large particles only: subjects only sensitized to allergens with large-sized particles; (2) medium-sized: subjects sensitized to medium-sized particles, possibly even to large particles, but not to small particles; and (3) small-sized particles: subjects sensitized to small particles regardless of whether they were sensitized to medium- or large-sized particles. Subjects who were non-atopics were used as reference group. Those who were sensitized only to food allergens were omitted from analyses, and those sensitized to bee ($n\text{Api m 1}$), were grouped in one group named 'others' and omitted from analyses (Table E2, available online).

Eosinophil cationic protein

Blood samples collected in ECRHS II (baseline) for the measurement of serum eosinophil cationic protein (S-ECP) were allowed to coagulate for 60 min at 24 °C before they were centrifuged. Sera were stored at –20 °C. The concentration of S-ECP was assayed using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden).

Measurements of exhaled NO

F_ENO measurements were performed according to American Thoracic Society/European Respiratory Society

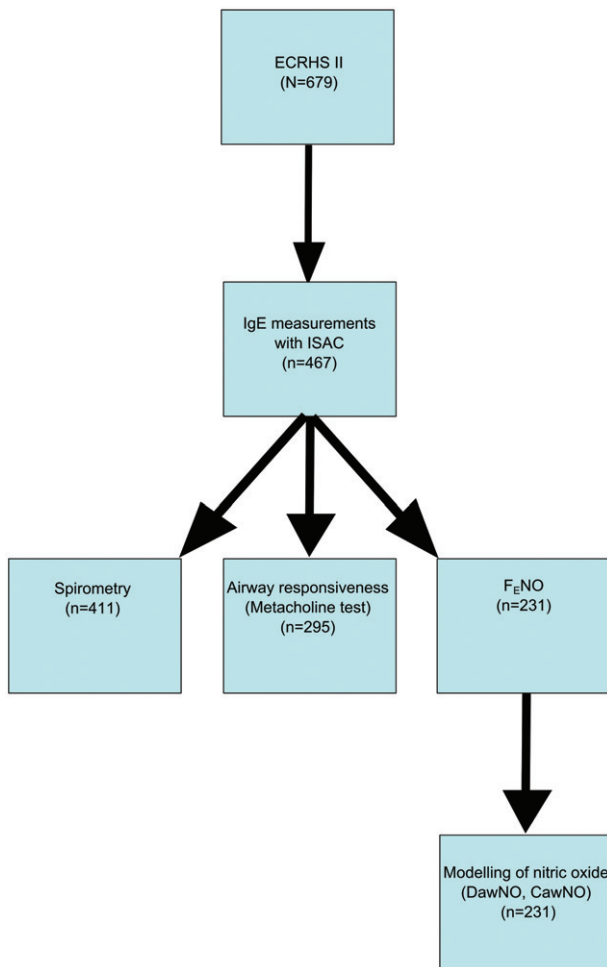


Figure 1. Flow chart of study population.

recommendations (28), apart from the use of three additional flow-rates (5, 100, and 500 mL s⁻¹). Mathematical modeling of pulmonary NO dynamics has been reviewed (29), and in this study the Högman and Meriläinen algorithm (HMA) has been applied (30). We used F_ENO collected at 5, 100, and 500 mL s⁻¹ exhalation flow-rates and an iteration algorithm, which estimates NO parameters in two compartments: 1) the conducting airways, and 2) the alveoli. NO in the conducting airways is assessed in two variables: tissue concentration of NO of the airway wall (C_{aw}NO) and airway compartment diffusing capacity of NO from the airway wall to the gas stream (D_{aw}NO). NO in the alveoli is estimated by steady-state alveolar NO concentration (C_ANO). Correction for axial back-diffusion of NO into the alveolar site was not applied (31). F_ENO at the exhalation flow-rate of 50 mL s⁻¹ (FeNO₅₀) is also presented throughout the article.

Lung function

Forced expiratory volume in 1 second (FEV₁) was measured using a dry rolling-seal spirometer (Model 2130; SensorMedics, Anaheim, CA, USA). American Thoracic Society recommendations were followed (32). The predicted values for FEV₁ were calculated on the basis of European Coal and Steel Union reference values (33,34).

Methacholine challenge test

Details of the methacholine challenge test have been described elsewhere (35). The level of airway responsiveness was expressed using a dose-response slope (36) where a lower value indicates a higher degree of airway responsiveness.

Questionnaires

The ECRHS II main questionnaire (<http://www.ecrhs.org>) (37) was used to obtain information about respiratory symptoms, exposure to investigated allergens, and smoking history.

Diagnosis of asthma or rhinitis

A person was recorded as having asthma if he/she had been diagnosed before with asthma and had had an asthma attack or one of the following symptoms during the last 12 months: nocturnal chest tightness, attack of shortness of breath, or chest wheezing or whistling (38). Furthermore participants with asthma were recorded as having asthma under treatment if he/she had used inhaled steroids at any time during the last 12 months.

A person was recorded as having rhinitis if he/she answered yes to the question 'Do you have any nasal allergies, including hay fever?' Subjects with rhinitis were recorded as having rhinitis under treatment if he/she had used antihistamines or nasal steroids during the last 12 months.

Statistics

Statistical analyses were performed by using STATA 11.0 software (Stata Corp, 2001, TX, USA). F_ENO₅₀, C_{aw}NO, D_{aw}NO, and C_ANO values were log-transformed before analysis. The chi-square test and unpaired *t* test were used when comparing non-atopics and sensitized subjects. Bonferroni correction was used to adjust for repeated comparisons. Linear regression was used when analyzing the correlation between the type of sensitization and F_ENO₅₀ or C_{aw}NO, or D_{aw}NO or C_ANO, or FEV₁ (%predicted), or FEV% or airway responsiveness or ECP. These models always included age, sex, height, weight, smoking history, presence of IgE sensitization to food allergens, and use of inhaled corticosteroids. Logistic regression was used in the bivariate analysis to analyze the correlation between the type of sensitization and asthma under treatment or rhinitis under treatment. The adjusted model included age, sex, height, weight, smoking history, IgE sensitization to food allergens, and use of inhaled corticosteroids (the last-mentioned was not included in the model with outcome treated asthma). A *P* value of <0.05 was considered statistically significant.

Ethics

All subjects gave their permission for the utilization of personal data for the purpose of this study. The study was approved by the Ethics Committee at the Medical Faculty at Uppsala University (Dnr 1998/495 and 1999/313).

Results

The study population consisted of 467 individuals (216 females) aged 29–55 years. The prevalence of atopy was 45% ($n=203$), of whom 69 were sensitized only to large aeroallergen particles, 26 sensitized to medium- but not small-sized aeroallergen particles, 79 sensitized to small aeroallergen particles, and 37 only to food allergens. The overlap of sensitization to aeroallergen particles of different size is shown in Figure 2.

Characteristics of participants grouped based on allergen particle size

Subjects sensitized to small particles ($P=0.01$) and subjects sensitized only to large particles ($P=0.04$) were younger than non-atopics (Table I). Sex, height, and weight did not differ between groups. Smoking history was similar in all groups compared with non-atopics except for the group of subjects sensitized only to large particles which had more subjects who had never smoked. Subjects sensitized to small particles used more inhaled corticosteroids than non-atopics ($P<0.001$), subjects sensitized to medium particles ($P=0.01$), and subjects sensitized only to large particles ($P<0.001$).

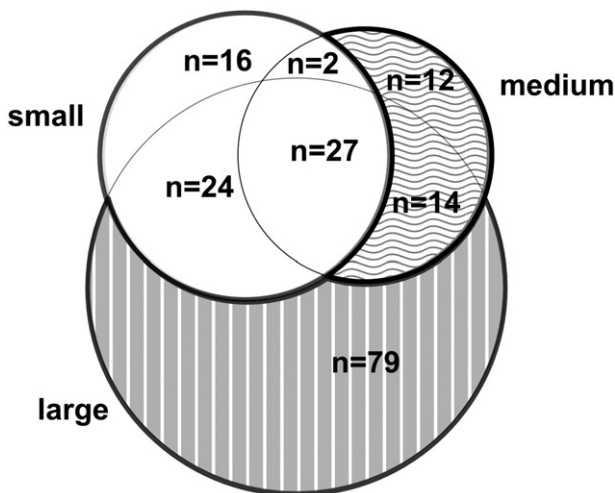


Figure 2. Overlap of sensitization to aeroallergen particles of different size.

Lung function and inflammation parameters in relation to sensitization pattern

Subjects sensitized to small particles had lower FEV₁ (%predicted) ($P=0.03$) than non-atopics, while forced vital capacity (FVC) and FEV% did not differ between the groups (Table II). Moreover, subjects sensitized to small particles ($P<0.001$), subjects sensitized to medium-sized particles ($P=0.04$), and subjects sensitized only to large particles ($P=0.02$) had higher F_ENO₅₀ levels than non-atopics. Likewise, subjects sensitized to small particles had higher D_{aw}NO levels ($P=0.001$), C_{aw}NO levels ($P=0.01$), and ECP levels ($P=0.01$) than non-atopics. C_ANO levels were similar in all groups. Finally, subjects sensitized to small particles had a higher degree of airway responsiveness (lower slope which mirrors a steeper curve in the methacholine test) than non-atopics ($P<0.001$) and subjects sensitized only to large particles ($P<0.001$).

We examined the independent effects of sensitization to particles of different size towards F_ENO₅₀, D_{aw}NO, C_{aw}NO, C_ANO, FEV₁ (%predicted), FEV%, airway responsiveness, S-ECP, asthma under treatment, and rhinitis under treatment (Table III, Figure 3). Sensitization to small particles was independently associated with higher ECP ($P=0.04$), F_ENO₅₀ ($P<0.001$), C_{aw}NO ($P=0.001$), and higher airway responsiveness ($P<0.001$) when compared with non-atopics. No differences were observed for D_{aw}NO, C_ANO, FEV₁ (%predicted), or FEV% ($P>0.05$). Sensitization to small particles was associated with higher levels of F_ENO₅₀ ($P=0.001$), C_{aw}NO ($P=0.008$), and higher airway responsiveness ($P=0.001$) compared with large particles. These models were adjusted for age, sex, height, weight, smoking history, IgE sensitization to food allergens, month of examination, and use of inhaled corticosteroids. There were no differences between subjects sensitized to small and medium-sized aeroallergen particles with regard to F_ENO₅₀, C_{aw}NO, D_{aw}NO, C_ANO, ECP, and airway responsiveness.

Prevalence of asthma and rhinitis in relation to sensitization pattern

The prevalence of asthma (with or without ongoing treatment) was higher in subjects sensitized to small particles than non-atopics ($P<0.001$, both) and subjects sensitized to large particles ($P<0.001$ and $P=0.001$, respectively). Rhinitis (with or without ongoing treatment) was more prevalent in subjects sensitized to small particles ($P<0.001$, both) and to large

Table I. Subjects sensitized to small particles were younger and used more inhaled corticosteroids than non-atopics. Sex, height, and weight did not differ in between groups.

	Non-atopics ($n=256$)	Small ($n=69$)	P value ^a	Medium ($n=26$)	P value ^a	P value ^b	Large ($n=79$)	P value ^a	P value ^b	P value ^c	Food ($n=37$)	P value ^a
Female (%)	48.1	42.0	0.37	34.6	0.19	0.51	44.3	0.56	0.78	0.38	54.1	0.49
Age (years)	43.5 ± 7.2	40.2 ± 6.9	0.011	42.9 ± 7.0	1	1	40.8 ± 7.7	0.044	1	1	45.0 ± 7.2	1
Height (cm)	174 ± 10	174 ± 9	1	180 ± 10	1	1	175 ± 11	0.51	1	1	173 ± 10	1
Weight (kg)	76 ± 16.0	78.4 ± 15.3	1	77.2 ± 14.2	1	1	76.1 ± 14.8	1	1	1	76.0 ± 15.3	
Smoking history (%)			0.34		0.22			0.01				0.92
Never	43.0	51.5		60.0			60.8				41.7	
Ex	40.2	30.9		32.0			22.8				38.9	
Current	16.8	17.7		8.0			16.5				19.4	

^aAgainst 'non-atopics'.

^bAgainst 'small'.

^cAgainst 'medium'.

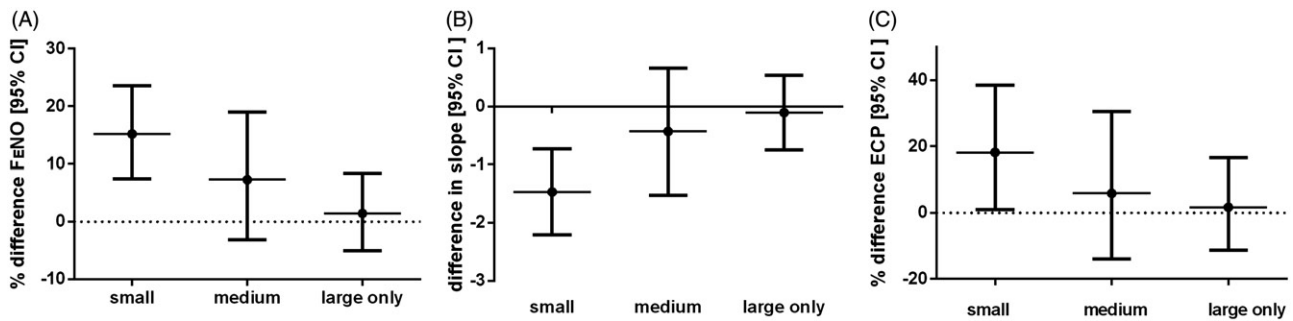


Figure 3. Independent effect of IgE sensitization to various groups (grouped according to their size) of aeroallergens (compared to non-atopics) to $F_{E}NO$ and airway responsiveness (where a lower value indicates more responsiveness) and S-ECP. Adjusted for age, sex, height, weight, smoking history, presence of IgE sensitization to food allergens, month of examination, and use of inhalant corticosteroids.

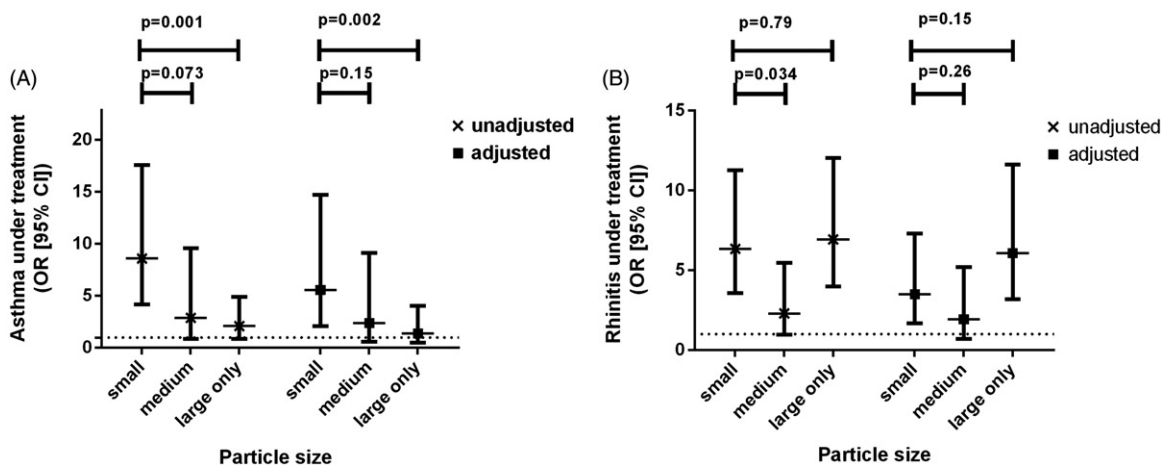


Figure 4. Independent effect of IgE sensitization to various groups of aeroallergens on risk for asthma (compared to non-atopics) and rhinitis. The adjusted model took into consideration age, sex, height, weight, smoking history, month of examination, use of inhaled corticosteroids (but not for the adjusted model of asthma under treatment), and presence of IgE sensitization to food allergens.

Table III. Sensitization to small particles is associated to higher tissue concentration of NO of the airway wall ($C_{aw}NO$) compared to non-atopics.

	% Difference $D_{aw}NO$ (95% CI) ^a	<i>P</i> value	% Difference $C_{aw}NO$ (95% CI) ^a	<i>P</i> value	% Difference $C_A NO$ (95% CI) ^a	<i>P</i> value
Small	5.0 (-1.7, 12.1)	0.15	11.7 (4.2, 18.9)	0.001	-0.3 (-9.8, 10.1)	0.97
Medium	1.4 (-8.0, 11.8)	0.76	6.4 (-3.5, 17.5)	0.19	6.7 (-7.9, 23.6)	0.31
Large only	-0.4 (-6.5, 6.1)	0.93	1.7 (-4.6, 8.4)	0.51	-1.3 (-10.2, 8.6)	0.98

^aAdjusted for age, sex, height, weight, smoking history, IgE sensitization to food allergens, and use of inhaled corticosteroids.

$C_A NO$ = fractional concentration of NO in the gas phase of the alveolar or acinar region (ppb); $C_{aw}NO$ = tissue concentration of NO of the airway wall (ppb); $D_{aw}NO$ = airway compartment diffusing capacity of NO from the airway wall to the gas stream ($mL s^{-1}$).

dog) leading to an increase of $F_{E}NO_{50}$, mainly explained by an increase in airway concentration of NO but no effect on alveolar contribution to NO. This is consistent with results of our previous study where IgE sensitization to cat allergens was associated with higher levels of $F_{E}NO_{50}$, $C_{aw}NO$, and $D_{aw}NO$, but not with $C_A NO$ (39). These results are also in line with previous data on this material, when only data on IgE sensitization to cat, timothy, mold, and mite were available and only IgE sensitization to cat extract independently related to $F_{E}NO_{50}$, $C_{aw}NO$, and $D_{aw}NO$. Furthermore, ECP, a marker of systemic eosinophil inflammation and asthma severity (44), was associated with sensitization to small particles in the present study. In a previous study (5), ECP levels were higher in

subjects sensitized to perennial allergens (including cat, dog, and mite allergens) than to seasonal allergens. Furthermore, exposure to small allergen particles produces an increase in interleukin-5 concentrations in sputum and blood (43), which should result in more activation of eosinophils and increased ECP levels. However, in our material, no differences between sensitization to large and small particles were found with regard to ECP, contrary to the IL-5 findings (43).

Subjects sensitized to small particles were more likely to have higher airway responsiveness compared with subjects sensitized only to large particles. That contradicts results of previous studies where larger aerosols of adenosine monophosphate (41), methacholine (14), cat allergen (42), and mite

allergen (43) gave more immediate responses than smaller particles. The same quantity could be delivered in both large and small airways with the nebulizer, but the concentration relative to the area of deposition in the larger airways would be higher as deposition of the same dose in the small airways could be insufficient to achieve similar airway bronchoconstriction due to the larger area of the small airways. However, in our study we found that the methacholine reactivity was higher in patients sensitized to small particle allergens compared with patients sensitized to only large particle allergens, whereas $F_{E}NO$ and FEV_1 did not differ between these two groups. This may support the view that small particle allergens affect small airways more than large particle allergens. This hypothesis is further supported by Zeidler et al. showing that naturalistic exposure to cat allergen caused a significant decline in small airway function including air trapping and increased small airway responsiveness to methacholine, but not in large airway function (FEV_1), in cat-sensitized asthmatics (45).

Similar levels of alveolar NO were found in all patient groups, disregarding presence of IgE sensitization and allergen particle size. This might be due to the absence of an increase of small airway inflammation, or the limited utility of alveolar NO as a marker of small airway inflammation in the presence of obstruction (46), which was not assessed. However, the present study was limited by not including other methods to assess small airway function, such as measurements of FEF_{25-75} , impulse oscillometry, and nitrogen oxide washout (7,17). We were therefore not able to study small airway functional impairment in this cohort in relation to IgE sensitization and allergen particle size. Moreover, our results were consistent also when further adjustment for the month of taking measurements was introduced in the multivariate analyses.

One strength of the present study was the availability of multiplex, molecular-based IgE antibody determinations of a large number of allergen components, which made possible the formation of subject groups with regard to allergen particle size. One more strength was the combined measurement of lung function, airway responsiveness, ECP, and airway inflammation, both as a global measure ($F_{E}NO$) and extended measurements, allowing assessment of bronchial and alveolar inflammation. In the present study we focused on the particle size and therefore assessed independently the effect of pet IgE sensitization (small allergen particles) versus mite, mold, and mouse sensitization (medium-sized allergen particles). Furthermore, we analyzed the relation of the different sensitization patterns with regard to asthma and rhinitis under treatment, a marker of systemic eosinophilic inflammation (ECP) (1) and nitric oxide (NO) flow-independent parameters which reflect NO contribution from central and peripheral airways, outcomes that were not used in our previous study.

However, it has to be acknowledged as a weakness that the group of subjects sensitized to medium-sized allergen particles was relatively small, and therefore we cannot exclude that these specific analyses were underpowered. Moreover, the present results can be difficult to transfer to a more general population due to the enrichment of the studied people with subjects with respiratory symptoms and due to the loss of eligible subjects due to lack of blood samples or different tests

(1). Our groups were heterogeneous, and most of the significant differences were observed in comparisons with non-asthmatic, non-atopic controls but not between groups of sensitized individuals, stratified according to the particle size division.

In conclusion, this study demonstrated that sensitization to small aeroallergens, as assessed by means of multiplex techniques, was associated with more local airway and systemic inflammation, airway responsiveness, and higher prevalence of asthma. Interestingly, sensitization to medium-sized and/or large allergen particles was not related to these asthma characteristics. However, we were not able to find a relation between the size of allergen particles and inflammation in the small airways, as assessed by alveolar NO. Future studies should incorporate functional methods to look at the small airways as well as longitudinal studies to assess if small allergen particles are more likely to lead to the development of asthma and airway responsiveness than medium-sized or large allergen particles.

Declaration of interest

Kjell Alving is a minority shareholder of Aerocrine AB and has received funds for research from the same company. Magnus Borres is an employee of Thermo Fisher Scientific (which manufactures kits to measure IgE antibodies against allergens). The rest of the authors had no financial or other conflicts of interest.

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