

Relationship of asthma, atopy, and bronchial responsiveness to serum eosinophil cationic proteins in early childhood

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Background: The relationship between atopy, asthma, and eosinophilic inflammation is less clear in early childhood than later in life.

Objective: We sought to determine the relationships between asthma, atopy, and serum eosinophil cationic protein (ECP), a biomarker of eosinophil activation, in 6-year-old children.

Methods: Serum ECP levels were available from 968 six-year-old children who were part of a longitudinal birth cohort being assessed for asthma and atopy. Detailed clinical history and examination, lung function testing, methacholine challenge, and skin prick testing to 4 common allergens were undertaken. Subgroups of the children were compared by using *t* tests, ANOVA, χ^2 tests, and regression analysis.

Results: One hundred ninety-one (19.7%) children had current asthma, with 114 (59.7%) of these being atopic. The mean serum ECP level for the entire group was 18.0 $\mu\text{g/L}$ (range, 2.0–146.0 $\mu\text{g/L}$), with no difference between male and female patients. Serum ECP was higher in atopic children (20.5 ± 18.4), those with asthma (22.4 ± 19.6), and those with asthma and atopy (26.6 ± 22.4 ; all $P < .001$ compared with children with no asthma or atopy [16.1 ± 15.9]). Serum ECP levels were highest in children with severe asthma ($P < .001$), especially in those with concurrent atopy. Severity of atopy, judged on the basis of wheal size or derived variables combining wheal size and the number of positive skin tests, was a major determinant of serum ECP. Heightened methacholine responsiveness was not associated with increased serum ECP levels.

Conclusion: The higher serum ECP levels seen in 6-year-old children with current asthma and more severe atopy suggest that atopy and eosinophilic inflammation are important in driving this clinical phenotype and that this might represent asthma that persists. (*J Allergy Clin Immunol* 2004;114:1040–5.)

Key words: *Childhood asthma, eosinophilic cationic protein, atopy, skin prick tests, methacholine challenge*

Abbreviations used

BHR: Bronchial hyperresponsiveness

ECP: Eosinophil cationic protein

OR: Odds ratio

PC₁₅FEV₁: Concentration (of methacholine) causing a 15% decrease in FEV₁

SPT: Skin prick test

Asthma in young children is more appropriately described as the number of wheezing phenotypes, with many children appearing to grow out of their symptoms. Longitudinal cohort studies suggest that the relationship between symptoms of asthma and atopy is not as strong in early childhood as it is later in life.^{1,2} The proportion of wheeze attributable to atopy in 6-year-old children is approximately equal to that attributable to viral infections.³ Asthma associated with atopy appears to be more likely to persist into adult life than viral-associated asthma.⁴ However, atopy is far more common than asthma,⁵ suggesting that simple allergy tests, such as skin prick tests (SPTs), might not be sufficient for determining the true relationship between atopy and asthma in young children.

Airway inflammation with increased numbers of eosinophils is a characteristic feature of asthma in adults. Activated eosinophils release a variety of proteins, including eosinophil cationic protein (ECP). Serum levels of ECP are increased in adults and older children with asthma and correlate with disease activity and compliance with inhaled steroid therapy.^{6–8} Bronchial hyperresponsiveness (BHR), another characteristic feature of adult asthma, is increased in the presence of airway inflammation and correlates with disease activity.^{9–11} However, the relationship between BHR and serum ECP is less clear,¹² especially in young children.

The present study was conducted to determine the relationships between serum ECP levels and clinical asthma, atopy, and BHR in 6-year-old children followed as part of a longitudinal birth cohort. In addition, we sought to determine whether measuring serum ECP helped clarify the relationship between atopy and asthma in these children.

METHODS

The study population consisted of children from a community-based longitudinal birth cohort from Perth, Western Australia.¹³

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Mothers were recruited antenatally and were not selected on the basis of any asthma or atopy criteria. The cohort initially consisted of 2860 live births, with 2537 potentially available for follow-up when the children were 6 years old. Follow-up data included physical examinations at birth and 1 and 6 years of age and comprehensive questionnaires at 1, 2, 3, and 6 years of age, including data on social and demographic factors, number of siblings, family history of asthma, atopy, lower respiratory tract illness, exposure to environmental tobacco smoke and wheezing histories, physician diagnoses of asthma, and use of reliever and preventive medications. The 6-year follow-up included the following: physical examination; lung function tests (reported elsewhere¹⁴); methacholine challenge; SPTs to 4 common allergens (dust mite, rye grass, cat dander, and mold); and measurement of serum ECP levels.

ECP

Whole blood was drawn by means of venipuncture and handled according to the manufacturer's instructions. Total serum ECP levels were determined by using the Pharmacia CAP system fluoroenzyme immunoassay, according to the manufacturer's standard procedures.

SPTs

SPTs were conducted on the volar aspect of the forearm by using standard methodology¹⁵ to house dust mite, mixed grass, cat dander, and mixed mold (Bayer Corporation, Elkhart, Ind), together with negative (saline) and positive (histamine HCl) controls. Anti-histamine preparations were withheld for at least 7 days before the test. Wheal size was measured after 15 minutes. A positive reaction was defined as a wheal of 2 mm or larger than that produced by the negative control.¹⁶ Children were considered to be atopic if they had at least one positive SPT response. In addition to the binary outcome (positive or negative) and the continuous wheal size, 2 categoric variables were constructed in an attempt to grade atopy. First, severity of atopy was defined as mild (1), moderate (2), or severe (3) according to wheal size. A wheal of 2 to 3 mm was defined as mild, 4 to 7 mm was defined as moderate, and greater than 7 mm was defined as severe. A score was given separately for each allergen tested. Second, the combined allergic severity grade was determined by adding the severity of allergy-specific atopy categories (0-3) for each of the 4 allergens. This score, which ranged from 0 to 12, was categorized as follows: grade 1, 1-5; grade 2, 6-12. By default, all nonatopic subjects had a grade of zero.

Lung function

Lung function was measured by using standard spirometry (Model 6100; Welch Allyn, Skaneateles Falls, NY) performed to American Thoracic Society standards with the children standing. The methodology and results of pulmonary function testing have previously been reported¹⁴ and are not included here.

Bronchial responsiveness

A random sample of the cohort ($n = 711$) was offered methacholine challenge. Within this sample, children were eligible if they could perform reproducible spirometry, had an FEV₁ of at least 80% of predicted value,¹⁷ and had withheld asthma medication for the standard periods (ie, bronchodilators withheld for 6 hours). The methodology and results of methacholine challenge have been reported previously.¹⁴ Briefly, methacholine challenge was performed by using a modified Yan technique.¹⁸ After saline inhalation, doses of methacholine (0.06, 0.10, 0.20, 0.50, 1.0, 1.8, 3.9, and 7.8 mg/mL) were delivered through De Vilbiss glass nebulizers until the FEV₁ decreased by 20% or greater or the highest dose was administered. On the basis of our previous study,¹⁴ the concentration of methacholine causing a 15% decrease in FEV₁ (PC₁₅FEV₁) was

determined, and a challenge was considered positive if the PC₁₅FEV₁ was 1.8 mg/mL or less.

Clinical definitions

Current asthma at age 6 years was defined as wheezing or cough without a cold in the preceding 12 months with a physician's diagnosis ever and current use of reliever or preventive medication.²

Severity of asthma was defined as mild, moderate, or severe on the basis of the main symptoms of episodes of wheezing per year, episodic wheezing causing speech interruption, or nocturnal waking.¹¹ Asthma was considered to be mild if 1 to 2 episodes of wheeze were reported in the past 12 months. Asthma was considered to be moderate if 3 to 12 wheezing episodes and not more than one occasion of nocturnal waking per week (on average) caused by wheeze had been reported during the past 12 months. Severe asthma was defined as at least one of the following features: more than 12 episodes of wheeze, one or more episodes of acute asthma limiting speech, or more than one occasion of nocturnal waking per week (on average) throughout the past 12 months.

Statistical analysis

Serum ECP values were positively skewed and therefore were log transformed for analysis. Analysis was mainly confined to 4 mutually exclusive groups: (1) no asthma or atopy; (2) atopic nonasthma; (3) nonatopic asthma; and (4) atopic asthma. ANOVA, *t* tests, and cross-tabulations were used to examine distributions in the different groups. Multiple linear regressions were used to estimate the associations between asthma, atopy, parental history of asthma, severity of asthma, grade-severity of atopic sensitivity, and serum ECP levels. Multiple logistic regressions were used to estimate the importance of various factors in distinguishing atopic from nonatopic asthma. The Statistical Package for the Social Sciences software (version 11.0, SPSS Inc, Chicago, Ill) was used throughout.

RESULTS

Serum ECP levels were available from 968 subjects who consented to blood samples, and they form the basis of the present report. These subjects were generally representative of those participating in the 6-year follow-up (Table I). The mean age of this group was 71.0 months (SD 2.4), and 56% ($n = 545$) were male. Twenty percent (191) had current asthma, 43% (415) were atopic, 12% (114) had atopy and asthma, and 8% (77) had asthma not associated with atopy.

For the total cohort, the group mean serum ECP level was 18.0 μ g/L and ranged from 2.0 to 146.0 μ g/L. Serum ECP levels did not differ with sex ($P = .56$) but were higher in children with atopy ($n = 415$; group mean, 20.5 ± 18.4 μ g/L) and those with asthma ($n = 191$; group mean, 22.4 ± 19.6 μ g/L; $P < .001$). Children with asthma but without atopy had serum ECP levels ($n = 77$; group mean, 16.2 ± 13.0 μ g/L) that were very similar to levels seen in those without asthma or atopy ($n = 476$; group mean, 16.1 ± 15.9 μ g/L). Children with asthma and atopy had higher levels ($n = 114$; group mean, 26.6 ± 22.4 μ g/L) that were significantly different ($P < .001$) from those of other groups. Serum ECP levels increased with increasing levels of reaction to each allergen (Table II). The unadjusted odds ratio (OR) for increased serum ECP

TABLE I. Participant characteristics

Characteristic	Data available	Participants (n = 968)	Nonparticipants (n = 1269)	P value
Birth weight (<2500 g)	2840	8%	9%	.60
Gestational age (<37 wk)	2840	9%	11%	.21
Maternal age at birth (<20 y)	2860	6%	11%	<.001
Sex (% male)	2860	56%	47%	<.001
Examination age, mean (mo)	1627	71.0	71.2	.095
Asthma status (% asthmatic)	2150	19%	20%	.82
Atopy	1584	42%	39%	.14
Height, mean (cm)	2019	115.9	115.9	.79
PC ₁₅ FEV ₁ <1.8 mg/mL	537	51%	56%	.28
Older siblings	2200	84%	66%	<.001
HDM SPT positive	1586	31%	27%	.14
Grass SPT positive	1586	25%	25%	.84
Cat dander SPT positive	1587	14%	9%	<.005
Mold SPT positive	1588	13%	11%	.44

TABLE II. Serum ECP level (log and geometric mean) increased with increasing severity of allergen sensitization, as determined on the basis of wheal size

Allergen	Nonsensitized	Mild	Moderate	Severe	P value*
House dust mite					
n	670 (69.2%)	92 (9.5%)	107 (11.1%)	99 (10.2%)	<.001
Log (mean $\pm$ SD)	1.08 $\pm$ 0.32	1.13 $\pm$ 0.38	1.17 $\pm$ 0.30	1.31 $\pm$ 0.34	
Geometric mean (μ g/L)	12.1	13.4	14.8	20.2	
Cat					
n	830 (85.7%)	71 (7.3%)	51 (5.3%)	16 (1.7%)	<.001
Log (mean $\pm$ SD)	1.10 $\pm$ 0.33	1.24 $\pm$ 0.36	1.28 $\pm$ 0.36	1.37 $\pm$ 0.24	
Geometric mean (μ g/L)	12.6	17.5	16.1	23.4	
Grass					
n	729 (75.3%)	77 (8.0%)	75 (7.7%)	87 (9.0%)	<.001
Log (mean $\pm$ SD)	1.10 $\pm$ 0.33	1.11 $\pm$ 0.35	1.18 $\pm$ 0.32	1.24 $\pm$ 0.37	
Geometric mean (μ g/L)	12.6	12.8	15.0	17.4	
Mold					
n	841 (86.9%)	66 (6.8%)	57 (5.9%)	4 (0.4%)	<.001
Log (mean $\pm$ SD)	1.11 $\pm$ 0.34	1.20 $\pm$ 0.36	1.22 $\pm$ 0.28	1.40 $\pm$ 0.46	
Geometric mean (μ g/L)	12.8	15.9	16.4	24.9	

*P value for linear trend by means of ANOVA for each allergen.

levels caused by allergic sensitization grade was an OR of 1.7 (95% CI, 1.4-2.0). Linear trends were seen between serum ECP levels and SPT allergy severity for house dust mite ($F = 37.4$, $P < .001$), rye grass ($F = 13.7$, $P < .001$), cat dander ($F = 16.3$, $P < .001$), and molds ($F = 8.4$, $P < .001$). This relationship also held true for our derived sensitization grade (0-2; $P < .001$, $F = 24.05$; Fig 1). Although in the group as a whole the Serum ECP mean levels were significantly different between grade 1 and grade 2 ($P < .001$; 95% CI, -0.199 to -0.050), this relationship was driven by those with asthma. No differences were seen between the sensitization groups in atopic children without asthma, whereas significant differences in serum ECP levels were seen between the sensitization groups in children with current asthma. Serum ECP levels increased progressively with asthma severity (mild, moderate, and severe; unadjusted OR, 1.5; 95% CI, 1.24-1.72; $P < .001$; Fig 2). This relationship was entirely a result of atopic children with asthma ($F = 13.8$, $P <$

.001), with no linear trend seen in nonatopic children with asthma ($F = 0.38$, $P = .58$).

Measurements of methacholine responsiveness were available from 354 of the children in the study population, with 182 having positive results (PC₁₅FEV₁ <1.8 mg/mL) and 172 having negative results. Although the mean serum ECP level was higher in those with positive methacholine challenge results (18.5 ± 19.0 μ g/L) than in those without such results (16.6 ± 15.0 μ g/L), the difference did not reach statistical significance (mean difference [log ECP], 0.04; $P = .242$; 95% CI, -0.02 to 0.11). Similarly, if the dose-response slope for methacholine responsiveness was used as a continuous variable, no relationship was seen between the dose-response slope and serum ECP levels.

Phenotypic variables that predicted serum ECP levels in linear regression analysis were as follows: age (0.148 μ g/L per month; $P < .001$); asthma (binary variable, $P < .001$) and atopy (binary variable, $P < .001$); and severity of dust mite atopy (0.133 μ g/L per category unit;

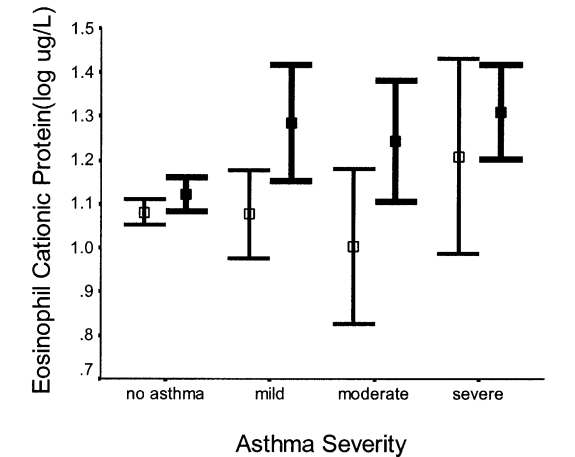


FIG 1. Serum ECP level (group mean log value and 95% CIs) related to sensitization grade (see text for clinical definitions). Data for children with asthma (*thick bars*) are shown separately from data for children without asthma (*thin bars*).

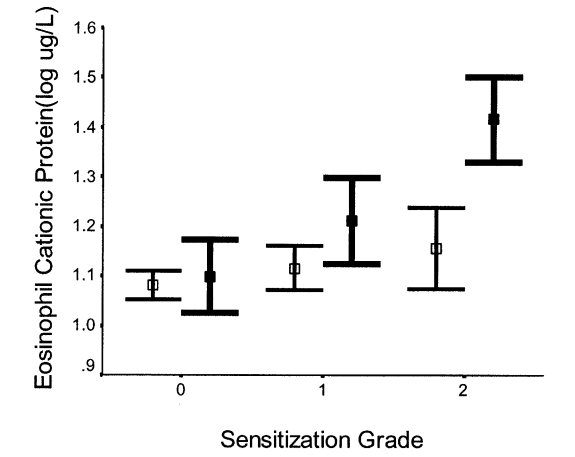


FIG 2. Serum ECP level (group mean log value and 95% CIs) related to clinical asthma severity (see text for clinical definitions). Data for asthma plus atopy (*thick bars*) are shown separately from data for asthma without atopy (*thin bars*).

Table III). Sensitization severity to remaining allergens and current asthma were not significant in the regression model. No family history variables significantly predicted serum ECP levels.

DISCUSSION

The results of the present study showed that serum ECP levels were greater in 6-year-old children with atopy and asthma associated with atopy but were not related to bronchial responsiveness, as judged on the basis of methacholine challenge. Furthermore, serum ECP levels correlated with clinical asthma severity in atopic but not in nonatopic children and with our derived atopy severity variables. The highest levels of serum ECP were seen in children with both current asthma and atopy, suggesting that atopy was a significant driver of asthma in these 6-year-old children.

Asthma in preschoolers is not as closely related to atopy^{1,4} as it is in older children and adults. Not all atopic children have asthma, and nonatopic asthma might well represent the type of asthma that will not persist into adult life. Longitudinal cohort studies have suggested that atopy is responsible for only 50% of wheeze in 6-year-old children¹; indeed, we have previously published data showing that the overlap between asthma and atopy in the cohort studied here is approximately 50%.² Atopic asthma in adults and older children is characterized by persistent airway inflammation, with an increase of eosinophils infiltrating the airway wall and an increase in the levels of markers of eosinophil activation (eg, serum ECP levels). The data presented here suggest that, as a group, children with current asthma associated with atopy are likely to have the type of asthma that persists into adult life. This premise is supported by the dose-response relationship between asthma severity and serum ECP

TABLE III. Significant associations with (log) serum ECP levels on linear regression analysis

	<i>P</i> value	Regression coefficient	95% CI
Age (mo)	<.01	−0.01	−0.02 to −0.01
HDM wheal size (mm)	<.01	0.01	0.00 to 0.02
Atopy and asthma	<.01	0.12	0.04 to 0.19

level that is dominated by those children with current atopy associated with asthma.

The data from the present study support an additional concept, namely that the severity of atopy is an important determinant of clinical outcome. Although this concept is not entirely new,¹⁹ it has not been demonstrated previously in 6-year-old children. In addition, we have shown here that both wheal size, considered as a continuous variable, and our derived variable, sensitization grade, which was created by adding together the severity scores for each of the 4 individual allergen SPT results, are related to serum ECP levels. Although this does not prove our notion that the severity of atopy determines the likelihood that asthma in an individual child is driven by atopy, it certainly warrants further investigation. Further longitudinal follow-up of this cohort will answer this question. Our results also suggest that considering atopy as a binary variable, with individuals having either positive or negative results, might risk underestimating the role atopy plays in the condition under study. This is consistent with the study in older children¹⁹ that showed that atopy considered as a binary variable was not a predictor of asthma persisting through puberty, whereas a derived atopic index, calculated by adding the number of positive test results from the 9 allergens tested, was significantly related to the persistence of asthma (*P* = .04). In the present study we used the

conservative cutoff point of a wheal size of 2 mm or larger for our binary classification. This value is used for infants and young children, whereas a value of 3 mm or greater is generally used for older children and adults. Use of a smaller wheal size would tend to bias against any relationship between atopic status and the variable under consideration (serum ECP level in our case). However, the results of our analyses treating atopic status as a continuous variable, either as wheal size or our derived classification of atopic severity, argues that any single cutoff point is arbitrary and that atopy should be considered as a continuous process. This approach allows the examination of dose-response relationships either for single allergens or multiple sensitizations.

Heightened bronchial responsiveness is also a characteristic feature of persistent asthma in adults. The data from the present study do not show a relationship between BHR, as determined by means of methacholine challenge, and serum ECP levels. There are a number of potential explanations for these findings. Our measure of BHR was determined by examining the relationship between $PC_{15}FEV_1$ and current asthma, defined as all 3 of a physician diagnosis of asthma, asthma symptoms within the previous 12 months, and current use of asthma medication.^{14,20} We have previously reported that the optimal cutoff point for a positive methacholine challenge, determined from receiver operating characteristic curve analysis, was a $PC_{15}FEV_1$ of less than 1.8 mg/mL.¹⁴ Methacholine challenges are not easy to perform in 6-year-old children, and it is possible that our tests lacked the precision associated with methacholine challenges in adults. However, other reports⁹ suggest that genetically distinct pathways determine atopic asthma and BHR. Adults with persistent asthma have chronic airway inflammation with associated thickening of the airway wall,^{10,21,22} which can result in BHR on geometric grounds.²³ This airway remodeling is thought to be a major factor in persistent asthma in adults.^{10,21,22} However, there is little or no evidence that asthma in young children is associated with airway remodeling. The lack of association between serum ECP levels and BHR in the present study is consistent with dissociation between the expression of atopic disease and BHR.

As well as examining the relationships between serum ECP levels and atopic asthma, the results of the present study serve to present normal values of serum ECP in a community sample of 6-year-old children. The birth cohort studied here was recruited without reference to the atopic or asthmatic status of the parents and is reasonably representative of the population of Perth, Western Australia. These data show that a wide range of serum ECP values are seen at this age but that there is no difference between male and female patients. Children in the present study were assessed when they were healthy and had been free of respiratory infections for at least 14 days. Thus we are reporting baseline levels and not those associated with exacerbations of respiratory disease. Although the levels of serum ECP are higher in children with atopy and asthma and are an indication of a differing phenotype, the wide

ranges seen are likely to limit the utility of measuring serum ECP levels in individual children as a guide to the diagnosis or management of asthma at this age.

In conclusion, the data from the present study suggest that current asthma associated with atopy in 6-year-old children is likely to represent the type of asthma that persists into adult life. In addition, our data suggest that atopy should be considered as a continuous variable and that the more severe the atopy, either defined by the wheal size of individual allergen SPTs or by our derived variable of sensitization grade, the more likely that atopy is driving the asthma in an individual child. These data suggest that 6-year-old children with current asthma and positive SPT responses should be considered as having atopic asthma and that the larger the wheal or the more allergens to which the child is sensitized, the more likely that this represents persistent asthma.

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