

Clinical significance of bronchoalveolar eosinophils in childhood asthma

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Asthma, rhinitis,
other respiratory
diseases

To evaluate the relationship between clinical parameters and differential cell counts, bronchoalveolar lavage was performed in 79 asthmatic infants and children with unusual asthma. Multivariate analysis showed significant associations between (1) allergic asthma and the presence of alveolar eosinophils and (2) persistent and longer asthma and an increased number of alveolar neutrophils. Our results provide the first evidence that in asthmatic infants and children eosinophilic inflammation is related to allergic sensitization. (*J Allergy Clin Immunol* 2002;110:42-4.)

Key words: Asthma, child, infant, bronchoalveolar lavage, eosinophils, neutrophils

Asthma is a chronic bronchial inflammatory disease characterized by a mucosal eosinophil and mast cell infiltrate controlled by cytokines from T_H2 lymphocytes. In adult asthma, a close relationship exists between eosinophilic inflammation in bronchial biopsy specimens and bronchoalveolar lavage fluid (BALF) and disease severity.¹ Furthermore, an important role for neutrophils has been demonstrated in acute severe asthma.² In children, the recruitment of eosinophils in BALF is associated with atopic asthma but is not seen in atopic diseases without asthma or viral-associated wheeze³; this suggests different pathophysiologic mechanisms in wheezing children.

We used multivariate analysis to evaluate the relationship between clinical parameters (age, asthma duration, allergic sensitization, bacterial airway infection, and asthma activity) and differential alveolar cell counts in a large population of asthmatic infants and children.

METHODS

Study design

This study, both prospective (1994-1999) and retrospective (1991-1993), involved infants and children with potentially unusual

Abbreviations used

AA: Allergic asthma
BAL: Bronchoalveolar lavage
BALF: Bronchoalveolar lavage fluid
IC: Inhaled corticosteroid

asthma—ie, with (1) recurrent or persistent wheezing resisting conventional treatment, (2) wheezing associated with daily cough with expectoration, and (3) persistent parenchymal shadowing. Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) was performed to eliminate the possibility of other diseases known to provoke wheezing, leading to the exclusion of 9 patients with aspiration of foreign body, tracheomalacia, vascular or pulmonary malformations, or cystic fibrosis and 6 patients with viral respiratory infection detected in BALF. In infants ≤ 30 months old, the criteria used to characterize asthma were (1) those of Tabachnik and Levison⁴ and (2) the Martinez⁵ parameters associated with a high risk of asthma persisting from infancy to childhood—ie, (a) elevated total serum IgE, (b) parental or personal atopic history (eg, atopic dermatitis and/or seasonal rhinitis), and (c) persistent wheezing at the age of 4 years. Twenty wheezing infants without these criteria were excluded from BALF analysis. In children, asthma diagnosis was supported by a history of recurrent wheezing and dyspnea attacks with proven β_2 -agonist reversible flow limitation. The prospective study was approved by the Institutional Review Board.

Data collection

Information on each patient's age, sex, asthma duration, parental and personal atopic history, total serum IgE, and inhaled corticosteroid (IC) treatment was collected. Allergic asthma (AA) was defined as an asthma with allergic sensitization as evidenced by positive skin prick test results and/or specific IgE against common aeroallergens, as well as symptoms usually triggered by these allergens. Asthma activity during the preceding year was characterized as episodic (with extended asymptomatic intervals between asthma attacks) or persistent (with persisting symptoms occurring regularly between asthma attacks).

BAL procedure

Fibreoptic bronchoscopy and BAL were carried out as described previously.⁶ Bacterial airway infection was defined by $\geq 10^5$ colony-forming units of bacteria per milliliter with $\geq 10^4$ neutrophils per milliliter in BALF.⁶ Differential cell counts were performed on BALF cytopsin preparations stained with May Grünwald Giemsa and hematoxylin-eosin. Normative values for differential cell counts were as defined previously.⁷

Statistical analysis

Results are expressed as means $\pm$ SDs. Statistical differences were assessed through use of χ^2 testing or ANOVA and Mann and

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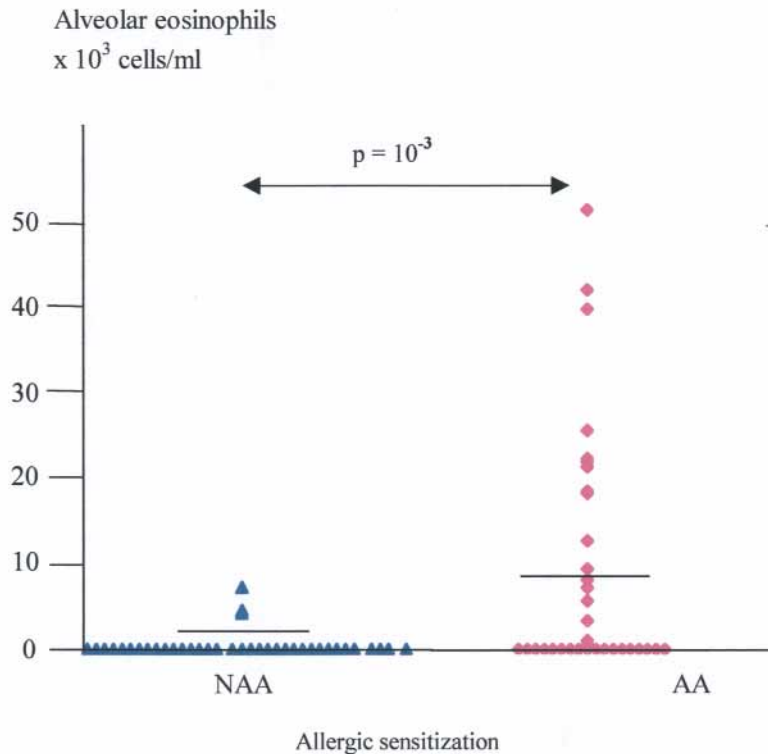
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FIG 1. Alveolar eosinophil counts related to allergic sensitization. NAA, Nonallergic asthma; AA, allergic asthma. The line represents the mean results.

TABLE I. Differential alveolar cell counts according to age, type of asthma (nonallergic vs allergic), bacterial airway infection, asthma activity, and inhaled corticosteroid treatment

	Alveolar cell count				
	Total 10 ³ /mL	Macrophages 10 ³ /mL	Lymphocytes 10 ³ /mL	Neutrophils 10 ³ /mL	Eosinophils 10 ³ /mL
Total population (n = 79)	257.7 ± 147.7	196.0 ± 120.3	41.6 ± 38.5	17.2 ± 47.4	4.8 ± 10.6
Infants (n = 21)	292.9 ± 142.4*	235.3 ± 123.4*	38.7 ± 38.9	18.8 ± 51.4	± 0.2‡
Children (n = 58)	244.7 ± 148.7	178.4 ± 118.3	41.9 ± 38.7	13.3 ± 39.9	6.4 ± 11.9
Asthma					
Nonallergic (n = 41)	251.3 ± 130.3	191.8 ± 107.3	41.7 ± 36.9	17.5 ± 56.9	0.4 ± 1.5§
Allergic (n = 38)	273.4 ± 170.7	199.6 ± 140.9	43.9 ± 41.9	13.0 ± 25.6	8.4 ± 13.5
Bacterial airway infection					
No (n = 66)	261.2 ± 154.6	193.3 ± 128.2	43.6 ± 41.0	15.8 ± 46.8	4.4 ± 9.4
Yes (n = 13)	240.0 ± 109.8	195.0 ± 83.9	28.3 ± 17.3	9.8 ± 9.3	6.5 ± 15.6
Asthma activity					
Episodic (n = 45)	267.7 ± 154.6	212.5 ± 120.4	44.6 ± 42.8	6.1 ± 6.5*	4.4 ± 10.1
Persistent (n = 34)	246.1 ± 141.3	171.0 ± 122.0	35.6 ± 32.2	26.4 ± 63.7	5.3 ± 11.4
Inhaled corticosteroid treatment					
No (n = 19)	313.9 ± 167.3*	239.8 ± 123.5*	61.0 ± 51.4†	5.2 ± 6.4	7.7 ± 13.8
Yes (n = 60)	239.7 ± 137.5	178.9 ± 118.2	34.7 ± 31.4	17.8 ± 48.8	3.8 ± 9.2

Differential alveolar cell counts were in infants, nonallergic asthma, no bacterial airway infection, episodic asthma, and no inhaled corticosteroid treatment versus children, allergic asthma, bacterial airway infection, persistent asthma, and inhaled corticosteroid treatment, respectively.

* $P \leq .05$ in bivariate analysis.

† $P \leq .01$ in bivariate analysis.

‡ $P \leq .00001$ in bivariate analysis.

§ $P \leq .001$ in bivariate analysis.

Whitney tests. The multiple linear regression model included clinical parameters and differential cell counts as dependent and independent variables, respectively. In addition, a stepwise regression analysis was performed (BMDP software package, Los Angeles, Calif). P values less than .05 were considered significant.

RESULTS

Patient characteristics

Of 6513 asthmatic patients hospitalized, 88 underwent fiberoptic bronchoscopy (transient bronchospasm occurred

in 12% of the patients without major complications). Seventy-nine patients (21 infants aged 15 ± 7.2 months and 58 children aged 92.9 ± 56 months) were further analyzed. Parameters associated with age—asthma duration (5.2 ± 7.6 months and 32.8 ± 38.2 months for infants and children; $P < .00001$), total serum IgE (252 ± 981 IU/L and 696 ± 1456 IU/L, respectively; $P < .001$), and AA frequency (14% and 61%; $P = 2 \times .0002$)—were higher in children. Conversely, these populations of infants and children were homogenous according to parental (62% and 69%, respectively) and personal (62% and 41%, respectively) atopic history as well as bacterial airway infection (19% and 16%, respectively). Among the 13 infected BAL, 4 had resistant bacteria such as β -lactamase-producing *Hemophilus influenzae* and *Morexalla catarrhalis* as well as *Streptococcus pneumoniae* with intermediate sensitivity to ampicillin. Although persistent asthma was less common in infants than in children (33% and 47%, respectively; $P = .002$), the percentages of infants and children (67% and 79%, respectively) treated with IC (attesting the disease severity) were identical.

Total and differential alveolar cell counts

Bivariate analysis (Table I) showed that in contrast to asthmatic children, asthmatic infants had a global BALF hypercellularity, mainly represented by alveolar macrophages. The number of alveolar eosinophils in BALF was significantly higher in asthmatic children than in their infant counterparts ($P \leq .00001$). Elevated alveolar eosinophil counts were found in AA but not in nonallergic asthma ($P \leq .001$; Fig 1). Elevated alveolar neutrophil counts were found in persistent as compared to episodic asthma ($P \leq .05$). A highly significant decrease in the number of total alveolar cells, affecting alveolar macrophages and lymphocytes, was observed in IC-treated patients as compared to their untreated counterparts.

Multivariate analysis with all clinical parameters for 73 patients, together with the expression of differential alveolar cell counts, showed significant associations between AA and the presence of alveolar eosinophils (with a partial regression coefficient [β] $\pm$ SE of 8.92 ± 2.65 [$P = .001$] and a coefficient of determination of 0.22). Asthma duration and persistent asthma were significantly associated with an increased number of alveolar neutrophils ($\beta \pm$ SE, 0.59 ± 0.20 [$P = .001$] and 21.86 ± 10.77 [$P = .05$], respectively; coefficient of determination, 0.20).

DISCUSSION

Inasmuch as our study included selected asthmatic infants with a high risk of persistence of asthma into childhood, it showed that airway inflammation, involving eosinophils and neutrophils, was related to different asthma phenotypes.

The main result of the study is that in asthmatic children, eosinophilic inflammation is related to allergic sensitization. We found that allergic sensitization was more frequent in asthmatic children than in their infant counterparts, in whom inflammation was transient and related to unspicif-

ic cells. However, a step-by-step multiple regression analysis indicated that eosinophilic inflammation was related neither to age nor to asthma activity. This might reflect a progressive allergic sensitization under environmental pressures in predisposed subjects, and it contrasts with what is seen in adults, in whom airway eosinophilia is related to asthma severity.¹ The former possibly reflects the fact that most of our patients were treated with ICs, resulting in a downregulation of eosinophilic inflammation. However, this is unlikely, because alveolar eosinophil numbers were not higher in treated patients than in untreated patients. It should also be noted that increased sputum eosinophilia in asthmatic children persists even with high doses of IC.⁸ Moreover, in asthmatic adults,⁹ IL-5 antibodies reduce sputum eosinophilia without modifying airway hyperresponsiveness. This, together with our results, brings into question the likelihood of a widely hypothesized causal relationship between airway eosinophilia and bronchial hyperresponsiveness.

In our study, the high percentage of bacterial infection might be a feature of a highly selected asthmatic population. Our analysis showed that neutrophilic inflammation was not statistically related to bacterial airway infection and was weakly but significantly related to persistent asthma and asthma duration (which might reflect asthma persisting after childhood, representing the most severe forms of the disease). Our results were in agreement with published data reporting increased sputum neutrophilia in asthmatic adults with acute as well as chronic persistent severe asthma.¹⁰

Further studies are required to assess the importance of our findings with regard to new drugs designed to inhibit neutrophil and eosinophil chemotaxis in the airways.

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