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ORIGINAL ARTICLE

Presence of Eosinophils in Nasal Secretion during Acute Respiratory Tract Infection in Young Children Predicts Subsequent Wheezing within Two Months

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ABSTRACT

Background: In young children with wheezing or bronchiolitis, especially with respiratory syncitial virus, blood eosinophilia and a high eosinophil cationic protein level in nasal secretions predicts subsequent wheezing in later childhood. However, whether eosinophil activation results from virus-induced inflammation or local eosinophilia per se precedes the onset of wheezing remains unknown. In the present study, we examined the association between the presence of nasal eosinophils during respiratory tract infection (RTI) and subsequent wheezing in young children.

Methods: A total of 35 young children less than 3 years of age who visited our outpatient clinic with rhinorrhea between April and July 2004 were enrolled in this prospective cohort study. Subjects who were given diagnoses of allergic rhinitis were excluded. In all the subjects, the presence of eosinophils in nasal secretions was determined. The subjects were followed, and the cumulative incidences of wheezing during the subsequent 2- and 12-month periods were examined.

Results: According to a logistic regression analysis adjusted for age, sex, family history, allergies, and wheezing at entry, young children with nasal eosinophil infiltration during acute RTI had a significantly higher risk of wheezing during the subsequent 2 months, compared with those without nasal eosinophil infiltration (adjusted odds ratio, 27.618, $p = 0.016$).

Conclusions: Our findings not only suggest that nasal eosinophil testing may serve as a convenient clinical marker for identifying young children at risk for subsequent wheezing, but also shed new light on the role of eosinophils in the onset of wheezing in young children.

KEY WORDS

eosinophils, nasal secretion, respiratory tract infection, wheezing, young children

INTRODUCTION

Wheezing is a very common symptom of lower respiratory tract infection (RTI) in infants; 34% of infants experience at least one wheezing episode by the age of 3 years, while 49% experience at least one episode by the age of 6.[1] The small absolute size of the airways, airway edema, mucus hypersecretions, impaired humoral protections and immature immune responses render infants and young children particularly susceptible to airway obstruction and can cause wheezing.[2,3] The majority of infants with wheezing have transient conditions associated with diminished airway function at birth and do not have increased risks of asthma or allergies later in life. In a substantial minority of infants, however, wheezing episodes

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are probably related to a predisposition to asthma.[1] Stein *et al*. have reported three different wheezing phenotypes in childhood: "transient early wheezing", "non-atopic wheezing" and "IgE-associated wheeze/asthma".[4] They also showed that only one third of wheezing infants exhibit persistent wheezing at any age with methacholine hyperresponsiveness, peak flow variability, and markers of atopy.

Bronchiolitis is one of the most severe types of lower RTI in infancy and is the most common disease requiring the hospitalization of infants among developed countries.[5] Approximately 70% of viral bronchiolitis is caused by respiratory syncytial virus (RSV) infection.[6] However, RSV is a common pathogen for RTIs among all age groups, especially during the winter season, and the symptoms of most patients with RSV infection are restricted to within the upper airways.[7,8] Thus, additional factors, such as Th2-deviated immune responses[9] or allergic predispositions,[10] are thought to play some roles in the onset of RSV-induced lower RTI, including bronchiolitis. RSV bronchiolitis in early childhood is an important risk factor for the subsequent development of asthma.[11,12]

In infants with wheezing or bronchiolitis, it is extremely important to investigate useful clinical markers capable of distinguishing early asthma patients from transient early wheezers to initiate possible early intervention or primary prevention strategies. For this purpose, a clinical index for defining the risk of asthma in young children with recurrent wheezing has been investigated.[13] Along with a familial history of asthma and a past history of atopic dermatitis, peripheral blood eosinophilia has been reported to be a valuable marker for predicting subsequent wheezing.[13] In addition, the serum eosinophil cationic protein (ECP) levels at the time of the first wheezing episode[14] and the nasal ECP levels[15,16] were also reported to be strong predictors of subsequent wheezing and asthma.

In infants with bronchiolitis, a number of investigators have reported similar findings-namely, that blood eosinophilia[17-19] and elevated serum ECP levels predict the onset of subsequent wheezing,[20-23] even though neutrophil activation is reportedly correlated with disease severity.[24,25] However, some controversial observations have also been reported.[22,26] Such controversy may be due to differences between local immune responses and peripheral blood testing.[27-30] For example, in patients with abundant eosinophil accumulation in the lung during the acute phase of bronchiolitis, the eosinophil ratio in the peripheral blood may be relatively low. Therefore, markers of local eosinophil activation should be expected; ECP[22,31] or RANTES[32] concentrations in nasal lavage fluid or nasal discharge during bronchiolitis have also been reported to be highly predictive of subsequent wheezing.

These findings strongly suggest that local eosinophilic inflammation during wheezing or bronchiolitis is positively associated with subsequent wheezing in young children with wheezing episodes or bronchiolitis. However, whether eosinophil activation results from virus-induced inflammation or local eosinophilia per se precedes the onset of wheezing remains unknown. This point is of particular interest for determining the role of eosinophils in the formation of wheezing in young children. In the present study, we prospectively examined the association between the presence of nasal eosinophils during RTI and subsequent wheezing in young children.

METHODS

SUBJECTS

A total of 35 young children (19 boys and 16 girls, age 6–33 months) who visited our outpatient clinic in Kochi, Japan, between April and July, 2004, and whose chief complaint was rhinorrhea were enrolled in this prospective cohort study. None of the subjects had experienced more than one wheezing episode prior to enrollment. Subjects who had been given diagnoses of allergic rhinitis by a physician were excluded from the study.

Informed consent was obtained from the parents of all the participating subjects, and the study was approved by the Ethics Review Board of Kochi Medical School.

METHODS

At the time of entry, the parents of the subjects answered a questionnaire regarding the age, sex, and the history of physician-diagnosed allergic diseases, including atopic dermatitis, food allergy, asthma, allergic conjunctivitis and urticaria, of both the subjects and their parents.

Nasal secretions were sampled during the first visit by swabbing the bilateral middle one-third inferior turbinates with cotton swabs. The samples were spread onto a slide glass, air-dried, fixed, and stained with Wright-Giemsa solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The samples were then evaluated for the presence or absence of eosinophils by examining 5 high power fields (HPFs) with a light microscope (Olympus, Tokyo, Japan).

The subjects were further examined by the same pediatrician 2 and 12 months after the first visit. The cumulative incidences of wheezing during the follow-up periods were then determined.

STATISTICS

A logistic regression model adjusted for age, sex, family history, allergic diseases in the subjects, wheezing at study entry, and eosinophils in the nasal secretion was analyzed using STATA software (StataCorp LP, College Station, TX, USA) and considered to be significant when $p < 0.05$. The post-estimated goodness of fit (Hosmer-Lemeshow) was confirmed

Table 1 Descriptive characteristics of the subjects at study entry and the presence or absence of eosinophils in nasal secretions

Characteristics †	Total		Eosinophils in nasal secretions				*p* value
	(*N* = 35)		Yes (*N* = 16)		No (*N* = 19)		
Age (months) ‡	16.5	(6−33)	18.2	(6−32)	15.0	(6−33)	0.407
Sex (M/F) §	19/16	(54)	10/6	(63)	9/10	(47)	0.371
Wheezing at study entry (Y/N) §	14/21	(40)	8/8	(50)	6/13	(32)	0.268
Family history §	16/19	(46)	8/8	(50)	8/11	(42)	0.640
Allergic diseases in the subject ¶							
Atopic dermatitis	5/30	(14)	1/15	(7)	4/15	(21)	0.347
Food allergy	7/28	(20)	2/14	(13)	5/14	(26)	0.415
Asthma	4/31	(11)	2/14	(13)	2/17	(11)	1.000

† Mean value or the number in the population, and the percentage or range.
‡ Two-sample Wilcoxon rank-sum (Mann-Whitney) test.
§ χ^2 test.
¶ Fisher's exact test.

Table 2 Laboratory findings for peripheral blood samples at study entry and the presence or absence of eosinophils in nasal secretions

Laboratory findings †	Total	Eosinophils in nasal secretions		*p* value ‡
		Yes	No	
	(*N* = 21)	(*N* = 11)	(*N* = 10)	
Total IgE (IU/mL)	472.1 (5−2700)	546.8 (5−2700)	667.6 (5−2100)	0.898
Percent of eosinophil (%)	4.9 (0−32)	5.3 (0−32)	4.4 (0.7−10)	0.934
Number of eosinophils (/mm^3)	486.5 (0−3648)	537.3 (0−3648)	430.6 (48.3−1150)	0.933

† Mean value or the number in the population and the percentage or range are shown.
‡ Two-sample Wilcoxon rank-sum (Mann-Whitney) test.

for all logistic regression analyses. The χ^2 test, Fisher's exact test and Wilcoxon rank-sum (Mann-Whitney) test were also performed using STATA software.

RESULTS

All 35 subjects completed the 12-month study. The descriptive characteristics of the subjects at entry are shown in Table 1. Age, sex, presence of wheezing at entry, family history of allergic diseases and allergic diseases in the subject were not associated with the presence or absence of eosinophils in the nasal secretions at entry (Table 1). The total IgE level, peripheral blood eosinophil ratio, and the number of eosinophils also were not associated with the presence or absence of eosinophils in the nasal secretions at entry (Table 2). In addition, both the maternal and paternal histories of any allergic diseases, particularly allergic rhinitis or asthma, were not associated with the presence or absence of eosinophils in the nasal secretion at entry (data not shown).

The age of infection has been reported to regulate cytokine production and disease patterns later in life.[33] Sex and a family history of allergic diseases are well-known to be associated with the risk of allergic diseases during childhood.[4] In addition, children with wheezing episodes tend to wheeze more than those without episodes.[1] To evaluate the effects of these confounding factors, a logistic regression model adjusted for age, sex, family history, allergic diseases in the subject and presence or absence of wheezing at entry was analyzed (Table 3). According to the logistic regression analysis, young children with nasal eosinophil infiltration during acute RTI had a significantly higher risk of wheezing during the subsequent 2-month period than those without nasal eosinophil infiltration (adjusted odds ratio (OR) 27.618; p = 0.016). The presence of wheezing at study entry was also a strong risk factor for subsequent wheezing (adjusted OR, 20.324; p = 0.024), as expected.[1]

In contrast, nasal eosinophil infiltration during acute RTI was not associated with the risk of wheezing during the subsequent 12-month period (adjusted OR, 4.099; p = 0.161; Table 4).

Table 3 Effect of age, sex, allergic diseases in the subject, family history, eosinophils in nasal secretions and wheezing at study entry on the risk of wheezing during the subsequent 2 months

Variable	Total	Wheezing positive	(%)	Crude OR (95% CI)	†Adjusted OR (95% CI)	*p* value
Age	35	15	(43)	1.012 (0.939－1.090)	1.048 (0.929－1.181)	0.445
Sex						
Male	19	7	(37)	1.000	1.000	
Female	16	8	(50)	1.714 (0.443－6.629)	1.240 (0.186－8.254)	0.824
Allergic diseases in the subjects						
No	21	10	(48)	1.000	1.000	
Yes	14	7	(50)	1.000 (0.284－4.256)	3.919 (0.293－52.414)	0.302
Family history of allergic diseases						
No	19	10	(53)	1.000	1.000	
Yes	16	5	(31)	0.409 (0.102－1.640)	0.267 (0.032－2.218)	0.222
Wheezing at study entry						
No	21	7	(33)	1.000	1.000	
Yes	14	8	(57)	2.667 (0.661－10.751)	20.342 (1.480－279.619)	0.024 *
Eosinophils in nasal secretions						
No	19	7	(37)	1.000	1.000	
Yes	16	8	(50)	1.714 (0.443－6.629)	27.618 (1.859－410.201)	0.016 *

†Adjusted for age, sex, family history, allergic diseases in the subject, eosinophils in nasal secretions and wheezing at study entry.
*$p < 0.05$.

Table 4 Effect of age, sex, allergic diseases in the subject, family history, eosinophils in nasal secretions and wheezing at study entry on the risk of wheezing during the subsequent 12 months

Variable	Total	Wheezing positive	(%)	Crude OR (95% CI)	†Adjusted OR (95% CI)	*p* value
Age	35	15	(43)	1.012 (0.939－1.090)	1.001	0.977
Sex						
Male	19	7	(37)	1.000	1.000	
Female	16	8	(50)	1.714 (0.443－6.629)	3.287 (0.582－18.571)	0.178
Allergic diseases in the subject						
No	21	7	(33)	1.000	1.000	
Yes	14	8	(57)	2.667 (0.661－10.751)	5.128 (0.751－35.020)	0.095
Family history of allergic diseases						
No	19	10	(53)	1.000	1.000	
Yes	16	5	(31)	0.409 (0.102－1.640)	0.296 (0.060－1.458)	0.161
Wheezing at study entry						
No	21	7	(33)	1.000	1.000	
Yes	14	8	(57)	2.667 (0.661－10.751)	2.488 (0.438－14.127)	0.304
Eosinophils in nasal secretions						
No	19	7	(37)	1.000	1.000	
Yes	16	8	(50)	1.714 (0.443－6.629)	4.099 (0.577－29.500)	0.161

†Adjusted for age, sex, family history, allergic diseases in the subject, eosinophils in nasal secretions, and wheezing at study entry.

DISCUSSION

In young children with wheezing and in young children with bronchiolitis, especially as a result of RSV infection, blood eosinophilia and nasal ECP predict subsequent wheezing. To test whether eosinophil activation is a result of virus-induced inflammation or local eosinophilia per se precedes the formation of wheezing, we prospectively examined the association between the presence of nasal eosinophils during RTI and subsequent wheezing in young children. As a result, we found that children with eosinophil infiltration in their nasal secretions during RTI were more likely to experience episodes of wheezing during the subsequent 2 months. The lack of association between the number of peripheral blood eosinophils

and the presence of nasal eosinophils (Table 2) suggests the presence of some specific mechanisms attracting eosinophils to the nasal mucosa during RTI in young children.

Nasal eosinophils probably do not play a direct role in the lower airway. However, recent studies have demonstrated that allergic inflammation in the upper airway is closely related to allergic inflammation in the lower airway; this phenomenon is now referred to as "One Airway, One Disease".[34] In other words, patients with nasal eosinophil infiltration tend to have eosinophil infiltration in their lungs as well.

Among the virus-induced chemokines that are present in the lung,[35] RANTES is known to recruit,[36] prime and activate eosinophils *in vitro*.[37] Eosinophil activation, especially leukotriene production, may participate in the formation of allergic inflammation and may cause wheezing. As a matter of fact, the levels of leukotrienes, but not of prostaglandin D2, are elevated in the bronchoalveolar lavage fluid of young children with persistent wheezing, suggesting that eosinophil activation, but not mast cell activation, plays critical roles in the formation of inflammatory reactions in the lung, particularly in young children.[38] In addition, anti-inflammatory therapy with oral corticosteroid[39] or with leukotriene antagonists[40] reduced the incidence of subsequent wheezing in children with bronchiolitis. However, a recent early interventional study with intermittent inhaled corticosteroids from infancy failed to alter the onset of asthma.[41] Further investigations are surely needed to determine whether treatment with corticosteroids or leukotriene antagonists can modify the natural course of asthma in young children.

Our findings strongly suggest that the detection of nasal eosinophils during RTI may be a clinically useful marker of the risk of subsequent wheezing. Young children with nasal eosinophil infiltration during acute RTI should receive early interventions; practically speaking, this means that the avoidance of irritants, including passive smoke,[7] and the avoidance of the aeroallergen exposure to diminish allergic sensitization[42] should be strongly recommended.

In a previous study, the effect of eosinophil activation during bronchiolitis on the increased risk of asthma persisted until adulthood.[27] In our study, however, nasal eosinophil infiltration during RTI showed a positive association only with the near-term risk of wheezing (2 months), but not with the long-term risk of wheezing (12 months). This may reflect the fact that eosinophils accumulating in the airway survive only a short period of time and require an additional supply from the peripheral blood to maintain the allergic inflammatory reactions that cause recurrent wheezing.

This study has some limitations. First, virus detection was not performed in any of the subjects, although the study was performed during the non-RSV season.[8] Host immune responses against individual viruses are not the same[16,43,44]; eosinophil-recruiting chemokines are strongly produced and released from bronchial epithelial cells after *in vitro* stimulation with RSV.[45,46] Eosinophils have been reported to be activated by RSV-induced chemokines,[37] by RSV directly,[47] and by RSV-infected epithelial cells through CD18-mediated interactions.[48] Thus, whether the association between nasal eosinophils and subsequent wheezing is affected by different types of viruses should be further examined. Second, the number of eosinophils in the nasal secretions was only qualified, but not quantified. Determining whether an association exists between the number of infiltrating eosinophils and subsequent wheezing would be a point of interest. Finally, 14 subjects exhibited wheezing at study entry, even though the number of wheezing episodes had been no more than one. In this context, the subjects were not considered to have "upper respiratory tract infections".[15] However, the subject population was typical of that visiting private outpatient clinics; as mentioned earlier, wheezing is a very common symptom in infancy and occurs in approximately one third of infants under 3 years of age.[1] In addition, our logistic regression model for analyzing the effect of the presence of nasal eosinophils on subsequent wheezing included an adjustment for the effect of wheezing at study entry.

Our findings strongly suggest that eosinophil activation precedes an allergic symptom, wheezing, in subjects with RTI. Our findings were compatible with our previous observation that eosinophilia in the cord blood preceded the onset of infantile eczema.[49] However, whether eosinophils play important roles in the onset of allergic diseases or are just a marker of allergic symptoms remains uncertain, even though eosinophils certainly play critical roles in airway remodeling.[50] In conclusion, the present findings not only suggest that nasal eosinophil testing may serve as a convenient clinical marker for identifying young children at risk for subsequent wheezing, but also shed new light on the role of eosinophils in the onset of wheezing in young children.

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