

Eosinophils and basophilic cells in sputum and nasal smears taken from infants and young children during acute asthma

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To examine the increase in eosinophils and basophilic cells in the respiratory areas of infants and young children with asthma ($n = 111$), we analyzed the numbers of eosinophils and basophilic cells in sputum and nasal smears. The number of children with eosinophilia grades of 2+ or greater (≥ 11 cells/five fields in $\times 1000$ magnification) in sputum and nasal smears, respectively, in each age group was as follows: 33% and 21% under 1 year, 59% and 64% at 1 year, and 79–80% and 75–78% at 2–3 years. An increased infiltration of basophilic cells according to age was also observed. Infiltration of these cells began earlier than the appearance of IgE antibodies to mite in blood.

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In adult asthma, studies employing fiberoptic bronchoscopy and examination of bronchoalveolar lavage (BAL) fluid have revealed serial changes in inflammatory cells and chemical substances (1–5). Such studies have shown mast cells, whose appearance precedes infiltration by eosinophils, to be the primary effector cells and the primary contributor to the asthmatic response (6). In childhood asthma, Ferguson & Wong (7), using BAL techniques, showed that an increased eosinophil count is closely correlated with histamine hyperresponsiveness. However, the mean age of their subjects was over 10 years, and it is difficult to apply their techniques to younger children. Several reports have discussed the use of sputum samples for the evaluation of cell components (8–12). Frigas et al. (9) demonstrated that in the sputa of patients with exacerbated asthma, eosinophil-derived major basic protein was markedly elevated. Pin et al. (10, 12) demonstrated a relationship between increased eosinophil and basophilic cell counts in sputa and histamine airway hyperresponsiveness.

Although common mechanisms may induce exacerbated asthma in adults and children, some etiologic characteristics differ between adult and childhood asthma. For example, many viral respiratory infections which may trigger wheezing, and asthma attacks often occur during childhood (13–16). The activation of eosinophils in nasal smears taken from infants with respiratory syncytial virus (RSV) infec-

tion or bronchiolitis has also been reported (17, 18). In a previous study, we demonstrated infiltration of eosinophils and basophilic cells in nasal smears taken from infants under 1 year of age with bronchiolitis, and the number of these cells increased in children with recurrent bouts of wheezing (19). Borres et al. (21) stated that the appearance of nasal metachromatic cells in infants was associated with atopic propensity. On the other hand, eosinophils were rare in the nasal smears of normal infants (20). On the basis of these results, an activation and increase in the number of these cells seem to be a key point in the development of asthma.

The purpose of the present study was to demonstrate the development of the inflammatory state as characterized by the accumulation of eosinophils and basophilic cells in the upper and lower respiratory tracts of infants and young children who had been diagnosed as having asthma.

Material and methods

Subjects

The subjects were asthmatic children who had experienced more than two bouts of recurrent wheezing with respiratory distress. They were treated at Chiba Children's Hospital, Chiba Prefecture, Japan, with various bronchodilators, such as xanthine or beta-agonists, according to the severity of the wheezing

symptoms. Bronchial provocation tests were not performed due to their ages. Children with other organic complaints, such as respiratory tract obstruction, cardiac disease, congenital respiratory anomaly, or foreign bodies, were excluded. A total of 111 patients were examined: 26 were younger than 1 year of age, 39 were 1 year of age, 21 were 2 years of age, and 25 were 3 years of age. Nasal-smear, sputum, and blood-samples were collected during routine outpatient checkups. Although the time lag from the onset of wheezing to sampling was variable, none of the patients experienced chronic wheezing. The younger the patient, the higher was the probability of being hospitalized after medical consultation. Since wheezing was improved by inpatient treatment and secretion was reduced, it was important to collect adequate sample volumes before starting the treatment. Cyto-diagnosis, RSV testing, and bacterial culture were also performed on each sample. In exceptional cases when an adequate amount of sample was not available, sampling was done a few hours after transfusion or treatment, but steroid medication was withheld during testing.

Examination of sputum and nasal smears

Nasal smears were obtained from the subjects by passing a straight cotton swab along the base of the nose and drawing it under the inferior turbinate with a twisting motion (19). Sputum was obtained with a suction tube connected to the aspirator system installed on the wall over the bed head. When aspirating sputum, the examiner rubbed the lower portion of the patient's cricoid cartilage to initiate the coughing reflex. Coughing drove sputum up along the trachea, where it was collected by the aspirator. If the infant patient was crying vehemently, this stimulation would be ineffective. In such a case, it was necessary to take sputum quickly at the moment when coughing was triggered by the crying or the aspiration.

In any case, it was essential that the patient produce wet cough. Sputum and nasal materials were smeared over the entire surface of a glass slide and dried either spontaneously or with a fan. Samples were stained with Hansel's stain. For sputum, the presence of alveolar macrophages in the specimen was considered evidence of secretion from the lower respiratory tract. For nasal smears, specimens with many squamous epithelial cells and few other cellular components were excluded from the study. Thus, the percentages of samples suitable for histologic examination were 84.7% (94/111) for sputa and 90.1% (100/111) for nasal smears.

Distribution and cell densities were not always regular. We recorded eosinophil concentrations in nasal smears and sputa by means of an approximate grading system (–, ±, +, 2+, 3+, 4+) in which total eosinophils in five microscopic fields of $\times 1000$ magnification were counted. Classification and gradation procedures for eosinophils have been previously described (19).

Actual microscopic observation was done as follows. After having made sure that an adequate number of cells was deposited on a slide glass, a quick observation was made of the entire sample under $\times 100$ magnification. If the allergic state was in progress, many eosinophils would be recognized in the microscopic field. Otherwise, the distribution of eosinophils was surveyed at $\times 100$ magnification to seek aggregations of these cells. The cells were thus quantified under $\times 1000$ magnification. Up to five aggregated fields were selected to determine the total number of cells. For basophilic staining cells not basophils, we did not scan the whole slide but used the average number of these cells from five microscopic fields, at $\times 1000$ magnification, in which basophilic cells seemed to be especially prevalent. Shioda & Mishima (22) and Kajosaari et al. (23) scanned whole slides and recorded the absolute total number of mast cells.

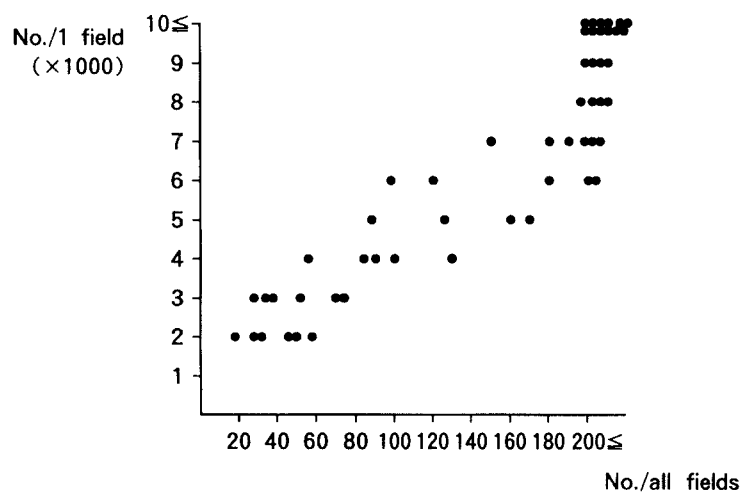


Fig. 1. Relation between number of basophilic cells in one field and all fields.

Table 1. Frequency of eosinophils in sputum by age

Eosinophil grade	Age group (years)			
	<1 n = 18	1 n = 37	2 n = 20 (%)	3 n = 19
≥4+	2 (11.1)	8 (21.6)	13 (65.0)	12 (63.2)
3+	0	9 (24.3)	1 (5.0)	3 (15.8)
2+	4 (22.2)	5 (13.5)	2 (10.0)	0
1+	4 (22.2)	4 (10.8)	1 (5.0)	0
±, -	8 (44.4)	11 (29.7)	3 (15.0)	4 (21.1)

Table 2. Frequency of eosinophils in nasal smear by age

Eosinophil grade	Age group (years)			
	<1 n = 24	1 n = 33	2 n = 20 (%)	3 n = 23
≥4+	1 (4.2)	7 (21.2)	7 (35.0)	14 (60.9)
3+	2 (8.3)	5 (15.2)	6 (30.0)	3 (13.0)
2+	2 (8.3)	9 (27.3)	2 (10.0)	1 (4.3)
1+	4 (16.7)	5 (15.2)	1 (5.0)	1 (4.3)
±, -	15 (62.5)	7 (21.2)	4 (20.0)	4 (17.4)

In this study, an average number of basophilic cells was used for the gradation. Before adopting this gradation technique, we compared the total number of basophilic cells on one slide with the average number of basophilic cells from selected fields. The relationship between the numbers of cells obtained using the two methods is shown in Fig. 1. When the number of basophilic cells exceeded 3 in one field by our criteria, it corresponded to Shioda & Mishima's criterion (22) of mast cells being over 20 in number on a whole slide. The criterion of over 20 on a whole slide could be used to indicate increased basophilic cells in the nasal smear.

Measurement of serum specific mite and egg-white IgE

Quantitation of IgE antibodies in serum was done with the radioallergosorbent test (RAST), using the

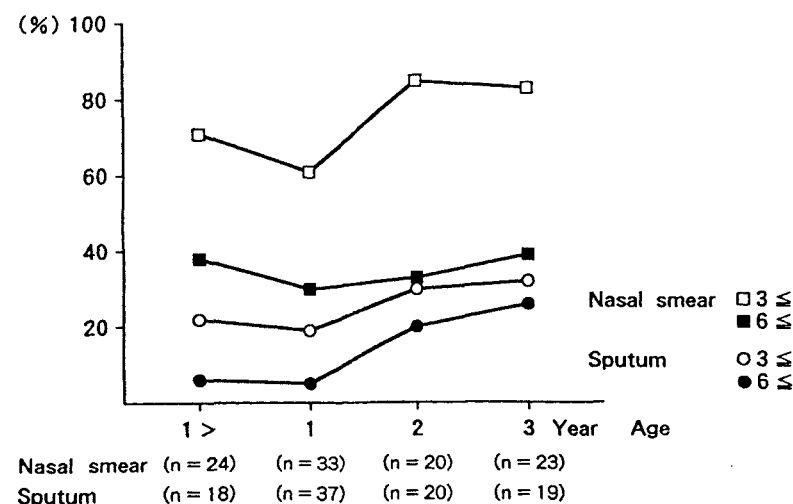


Fig. 2. Basophilic cells in sputum and nasal smear. Open symbol represents three or more basophilic cells in one microscopic field at $\times 1000$ magnification. Closed symbol represents six or more basophilic cells in one microscopic field at $\times 1000$ magnification.

Phadebas RAST kit (Pharmacia Diagnostics, Uppsala, Sweden). Measurement of house-dust-mite and egg-white IgE was carried out in all subjects, and RAST scores of $\geq 2+$ were regarded as positive.

Results

Frequency of increased eosinophils in sputum and nasal smears

The proportion of children with eosinophilia in sputum increased with age (Table 1). Pronounced eosinophilia, i.e., a grade of 4+, was more common from 2 years of age than in younger children. Similarly, eosinophilic infiltration in nasal smears increased with age (Table 2). Eosinophils generally appeared earlier in sputum than in nasal smears.

Frequency of children with infiltrated basophilic cells in sputum and nasal smears

A gradually increased infiltration of basophilic cells in sputum according to age was also observed. The percentage of infants of each age with infiltrated basophilic cells in nasal smears was high, and the differences between ages were not apparent (Fig. 2).

Eosinophils in sputum and nasal smears and RAST scores

The proportion of positive egg-white RAST scores was unaffected by age, whereas positive house-dust-mite RAST scores increased with age (Fig. 3). The proportion of positive RAST scores was always smaller than the proportion of children with eosinophil grades of 2+ or greater for all age groups.

Discussion

The subjects in the present study were infants and children with recurrent wheezing episodes and especially high frequencies of positive RAST scores to egg white and mite. They also showed rapid infiltration by eosinophils and basophilic cells. In sputum,

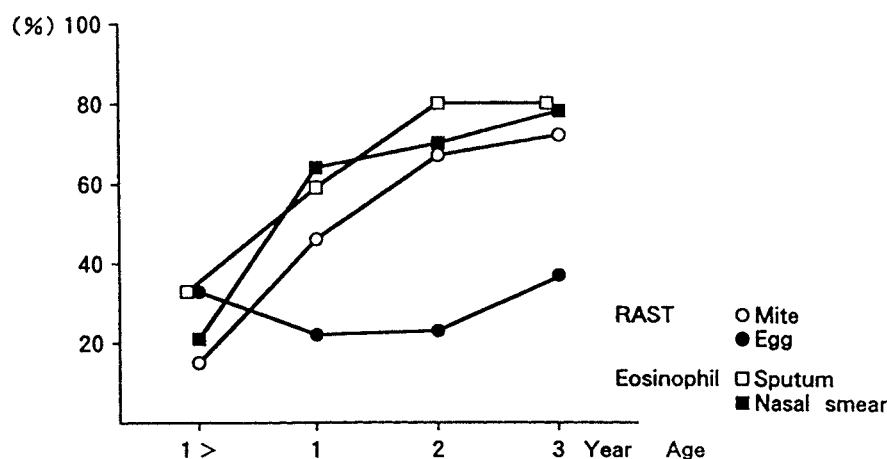


Fig. 3. Eosinophil grade and RAST score. Circle represents RAST score. Square represents eosinophils in sputum or nasal smear.

eosinophilia increased until 2 years of age, and pronounced eosinophilia, i.e., a grade of 4+, was more common after 2 years than in younger children. On the other hand, eosinophils in nasal smears increased gradually. In children, activation of eosinophils in the nasal region during respiratory infection has been reported (17, 18). Colacho Zelaya et al. (17) demonstrated an increase in eosinophil cationic protein (ECP) in nasopharyngeal specimens from wheezy infants infected with RSV (17). Garofalo et al. reported elevated ECP in bronchiolitis (18).

In this study, basophilic cells increased differently in nasal smears and sputum. Basophilic cells appeared in nasal smears during early infancy, and cell numbers were unaffected by age. In sputa, basophilic cells tended to increase gradually with age. In an animal model, it has been reported that mast cells in BAL increase during influenza virus infection and that the number of these cells is affected by bronchial sensitivity (24). Children with increased eosinophils or basophilic cells in the airways, triggered by viral infections, may show greater susceptibility to infection than healthy children. In comparison to sputum, the accumulation of basophilic cells in nasal smears begins during early infancy. The nasal cavity, through which antigens can enter and invade the respiratory tract, may serve as the first reactive site for inflammation. Infiltration by basophilic cells may increase during wheezing episodes when respiratory infections from unknown pathogens are also present. Although basophilic cells may have a potential role in allergic inflammation, the transitory accumulation of basophilic cells does not necessarily denote a clinical nasal allergic manifestation. Only afterward, when the allergic inflammatory process has been completed, may typical nasal allergic manifestations occur.

It would have been useful to compare the secretions of these children with those of healthy children; however, sampling would have been difficult. Many asthmatics in a stable state and healthy subjects are

unable to produce sputum spontaneously. In regard to sputa, samples without macrophages cannot be regarded as being secretions from the lower respiratory tract. As nasal smears are likely to contain a mixture of secreted free cells and scraped-off epithelial cells, only a few cells per slide may be seen when specimens have been taken from subjects without rhinitis. In a study of the cell influx into nasal washings after antigen challenge, Bascom et al. (25) showed the paucity of inflammatory cells in normal nonallergic subjects. Other studies have shown the presence of only a few eosinophils and neutrophils in normal control BAL fluid (26) or nasal smears (27). Fahy et al. (8) obtained sputum samples from asthmatic adults by inhalation of hypertonic saline, but this method is difficult to apply to children. Our experience has shown that suctioned samples from children without a moist cough usually contain only squamous cells and other debris.

The diagnostic procedures used during this study are repeatable and noninvasive. Infiltration by these cells began earlier than the appearance of specific IgE to mite. Although there seems to be no clear distinction between infectious and allergic wheezing, increases in eosinophils or basophilic cells may indicate a progression of allergic inflammation during respiratory lesions in childhood.

In conclusion, the microscopic examination of sputum and nasal smears taken from asthmatic infants and children demonstrated the progressive accumulation of eosinophils and basophilic cells with age.

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