

Age and sex as factors of response to RSV infections among those with previous history of wheezing

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Although enhanced immune reaction caused by the respiratory syncytial virus (RSV) in allergen-sensitized animal model has been reported, RSV illnesses in children already sensitized or having recurrent wheezing episodes have not been completely studied. In addition, the reason for male dominances in RSV infection at young ages was also inconclusive. Therefore, gender analysis in recurrent wheezing children with RSV infection can shed light on asthma pathogenesis. We studied the clinical features and the laboratory data of RSV infections in children who had recurrent wheezing histories. The subjects with RSV infection consisted of 98 boys and 58 girls. The children under 4 yr of age were 123 (78.8%) in number. Children with pneumonia were 78 and those with febrile episode were 119. Children above 1 yr of age were highly sensitized with mite antigen (75/96, 78.1%). The clinical symptoms and signs differed according to their ages. Children in each age group behaved differently in their immune reaction to RSV. Above all, 3-yr-old children deteriorated clinically during acute RSV infection, accompanied by transient elevated C-reactive protein (CRP) and suppressed blood eosinophil counts. Clinical features differed in several points between boys and girls. In general, the white blood cell count and the CRP levels were higher in girls in every age group. Blood eosinophil counts at the acute illness were significantly higher in boys than girls aged 2 and 3 < yr. Age and gender comparison in already sensitized children might suggest a clue to asthma pathogenesis.

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Respiratory syncytial virus (RSV) is a major cause of respiratory disease early in life and is believed to cause wheezing in infants and young children. A pivotal role of RSV infection in the development of recurrent episodes or asthma progression has been discussed (1–3). In recent times, new approaches for preventing RSV disease have been developed (4). However, there may be possibilities of the altered immune response to RSV playing a key role as a determinant of asthma progression (5, 6). RSV, in spite of RNA virus, was reported to persist in animal model (7). RSV infection in mice can enhance the consequences of allergic airway

sensitization, resulting in lung eosinophilia and the development of airway hyper-responsiveness to inhaled methacholine (8). In contrast, prior airway exposure to allergen increased virus-induced airway hyper-responsiveness (9). These data suggest that prior airway exposure to allergen may predispose sensitized hosts to a greater degree of altered airway function upon subsequent respiratory viral infection. The mechanisms leading to accumulation of eosinophils have been a topic of asthma establishment. However, the impact of acute RSV infection on children who had had episodes of recurrent wheezing in the past time or already sensitized

individuals has not been elucidated. Thus, it has been of particular interest to study the clinical features of RSV infections among recurrent wheezing children, namely asthma. There are most probable multiple factors of innate and adaptive immunity that induce diffuse airway narrowing at young ages. A variety of factors including various inflammatory cells, plasma extravasations, reduction of airway caliber in baseline, neurogenic inflammation (10) and massive goblet hyperplasia (11) might act differently depending on host predisposition. Considering the male predominance of morbidities in number among young ages, boys and girls might react differently to respiratory antigens; thus, gender analysis also can provide us information about RSV infection and asthma progression. To delineate inflammatory response due to RSV infection, we assessed clinical features and laboratory examinations among Japanese children who had had recurrent wheezing episodes before RSV infection.

Methods

Subjects

The subjects were required to have a confirmed diagnosis of RSV lower respiratory tract infection. They had had a history of three or more episodes of wheezing with respiratory distress, including this episode. Subjects with underlying condition, such as neural, cardiac diseases or otolaryngeal diseases or congenital anomalies were excluded from the study. All patients except eight had been followed prior to contracting RSV infection. The major parts of children were treated for asthma with oral or inhaled medicine. The main oral medicine was sustained theophylline as a controller intermittently or continuously. The rate of oral sustained theophylline for at least half a year was 101/156 (64.7%). Inhaled disodium cromoglycate was used in 66.6% (103/156). Inhaled steroid was given in 28 children in our hospital prior to contracting RSV infection.

Blood specimen

White blood cell (WBC) was counted mechanically. The COULTER STKR system (Beckman Coulter, CA, USA) was used for counting WBC by the method of electric impedance. Blood eosinophil count was calculated by total WBC count and eosinophil fraction percentage. The first blood test was performed at positive RSV antigen. The amount of serum C-reactive protein (CRP) was determined by the turbidity photo-

metrically by using commercial kit of LPIACE CRP-L (Mitsubishi-Chemical, Tokyo, Japan). The number of blood specimens was 155 at the time of positive RSV antigen, but 78 at the second time within 1 month.

The measurement of specific IgE for mite, egg white, milk and cockroach in serum was done by immunoallergosorbent assay using Pharmacia CAP RAST (radioallergosorbent test) system (Pharmacia Pharmaceutical, Uppseda, Sweden). We considered scores of 2 and more for mite and scores of 1 and more for foods (egg white or milk) as positive.

RSV infection was confirmed by the antigen-detecting method using Abbott TEST PACK RSV test (Abbott Labs, IL, USA).

Statistical analysis

Continuous variables were compared by students' *t*-test or by analysis of variance with Turkey's test or multiple comparisons. Categorical variables were compared by using chi-square analysis. When the samples were small, Fisher's tow samples were employed. Statistical significance was defined as $p < 0.05$. All data are reported as means $\pm$ s.d. unless otherwise noted.

This study protocol was approved by the Ethics Institutional review boards.

Results

Table 1 shows age distribution of 156 subjects: 98 boys and 58 girls, in combination with their clinical symptoms namely, fever and abnormal shadow in chest roentgenogram at acute RSV infection. Most children revealed acute infectious symptoms at positive RSV antigen. Febrile illness was seen in 74/98 (75.5%) and 45/58 (77.6%) in boys and girls, respectively. Chest roentgenogram was examined in 128 children. Abnormal pneumonic shadows on their chest

Table 1. Clinical features of respiratory syncytial virus (RSV) infections in recurrent wheezy children

Age (yr)	0-1	2	3	4-5	5<	Total
Boys						
Febrile	30/36	14/19	20/24	7/9	3/10	74/98
Abnormal roentgenogram	21/31	10/15	14/20	3/7	2/7	50/80
Specific IgE for mite	13/35	16/19	17/23	7/7	8/9	61/93
Specific IgE for foods	11/31	6/14	8/20	5/9	2/4	32/78
Girls						
Febrile	17/18	12/15	8/11	7/11	1/3	45/58
Abnormal roentgenogram	8/16	6/11	6/9	7/9	1/3	28/48
Specific IgE for mite	6/17	8/15	8/10	9/10	2/3	33/55
Specific IgE for foods	5/14	5/12	2/6	2/5	0/0	14/37

roentgenogram were observed in 50/80 (62.5%) and 28/48 (58.3%) of boys and girls, respectively. In boys, the incidence of pneumonia and febrile episode reached the highest levels at 3 yr of age, while pneumonic shadow in girls increased with ages.

Laboratory examination

The results of serum-specific IgE antibody for mite, egg white or milk are shown in Table 1. Recurrent wheezing children in this study were highly sensitized to mite or foods. The positive rate of CAP RAST for mite became higher with age. The positive rate of specific IgE for mite in 0–1-yr age group was 13/35 (37.1%) and 6/17 (35.3%) in boys and girls, respectively. After 1 yr of age, recurrent wheezing children possessed specific IgE for mite in high frequency. The positive rate for mite in children over 1 yr of age was 48/58 (82.8%) and 27/38 (71.1%) in boys and girls, respectively. On the other hand, their positive rate of RAST for foods was 21/47 (44.7%) and 9/23 (39.1%) in boys and girls, respectively, over 1 yr of age.

The WBC count was examined in 155 children at positive RSV antigen. The mean WBC count was not different between sexes and ages. Although there was no significant difference in the WBC count, it tended to be higher in girls than in boys in all age groups. Serum CRP levels by sex and age at positive RSV antigen are shown in Fig. 1. Similarly, CRP levels tended to be higher in girls than in boys in each age group. CRP levels were significantly higher in boys aged 3 yr (3.59 ± 5.17) than boys aged 0–1 yr (1.59 ± 2.38 , *t*-statistics, $p < 0.05$) and 2 yr (0.86 ± 0.98 , *t*-statistics $p < 0.05$).

The first test for blood eosinophil counts by sex and age at positive RSV antigen is shown in Fig. 2. The blood eosinophil counts in each age

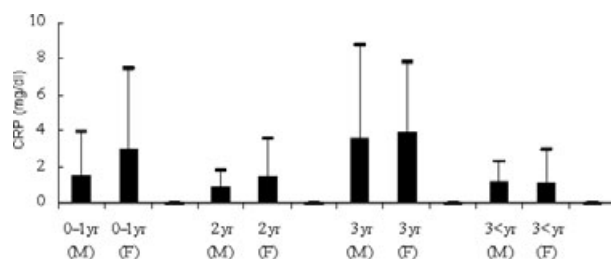


Fig. 1. Serum C-reactive protein (CRP) levels (mg/dl) in boys and girls in four age groups at the time of positive respiratory syncytial virus (RSV) antigen. Horizontal line indicates each age group. M represents male and F represents female. Box represents the mean value of serum CRP levels and bar represents $1 + \text{s.d.}$

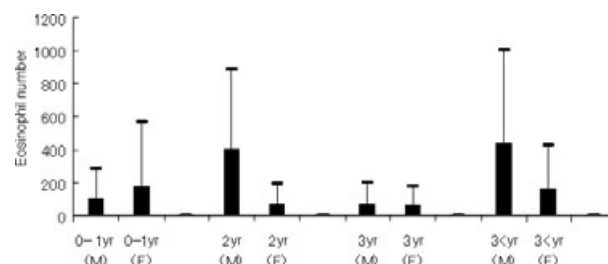


Fig. 2. Blood eosinophil counts in boys and girls in four age groups at the time of positive respiratory syncytial virus (RSV) antigen. Horizontal line indicates each age group. M represents male and F represents female. Box represents mean value of blood eosinophil counts and bar represents $1 + \text{s.d.}$

group differed between boys and girls. The eosinophil counts in 2 yr-old boys (408.39 ± 478.83) increased significantly compared with those in the girls of the same age (71.0 ± 120.72) (*t*-statistics, $p < 0.01$). Similarly, a significant difference was observed between boys (445.32 ± 553.49) and girls (166.21 ± 260.75) in the age group above 3 yr (*t*-statistics, $p < 0.05$). The boys' eosinophil count dropped significantly from 2 yr (408.39 ± 478.83) to 3 yr of age (79.17 ± 117.49) (*t*-statistics, $p < 0.05$). However, there was no further eosinophil suppression if the boys were older than 3 yr at the time of positive RSV, while the girls' blood eosinophil counts at positive RSV were suppressed throughout 0–3 yr of age. Similar to boys, girls' blood eosinophil count also dropped at 3 yr of age. In contrast, the second blood eosinophil count was evaluated in 78 samples. The second blood eosinophil count in boys and girls aged 3 yr was 313.08 ± 446.36 and 119.50 ± 222.97 , respectively. There was no further suppression in blood eosinophil counts at the convalescent stage in both sexes.

Discussion

Although much attention has been paid to the link between RSV infection and asthma onset, eosinophilic activation during RSV infection might be crucial for asthma progression. Usually through experience of RSV infections, healthy children become resistant to this agent after 2 yr of age (12). Subsequent RSV infections are generally milder. However, children with underlying conditions are considered to be different. During the 12-yr observation at our hospital, 156 children who had had recurrent wheezing episodes revealed positive RSV antigen. Most of

them showed acute respiratory illness with wheezy distress. RSV infection in children already affected by asthma has not been previously evaluated. Our observation of recurrent wheezing children showed three findings. First, eosinophil count was higher in boys than girls. Second, the girls' responses in WBC count and CRP level were stronger than those of the boys at the acute illness. The last was that the clinical features and laboratory data differed unexpectedly according to their ages and sexes. The comparison of clinical pictures among four age groups showed clearly that 3-yr-old children deteriorated, mostly characterized by the high pneumonic and febrile rates. Among 3-yr-old children, exaggerated immune reaction characterized by CRP elevation and suppressed eosinophil count was observed. This inflammatory pattern might not have been reported in the previous papers. The exact reason for clinical deterioration accompanied by eosinophilic suppression is still unknown. One speculation was that high viral load or proliferation might occur due to immunosuppression triggered by RSV. Otherwise, the imbalanced immune functions in the course of resolving RSV infection, such as innate vs. acquired immunities or helper T vs. regulatory T-cell imbalance, might cause the clinical deterioration. Nevertheless, children aged 3 yr responded intensely to RSV antigen. In general, eosinophil disappearance was usually observed at severe inflammatory states during acute infections. Chen et al. (13) showed that RSV-infected infants had a more frequent interferon (IFN)-gamma expression in CD3+CD8+ cells than controls, and they also showed decreased interleukin 4 (IL-4) and elevated IFN-gamma in RSV bronchiolitis. Regarding the relation between the age of the host and the disease severity, the deficient RSV-specific cytotoxic lymphocytes (CTL) may also account for the increased morbidity from RSV infections in animal model (14). Splenic lymphocytes from old mice exhibited significantly lower RSV-specific CTL responses and reduced IFN production than young mice. These results demonstrate that the severity of RSV illness might be associated with diminished RSV virus-specific CTL responses and IFN-gamma production. This immunomodulation could be executed by RSV itself for the viral survival. There are possibilities that CTL suppression might occur in allergen-sensitized children exclusively.

Most of our subjects were already sensitized by mite antigen. Until now, predominant T-helper-2-like response during RSV infection has been reported (15). RSV bronchiolitis in infancy may

increase the risk of allergic sensitization by providing a local IL-4-rich environment. RSV primes the memory T-cells that make IFN-gamma, but subsequently IL-4-producing T cells are also frequently primed in bronchiolitis. In animal models, bronchial inflammation and hypersensitivity by RSV differed between sensitized and non-sensitized animals (8, 9, 11). A variety of eosinophilic response in each age group might reflect the imbalance of protective and disease-enhancing immune responses to RSV. In contrast, peripheral blood lymphocytes from children who had been hospitalized with RSV bronchiolitis in infancy, and had grown up to 7–8-yr-old, showed more frequent IL-4 producing T cells to RSV, while the IFN-gamma response to RSV was equally strong compared with controls (16). The time of eosinophil activation relevant to RSV infection, known by the past clinical reports was still controversial (17–25). Rakes et al. (23) indicated the association between rhinovirus infection and atopic diathesis characterized by nasal eosinophils and RAST positive. RSV is rarely isolated from wheezy children over 2 yr in their study. Martinez et al. (18) showed that the persistent wheezers aged 6 yr had eosinophilia during their lower respiratory infection before 3 yr of age, whereas, transient early wheezers, who stopped wheezing by 6 yr had not exhibited eosinophilia during their past infections. Children with acute RSV bronchiolitis had significantly higher levels of serum eosinophilic cationic protein (ECP) (19). On the other hand, the total eosinophil counts significantly decreased in acute bronchiolitis (18). The relationship between the immune response to RSV pathogenesis and their age deserves further analysis.

In our study, boys tended to show higher blood eosinophil count compared with girls in all age groups. It is well known that asthma is a chronic inflammatory state characterized by eosinophilia in the lower respiratory tract. Heterogeneity of eosinophil fluctuation was observed in bronchoalveolar lavage (BAL) samples taken from asthmatic children or virus-proven wheezing (24). We already showed the relation between respiratory eosinophilia and ECP levels in sputum in infants and young children (25). There was a discrepancy between the eosinophil count and the ECP levels. Eosinophil was considered one of the effector cells during acute respiratory virus infection. Furukawa et al. (26) showed eosinophil chemotaxis by added supernatant infected with rhinovirus. Rhinovirus infection stimulated the lymphocytes to induce IFN and T-cell proliferation by the activation of eosin-

ophils (27, 28) and induced up-regulation of eotaxin expression in bronchial epithelial cells (29). BAL obtained from asthmatic children had high level of I-L8 and neutrophil activation (24). Increased leukotriene B₄ in exhaled breath condensate in childhood asthma was associated with asthma severity more closely than cystenyl leukotriene (30). From these evidences, the altered immune function to viral antigen might be closely linked to asthma progression.

Astonishingly, the high sensitization rate to inhalant allergen among Japanese recurrent wheezy children was shown in this study. This allergic propensity may be attributable to a high moisture climate in Japan. In addition, Japanese medical circumstances enable access to even mild wheezing children for treatment. Reasonably, these factors also might provide unique outcomes of wheezing children in Japan. We speculate that the clinical outcome in Japanese wheezy children may be distinguished from other counterparts in the United States or Europe. An inevitable respiratory infection and pre-existing susceptibility of asthma diathesis are not mutually exclusive. Comparison of wheezing children in multiple countries is informative and useful. Multivalent effects of acute respiratory infections on asthma airway are further investigated seeking its linkage to asthma progression.

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