

Creola bodies in wheezing infants predict the development of asthma

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Creola bodies (CrBs) have been shown to be present specifically in the sputum of adults with asthma, and are clinically useful in the estimation of airway hyper-responsiveness through correlation with the levels of eosinophil cationic protein in the sputum. Our aim was to investigate whether CrBs can be detected in the sputum of wheezing infants, and the clinical significance of CrBs in wheezing infants in the development of asthma. Twenty-three wheezing infant patients, mean age 5.3 months, were studied. The presence of CrBs in the sputum was determined by Papanicolau's stain. The patients were divided into two groups: CrB positive [CrB(+)] and CrB negative [CrB(-)]. In addition, we examined the relationship between the presence of CrBs and the asthma symptom scores that occurred during a 1-month post-discharge period. The following results were obtained: (i) CrBs were detected in 15 of 23 (65%) wheezing infants; (ii) the asthma symptom scores after discharge was significantly higher in the CrB(+) group than in the CrB(-) group ($p < 0.001$); (iii) in the infants experiencing their first episode of wheezing, the asthma symptom scores during the post-discharge period was also significantly higher in the CrB(+) group than in the CrB(-) group ($p < 0.05$). Furthermore, infantile asthma was diagnosed in 12 (80.0%) infants in the CrB(+) group during the following 2-yr period, but none in the CrB(-) group. These findings suggest that CrBs detected in the sputum of wheezing infants may be used to identify the progression to infantile asthma.

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The onset of childhood asthma often occurs before 3 yr of age (1). Recently, there has been a tendency for asthma to occur at earlier ages and for the prevalence of infantile asthma to increase (2). Our understanding of the pathology of infantile asthma has not progressed to the same extent as in adults. The reasons for this are the difficulties in studying bronchial biopsies, and evaluating objectively respiratory hyper-responsiveness in infancy. However, bronchoalveolar lavage has shown the presence of airway inflammation (3). A further problem is that because of the special anatomical and physiological characteristics of the lower airway in infants, it is easy to induce airway constriction. In wheezing infants, some will stop wheezing at an early age, but some will progress to asthma (4). Up to now no variable that could predict whether a wheezing infant is likely to develop asthma subsequently has been identified. A diagnosis of infantile asthma, therefore, must be

based on the family history, the presence of other atopic diseases, and clinical judgment based on the physical findings, such as recurrent wheezing. Recently, it has been recommended that children who continue to experience wheezing should be diagnosed with asthma after more than three episodes of wheezing (4).

Naylor et al. described and named clusters of bronchial epithelial cells in sputum 'Creola bodies' (CrBs) and reported that their occurrence in the sputum is specific to adult bronchial asthma (5). There are also reports that CrBs are correlated with eosinophil cationic protein (ECP) and major basic protein in sputum, and also with airway hyper-responsiveness (6–8). However, there have been no reports of CrBs in the sputum of children with asthma, particularly infants.

It is possible that CrBs can be assessed in sputum non-invasively in infants and at the same time indirectly evaluating airway

hyper-responsiveness. In the present study, we have collected the sputum of wheezing infants and investigated the clinical significance of CrBs.

Patients and methods

Patients

This study was designed as a randomized study between February 1997 and June 1999. Wheezing infants who had been hospitalized in the department of pediatrics of the University of Dokkyo were selected. All subjects had documented evidence of wheezing by auscultation, and an effect of inhaled beta-agonists on admission ($>2\%$ increase in SpO_2). None had any other organic complaint, such as congenital respiratory anomaly, cardiac disease, or the presence of foreign bodies. Furthermore, subjects with bacterial infection of the respiratory tract as suggested by radiograph, increase of C-reactive protein (<0.4 mg/dl), and/or bacterial culture were excluded. Therefore, of the 30 subjects selected, 28 wheezing infants (aged 1–10 months) for whom written informed consent was obtained from their parents or guardians were included in this study. Furthermore, 23 wheezing infants (mean age 5.3 months) with appropriate cell components in the sputum were enrolled in this study. Five subjects with diffuse massive squamous epithelial cells or no macrophages in the sputum were excluded from this study.

Examination of sputum

Sputum was obtained from the subjects by using an aspirator (9) at the time of admission. The following procedure was used to obtain sputum from the infants: The suction device was constituted by a two-way tube with a trap. One end of this tube was connected to the aspirator equipment on the wall or an aspirator machine. As coughing drives sputum up the trachea, coughing was induced by rubbing the anterior neck of the infant over the upper section of the cricoid cartilage. A tube was inserted orally and a specimen taken quickly during coughing. If the infant patient cried vigorously, this stimulation would be ineffective. In such a case, it was necessary to take the sputum quickly at the moment when coughing was triggered by the crying or the aspiration. Smears of 100 μl of sputum were spread on pairs of glass slides without the addition of diluent solution. The slides were fixed with 95% ethanol. One slide was stained with Papanicolaou's stain to detect CrBs on the whole glass slide. Fig. 1 shows CrBs in the

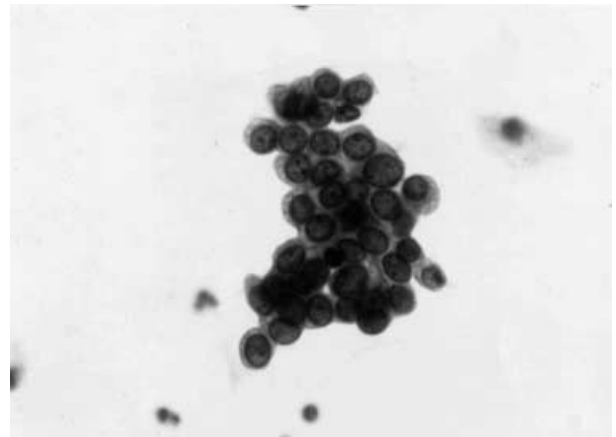


Fig. 1. Creola body in the sputum of a patient with childhood asthma.

sputum. CrBs were detected by the presence of clusters of more than 10 desquamated respiratory epithelial cells (10). The second slide was stained with Hancel's stain and analyzed for cell components. The presence of alveolar macrophages in the specimen was considered evidence that the secretions came from the lower respiratory tract. Specimens with diffuse massive squamous epithelial cells or no macrophages were excluded from the study, because those came from the upper respiratory tract.

Assessment of patients

Each slide was examined for the presence of CrBs over the whole slide, and the slides were classified into two groups: CrB positive [CrB(+)] or negative [CrB(-)]. At the time of entry into the study, the parents of the infants were instructed on the use of a daily diary that recorded symptoms such as wheezing, cough, and breathlessness. The relationship between the presence of CrBs and the asthma symptom scores that occurred during the 1-month post-discharge period after hospitalization was examined. The asthma symptom scores were defined by the guidelines of the Japanese Society of Allergology, which uses a scale of 0–9 (0 = no symptoms, 0.5 = frequent cough, 1 = wheezing, 3 = mild attacks, 6 = moderate attacks, 9 = severe attacks). Daily totals were calculated during the month following discharge. Each infant was followed-up for 2 yr after the examination.

Statistical analysis

Results were expressed as the mean $\pm$ s.e.m. Statistical comparison between groups was performed using the Mann–Whitney two-sample

U-test for unpaired data. A p-value of <0.05 was considered to be statistically significant.

Ethics

The study was approved by the Regional Ethics Committee for Human Research at the Hospital of Dokkyo University School of Medicine.

Results

The characteristics of the 23 infants with respect to gender, age, and history of familial allergic disease are shown in Table 1. Fifteen infants had a family history of atopy. The number experiencing their first episode of wheezing was 16. CrBs were detected [CrB(+)] in 15 (65%) of the sputum samples from wheezing infants and not detected [CrB(-)] in 8 (35%) (Table 1, Fig. 1). The asthma symptom scores during the 1-month post-discharge period was significantly higher in the CrB(+) group than in the CrB(-) group (33.7 ± 5.4 vs. 4.3 ± 0.9 , $p < 0.001$) (Fig. 2). Also, the symptom scores during the 1-month post-discharge period in patients experiencing their first episode of wheezing was significantly higher in the CrB(+) group than in the CrB(-)

Table 1. Characterization of wheezing infants with and without creola bodies in the sputum

No.	Gender*	Age (months)	Familial atopy†	Episode of wheezing‡	Creola body
1	M	5	+	-	-
2	M	6	+	-	-
3	F	7	-	-	-
4	F	7	+	+	-
5	F	5	+	+	-
6	F	3	-	-	-
7	M	10	+	-	-
8	M	10	+	-	-
9	M	3	+	-	+
10	M	5	-	-	+
11	F	1	+	-	+
12	F	1	-	-	+
13	M	9	-	-	+
14	M	4	+	-	+
15	F	3	+	-	+
16	M	4	+	+	+
17	M	4	+	+	+
18	M	8	-	+	+
19	M	7	+	+	+
20	M	5	-	+	+
21	M	4	-	-	+
22	F	4	+	-	+
23	M	8	+	-	+

*F, female; M, male.

†A history of familial atopy was considered as positive if there were atopic-related diseases (allergic rhinitis, asthma, or eczema) in one or both parents.

‡An episode of wheezing was considered as positive with more than two episodes of wheezing.

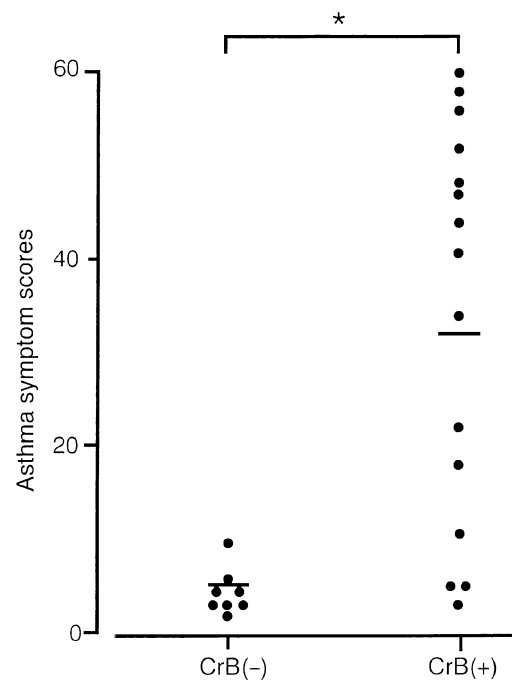


Fig. 2. Comparison of asthma symptom scores during the 1-month post-discharge period in the creola body positive [CrB(+)] group ($n = 15$) and the creola body negative [CrB(-)] group ($n = 8$) in wheezing infants. Bars indicate mean value; * $p < 0.001$.

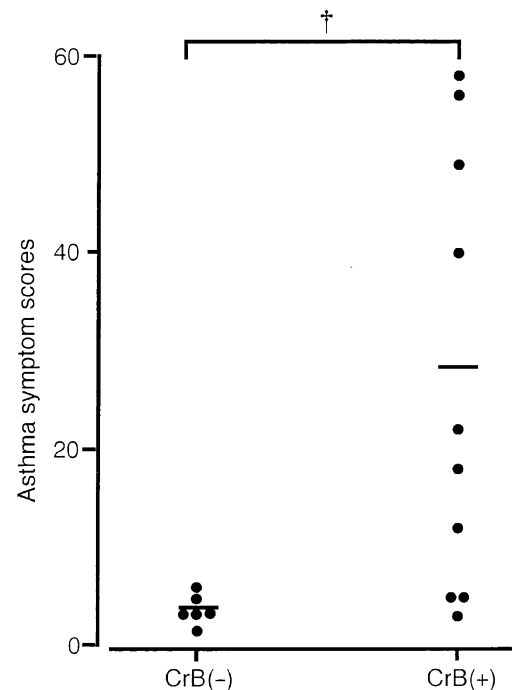


Fig. 3. Comparison of asthma symptom scores during the 1-month post-discharge period in the creola body positive [CrB(+)] group ($n = 10$) and the creola body negative [CrB(-)] group ($n = 6$) in patients experiencing their first episode of wheezing. Bars indicate mean value; † $p < 0.05$.

group (26.8 ± 6.9 vs. 3.3 ± 0.4 , $p < 0.05$) (Fig. 3). During the 2-yr period after the examination, infantile asthma was diagnosed in 12 (80.0%) infants in the CrB(+) group, but none in the CrB(-) group.

Discussion

The major finding of the present study indicated that CrBs are detected in the sputum of wheezing infants, an effect that is caused by epithelial damage. The CrB(+) group had significantly elevated asthma symptom scores during the 1-month post-discharge period compared with the score for the CrB(-) group in wheezing infants. Similar findings occurred in patients experiencing their first episode of wheezing. Furthermore, infantile asthma was diagnosed in a greater percentage of CrB(+) group infants than in the CrB(-) group infants during the following 2-yr period. These findings suggest that the detection of CrBs may be helpful in predicting the development of infantile asthma in wheezing infants.

Recently, the presence of CrBs has been specifically recognized in the sputum of adults with asthma and correlated with airway hyper-responsiveness (6, 7). The relationship between airway hyper-responsiveness and respiratory epithelium damage indicates that in humans the number of epithelial cells in the bronchoalveolar lavage fluid is correlated with airway hyper-responsiveness (11). Taken together, respiratory epithelial damage due to airway inflammation as characterized by the presence of inflammatory cells, such as eosinophils, has recently been identified as an important factor contributing to increased airway hyper-responsiveness (12, 13). More specifically, respiratory epithelial damage is thought to make it easier for irritant substances to reach airway smooth muscle, blood vessels, and nerves, to bring about a decrease in endogenous airway dilator substances and to attenuate the ability to degrade airway constrictor substances. To assess airway responsiveness, inhalation challenge tests using acetylcholine or methacholine are clinically useful. However, it is especially difficult to use these tests with infants. Sputum can be easily and non-invasively collected from infants, and the presence of CrBs in sputum appears to indirectly indicate airway hyper-responsiveness.

Currently in young children, ECP and soluble interleukin-2 (IL-2R) were found to be helpful in predicting the outcome of asthma, as increased levels were associated with a persistence of symptoms (14). Although in this study there is

no investigation of the relation between CrBs and these inflammatory markers in the sputum, evidence suggests that CrBs are correlated with ECP in adults with asthma (8). It will be a necessary future step to detect the prognostic factor of CrBs more accurately in childhood asthma.

In conclusion, this study demonstrates that CrBs can be detected in the sputum of wheezing infants, and their presence may predict persistent wheezing and progression to infantile asthma.

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