

Eosinophil cationic protein and interleukin-8 levels in bronchial lavage fluid from children with asthma and infantile wheeze

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It has been shown previously that airway eosinophils characterize childhood asthma and neutrophils contribute to the pathophysiology of both infantile wheezing and asthma. Therefore, eosinophil cationic protein (ECP) and interleukin-8 (IL-8) levels in bronchoalveolar lavage fluid (BALF) from asthmatics ($n=16$) and infantile wheezers ($n=30$) were analyzed as markers of eosinophil- and neutrophil-mediated inflammation. To aid the interpretation, a control group of children ($n=10$) with no lower airway pathology were included. Disease severity was assessed by using a symptom score. Surprisingly, no significant difference was found in IL-8 or ECP levels among asthma, infantile wheeze, and control groups. Asthma was characterized by: a correlation between ECP levels and eosinophil counts ($r=0.618$, $p=0.014$); a correlation between neutrophil number and IL-8 levels ($r=0.747$, $p=0.002$); and increasing IL-8 levels with symptom score ($p=0.03$). In infantile wheezers, IL-8 levels were poorly related to neutrophil number but were significantly increased when neutrophils were $>10\%$. Although detectable levels were found in all but one symptomatic infant, IL-8 concentrations did not reflect the symptom score in infantile wheeze.

ECP was unexpectedly correlated to neutrophil percentages ($Rho=0.832$, $p=0.001$), and a threshold of $ECP>20$ ng/ml was associated with persistent symptoms in these infantile wheezers. Hence, in accordance with BALF cellularity, activation of eosinophils was suggested by raised levels of ECP in childhood asthma, but not in infantile wheeze. Neutrophil-mediated inflammation appeared to better reflect the severity of asthma than that of infantile wheeze. Although its meaning remains to be elucidated, ECP was suggested to be a helpful indicator of persistent infantile wheeze. However, its utility as a marker predicting ongoing asthma remains to be established.

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There have been considerable advances in understanding the immunopathology of adult asthma but less information is available on pathophysiological mechanisms in airway diseases of childhood (1). In adults, the eosinophil is believed to be a key effector cell in asthma (2). We (3), and others (4), have shown that the same is true in childhood asthma, but not in infantile wheeze (3,5). Infantile wheeze is not a single condition because it occurs in infants with smaller airways during viral

infections and in others with immunoglobulin E (IgE)-mediated sensitization (6). We recently demonstrated neutrophilic but not eosinophilic inflammation in infantile wheezers, which was only sometimes associated with bacterial colonization of the airway (3). We also reported that the neutrophilic inflammation was related to disease activity in both asthma and infantile wheeze.

Interleukin-8 (IL-8) is a C-X-C chemokine produced by all leukocytes, macrophages, and

epithelial cells in asthma (7,8). IL-8 is considered to be the most potent chemoattractant cytokine for neutrophils (9) but also, to a lesser extent, for lymphocytes and eosinophils (10). Levels of IL-8 have been related to severity of adult asthma (11), and IL-8 has been demonstrated to contribute to virus-induced bronchial inflammation both in adults (12) and in children (13). Viruses have been implicated as the principal cause of exacerbation in asthmatic children and infantile wheezers. In such circumstances, up-regulation of IL-8 would be expected in the bronchoalveolar lavage fluid (BALF) from these patients.

Eosinophil cationic protein (ECP) has been suggested as a marker of eosinophil activation in adult asthma, and levels of ECP correlated with the activity of the disease (2). ECP was shown to be increased in airway secretion from mild asthmatic children (4,14–16) but not significantly in infantile wheezers (15), although high levels of ECP were measured in infants with viral acute bronchiolitis (17).

We hypothesized that ECP would characterize asthma, and that IL-8 would characterize both asthma and infantile wheeze, and therefore measured these markers in the BALF from such patients in relation to clinical severity of the condition. We compared results with a group of controls with no lower airway disease.

Patients and methods

Fibre optic bronchoscopy was performed on 56 infants and children, and these patients were categorized into three groups according to the diagnosis at the time of the investigation. Sixteen children aged between 4 and 15 years had established asthma (group A). These children had a history of recurrent wheezing associated with evidence of β_2 -agonist reversibility of airflow limitation. The indications for bronchoscopy were: persistent radiologic abnormalities (seven atelectasis and two asymmetrical lung fields), suspected extrinsic allergic alveolitis (two patients), hemoptysis (one patient), and unexplained failure of treatment with steroids (four patients). The second group of 30 children aged between 5 and 46 months were infants with recurrent wheezing (group W). All had at least three episodes of wheeze and cough during presumed viral infection. Bronchoscopy was justified by persistent atelectasis ($n=10$), asymmetrical lung fields on radiograph ($n=5$), suspected lung malformation ($n=1$), unexplained failure of steroids ($n=7$), uncertain diagnosis ($n=5$), and associated stridor ($n=2$). The final group (aged from 1.5–14 years) acting as controls

involved 10 children without any known chronic lower airway disease. They had tracheal or lung malformation ($n=5$), stridor ($n=1$), psychogenic cough ($n=1$), a suspicion of lung malformation ($n=2$), or suspected primary tuberculosis ($n=1$). Standard clinical histories and examination were performed with the addition of chest radiographs. Allergy prick tests and serum IgE assays were routinely performed on most of the patients from groups A and W. The characteristics of the patients are summarized in Table 1.

Twenty-eight of the children were taking inhaled steroids and all were treated for at least 1 month (Table 1). We classified patients of groups A and W according to existing symptoms (night cough, wheezing or dyspnea) at the time of bronchoscopy (Table 1). The nine asymptomatic patients (S0) were defined as children without any symptoms for at least 1 month before and after the bronchoscopy. Children who suffered from at least weekly respiratory symptoms significantly affecting their quality of life (sleep, behavior, exercise tolerance, and school attendance), and/or causing failure to thrive, were graded as (S2). The remaining symptomatic children (S1) had exercise-induced symptoms or mild-to-moderate exacerbations with little effect on their quality of life.

Bronchoscopy procedure

All children were admitted to the day ward before the bronchoscopy procedure was carried out. A transnasal fibre optic bronchoscopy (Olympus BF 3C20, or for teenagers an Olympus BF P10; Keymed Ltd, Southend-on-Sea, UK) was performed under monitoring with continuous oximetry assessment and clinical evaluation. Premedication consisted of midazolam, pethidine, and atropine sulfate (0.01–0.02 mg/kg, but less than 1 mg in total). Topical anesthesia consisted of lignocaine 2% in the upper airways and 1% in the bronchial tree. Preventive salbutamol nebulization was prescribed in 23 patients, and four infants were investigated under oxygen supplementation via nasal canulae. The bronchoscope was wedged in a segmental bronchus. The site depended on the localization of abnormality from chest radiographs but if there was no local disease, then the right middle lobe was selected. Aliquots of 10 ml of warmed, sterile 0.9% saline solution were injected and aspirated. The total volume ranged between 2 and 3 ml/kg and the specimens collected were $49 \pm 19\%$ of the original injected volume. The BALF recovered was pooled in fresh sterile containers and divided into aliquots for the various investigations. In

Table 1. Characteristics of the 56 studied children

Characteristic	Group		
	A (n=16)	W (n=30)	C (n=10)
Age in months (range)	90 (49–180)	25 (5–46)	97.8 (17–190)
Age at onset (months) (range)	22 (1–150)	5.4 (1–30)	–
Gender ratio (M/F)	13/3	17/13	5/5
Familial asthma history (%)	44	30	30
Familial atopy history (%)	37	33	10
Atopic dermatitis (%)	56	10	0
Positive skin test (%)	60	33*	–
High total IgE level (%)	38	36*	–
Proven allergy (%)	47	–	–
Symptom score S0/S1/S2	4/9/3	5/17/8	–
Inhaled steroids	10	18	–
Positive/available BALF cultures	5/13	15/278	2/10
<i>Haemophilus influenzae</i>	2	8	2
<i>Streptococcus pneumoniae</i>	1	1	1
<i>Moraxella catarrhalis</i>	2	4	–
<i>Streptococcus</i> sp.	3	6	1
<i>Escherichia coli</i>	–	1	–

A, established asthma; C, control; W, recurrent infantile wheeze.

BALF, bronchial lavage fluid; IgE, immunoglobulin E; S0, asymptomatic; S1, symptomatic, but with little effect on quality of life; S2, symptomatic with respiratory symptoms significantly affecting quality of life.

*Data available in 22 infants.

order to reduce the risk of contamination from the upper airways, no suction was used until the tip of the bronchoscope had been wedged into the tracheal lumen. Written consent was obtained from the parents for the procedure, which in all cases was based on a clinical imperative. The Hospital Ethics Committee approved additional use of the lavage specimens for research purposes.

Cell count

The lavage fluid was not filtered but was centrifuged for 10 min at 1000×g at 4°C. The pellet was re-suspended in phosphate-buffered saline (PBS) (5 ml) containing 10% fetal calf serum (FCS). The total cell count was performed using a hemocytometer. Viability was assessed by using Trypan Blue exclusion. Differential cell counts were performed on cytopspin slide preparations stained with May-Grünwald-Giemsa. At least two slides and 200 cells were counted for each subject. Total

cells and differential counts were as detailed previously (3) and are presented in Table 2.

Mediator assays

The assay for IL-8 was based on an in-house enzyme-linked immunosorbent assay (ELISA), as described previously (18), with a sensitivity of 20 pg/ml. ECP was measured by using a radio-immunoassay (Pharmacia Diagnostics, Uppsala, Sweden) with a detection limit of <2 ng/ml. The assays were performed blind with no knowledge of the patient's diagnosis.

Microbiological analysis

Quantitative cultures were carried out to determine the presence of the most common aerobic micro-organisms: *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Streptococcus* sp. A significant result was defined as growth of >10³ colony-forming units (CFU)/ml.

Table 2. Bronchial lavage fluid (BALF) cellularity*

	Asthma	Infantile wheeze	Control
Total cells (×10 ⁶ /ml)	0.521 (0.400–0.700)	0.705 (0.291–0.930)	0.490 (0.410–0.560)
Epithelial cells (%)	13.5 (8–20)	12 (4–33)	6 (3–14)
Lymphocytes (%)	6.5 (4–10)	8 (4–9)	7 (5–8)
Macrophages (%)	62 (53–70)§	47 (30–63)§	73 (66–84)
Neutrophils (%)	4 (1–12)	12 (3–57)†§	2 (1.5–3)
Eosinophils (%)	3 (0–9)‡§	0 (0–1)	0 (0–1)

*Data are expressed as median and (25th–75th quartile) or (range).

†p<0.05 with asthma, ‡p<0.05 with infantile wheezers, §p<0.05 with controls.

Data analysis

All results were presented as medians and inter-quartile 25th–75th ranges. IL-8 and ECP levels were expressed per ml of the pooled retrieved liquid, and no dilution markers were used, as previously discussed (3).

Statistical analysis

Statistical analysis was carried out using Spearman's two-tailed ranked correlation (Rho) in addition to Pearson's linear regression two-tailed analysis (r). When applicable, differences between groups were determined using the Mann–Whitney U -test or unpaired t -test. Fisher's exact test was used for qualitative analysis between the groups. A p -value of <0.05 was considered statistically significant, while trends were considered to be present with a p -value of <0.1 . Statistics were performed using statview 5.0 software (SAS Institute Inc., Berkeley, CA, USA).

Results

IL-8 concentrations in BALF

No significant difference in the IL-8 concentrations in BALF was found among the groups ($p=0.767$). IL-8 levels were undetectable in seven controls, five asthmatics, and 18 infantile wheezers. Only in infantile wheezers did steroid therapy reduce IL-8 levels significantly (Fig. 1). A linear correlation between the IL-8 levels and neutrophil percentages was found in asthma ($r=0.747$,

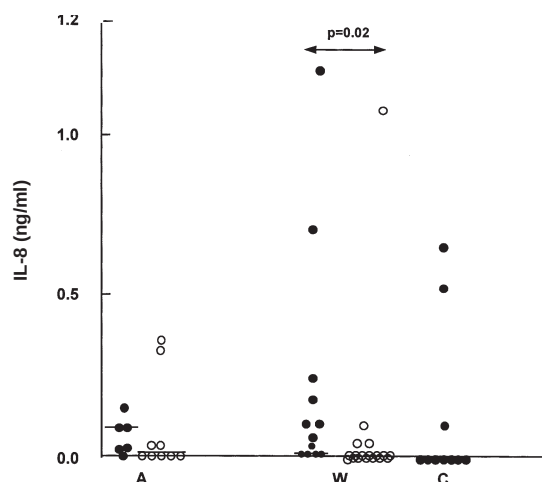


Fig. 1. Interleukin-8 (IL-8) levels in bronchial lavage fluid (BALF) from children with asthma (A, $n=15$), infantile wheeze (W, $n=30$), and in BALF from control children (C, $n=10$). Children receiving treatment (•) or untreated (○) with steroids are shown. Horizontal bars indicate the median for each group of patients. A significant difference was found only within infantile wheezers and not between the studied groups.

$p=0.002$) (Fig. 2a) and a trend was observed in infantile wheezers ($r=0.350$; $p=0.079$). IL-8 levels were, however, significantly increased in BALF from infantile wheezers with a neutrophil percentage of $>10\%$ (Fig. 3). IL-8 concentrations were not related to the percentage of eosinophils or lymphocytes. IL-8 levels were comparable in BALF, which was either positive or negative for bacteria, in asthmatics (0.036 ng/ml, range 0.003–0.157 ng/ml vs. 0.002 ng/ml, range 0–0.058 ng/ml; $p=0.4$) and infantile wheezers (0.002 ng/ml, range 0–0.18 ng/ml vs. 0.014 ng/ml, range 0–0.130 ng/ml; $p=0.29$).

ECP levels in BALF

No significant difference was found in ECP levels among the different groups of children (Fig. 4). ECP levels were ≤ 2 ng/ml in six controls, five asthmatics, and 10 infantile wheezers. Unexpectedly high levels of ECP were found in two children from the control group. Eosinophils were found in BALF (2%) from one of these controls. ECP levels were similar in BALF from children, treated or untreated with steroids, in the group of asthmatics (7 ng/ml, range 2–49 ng/ml vs. 9 ng/ml, range 4–45 ng/ml; $p=0.89$) and in the group of infantile wheezers (5 ng/ml, range 2–40 ng/ml vs. 3 ng/ml, range 2–8.5 ng/ml; $p=0.23$). In asthmatics, but not in infantile wheezers, ECP levels were correlated with eosinophil percentages ($r=0.618$; $p=0.014$) (Fig. 2b) and were significantly higher when eosinophils were detected in BALF than when they were not (37 ng/ml, range 4–73 ng/ml vs. 2 ng/ml, range 2–8 ng/ml; $p=0.017$). No difference was found between atopic and non-atopic asthmatics: 40 ng/ml (range 3–66 ng/ml) vs. 7.5 ng/ml (range 3–22 ng/ml); $p=0.56$. ECP levels were correlated with the number of neutrophils in infantile wheeze ($Rho=0.832$, $p=0.001$), being significantly higher in those with a high neutrophil percentage ($>10\%$) (Fig. 3). The ECP levels were unaffected by the bacterial colonization of BALF.

IL-8, ECP levels, and symptomatic status

IL-8 levels increased with the symptom score in asthmatics but not in infantile wheezers. No significant variation of ECP levels was found in relation to the symptom score in asthma. In infantile wheezers, detectable IL-8 and ECP levels >20 ng/ml were measured in all but one symptomatic infant. The percentages of wheezy infants with detectable IL-8 levels were 20% in S0, 47% in S1, and 38% in S2 ($p=0.55$). The percentages of infants with ECP levels >20 ng/ml were 0% in S0, 12% in S1, and 62.5% in S2, with a significant

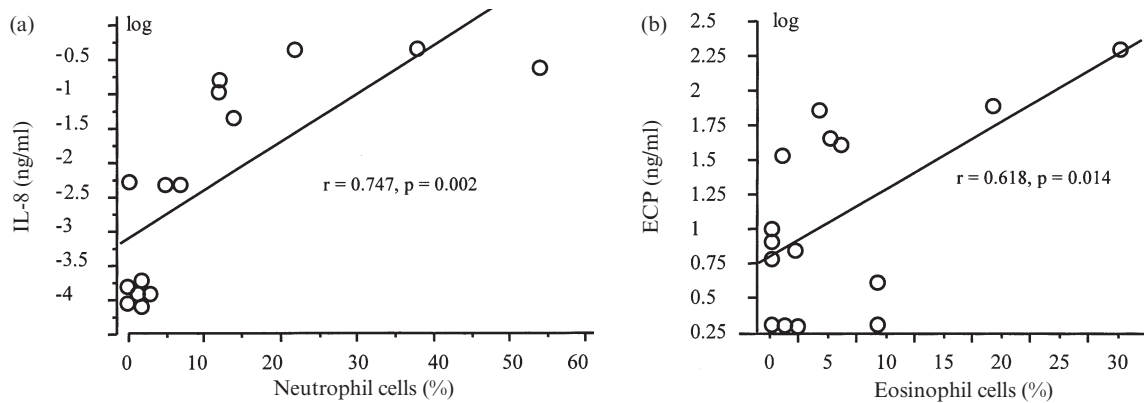


Fig. 2. Relationships between interleukin-8 (IL-8) and eosinophil cationic protein (ECP) levels and bronchial lavage fluid (BALF) cellularity in asthma. IL-8 levels and ECP levels were, respectively, correlated to neutrophil (a) and eosinophil (b) percentages.

difference between S1 and S2 ($p=0.016$) and a trend between S0 and S2 ($p=0.07$).

Discussion

BALF cell profiles have been discussed in detail previously (3), and the results suggested that airway eosinophils characterized childhood asthma, and neutrophils contributed to the pathophysiology of both infantile wheezing and asthma. We therefore considered that ECP and IL-8 levels in BALF would reflect eosinophil- and leukocyte-mediated inflammation. Unlike BALF cellularity (3), we found no difference in cytokine levels between the various groups studied. Nevertheless, some differences between asthmatics and infantile wheezers were highlighted in this study.

Unexpectedly (14–16), ECP levels in asthmatics were not different from that of control children. Our control group included children who had no obvious lower airway pathology but they could have had occult underlying bronchial inflammation. The asthmatics were, however, characterized

by ECP levels correlating to eosinophil percentages (3). This provides evidence for eosinophil activation similar to that seen in adult asthma (2). We found no variation in the ECP levels in relation to allergic status, which is different from two other studies of atopic asthmatic children with mild disease (4,15). Furthermore, we found that ECP in BALF was not a reliable marker of severity of the disease, a finding that contrasted with studies in adult asthma (2).

IL-8 contributed to the clinical severity of asthma: the chemokine levels were not only strongly correlated with neutrophil counts but also increased with the symptom score. This is in keeping with the observations of Teran et al. (13), who demonstrated a similar relationship with upper respiratory tract symptoms in children with virus-induced asthma exacerbations. Moreover, neutrophil-mediated inflammation has been involved in adults, both in the severity of acute asthma (11) and in virus-induced exacerbations (12). In this respect, our findings are very much in agreement with adult studies that relate neutrophilic inflammation, IL-8, and neutrophil myelo-

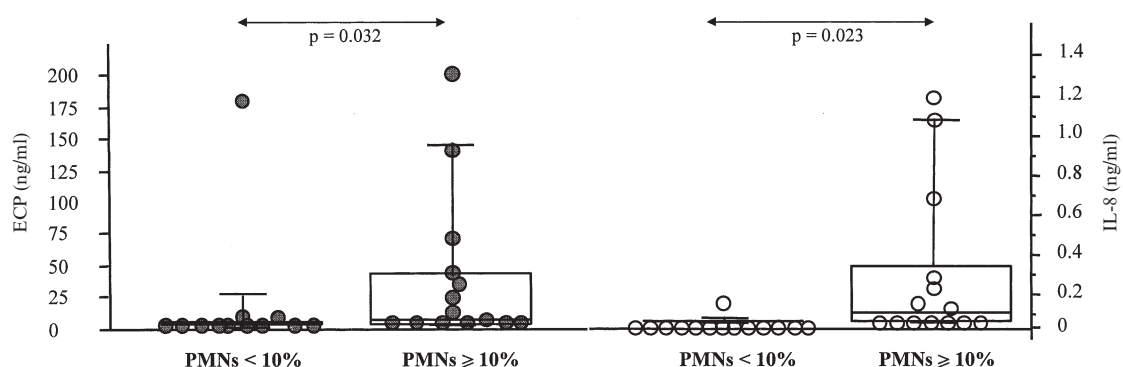


Fig. 3. Relationships between neutrophil counts and markers of inflammation in bronchial lavage fluid (BALF) from infantile wheezers. The population of infantile wheeze could be divided into two equal groups of infants according to a low and high neutrophil ratio. Interleukin-8 (IL-8) (open bar) and eosinophil cationic protein (ECP) (closed bar) levels are expressed as box and whiskers plots, and respective values (○), (●) are shown.

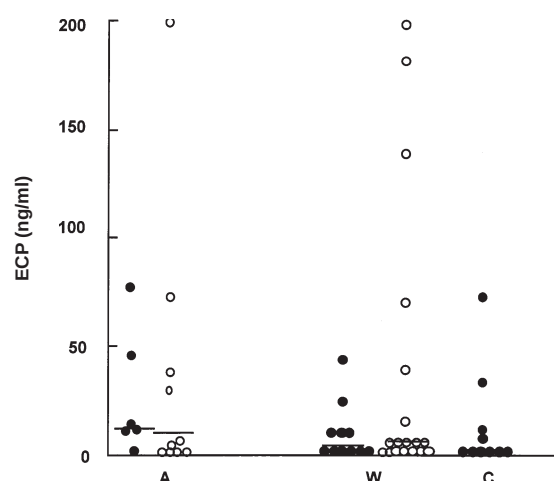


Fig. 4. Eosinophil cationic protein (ECP) levels in bronchial lavage fluid (BALF) from children with asthma (A, $n=16$), infantile wheeze (W, $n=29$) and in BALF from control children (C, $n=10$). Children receiving treatment (●) or untreated (○) with steroids are shown. Horizontal bars indicate the median for each group of patients. No significant difference was found within and between the studied groups.

peroxidase with severe, persistent disease (19). Although IL-8 is also considered as a chemoattractant for eosinophils, no correlation with either eosinophil number or ECP levels was established in this study.

Several cohort studies have shown that infantile wheeze is a syndrome that differs from asthma, per se (6). Viruses have been mainly involved in its pathophysiology and could induce a neutrophilic inflammation by up-regulation of IL-8 (20) and recruitment of neutrophils (21), but have also been associated with high ECP levels (22). We have shown previously that neutrophils contributed to the persistence of symptoms in these infants (3) and now report that the increased IL-8 production was associated with the intra-luminal neutrophil recruitment, but did not add to the severity of the disease, in contrast to our findings in asthma. However, several infants were receiving treatment with inhaled steroids, which down-regulate IL-8 production, although this was insufficient to prevent recurrent wheeze.

Although eosinophils were rarely found in BALF from patients with infantile wheeze (3,5,23), ECP was detected at levels comparable to that of controls or found in asthma (15). Furthermore, we found that a threshold of 20 ng/ml was associated with the persistence of symptoms. This is similar to the threshold of 50 ng/ml in nasal secretion (17) or 20 ng/ml in serum (24), which were reported as risk factors of recurrent wheezing in infants with bronchiolitis (17). Unlike asthma, ECP levels were correlated with the number of neutrophils, but not eosinophils, suggesting that ECP could be a non-specific marker of leuko-

cyte inflammation in infantile wheeze. This observation is also similar to studies of adult asthma where raised levels of ECP and neutrophils, but not eosinophils, were specifically associated with severe, persistent disease (19). Relationships between ECP production and neutrophil numbers have been previously reported. It has been suggested that ECP might reflect eosinophil turnover, with neutrophils phagocytosing eosinophil granules, thereby leading to an underestimation of eosinophil counts (25–27).

In conclusion, the measurement of neutrophil-related IL-8 levels in asthmatics and infantile wheezers is of clinical relevance, although raised levels of IL-8 are common in various bronchial diseases (28,29). IL-8 has been shown to maintain bronchial hyper-responsiveness (29) and to be associated with chronic obstructive disease in premature infants (27). However, an association of IL-8 levels with the symptom status was more evident in asthma than in infantile wheeze. Therefore, IL-8 would be a better target for therapeutic intervention in asthma than in infantile wheeze. Eosinophil-related ECP levels were detected only in childhood asthma, as described in adults. We, however, suggest that measurement of the level of airway ECP might be helpful in assessing bronchial inflammation in infantile wheezers, but follow-up studies are required to establish whether it predicts ongoing asthma.

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