

Mast cell activation and leukotriene secretion in wheezing infants. Relation to respiratory syncytial virus and outcome

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The persistence of wheezing after early wheezing episodes in infancy may be related to the virus involved and to the type of inflammation during the initial wheezing. The role of mast cell activation and leukotriene secretion in wheezing, and the relation to outcome, is not known. Our objective was to study markers of mast cell activation and leukotriene secretion from wheezing infants, and the relation to respiratory syncytial virus (RSV) infection and persistent wheezing. Urinary $9\alpha,11\beta$ -PGF₂, a marker of mast cell activation, and urinary leukotriene E4 were measured in 106 infants hospitalized for wheezing during their first year of life. Results were related to the presence of RSV infection and the persistence of wheezing at follow-up 20 months later. Levels of $9\alpha,11\beta$ -PGF₂ were higher in infants positive for RSV than in those with RSV negative wheezing, but both groups had higher levels than controls. Leukotriene E4 levels were higher in wheezing infants than in controls. Urinary $9\alpha,11\beta$ -PGF₂ levels were higher in infants with transient compared with persistent wheezing. We found a positive correlation between $9\alpha,11\beta$ -PGF₂ and leukotriene E4, strongest in infants with RSV negative disease and in infants with persistent wheezing. The results suggest that mast cells play an important role in infant wheezing, and may be a major source of leukotriene secretion in these infants. Mast cell activation and leukotriene secretion were not associated with persistent wheezing.

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Wheezing episodes in early childhood are common, but represent heterogeneous conditions with differential long time outcome. Several studies have suggested that the type of virus involved, but also differences in the underlying pathophysiology may have implications for the frequency of subsequent wheezing after the initial episode (1–3). Infants with persistent wheezing may during the initial episodes have increased levels of eosinophils in bronchoalveolar lavage (2), higher levels of eosinophil counts (3, 4) and levels of eosinophil cationic protein in serum (S-ECP) compared with infants with transient wheezing (5). These data suggest that eosinophil inflammation and activation may play a part in the airway hyperreactivity observed in infants with persistent wheezing.

Infiltration of mast cells is together with eosinophils, a hallmark of the inflammation in

asthma (6). However, far less is known about the role of mast cell activation during episodes of early wheezing, and especially the implication of mast cell involvement and long time outcome. Prostaglandin D₂ (PGD₂) is the major cyclooxygenase product of mast cells, and a specific marker of mast cell activity (7). Recently, measuring to $9\alpha,11\beta$ -PGF₂ (PGF₂) in urine by direct enzyme immunoassay has been demonstrated sensitive and sufficiently specific to monitor activation of the PGD₂ pathway (8). U-PGF₂ has been suggested as a marker of mast cell activation in children with asthma (7), but the role of U-PGF₂ in wheezing infants is not known.

During activation, mast cells may release several inflammatory mediators, among these leukotrienes. Leukotriene B4 (LTB4) and the

cystinyl-leukotrienes LTC₄, LTD₄ and LTE₄ arise from the oxidative metabolism of cell membrane arachidonic acid (9). In addition to mast cells, leukotrienes are also secreted from eosinophils and macrophages (9). Clinical studies have demonstrated a correlation between the severity of respiratory syncytial virus (RSV) respiratory disease in infants and the concentration of LTC₄ in their respiratory secretions (10). Recently, a relationship between LTC₄ and ECP in nasal lavage from infants with RSV bronchiolitis was demonstrated, suggesting that eosinophils were the most likely source of leukotriene secretion in these infants (11). However, in RSV infected rats, increase of cysteinyl-leukotriene synthesis in the lungs was associated with a histopathological expanded mast cell population, suggesting the mast cells were the main source of leukotriene production (12). Little is known about the secretion of leukotrienes from mast cells in wheezing infants.

Levels of LTE₄ measured in urine (U-LTE₄) may be a useful measure of whole-body cysteinyl-leukotriene production *in vivo*, as LTE₄ is a stable urinary metabolite of LTC₄ and LTD₄. Studies have demonstrated increased levels of U-LTE₄ in children with asthma, and the parameter has been suggested as a tool for monitoring disease activity (13).

We aimed to study the role of mast cell activation and leukotriene production in infants hospitalized for severe wheezing with or without RSV infection. Levels of U-PGF₂ and U-LTE₄ were assessed and related to markers of eosinophil activation, to the presence or absence of RSV infection, and to the persistence of wheezing at follow-up 20 months later.

Material and methods

Study subjects

One hundred and six infants subsequently admitted to hospital for wheezing during their first

year of life were enrolled (Table 1). Wheezing was defined as acute respiratory illness with tachypnoe, dyspnoe, prolonged expiration and wheezing on auscultation of the chest. All infants had fever, suggestive of an airway infection.

Nasopharyngeal mucus was examined for RSV by direct immunofluorescence in all patients (BioMerieux, France). Sixty-seven of the patients were RSV-positive and 39 RSV-negative. Infants with RSV infection were younger than those who were RSV-negative (Table 1). Eighty-eight of the infants experienced wheezing for the first time. Eighteen of the infants had experienced symptoms of cough and wheeze before, but less than three episodes and therefore not fulfilling the criteria for having asthma (14). None of the infants had been hospitalized for wheezing before, and none had been treated with inhaled corticosteroids. Infants with previous or current atopic dermatitis, pre-existing lung disease (e.g. bronchopulmonary dysplasia), or signs of bacterial infection were not included.

Twenty-three healthy infants of the same age, with no family history of atopic disease or symptoms, and without a history infection in the last 2 wk served as controls (Table 1).

The study was approved by the Regional ethical committee, and signed statements of informed consent were obtained from all parents.

Laboratory analysis

Urine was collected within 4 h after admission and kept in a refrigerator until aliquoting and freezing (−20°C) within 10 h. U-PGF₂ and U-LTE₄ were analyzed by a specific enzyme-linked immunoassay (Cayman Chemical, Ann Arbor, MI, USA). The detection limit in the assay was < 5 ng/l for U-PGF₂ and < 10 ng/l for U-LTE₄, and the inter-assay variation was < 12%. Levels of urinary eosinophil protein X [U-EPX, results published earlier (4)], were analyzed by a specific radioim-

Table 1. Number of subjects in different groups, gender, age and the levels of urinary (U) 9 α ,11 β -prostaglandin F₂ (U-PGF₂), U-leukotriene E₄ (U-LTE₄), U-eosinophil protein X (U-EPX) and total eosinophil count in 106 children hospitalized for wheezing during the first year of life. Results are given as nanogram and microgram per millimole creatinine (cr), and are presented as median and quartiles

	Number	Boys/girls	Age (months)	U-PGF ₂ (ng/mmol cr)	U-LTE ₄ (ng/mmol cr)	U-EPX (μg/mmol cr)	Eosinophil counts × 10 ⁹ /l
Wheezing	106	66/40	5; 2–8	504; 306–923*	86; 46–150†	97; 63–129	0.14; 0.04–0.34
RSV positive	67	39/28	3; 1–5‡	534; 353–976†,§	83; 47–144*	98; 70–137	0.07; 0.04–0.27‡
RSV negative	39	27/12	9; 6–11	402; 195–785	99; 44–159*	88; 61–117	0.19; 0.12–0.51
Controls	23	14/9	5; 3–8	277; 180–496	47; 24–83	107; 51–224	

*p < 0.01 compared with controls.

†p < 0.001 compared with controls.

‡p < 0.001 compared with RSV negative.

§p < 0.05 compared with RSV negative.

immunoassay (Pharmacia, Uppsala, Sweden). The detection limit in the assay was $< 3 \mu\text{g/l}$ and the coefficient of variation was $< 5\%$ within the assay and $< 10\%$ between the assays. Urine creatinine levels were measured by Vitros 250 system (Ortho Clinical Diagnostics, Inc., Rochester, NY, USA). All measurements were carried out in duplicate. U-PGF₂, U-LTE4 and U-EPX levels are presented as nanogram per millimole creatinine. Blood was drawn immediately after admission, and eosinophil counts determined with Technicon H*2/H*3 or Coulter STKS (4).

Follow-up

Twenty months (range 19–23 months) after hospitalization for wheezing, 103 infants (97%) were available for follow-up (mean age 25 months, range 22–31 months). Of these, 65 infants were RSV-positive and 38 RSV-negative at the time of hospitalization. At follow-up, a standardized questionnaire was used to interview all parents. The history of wheezing of the child and the family history of atopy were questioned. Parental atopy was defined as one or both of the parents having atopic disease (atopic dermatitis, rhinoconjunctivitis, asthma). To identify episodes of wheezing, the parents were asked if the child had demonstrated symptoms similar to the symptoms when hospitalized for wheezing. Both wheezing episodes recorded by a physician or reported by parents were considered. One or more consecutive days of wheezing followed and preceded by a healthy period of 1 wk, were counted as one wheezing episode. Furthermore, any hospitalization of the child because of wheezing after the initial episode was noted according to the medical record. According to guidelines, children were considered to have persistent wheezing if they had experienced at least three wheezing episodes including the first hospitalization, with at least one of the episodes during the last 6 months which was during the winter season (14). The remaining children were classified as having transient wheezing.

Statistical methods

Data were analyzed using the SPSS 11.0 statistical package (SPSS Inc, Chicago, IL, USA). Comparisons between groups were made with the nonparametric Mann–Whitney *U*-test and results are presented as median and quartiles.

Logistic regression models were used to calculate the odds ratios (OR) with 95% confidence intervals (CI) for estimating the associations between potential risk factors and persistent wheezing. Each variable was entered separately into univariate models. As the parameters were not normally distributed, Pearson's correlation analyses were carried out by after logarithmic transformation. All analyses were two-tailed.

Results

Levels of U-PGF₂, U-LTE4, U-EPX and total eosinophil counts are presented in Table 1. For U-PGF₂, levels were higher in both RSV positive and RSV negative wheezing compared with controls, and levels were higher in those with RSV positive disease compared to those with RSV negative disease. Levels of U-LTE4 were higher in infants with both RSV positive and RSV negative wheezing compared with controls, but were similar in infants with RSV positive and RSV negative disease. Levels of U-EPX did not differ between infants with wheezing and controls, whereas total eosinophil counts were lower in infants with RSV positive disease compared to those with RSV negative disease (4).

Infants with transient wheezing had higher levels of U-PGF₂ and lower total eosinophil counts at the time of admission compared to those with persistent wheezing (Table 2). There was a tendency towards higher levels of U-LTE4 in those with transient wheezing (n.s.), whereas levels of U-EPX did not differ between the two groups (Table 2).

When measured by univariate regression analysis, increasing age and eosinophil counts, RSV negative disease, parental atopy and male gender

Table 2. Levels of urinary (U) 9 α ,11 β -prostaglandin F₂ (U-PGF₂), U-leukotriene E₄ (U-LTE₄), U-eosinophil protein X (U-EPX) and total eosinophil count in 103 children hospitalized for wheezing during the first year of life and who after 20 months (range 19–23) had persistent wheezing or not (transient wheezing). Results are presented as median and quartiles

	Number	U-PGF ₂ (ng/mmol cr)	U-LTE ₄ (ng/mmol cr)	U-EPX (μg /mmol cr)	Eosinophil counts ($\times 10^9/\text{l}$)
Persistent wheezing	39	381; 181–737*	75; 42–134†	108; 71–164	0.20; 0.09–0.47*
Transient wheezing	64	575; 364–932	99; 47–153	94; 63–126	0.09; 0.04–0.22

* $p < 0.01$ compared with transient wheezing.

† $p = 0.14$ compared with transient wheezing.

Table 3. Predictive factors for persistent wheezing in 103 children 20 months (range 19–23) after being hospitalized for wheezing during the first year of life. Results of univariate analysis

Variable	p-value	OR	95% CI
Age in months	<0.001	1.3	1.2–1.5
RSV negative wheezing	<0.001	5.7	2.4–13.7
Eosinophil count	0.02	9.6	1.5–60
Parental atopy	0.002	4.0	1.7–9.3
Male	0.02	3.0	1.2–7.4
Urinary PGF ₂	0.02	<1.0	0.99–1.01
Urinary LTE4	0.12	<1.0	0.99–1.01
Urinary EPX	0.4	1.0	0.99–1.01

were positive predictive factors for persistent wheezing (Table 3). Furthermore, the level of U-PGF₂ was a negative predictive factor for persistent wheezing and there was a tendency towards the level of U-LTE4 as a negative predictive factor for persistent wheezing.

At the time of admission there was a significant correlation between the logarithmic levels of U-PGF₂ and U-LTE4 ($r = 0.2$; $p < 0.05$). However, there was an even stronger correlation between these variables in RSV negative infants ($r = 0.6$; $p < 0.001$) and in infants with persistent wheezing ($r = 0.42$; $p < 0.001$), whereas no correlation was found in RSV positive infants ($r = 0.06$; $p = 0.6$) nor in infants with transient wheezing ($r = 0.09$; $p = 0.5$) (Fig. 1a,b). No other significant correlations were demonstrated between the logarithmic levels of U-PGF₂, U-LTE4, U-EPX and total eosinophil count in any of the groups.

Discussion

The results of this study strongly support a role for mast cell activation in wheezing infants. Furthermore, levels of U-PGF₂ were higher in infants with RSV infection compared to those with RSV negative disease, suggesting differences with respect to the inflammatory pattern between these groups, with mast cell activation being more important in RSV induced wheezing. Levels of U-LTE4 were higher in wheezing infants compared with controls, but no differences were observed between those with RSV positive and negative disease. This suggests that leukotrienes are involved in the pathophysiology in both RSV positive and RSV negative wheezing.

Both PGD₂ and LTC₄ and LTD₄ are potent bronchoconstrictive agents, and as for LTE₄ and LTB₄ they stimulate mucus release in the airways and increase microvascular permeability and epithelia edema (10, 15). Our results may therefore indicate that mast cell activation through secretion of PGD₂ and the release of leukotrienes

may be important contributors to the wheezing observed in infants with viral infections. Possible, the higher levels of U-PGF₂ in RSV positive children may indicate a higher influence from mast cell activation in these infants compared to those with RSV negative infection.

It has been suggested that the enhanced release of leukotrienes from inflammatory cells may occur after sensitization by interferon gamma (10, 16). However, conflicting results have been published regarding the source of leukotrienes during wheezing, whether from activated eosinophils (9) or mast cells (10). In the present study, we demonstrated a correlation between levels of U-PGF₂ and U-LTE₄, suggesting that during early wheezing episodes the mast cell is a major source for leukotriene secretion.

However, in RSV positive wheezing there was no correlation between levels of U-LTE₄ and U-PGF₂. A higher degree of mast cell activation was observed in these infants, but not a corresponding increase in levels of U-LTE₄. Little is known about the mechanism of mast cell activation during infant wheezing. A possible explanation for our results may be that compared with other viruses, RSV may induce a stronger release of PGD₂, and that the mechanisms involved may not influence the secretion of leukotrienes to the same extent. As a consequence, the correlation between U-LTE₄ and U-PGF₂ may have been obscured in RSV positive children, whereas this correlation was highly present in children with RSV negative disease. As RSV infection was negatively associated with persistent wheezing, the correlation between U-LTE₄ and U-PGF₂ was present in children with subsequent persistent wheezing, but not in those with transient wheezing.

Infants with persistent wheezing had significantly lower levels of U-PGF₂ compared to those with transient wheezing, and U-PGF₂ was a negative predictive factor for persistent wheezing. This is consistent with Krawiec et al. (17) who studied bronchoalveolar lavage from young infants, and demonstrated that persistent wheezing was not associated with increased levels of the mast cell mediators PGD₂ and β -tryptase. Together, the results suggest that a high degree of mast cell activation in wheezing is typical for RSV infection, but not associated with persistent wheezing.

In contrast, persistent wheezing was associated with high levels of total eosinophil counts, consistent with other studies suggesting that an eosinophilic driven inflammation may be more typical already at the time of the first wheezing in infants with subsequent persistent wheezing (2–

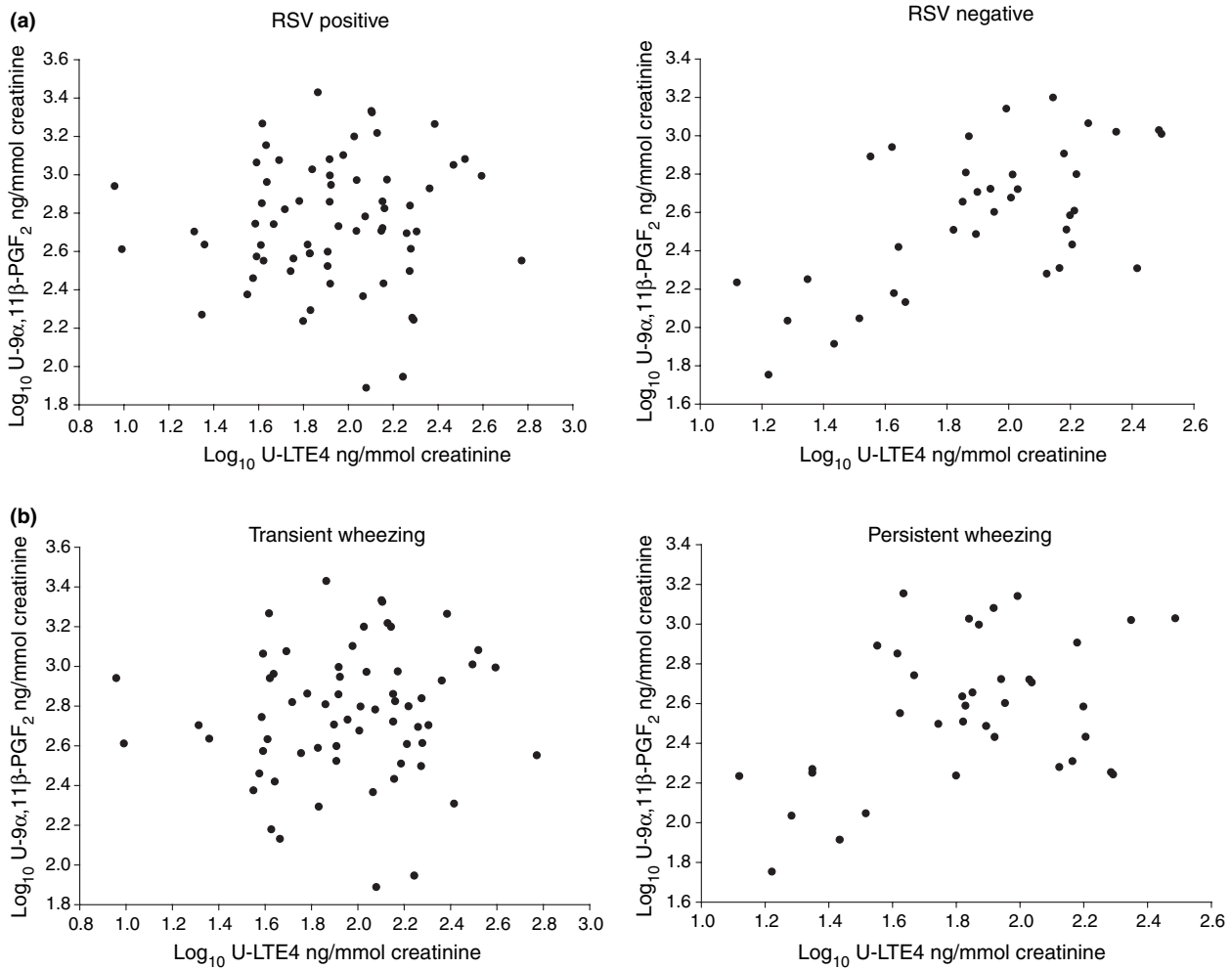


Fig. 1. (a) Correlation between the logarithm (LOG10) to levels of urinary (U) leukotriene E4 (U-LTE4) and U-9α,11β-prostaglandin F2 (U-9α,11β-PGF₂) in 106 children hospitalized for wheezing during the first year of life; 67 children were positive for respiratory syncytial virus (RSV; $r = 0.06$; $p = 0.6$) and 39 were negative for RSV ($r = 0.6$; $p < 0.001$). (b) Correlation between the logarithm (LOG10) to levels of urinary (U) leukotriene E4 (U-LTE4) and U-9α,11β-prostaglandin F2 (U-9α,11β-PGF₂) in 103 children hospitalized for wheezing during the first year of life with transient wheezing ($n = 64$; $r = 0.09$; $p = 0.5$) and persistent wheezing ($n = 39$; $r = 0.42$; $p < 0.001$) 20 months later.

4). However, there was no correlation between levels of U-LTE4 and markers of eosinophil activation, and there was a tendency towards lower levels of U-LTE4 in infants with persistent wheezing compared to those with transient wheezing. Our results may therefore suggest that the role of leukotrienes in these infants is neither associated with eosinophil inflammation nor with factors related to persistent wheezing.

Recent studies have suggested a role for leukotriene antagonists in the treatment of wheezing infants, and further a role for this treatment in the prevention of persistent wheezing (18, 19). Our study may support a role for leukotriene receptor antagonists during acute severe wheezing in infants. However, as levels of U-LTE4 were not related – or possible

negative related – to persistent wheezing, the results does not suggest a major role for leukotriene antagonists in preventing persistent wheezing.

Guidelines suggest that when a child has experiences three episodes of airway obstruction they may be classified as having asthma, and symptoms persisting after the age of 2 yr strongly suggests asthma (14). As our follow-up was at an early age (average 25 months), we carefully defined ongoing symptoms as persistent wheezing and not asthma. However, the symptoms in children with persistent wheezing strongly suggest the diagnosis of asthma, but only a later follow-up can clarify if the results for those with persistent wheezing in our study will apply for those with persistent asthma in later childhood.

In conclusion, during early episodes of severe wheezing, infants with transient wheezing and those with subsequent persistent wheezing differs regarding the inflammatory pattern observed. In children with transient wheezing and in RSV positive wheezing, mast cells seem to be involved more extensively than in those with persistent wheezing or RSV negative wheezing. Mast cells may be an important source for leukotriene secretion in infants with early severe wheezing, but mast cell activation and leukotriene secretion were not risk factors for persistent wheezing.

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