

Neutrophil but not eosinophil inflammation is related to the severity of a first acute epidemic bronchiolitis in young infants

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Acute bronchiolitis is the main cause of emergency visits and hospitalizations in infants. Recent data suggest that neutrophil- and eosinophil-mediated inflammations were part of bronchiolitis pathophysiology. Apart from the defined risk factors, few was known on the underlying pathophysiology, which might point out the differences observed in the severity of the disease. The aim of this study was to assess whether the clinical severity of acute epidemic bronchiolitis in young infants might be related to a specific underlying inflammatory process. Total and differential cell counts, IL-8, eotaxin, eosinophil cationic protein (ECP) and albumin levels were assessed at the time of admission in bronchial secretions from 37 infants (median age 17 wk) with acute bronchiolitis. Outcome severity variables were: hypoxemia, Silverman score, tachypnea, feeding alteration, and duration of hospitalization. Neutrophils predominated, and eosinophils were present in 54% of the infants. IL-8 levels strongly correlated with ECP and albumin levels. Albumin levels were correlated with ECP and eotaxin levels. IL-8 levels were higher in infants with hypoxemia and inversely related with SaO₂ levels. IL-8 and albumin levels significantly rose with respiratory rate, and Silverman score. IL-8, albumin and ECP levels were significantly higher in infants hospitalized ≥ 7 days. Furthermore, IL-8 levels were correlated with the duration of hospitalization. Neither cell counts nor eotaxin levels were related to the severity criteria studied. This study suggests that IL-8-associated airway inflammation significantly contributed to the severity of acute epidemic bronchiolitis.

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Acute bronchiolitis is the most common respiratory disease in infants under 2 yr of age. During the epidemic season, 90% of this acute wheezing disease is due to respiratory syncytial virus (RSV). Although most infants recover within 5 days (1), annual bronchiolitis hospitalization among infants younger than 1 yr has been assessed at 31.2/1000 (2). In RSV etiology, bronchial epithelium damage, excessive secretions of mucus and bronchial wall edema resulted in bronchiolar obstruction (1). This showed that

bronchial inflammation was involved in the severity of acute bronchiolitis. The role of the RSV-associated inflammatory process has been assessed by measuring *in vitro* production of inflammatory mediators by epithelial cells (3, 4), macrophages (5, 6) and in experimental animal models (7). Both neutrophil- and eosinophil-mediated inflammations have been involved in the pathophysiology of acute bronchiolitis. Neutrophil cell recruitment has been shown to be predominant in acute bronchiolitis (8, 9). High

levels of neutrophil chemokines were also measured in nasal (4, 10), tracheal fluid (11) from infants with RSV bronchiolitis. The role of mast and eosinophil cells has been suggested by respective airway production of tryptase and histamine (9, 12, 13) or eosinophil cationic protein (ECP) (14, 15). In addition, eosinophils might contribute to the severity as they were involved in experimental RSV-induced bronchial hyper-responsiveness (16). On the other hand, the above inflammatory process was demonstrated to be not RSV specific and associated with other viral airway infection (17, 18). By contrast, most clinical trials have demonstrated that oral steroids had no effect on the short-term outcome of acute viral bronchiolitis (19), which questioned the role played by bronchial inflammation. This discrepancy was highlighted by van Woensel et al. (20), who showed that a clinical effect of steroids could be expected only in cases of severe bronchiolitis.

Few studies have attempted to determine whether neutrophil- or eosinophil-mediated bronchial inflammation might point out the differences observed in the severity of acute bronchiolitis. We therefore analyzed either cell or cytokine profiles in airway secretions from infants with acute epidemic bronchiolitis, and researched whether they were related to each clinical parameter usually recommended to assess the severity of acute bronchiolitis in clinical practice. The results showed that the non-specific viral IL-8-associated inflammation appeared significantly related to the severity of the disease.

Study design, patients and methods

A prospective study was conducted during an epidemic season in 44 infants with a first acute episode of bronchiolitis. Inclusion criteria were infants aged from 1 month to 1 yr, requiring hospitalization for a first acute bronchiolitis with no steroids, bronchodilators, and antibiotics. Viral etiology was not an inclusion criterion. Exclusion criteria were any history of underlying disease (i.e., congenital heart disease, cystic fibrosis, immunodeficiency, premature birth, neonatal respiratory disease, chronic lung disease or associated laryngitis). The Hospital Ethics Committee approved additional use of sputum specimens for research purposes and informed consent was obtained from the parents.

Demographic data were collected. Diagnosis and severity of bronchiolitis were assessed in the Emergency department. The diagnosis was based on dyspnea and wheezing associated with

lung hyperinflation on chest X-ray. The severity of the disease was assessed with the standard criteria as previously described (21): respiratory rate, respiratory distress, difficulties in feeding at home and hypoxemia. Respiratory rate was categorized into three groups: $RR \leq 49/\text{min}$, $50/\text{min} \leq RR \leq 59/\text{min}$, and $RR \geq 60/\text{min}$ or occurrence of apnea. Respiratory distress was assessed with a restricted Silverman retraction score (xiphoid and intercostal retraction, nasal flaring, and chest movement synchronization) scaled from 0 to 8. Feeding at home was estimated as unchanged or altered (decrease in ounces of formula taken in the past 24 h). Oxygen saturation was recorded with an Ohmeda Biox 3740 pulse oxymeter (Louisville, CO, USA), in room air, for at least 5 min. The oxygen saturation value was recorded when the displayed wave of pulse monitoring was stabilized for at least 1 min. $\text{SaO}_2 \leq 92\%$ was considered a criterion of severity (22). In addition, the necessity of enteral or parenteral feeding, and the length of hospitalization were assessed as severity criteria (23). The duration of hospitalization attributed to bronchiolitis was estimated from the day of admission to the day of discharge. Discharge was defined when the following three criteria were obtained: normal feeding, $\text{SaO}_2 > 95\%$ and normalized RR. We did not take into account the persistence of other symptoms as cough, wheezing or auscultation signs.

Collection of airway secretions

Physiotherapy is routinely recommended and performed in the care of acute bronchiolitis in France (24). A careful nasal lavage was performed by flushing 5 ml of sterile isotonic saline solution twice through each nostril. The nasal fluid and upper airway secretions were aspirated by introducing a suction catheter (6F; Vygon Corp., Ecouen France) through each nostril as far as the laryngo-pharynx, and then discarded. Then, the pediatric chest physiotherapist applied expiratory flow ventilation to the infants on an empty stomach and drained the bronchial secretions to the lower pharynx and provoked coughing by leaning the thumb on the trachea. Sputum was quickly collected into a sterile vial by gentle suction through the mouth (catheter AM10610P; Cair LGL Corp., Civrieux, France). The first sample was discarded and following samples were kept for analysis. All the samples were collected in the morning of the first day following admission. Samples were immediately sent to the laboratory. The safety of physiotherapy was

evaluated as good, poor (causing tiredness) or bad (hypoxemia).

Microbiological analysis

Respiratory syncytial virus was researched by RSV ELISA (Monofluoscreen RSV, Sanofi-Pasteur France) on nasopharyngeal secretions. Quantitative bacterial cultures were carried out for the growth of common aerobic agents, such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Streptococcus* sp. A significant result was defined as growth $> 10^5$ CFU/ml.

Cell counts

Laboratory tests were performed as previously recommended (25). The sputum was macroscopically identified, and mixed with an equal volume of dithiothreitol 0.1%. After gentle homogenization, a volume of phosphate-buffered saline (PBS) solution (pH 7.4) equal to four times the initial selected sputum was added, mixed by vortex and filtered (48- μ m nylon mesh). The total cell count was performed using a hemocytometer. Viability was assessed by trypan blue exclusion test. Differential cell counts were carried out on Cytospin slide preparations stained with May-Grünwald-Giemsa. Squamous epithelial cells were counted separately and differential cell counts were performed on 400 non-squamous cells. The supernatant was stored at -80°C until further analysis. The sputum sample was considered acceptable: (1) if the percentage of squamous epithelial cell contamination numbered less than 20% of total cell count and did not prevent the differential cell count (25); and (2) if macrophages were present.

Other assays

Human IL-8, one of the most potent neutrophil chemokine (26), and human eotaxin, a selective eosinophil chemokine (27) were determined by immunoassay (Quantikine®; R&D Systems, Abingdon, UK) with a sensitivity less than 10 and 5 pg/ml respectively. Released ECP, a marker of activation, was measured by a radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) with a detection limit < 2 ng/ml. Considered as a potential dilution markers, albumin levels were established by using Coomassie blue technique (Bio-Rad protein assay, München, Germany). The assays were performed by investigators blinded to the patient's diagnosis.

Data analysis

Cell counts were expressed as number per ml or as percentage of total cell counts which included epithelial cells. All other results were presented as medians and interquartile (25th–75th) ranges.

Statistical analysis

Pairwise correlation among differential cell counts, IL-8, eotaxin, ECP and albumin levels were assessed using Spearman's rank test. Univariate analyses were performed to assess the association between these variables and severity criteria. For dichotomous severity criteria, namely, SaO_2 ($\leq 92\%$ and $> 92\%$), feeding difficulties (yes and no), and enteral feeding (yes and no). A Mann-Whitney's test was also used. Jonckheere Terpstra's non-parametric test was used for ordinal severity criteria, namely respiratory rates (three ordered levels) and Silverman score (eight ordered levels). Additionally, Spearman's rank test was used to verify that results held when originally continuous severity criteria, namely SaO_2 , duration of hospitalization, were coded as continuous rather than categorical variables. Multivariate analyses were performed by using stepwise logistic regression and stepwise linear regression to select variables independently associated with severity criteria. In all the analyses, a value of $p < 0.05$ was considered statistically significant. The statistical analyses were performed using Statview 5.0 (SAS Institute Inc., Cary-NC CA, USA), StatXact 3 (Cytel Software Corp., Cambridge MA, USA), and BMDP (Statistical Software Inc., San Jose CA, USA).

Results

Sixty-eight eligible infants were hospitalized during the same RSV outbreak (January 1995 to March 1996) and 44 were included in the sputum study. Seven infants were subsequently withdrawn because squamous epithelial cell contamination exceeded 20% of total cell count. A total of 37 infants (male:female ratio: 2.08) with a median (interquartile) age of 17 (13–24) wk were included, with a median duration of symptoms before admission of 3 (2–5) days, and median SaO_2 of 93 (91–95)%. Five (13.5%) had atelectasis on chest X-ray and 17 (46%) had familial history of atopy or asthma. Blood eosinophil was 130 (0–180) cells/mm³. The median duration of hospitalization was of 6 (4–8) days. Physiotherapy was well tolerated in 23 infants (62%) and led to

Table 1. Cellularity and IL-8, eotaxin, ECP, and albumin levels in airway secretions from 37 infants with acute bronchiolitis

Epithelial cells (%)	2.0 (0–6.1)
Neutrophils (%)	85.4 (77.8–89.4)
Eosinophils (%)	1.0 (0–3.0)
Lymphocytes (%)	2.2 (1.0–4.3)
Macrophages (%)	6.0 (4.2–9.0)
IL-8 (pg/ml)	10,290 (5219–15,653)
Eotaxin (pg/ml)	11.5 (7.5–35.3)
ECP (μ g/ml)	376 (175–696)
Albumin (mg/l)	1331 (984–1823)

Results are expressed as median (interquartile range 25th–75th percentile).

tiredness but no hypoxemia in the remaining 14 infants (38%).

Bronchial secretions cell profiles and severity of the disease

The results of total and differential cell counts are summarized in Table 1. The median volume of bronchial aspiration was 0.3 (0.13–0.4) ml. The viability of the cells was 75 (40–80)%. Macrophages were present in all samples confirming the bronchial origin of the secretions (26). Neutrophils were the predominant cells in all infants. Eosinophils were found in 54% of the infants and the ratio ranged from 1.0% to 8.6%. The percentage of eosinophils was unexpectedly higher in the infants with no previous family or personal history of atopy than in those with such a history: 1.54 (0–4.1)% vs. 0 (0–1.03) %; $p = 0.01$. No difference was found in the differential cell counts between the severity parameters that were analyzed. The duration of symptoms preceding the admission did not influence the cell profiles.

Relationships between cell counts and IL-8, eotaxin, ECP and albumin levels

No correlation was found either between IL-8 levels and neutrophil ($\rho = 0.034$) or eosinophil ($\rho = -0.140$) percentages or between eosinophil percentages and ECP ($\rho = -0.129$) or eotaxin ($\rho = 0.039$) levels. Albumin levels correlated positively with IL-8 levels ($\rho = 0.756$; $p < 0.0001$), ECP levels ($\rho = 0.697$; $p < 0.0001$) and a trend was observed with eotaxin levels ($\rho = 0.281$; $p = 0.092$). This suggests an increase in airway wall permeability. ECP and IL-8 levels were positively related ($\rho = 0.567$ $p < 0.0001$). The levels of soluble markers did not depend on the duration of symptoms preceding the admission.

Inflammation markers and clinical bronchiolitis severity

IL-8 levels significantly increased in infants with $\text{SaO}_2 \leq 92\%$ (Fig. 1), rose with the respiratory

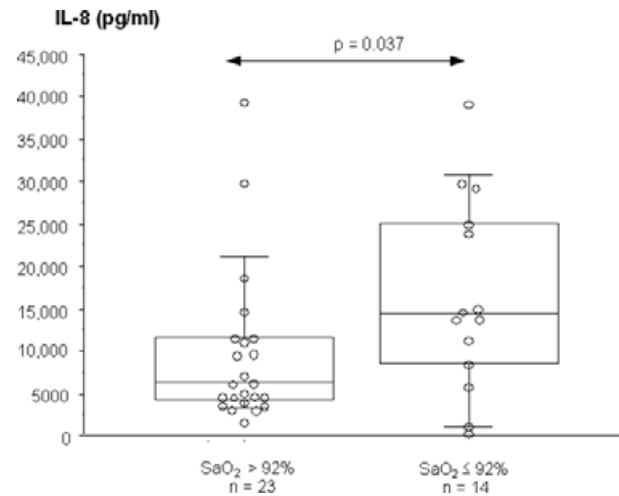


Fig. 1. IL-8 airway production in relation to hypoxemia in 37 infants with acute bronchiolitis. Hypoxemia defined as $\text{SaO}_2 \leq 92\%$ and IL-8 levels were assessed at the time of admission. Results are expressed as box and whisker plots: values, median, 10th, 25th, 75th, and 90th percentiles are displayed. Mann–Whitney's *U*-test was used for statistical analysis and showed higher IL-8 levels in hypoxemic infants.

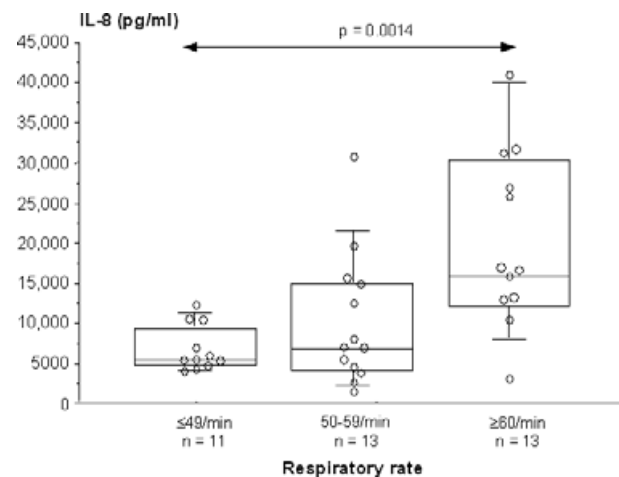


Fig. 2. IL-8 airway production in relation to respiratory rate in 37 infants with acute bronchiolitis. Respiratory rate and IL-8 levels were assessed at the time of admission. Results are expressed as box and whisker plots: values, median, 10th, 25th, 75th, and 90th percentiles are displayed. Jonckheere–Terpstra's ordinal test was used for statistical analysis and showed a positive relationship between IL-8 airway levels and tachypnea.

rate score (Fig. 2) and the Silverman score (Fig. 3). When coded as a continuous variable, SaO_2 (%) tended to inversely correlate with IL-8 levels ($\rho = -0.265$; $p = 0.093$), but not to other mediator levels (Fig. 4). Albumin levels increased with the respiratory rate score (Jonckheere–Terpstra test; $p = 0.0286$). Feeding difficulties were unrelated to the inflammatory markers analyzed. No difference was observed between

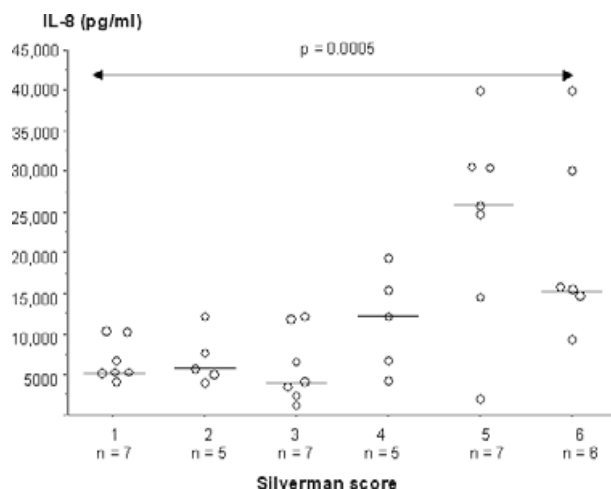


Fig. 3. IL-8 airway production in relation to Silverman score in 37 infants with acute bronchiolitis. Respiratory rate and IL-8 levels were assessed at the time of admission. Results are expressed as box and whisker plots: values, median, 10th, 25th, 75th, and 90th percentiles are displayed. Jonckheere Terpstra's ordinal test was used for statistical analysis and showed a positive relationship between the severity of respiratory distress and IL-8 airway levels.

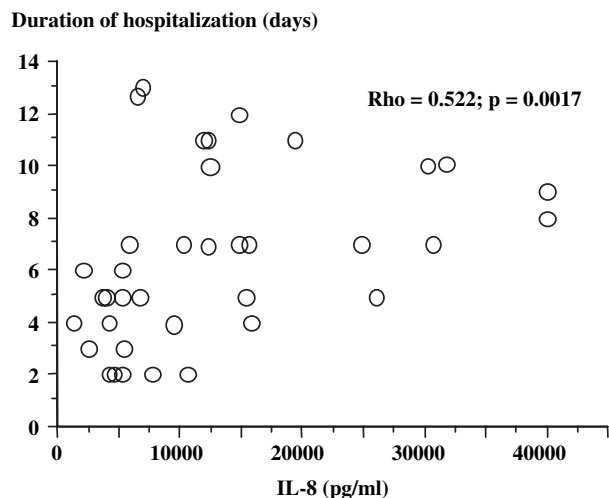


Fig. 4. Negative relationship between airway IL-8 levels and hypoxemia in 37 infants with acute epidemic bronchiolitis. Five patients (*) were outliers (three under 10th and two over 90th). When excluded, the Spearman's test became statistically significant ($\rho = -0.454$; $p = 0.0074$).

cell counts, eotaxin or ECP levels in the groups defined by each clinical parameters of severity that were studied.

Inflammation markers and duration of hospitalization

Hospitalization stay (Fig. 5) was correlated with IL-8 ($\rho = 0.522$; $p = 0.0017$), albumin levels ($\rho = 0.546$; $p = 0.001$), and ECP levels ($\rho = 0.346$; $p = 0.0379$), but not with eotaxin levels.

Duration of hospitalization (days)

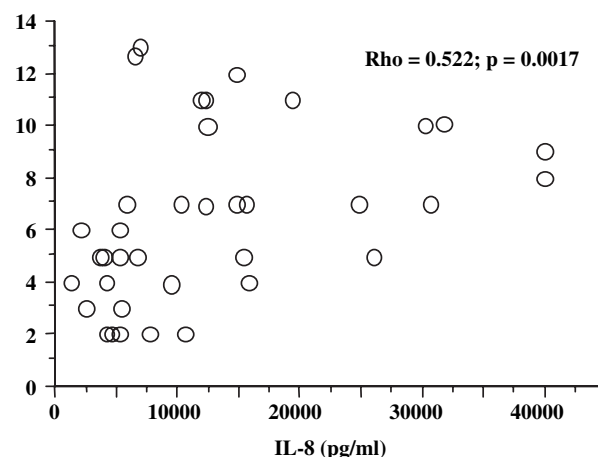


Fig. 5. Correlation between airway IL-8 levels at the time of admission and the duration of hospitalization.

The duration of hospitalization was not related to differential cell counts.

Multivariate analyses

Multivariate analyses showed that once IL-8 was controlled for, no factor had a significant and independent association with any of the severity criteria, because these factors were correlated with IL-8.

Influence of microbiological findings on cellularity, IL-8, eotaxin, ECP and albumin levels

A respiratory syncytial virus ELISA was performed in 27 infants and was positive in 48%. There was no difference between RSV+ ($n = 13$), RSV- ($n = 14$) and RSV not-determined patients, regarding any of demographic or severity criteria analyzed (data not shown). A lower percentage of epithelial cells was found in the sputum from the infants with detectable RSV than that from patients with undetectable RSV: 1.0 (0–1.4)% vs. 6.2 (2.0–10.2)% ($p < 0.001$). Conversely, the percentages of neutrophils tended to be higher in the infants with an RSV-positive sample: 88 (80.9–90.5)% vs. 82.8 (76.1–87.8)% ($p = 0.052$). The percentages of eosinophils were similar in both groups [1(0–3.4)% and 1(0–2.6)% respectively; $p = 0.84$] as well in the levels of the soluble markers that were measured. Bacterial culture was performed in 33 infants and positive in 25. Positive bacterial culture were similar in RSV+ (11/13) and RSV- (11/14) patients. All infants with fever ($n = 14$) had positive bacterial airway secretion culture. A significant relationship was observed between

fever and positive bacterial culture ($p = 0.010$, Fischer's exact test). There was no influence of the bacterial culture either on the total cell and differential cell counts or on the soluble component levels. The percentages of neutrophils in the group of infants with a positive or a negative sample culture were 86 (81–88)% and 84 (79–89)% respectively.

Discussion

The aim of this study was to evaluate the cell profiles and the respective concentrations of soluble components according to the clinical severity of epidemic bronchiolitis. This study demonstrated that IL-8 levels in sputum could reflect the severity of the disease observed at the time of admission. Furthermore, the study showed that the short-term outcome, assessed by the duration of hospitalization, was related to the initial airway inflammation.

Airway inflammation was evaluated by collecting secretions from pharyngeal aspiration immediately after chest physiotherapy. Sputum induction has shown to be an effective means to investigate bronchial inflammatory process in adult bronchial diseases (29) and has also been proposed in childhood asthma (27–30), and in acute infantile wheezing (9). All the procedures used in this study were the same routinely performed by chest physiotherapists in the recommended standard treatment guidelines for infants with acute bronchiolitis in France (24). A saline nebulization was not required because of the sufficient recovered amount of secretions. The secretions analyzed in this study were thought to primarily originate from lower airways: alveolar macrophages were constantly present in the sputum, and the differential count was similar to that found in bronchoalveolar lavage obtained from bronchial diseases associated with comparable high percentages of neutrophils (31). Oropharyngeal contamination was slight in our study, as squamous epithelial cells were less than 20% compared with the threshold of 50% (28) or 42% (32) reported in recent studies in asthmatic and control children. Moreover, the nasal lavage and suction of upper airway secretions performed before chest physiotherapy associated with a pharyngeal suction had limited nasal contamination of collected secretions. Nevertheless, the presence of slight nasal or pharyngeal contamination could not be completely excluded.

The main findings of this study are that the neutrophil cells were predominant in the airways of these infants, and that IL-8 levels in airway

secretions correlated positively with the severity of acute bronchiolitis. The percentages of neutrophil cells were similar to that found in tracheal aspirates from intubated infants (8, 11), and higher than that previously reported in pharyngeal secretions (9). However, in the latter study, secretions were obtained through a nasopharyngeal suction and squamous cell counts were not indicated. On the other hand, the difference in age of both populations could explain the discrepancy between the neutrophil counts reported in both studies, as neutrophil percentage was shown to inversely depend on age (33). However, no differences were observed in the cell profiles according to the bacterial colonization, fever or severity variables studied in contrast to results in other childhood bronchial diseases (31). This finding suggests that the overwhelming intraluminal neutrophil recruitment strongly reflected the viral-induced inflammatory process (34, 35).

Elevated IL-8 levels were measured in secretions from all infants, as recently demonstrated in nasal secretions (10, 11) or tracheal aspirates (4, 11) from infants with RSV bronchiolitis. Interestingly, our study supported the hypothesis that respiratory distress and hypoxemia at the time of admission and the subsequent duration of hospitalization were related to an underlying bronchial IL-8-mediated inflammation and therefore to neutrophil-related inflammation in acute bronchiolitis. Three recent studies have analyzed IL-8 levels in infants with acute RSV bronchiolitis according to the clinical severity of the disease. One study demonstrated that plasma IL-8 levels were higher in the intubated infants, and therefore suggested that plasma IL-8 levels were a marker of severe disease (36). Smyth et al. (37) studied a large population of VRS+ infants, in whom severity was defined by the need of oxygen therapy ($\text{SaO}_2 < 92\%$). They showed that nasopharyngeal mRNA IL-8 concentrations increased with the severity of the disease. The additional analysis of 24 intubated infants suggested that nasopharyngeal results reflected bronchoalveolar inflammation. In addition, the specific role of IL-8 in acute bronchiolitis was recently implicated, as the severity of illness might be genetically determined by a polymorphism in the promoter region of the IL-8 gene (37). On the other hand, Sheeran et al. (11) reported contrasting results on airway inflammation: IL-8 levels in tracheal aspirates were inversely correlated with the duration of assisted ventilation. IL-8 levels in nasal secretions were similar in ventilated and non-intubated infants, and were not related to the duration of hospitalization. However, infants requiring intubation belong to

a particularly severe subgroup, and therefore only non-intubated infants were enrolled in our study. This neutrophil recruitment observed in acute bronchiolitis was consistent with virus-induced epithelial damage (4, 26), which is one of the main alterations found in this disease. Epithelial damage was therefore enhanced in our study: the number of epithelial cells was fourfold elevated than that was in airway secretions from control children (28), and the strong correlation of albumin with other mediator levels suggested a chemokine-related albumin leakage. Otherwise, feeding difficulties, which are common arguments in the decision to hospitalize infants with acute bronchiolitis, were obviously not linked solely to airway inflammation. This suggests that other mechanisms could be involved in the severity of bronchiolitis (i.e., nasal obstruction).

The role of eosinophil-associated inflammation in the severity of acute bronchiolitis was also evaluated in the study. The eosinophil cells were observed in 54% of infants and this was not related to a previous family history of atopy. In addition, we observed increased ECP levels (>200 ng/ml) in two-thirds of patients and detected eotaxin in all but four infants. In contrast with a previous report (15), these data suggest a recruitment and activation of eosinophils in acute epidemic bronchiolitis, which was not related to the initial severity of the disease. The contribution of eosinophils in pathophysiology of infantile bronchiolitis still remains unclear. In experimental studies, RSV was shown to cause eosinophil influx (16). *In vivo*, the detection of eosinophils was not as common which ranged from 0% to 27% of the infants with bronchiolitis (8, 9, 39). The determination of ECP levels in airway secretions was considered as the involvement of eosinophils in viral airway diseases and more specifically in their bronchial localization and consequent severity (15, 39). On the other hand, IL-8 and ECP levels were correlated with each other and to the duration of hospitalization, suggesting that neutrophil- and eosinophil-mediated inflammations participated in a parallel manner in the pathophysiology of acute bronchiolitis. The interpretation of such high ECP levels in airway secretion remains to be highlighted, as various hypotheses on the relationships between ECP production and neutrophil-associated inflammation were previously reported. Eosinophil granules might stimulate the neutrophils IL-8 production (40) and other data have suggested a possible ECP secretion by neutrophil cells (41–43). Therefore, the interpretation of such high ECP levels in airway secretion remains to be highlighted. Eotaxin was previ-

ously detected in bronchial alveolar lavage from ventilated infants with RSV bronchiolitis (4). We found eotaxin in airway secretions from all but four infants, but no relationship with any of the severity variables was observed. The chemoattractant effect of eotaxin on eosinophils appeared to be linked to the presence of mast cells (44), which may be involved in RSV bronchiolitis (9, 12, 13). Thus, eotaxin might therefore contribute to the subsequent bronchial obstruction or hyper-reactivity that were frequently observed in the outcome of viral bronchiolitis (45).

To our knowledge, this is the first series to assess a relationship between neutrophil number and IL-8 levels, as well as eosinophil number and eotaxin levels in young infants with a first acute bronchiolitis. However, no correlation was found between soluble mediators and the corresponding dependent cell number. A similar lack of correlation was previously observed between ECP levels and eosinophil number in acute bronchiolitis (9) and between IL-8 levels and neutrophil number in infantile wheeze (41). This suggests that other mechanisms might be involved in the recruitment of neutrophil cells. Thus, Jones et al. (46) recently showed that a prolonged IL-8 independent survival of neutrophils could be expected in infant airways. Viral etiology is not required to assess the initial bronchiolitis severity in daily clinical practice and therefore was not an inclusion criterion. Because IL-8 was produced in a non-specific mechanism by various viruses (47), our results remained, however, relevant although bronchiolitis were partly RSV proven in a proportion similarly reported elsewhere (45). Thus, IL-8 production was shown to increase with the severity of rhinovirus or influenza community-acquired infection in infants (18), both viruses being involved in acute bronchiolitis, frequently as co infection with RSV (48). RSV might therefore be the principal virus implicated in the study, as all infants were enrolled during a RSV outbreak and were nearly 2-month olds (39). A negative RSV ELISA technique suggests that RSV load was low at the time of the nasopharyngeal aspiration or that other viruses, not researched in this study, were involved. As all infants were enrolled during an RSV outbreak and were nearly 2-month olds, RSV might therefore be the principal virus implicated in the study (39). This study showed an increase in neutrophil and a decrease in epithelial cell counts in the infants with a proven RSV infection relative to those with no identified virus, which contrasted with other reports (9, 39). This is consistent with the influence of RSV load on

intraluminal neutrophil recruitment (11) but not on epithelium shedding which has been observed even in the absence of viral replication (4).

In conclusion, this study demonstrates the role of both neutrophil- and eosinophil-related inflammations in epidemic acute viral bronchiolitis. In addition, the differences in the albumin levels, related to the differences in chemokine levels, might reflect an increasing capillary leak in the lungs with the severity of the disease. These results were not markedly different from that reported with other viral airway diseases (38, 47). The fact that only IL-8 associated inflammation was significantly related to the severity of the disease suggests that neutralizing IL-8 might constitute a novel therapeutic approach for acute viral bronchiolitis.

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