Human Neutrophil Elastase in RSV Bronchiolitis

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Abstract. Acute bronchiolitis is the most common lower respiratory tract infection in young children and may be life-threatening in those with underlying cardiac or respiratory conditions. We evaluated the nasal and serum levels of human neutrophil elastase (HNE) in patients with acute respiratory syncytial virus (RSV) bronchiolitis and investigated the correlation of these levels with illness severity. Fifty-one patients (28 boys, 23 girls) with acute bronchiolitis positive for RSV by direct immunoenzyme assay in nasal secretions (Group A) were studied. Thirty healthy children (17 boys, 13 girls) constituted the control group (Group B). Subjects in both groups were matched for age and gender. The ages (mean ± SE) in Groups A and B were 4.5 ± 0.41 and 5.0 ± 0.65 mo, respectively. Venous blood and nasal secretions were taken from patients in group A on 1, 5, and 15 days after admission and once from controls (Group B) for determinations of HNE in nasal lavage and serum, as well as white blood counts (WBC). The peripheral blood eosinophil and neutrophil counts were elevated in 22/51 patients (43.1%) and 15/51 patients (29.4%), respectively. In nasal lavage specimens, neutrophils represented ≥75% and eosinophils >2% of all cells in 42/51 (82.0%) patients and 11/51 (21.5%) patients, respectively. There was strong correlation between the level of HNE and the percentage of neutrophils in nasal lavage (r = 0.92). The mean nasal HNE concentrations of the patients on 1, 5, and 15 days after admission were higher than those of Group B (p <0.0001, p <0.001, p <0.001, respectively). Mean serum HNE concentrations on 1, 5, and 15 days after admission were higher in Group A than in Group B (p <0.0001, p <0.0001, p <0.0001, respectively). Nasal and serum HNE concentrations showed no correlations with the clinical score of disease severity (r = 0.28 and r = 0.29, respectively). This study shows that (a) serum and nasal HNE concentrations were significantly higher in RSV bronchiolitis patients than in controls, (b) they did not return to normal after the respiratory symptoms had improved, and (c) they showed no significant correlations with clinical score of severity. The results indicate that neutrophils contribute significantly to airway inflammation in these subjects and HNE levels in serum and nasal lavage may be useful markers of inflammation in acute RSV bronchiolitis.

Keywords: respiratory syncytial virus, bronchiolitis, neutrophils, human neutrophil elastase

Introduction

Acute bronchiolitis is the most common viral infection of the lower airways of infants and it is classically defined as the first episode of wheezing in children younger than 2 yr old [1-3]. The etiologic agents most often associated with acute bronchiolitis are respiratory syncytial virus (RSV), which accounts for 60-80% of cases, parainfluenza, influenza, and adenovirus [4,5]. Recently, other viruses have also been associated with this illness, including rhinovirus, enterovirus, metapneumovirus, and coronavirus [6-10].

Despite intensive efforts during the past 30 years, the inflammatory process in the respiratory tract of infants with RSV bronchiolitis has not been clearly characterized [3,4]. Respiratory viruses infect bronchial epithelial cells, resulting in epithelial activation and increased neutrophil recruitment and activation [11,12]. Neutrophils are
present in large numbers in the airways of infants with RSV bronchiolitis and represent >76% of all cells in lavage fluid obtained from the upper or lower respiratory tract [11]. There is evidence that neutrophil-mediated inflammation is involved in the pathogenesis of tissue destruction and the augmentation of bronchial reactivity in RSV bronchiolitis [12-14]. The neutrophil response plays a significant role in the airway obstruction through the release of human neutrophil elastase (HNE), as well as other inflammatory mediators [14,15].

HNE is a protease localized in azurophilic granules of polymorphonuclear granulocytes [16]. Its potential substrates include almost all components of the extracellular matrix, including proteins as diverse as clotting factors, complement, immunoglobulins, and cytokines. When neutrophils are activated, HNE is rapidly released from the cytoplasmic granules into the extracellular space [16,17]. HNE is a potent secretagogue in airway goblet cells; it induces hypersecretion of mucus and causes mucous plugs in airways [12].

The aim of this study was to assay the levels of HNE in serum and nasal lavage samples from patients with acute RSV bronchiolitis and to ascertain whether severity of the illness is correlated with HNE concentrations in serum and nasal secretions.

Materials and Methods

This study included 75 infants with acute bronchiolitis, who were under the medical care of the 2nd Department of Paediatics (Aristotle University, Thessaloniki, Greece) during the period from January 2002 to December 2004. Of these 75 infants, 51 (28 boys, 23 girls) had positive tests for RSV by direct immunoenzyme assay in nasal secretions and were categorized as Group A. The patients of Group A were characterized clinically by coryza, cough, hyperinflation, tachypnea, and widespread crepitation on auscultation with or without wheezing [1]. Patients with severe illness and non-severe illness were distinguished by the clinical score of disease severity [18] (Table 1). Inclusion criteria for hospital admission were lower respiratory tract symptoms, age <13 mo, ODS birth after 38 wk of gestation, and appropriate weight for gestational date. Excluded from Group A were children with bronchiolitis who had been treated with corticosteroids during the illness or who had underlying cardiopulmonary disease (eg, bronchopulmonary dysplasia, recurrent pneumonia, recurrent wheezing, cystic fibrosis, prematurity, or immunodeficiency). Each patient’s medical history and chart were reviewed to determine the date of onset, character of symptoms, examination findings, clinical score of severity, and results of chest X-rays. After admission to the hospital, all of these infants were treated with bronchodilators (nebulized salbutamol, with or without ipratropium) and some also received antibiotics.

Thirty infants (17 boys, 13 girls) comprised the control group (Group B). This group was drawn from children attending an ambulatory routine health check-up. They were non-atopic, had no pre-existing cardiorespiratory disease or acute respiratory illness, and had not recently received any medications. Subjects of Group A and Group B were matched for age and sex. Their ages (mean ± SE) were 4.5 ± 0.41 and 5.0 ± 0.65 mo (range 1.5-12 mo) in Groups A and B respectively. No infant in either group had been ventilated in the neonatal period. Informed consent from the parents was obtained by the Hospital Ethics Committee.

Venous blood samples were obtained from all patients of group A on 1, 5, and 15 days after admission and one sample was obtained from the control subjects (Group B) for determination of HNE and leukocyte count. Serum was separated and stored at -20°C until HNE assay.

Neutrophil elastase in serum and nasal washings was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Immuno Biological Laboratories, USA) in accordance with the manufacturer’s directions. Nasal lavage for detection of RSV and determination of HNE was performed on admission to the hospital (Group A) or at the time of entry to the study (Group B). The second and third nasal lavage samples were obtained from patients of Group A at 5 and 15 days after admission.

Before they were recruited to the study, the 51 infants in Group A all gave positive tests for RSV by an enzyme immunoassay for the rapid detection of RSV in nasopharyngeal washes (Test Pack RSV kit, Abbott Diagnostics, USA) [19]. Nasal lavage was performed with the patient held in a supine position. Two ml of phosphate-buffered saline (PBS) was gently instilled into each nostril. The wash fluid was simultaneously suctioned back from the anterior nares using a nasal catheter. The catheter was attached to a mucus collector (FG12 Riplast GmbH) connected to suction (Ultrasonic 2000, Switzerland) using a pressure of 2.7 - 6.7 kPa (20-50 mmHg). Samples were held on ice until arrival in the laboratory where the lavage fluid was centrifuged at 300 x G for 5 min. The supernatant was separated from the pelletted cellular debris and stored at -70°C.

Results are reported as mean ± SE. Significance of differences between Groups A and B was assessed by ANOVA. Pearson’s correlation test was used to calculate r-values. Statistical significance was assumed for p values <0.05.

Results

Elevated neutrophil counts in peripheral blood were defined as >5800 cells/mm³ [3,29] and elevated eosinophil cell counts in peripheral blood were defined as >400 cells/mm³ [5,20]. The serum C-reactive protein level (CRP, mean ± SE) on
admission for infants of Group A was 1.09 ± 0.23 mg/dl. The peripheral blood neutrophil count (mean ± SE) of Group A on admission was 4649 ± 428 cells/mm$^3$.

Respiratory syncytial virus was detected in 51/75 (55%) cases. The characteristics of patients with RSV bronchiolitis are shown in Table 2. The mean age of the patients of Group A was 4.5 ± 0.4 months. In Group A, 21/51 (41%) patients presented with severe bronchiolitis (clinical score ≥8), all of whom required oxygen therapy but none required automatic ventilation; 8/21 (38%) infants with severe bronchiolitis were <3 mo old. The average length of hospitalization was 4 days. Peripheral blood eosinophil counts were elevated in 22/51 patients (43.1%) and 6/22 (27.3%) of them had severe bronchiolitis (clinical score ≥8). There was no significant difference in the peripheral blood eosinophil count in patients who did or did not show presence of eosinophils in nasal lavage. Neutrophils in peripheral blood were elevated in 15/51 (29.4%) patients and 8/15 (53.3%) of them had severe bronchiolitis (clinical score ≥8). There was no significant difference in the concentration of serum HNE in patients with or without elevated neutrophil counts in peripheral blood.

Table 2. Characteristics of patients studied.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 51)</th>
<th>Group B (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>4.5 ± 0.41</td>
<td>5.0 ± 0.65</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>28 (55%)</td>
<td>17 (56%)</td>
</tr>
<tr>
<td>Race, Caucasian (%)</td>
<td>51 (100%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Smoking at home (%)</td>
<td>15 (29.4%)</td>
<td>8 (26.7%)</td>
</tr>
<tr>
<td>O$_2$ therapy required (%)</td>
<td>17 (33.3%)</td>
<td>-</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>4.0 ± 0.41</td>
<td>-</td>
</tr>
<tr>
<td>Mechanical ventilation (%)</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of atopic diseases</td>
<td>23 (45%)</td>
<td>0</td>
</tr>
<tr>
<td>Clinical score (&gt;6)</td>
<td>28 (54.9%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Neutrophils represented ≥75% of all cells in nasal lavage in 42/51 (82%) patients and eosinophils represented >2% of all cells in nasal lavage in 11/51 (21.5%) patients. There was strong correlation between the levels of HNE in nasal lavage and the percentage of neutrophils in the nasal lavage (r = 0.92). The mean concentrations of HNE in nasal lavage and serum samples in the 2 groups are shown in Fig. 1. The mean nasal HNE concentrations of the patients on admission (at the onset of illness) were significantly higher than those of the control subjects (474 ± 70 ng/ml vs 31.5 ± 3.5 ng/ml, respectively; p <0.0001).

The mean ± SE nasal HNE concentrations of patients in Group A on the 5th and 15th day after admission were 167 ± 33 ng/ml and 43.1 ± 7.3 ng/ml, respectively, and were significantly lower than on the 1st day of admission (p <0.05 and p<0.005, respectively). The nasal HNE concentrations were also significantly higher on the 5th and 15th day after admission in Group A compared to the controls in Group B (p <0.001 and p <0.001, respectively; Fig. 1). The serum HNE concentrations on the 1st, 5th, and 15th day after admission in Group A were 757 ± 65 ng/ml, 489 ± 63 ng/ml, and 289 ± 56 ng/ml, respectively (Fig. 1). These concentrations were all significantly higher than in control subjects (64.1 ± 12.9 ng/ml; p <0.0001). Nasal HNE concentrations showed no significant correlation with the clinical score of severity of disease (r = 0.28). Serum HNE concentrations also showed no significant correlation with the clinical score (r = 0.29).

Discussion

Acute bronchiolitis is generally a self-limiting condition, but it may be life-threatening in a few children, especially those with pre-existing cardiac or respiratory conditions [1,21,22]. Infants younger
than 3 mo without any underlying disease may be at high risk for severe infection [22]. In our study we found that the majority of patients presented with moderate to severe clinical score of severity of RSV bronchiolitis and that about one-third of those with severe bronchiolitis were younger than 3 mo. This means that young age remains among the high risk criteria for severe infection.

Our results suggest that neutrophils in nasal lavage, which represent >75% of all cells in lavage, contribute significantly to the airway inflammation of infants with RSV bronchiolitis. Neutrophils evidently play an important role in the causation of symptoms during viral respiratory tract infections. This neutrophil response is hypothesized to contribute significantly to airway obstruction through the release of inflammatory mediators that contribute to airway edema, mucus secretion, and excessive airway fluid. Our findings are in agreement with those of other investigators [11,14,23].

The activated neutrophils produce HNE. To our knowledge there has been only one study that compared HNE levels in nasal lavage in infants with bronchiolitis vs control infants [14] and one study that evaluated serum HNE level and the role of neutrophil-mediated inflammation in the pathogenesis of acute RSV bronchiolitis [24]. We measured HNE in both serum and nasal lavage in our patients and investigated the correlation of HNE levels with the severity of the disease. Our results showed that serum HNE concentrations in RSV bronchiolitis patients were significantly higher than in controls and did not return to normal after respiratory symptoms improved. The elevation of serum HNE concentration was observed in patients with high or low clinical scores of severity of RSV.

We found that nasal HNE concentrations in RSV bronchiolitis patients were significantly higher than in controls and did not return to normal after respiratory symptoms improved. A previous study also reported high levels of HNE in nasal secretions in patients with RSV bronchiolitis [14]. Elevation of nasal HNE concentration was noted in patients with high or low clinical score of severity of RSV bronchiolitis. In all patients with high nasal HNE levels (higher than levels in the controls), neutrophils comprised >75% of the cells in nasal lavage.

In the present study, samples were obtained from the upper respiratory tract of infants with RSV bronchiolitis using a lavage technique. This technique provides useful information that is relevant to the process occurring in the lungs of infants with bronchiolitis. Much remains to be learned about the effects of RSV on the host response. For respiratory viruses, the induction of neutrophils within the airways is more advantageous to the virus than the host. Neutrophil products stimulate the host to secrete mucus and they induce sneezing and coughing, which are important in transmission of the virus. Our study shows elevated levels of nasal neutrophil elastase in the upper respiratory tract in these patients and this is reflected by an elevation of HNE levels in serum as well. HNE may play a role in the intense...
inflammatory process within the airways of such infants as a sequel of the neutrophil recruitment, leading to airway obstruction and the development of respiratory symptoms during viral infections. These results indicate that neutrophils contribute to the airway inflammation of infants with RSV bronchiolitis and may enhance the respiratory morbidity in the years following acute RSV bronchiolitis [1,44].

HNE has been reported to be excreted in proportion to the activation of neutrophils and to correlate with peripheral blood neutrophil counts. Recent studies suggest that neutrophil elastase plays an important role in the pathophysiology of lung inflammation and acute lung injury, either by influencing neutrophil chemotaxis and cell adhesion or by inducing the release of inflammatory mediators [24-42]. Neutrophil elastase may possibly contribute to a decrease in respiratory function, not only by inducing alveolar edema but also by damaging alveolar surfactant [43].

Our results suggest that activation of neutrophils is involved in the pathogenesis of RSV bronchiolitis, leading to elevation in nasal and serum HNE levels. Moreover, our results indicate that HNE is released in the upper respiratory tract of infants with RSV bronchiolitis and that this may play a major role in promoting the intense inflammatory process evident in their airways.

In conclusion, this study shows that HNE was significantly elevated in nasal secretions and serum of infants with acute RSV bronchiolitis, compared to control subjects. The elevated HNE levels indicate that neutrophils contribute to airway inflammation in these infants and HNE levels may be useful markers of inflammation in acute RSV bronchiolitis. Further studies are required to elucidate the pathophysiologic mechanisms of bronchiolitis and the exact role of HNE in infants with RSV bronchiolitis.

References


