Chemokine Concentrations in Nasal Washings of Infants with Rhinovirus Illnesses

We determined RANTES (regulated on activation, normal T cell expressed and secreted) and interleukin-8 (IL-8) concentrations, and total white blood cell (WBC) and differential counts in nasal wash samples from rhinovirus-infected infants presenting with wheezing or acute upper respiratory illness alone and compared them with those from healthy infants. RANTES concentrations were significantly greater in acute samples from wheezy patients than in those from patients with acute upper respiratory illness only, or in control samples. IL-8 concentrations and WBC and neutrophil counts were significantly greater in acute samples from wheezy infants and patients with upper respiratory illness alone than in control samples, but they did not differ significantly between the 2 patient groups.

Rhinovirus infection in any age group usually causes only mild upper respiratory tract illness—that is, the common cold. Recent studies, however, have also highlighted the importance of this virus in causing lower respiratory tract disease among children. In particular, rhinoviruses have been associated with exacerbations of reactive airway symptoms in older children and with bronchiolitis in infants and young children [1]. The mechanism behind the close association between rhinovirus infection and wheezing attacks remains unclear. There is an early influx of polymorphonuclear leukocytes (PMNLs) into nose and nasal secretions in naturally acquired and experimental rhinovirus colds that is due, at least in part, to elaboration of interleukin-8 (IL-8) by infected epithelial cells [2]. On the other hand, experimental studies have shown bronchial epithelial lymphocyte and eosinophil infiltration during rhinovirus colds in asthmatic subjects [3]. Although PMNLs and possibly eosinophils may respond to α-chemokines such as IL-8, the recent discovery that several members of the β-chemokine family—in particular RANTES (regulated on activation, normal T cell expressed and secreted)—are potent chemotactants for monocytes, T lymphocytes, basophils, and eosinophils but have virtually no activity on PMNLs [4] raises the question about the role of these molecules in the context of wheezing associated with rhinovirus infection.

The objectives of the present study were to determine whether RANTES and IL-8 could be detected in the nasal wash samples of rhinovirus-infected infants with either acute wheezing illness or upper respiratory symptoms alone and to assess whether concentrations of these chemokines correlated with WBC counts in nasal wash samples.

The study population consisted of 25 patients aged 3–47 months with documented rhinovirus infection. The patients were recruited from children with clinical manifestations of acute respiratory tract infection who had been admitted between January 1997 and March 1999 to the acute care clinic of our institution and who had received neither inhaled nor systemic corticosteroids within the previous 7 days. None of the patients had undergone ventilation in the neonatal period and none had known underlying cardiac or pulmonary disease.

Fourteen of the 25 rhinovirus-infected patients presented with mild wheezing illness (group 1). Infants with wheezing were defined as those having prominent findings of bronchial obstruction, manifested primarily as expiratory wheezing of acute onset, preceded by or accompanied by fever, coryza, or both. On admission, 8 of the 14 patients were infants with first-time wheezing. All patients with wheezing fulfilled the previously validated criteria of Wang et al. [5] for a mild illness, having a median severity score of 6 points (range, 4–7). A value from 0 to 3 was assigned for each of the following measurements: wheeze score (0, no wheezing; 1, end expiratory wheeze only; 2, wheeze during entire expiratory phase or audible on expiration without stethoscope; 3, inspiratory and expiratory wheezing audible without stethoscope), accessory muscle score (0, no indrawing; 1, mild intercostal indrawing; 2, moderate indrawing with tracheosternal retractions; 3, severe retractions with nasal flaring), and respiratory rate (0, ≤30 breaths/min; 1, 30–45 breaths/min; 2, 46–60 breaths/min; 3, >60 breaths/min). In addition, the behavioral symptoms (decreased appetite, crankiness, and excessive sleepiness) were assessed. A score of 0 represented the patient’s normal general appearance (quiet alert or active alert), and a score of 3 represented the most severe illness. When outcome was also taken into account, diagnosis of mild illness was still consistent: All 14 infants required admission for observation without oxygen (or ventilation) requirement. The remaining 11 rhinovirus-infected patients (group 2) presented with an acute, self-limited upper respiratory disease (defined as the presence of new-onset rhinorrhea with or without fever and cough), lasting no longer than 6 days (median, 4) of the 3 weeks that followed. At study entry, 6 of the 14 group 1 patients as well as 4 of the 11 group 2 patients (42.8 vs. 36.3%; P = 1.0) had experienced 1 episode of previous wheezy illness. After resolution of their acute illness, no patient in either group developed episodes of acute respiratory illness during the entire follow-up period.

In addition, 30 healthy controls (group 3) were chosen...
The results of measurements of RANTES, IL-8, and albumin concentrations in acute nasal wash samples by form of rhinovirus illness are shown in figure 1. Median RANTES concentrations were significantly greater in nasal wash samples from patients with wheezing (group 1) (97 pg/mL; range, 27–950 pg/mL), but not in samples from patients with upper respiratory illness only (group 2) (8 pg/mL; range, 0–175 pg/mL), than in samples from control infants (group 3) (0.2 pg/mL; range, 0–43 pg/mL). Median IL-8 concentrations were significantly greater in nasal wash samples from patients with wheezing (16,196 pg/mL; range, 576–41,170 pg/mL) and patients with upper respiratory illness only (6000 pg/mL; range, 230–40,000 pg/mL) than in samples from controls (490 pg/mL; range, 3.3–8125 pg/mL). However, no significant differences in IL-8 concentrations in nasal wash samples between groups 1 and 2 were found. Median albumin concentrations were significantly greater in nasal wash samples from patients with wheezing (395.5 μg/mL; range, 32–1208 μg/mL; \( P < .0001 \)) and patients with upper respiratory illness only (112 μg/mL; range, 39–847 μg/mL; \( P = .006 \)) than in samples from controls (49 μg/mL; range, 1.5–279 μg/mL). Although albumin concentrations in nasal wash samples from patients with wheezing tended to be higher than those in samples from patients with upper respiratory illness only, the difference was not significant (\( P = .18 \)). If chemokine results among the groups were normalized to albumin concentration or expressed as ratios of RANTES to IL-8 for each nasal wash sample, all statistical conclusions pertaining to the chemokines remained valid. Median RANTES concentrations were significantly greater in nasal wash samples from patients with wheezing (0.18 pg/mL/μg of albumin; range, 0.07–1.6 pg/mL; \( P = .0004 \)), but not in samples from patients with upper respiratory illness only (0.01 pg/mL/μg of albumin; range, 0–0.3 pg/mL/μg of albumin), than in samples from controls (0.005 pg/mL/μg of albumin; range, 0–1.5 pg/mL/μg of albumin). Median IL-8 concentrations were still significantly greater in nasal wash samples from infants with wheezing (58.4 pg/mL/μg of albumin; range, 15–260 pg/mL/μg of albumin; \( P = .01 \)) and infants with upper respiratory illness only (65.4 pg/mL/μg of albumin; range, 17–392 pg/mL/μg of albumin; \( P = .01 \)) than in samples from controls (20 pg/mL/μg of albumin; range, 0.6–113 pg/mL/μg of albumin). However, no significant differences in IL-8 concentrations in nasal wash samples were found between groups 1 and 2. The median ratio of RANTES to IL-8 was significantly higher in nasal wash samples from patients with wheezing (0.006; range, 0.001–0.15) than in samples from patients with upper respiratory illness only (0.0007; range, 0–0.038; \( P = .0007 \)) or in samples from controls (0.0002; range, 0–0.10; \( P < .0001 \)).

Table 1 shows total WBC counts and differentials in acute nasal wash samples by form of rhinovirus illness. Median WBC and PMNL counts in nasal wash samples were sig-
Figure 1. Comparison of concentrations of RANTES (regulated on activation, normal T cell expressed and secreted) and interleukin-8 (IL-8) in acute nasal wash specimens from patients with wheezing rhinovirus illness (group 1), or upper respiratory illness alone (group 2), and in nasal wash samples from healthy controls (group 3). Filled circles, infants without history of previous episodes of wheezing; open circles, infants with such history. Horizontal lines represent medians. *P* values were derived by use of the Mann-Whitney test.
Table 1.  Cell counts and differentials in acute nasal wash samples from rhinovirus-infected infants with wheezing or with upper respiratory illness (URI) alone and from healthy controls.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Wheezing</th>
<th>URI alone</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells</td>
<td>9.9 (0.6–40.2)</td>
<td>4.7 (0.1–78.5)</td>
<td>0.3 (0.04–11.2)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>8.9 (0.1–37.7)</td>
<td>3.8 (0.1–75.2)</td>
<td>0.3 (0–9.5)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0 (0–0.7)</td>
<td>0.02 (0–3.1)</td>
<td>0 (0–0.4)</td>
</tr>
<tr>
<td>Mononuclear leukocytes</td>
<td>0.12 (0–20)</td>
<td>0 (0–1.1)</td>
<td>0 (0–0.6)</td>
</tr>
<tr>
<td>Squamous epithelium</td>
<td>0.16 (0–3)</td>
<td>0 (0–0.73)</td>
<td>0.15 (0–2)</td>
</tr>
</tbody>
</table>

NOTE.  Cell numbers are ×10⁶/mL, and are expressed as median (range).

a  P = .0001 vs. control infants; P = .6 vs. infants with URI.
b  P = .002 vs. control infants.
c  P = .002 vs. control infants; P = .5 vs. infants with URI.
d  P = .03 vs. control infants.

Significantly greater in patient groups than in controls. However, median WBC and PMNL counts in nasal wash samples did not differ significantly between the 2 patient groups. We also determined, whether during the acute phase of both rhinovirus-associated respiratory illnesses, concentrations of chemokines correlated with WBC counts in nasal wash samples. Among patients with wheezing, there was a positive correlation between IL-8 concentrations and WBC counts (r = .57, P = .03). Among patients with upper respiratory illness only, the same relationship was found (r = .66, P = .02). In contrast, RANTES did not correlate with WBC counts among patients with wheezing (r = .37, P = .18) or patients with upper respiratory illness only (r = .22, P = .49).

All acute and follow-up nasal wash samples were analyzed for paired comparisons of albumin and chemokine concentrations as well as of WBC counts. Patients with wheezing had significantly decreased median albumin (84.5 μg/mL; P = .007), RANTES (0.2 pg/mL; P = .0001), and IL-8 (826 pg/mL; P = .002) concentrations as well as median WBC counts (1.8 × 10⁶ cells/mL; P = .01) in follow-up samples. Patients with upper respiratory illness had significantly decreased median albumin (54.6 μg/mL; P = .01) and IL-8 (643 pg/mL; P = .01) concentrations as well as median WBC counts (1.3 × 10⁶ cells/mL; P = .03) in follow-up samples. As expected, acute and follow-up nasal wash samples from patients with upper respiratory illness did not differ significantly in median RANTES concentrations (0.2 pg/mL for each).

Viral respiratory infection is a common trigger of wheezing in early childhood and may have an association with subsequent asthmalike symptoms in susceptible subpopulations. Two recent studies compared responses to experimental rhinovirus infections in asthmatic or allergic rhinitic subjects and normal subjects, and both implicated an increased bronchial eosinophil infiltrate in the pathogenesis of virus-induced exacerbations of asthma [3, 8]. To our knowledge, this is the first study to document elevated nasal concentrations of the eosinophil chemoattractant RANTES during acute wheezy episodes associated with respiratory infection with rhinovirus in infants. The increased production of RANTES was not simply a nonspecific response to viral infection, because concentrations were much greater in infants with virus-induced wheezing than in those with upper respiratory illness alone attributable to rhinovirus infection. These findings echo those of Bonville et al. [9], who showed that RANTES was not present in the nasal samples of children aged 8 days to 10 years with upper respiratory tract infections caused by rhinovirus, and those of Teran et al. [10], who, in contrast, showed increased levels of RANTES in the upper airways of children aged 9–11 years with simultaneous rhinovirus-related colds and asthma exacerbations. Taken together, these observations suggest a prominent role for this chemokine in wheezing associated with rhinovirus infection. This is consistent with elegant in vitro studies that have demonstrated the ability of rhinovirus to induce RANTES secretion on infection of human bronchial epithelial cells [11].

In the present study, the finding of elevated nasal wash levels of RANTES in rhinovirus-infected infants with wheezing apparently conflicted with the concurrent predominance of PMNLs in nasal wash samples. In view of the known biological functions of this chemokine, this seems surprising. However, comparable data are available from the recent study by Rakes et al. [12] on eosinophil analysis of wheezing infants with respiratory syncytial virus infection: The finding of elevated levels of nasal wash eosinophil cationic protein conflicted with the concurrent absence of nasal eosinophilia in nearly all the wheezy patients. This is of interest because RANTES induces the release of eosinophil cationic protein [4]. A possible explanation for our findings and those of Rakes et al. [12] is that many eosinophils may have been activated and lost their characteristic granules. This is unlikely, because Rakes et al. [12] showed in the same study that, among children aged >2 years with rhinovirus-associated wheezing, nasal eosinophilia as well as elevated concentrations of eosinophil cationic protein were strongly associated with the illness. It is also reasonable to hypothesize that eosinophils, if attracted, could be localized in the nasal submucosa rather than found on the mucosal surface. A third possible explanation is that there are significant differences between the inflammatory infiltrate present in the upper airways and the infiltrate present in the distal airways, thereby explaining these discrepancies. However, Neilson and Yunis [13] did not observe, at autopsy, eosinophils in the bronchial walls of infants with fatal acute wheezing episodes due to respiratory syncytial virus. Finally, when looking at our patient population, it may be argued that rhinovirus infection may incite eosinophilic upper airway inflammation in the counterpart of infants with wheezing with severe clinical course. However, the results of Neilson and Yunis [13] suggest that eosinophil findings are independent of illness severity. A comparable observation has been recently reported by Sheeran et al. [14] in respiratory syncytial virus–infected infants. In that study, predominance of
PMNs in acute nasal wash and tracheal aspirate samples from patients with bronchiolitis (or pneumonia) appeared to be independent of the severity of illness.

It now is well known that a chemotactrant for PMNs (IL-8) is elaborated during colds and that both this chemotactrant and PMNs are associated with symptomatic infection [2]. Recent studies have also found increased concentrations of IL-8 in the nasal secretions of asthmatic subjects with either acquired viral infection or experimental rhinovirus colds [15, 16]. From our data, the finding of elevated IL-8 concentrations in nasal wash samples as well as of elevated WBC and PMNL counts, during the acute phase of rhinovirus illness appeared to be independent of the involvement of the respiratory tract, suggesting that release of neutrophil chemotactic factors into the upper airway may represent a general phenomenon during acute symptomatic respiratory infection with rhinovirus.

Because accurate calculation of the volume of epithelial lining fluid in respiratory lavage fluid is not possible with current techniques, particularly in the setting of inflammation and increased vascular permeability, our data were not corrected for dilution of nasal secretions by lavage fluid. One commonly used endogenous indicator of relative dilution of nasal secretions by lavage fluid is albumin [6]. Another strategy is to examine the ratio of RANTES to IL-8, circumventing the need to standardize to a nonchemokine reference lavage constituent. When the ratio of RANTES to IL-8 is determined in individual samples, the unit of measure is omitted and the ratio is, at the same time, independent of the dilution. Our conclusions regarding RANTES and IL-8 were strengthened by finding that the pattern of chemokines produced in response to rhinovirus illnesses was the same whether data were not standardized, standardized to albumin concentrations, or expressed as ratios of RANTES to IL-8.

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References


