Respiratory Syncytial Virus Induces Selective Production of the Chemokine RANTES by Upper Airway Epithelial Cells

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The presence of histamine and eosinophil cationic protein in nasopharyngeal secretions of infants with respiratory syncytial virus (RSV)-induced bronchiolitis implies the activation of basophil and eosinophil leukocytes, but the specific mechanism of their recruitment has not been elucidated. Chemokines are potent and selective leukocyte chemotactic molecules that are also expressed by airway epithelial cells. Therefore, the pattern of chemokines produced in response to RSV infection was investigated in primary cultures of human nose- and adenoid-derived epithelial cells. Interleukin-8, growth-related peptide-α, and monocyte chemotactic protein-1 were constitutively released by uninfected epithelial cells and were not further enhanced by infection with RSV. RANTES (regulated upon activation, normal T cell–expressed and –secreted), which was present in negligible concentrations in uninfected cultures, was strongly induced by RSV infection, in a dose- and time-dependent manner. Through the release of RANTES, epithelial cells may control the selective concentration and activation of basophils and eosinophils in RSV-infected airway mucosa.

Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis in infants [1] and a major pathogen in the elderly, as it is recognized by the epidemics of respiratory tract diseases that occur yearly in hospitals and nursing homes [2, 3]. In addition to its acute morbidity, RSV infection predisposes to the development of hyperreactive airway disease [4], and in children with asthma, wheezing is often triggered by upper respiratory tract infections [5]. A series of studies has suggested that in RSV-infected infants, the severity of the respiratory disease correlates with the presence and concentrations in nasopharyngeal secretions of RSV-specific IgE and the proinflammatory mediators histamine [6], leukotriene C<sub>4</sub> [7], and eosinophil cationic protein (ECP) [8, 9]. High levels of ECP were found to be associated with clinical evidence of bronchiolitis at the time of RSV infection [8, 9]. Infants who contracted or even died of RSV infection, despite immunization with a formalin-inactivated RSV vaccine, had increased numbers of eosinophils in the peripheral blood [10] and in the airways [11]. Animal studies that were designed to investigate the contribution of immunity to the augmentation of lung pathology have shown that in mice previously sensitized to the RSV major surface glycoprotein (G), up to 25% of bronchoalveolar lavage cells were in fact eosinophils [12]. Furthermore, transfer of G-specific T cells into naive RSV-infected mice induced lung hemorrhage and intense pulmonary eosinophilia [13]. Although these studies suggest that in naturally acquired RSV infection as well as in vaccine-induced enhanced disease, eosinophils and basophils may undergo activation in the airways, definitive mechanisms of their recruitment to the airway mucosa have not been elucidated.

Chemokines are a novel class of small chemotactic cytokines with discrete target cell selectivity that are able to activate leukocytes and therefore have a potential role as potent mediators of inflammation [14]. Two subfamilies, the CXC and the CC chemokines, are defined by the splicing of the conserved cysteine residues, which either are separated by one amino acid (CXC chemokines) or are adjacent (CC chemokines). Interleukin (IL)-8 and growth-related peptide (GRO)–α belong to the CXC family and are mainly chemotactic factors for neutrophils. On the other hand, CC chemokines, which include monocyte chemotactic protein (MCP)-1, the best characterized “histamine releasing factor,” macrophage inflammatory protein (MIP)–1α, and RANTES (regulated upon activation, normal T cell–expressed and –secreted) are chemotactic factors for monocytes, basophils, and eosinophils, with virtually no activity on neutrophils [15].

Under normal conditions, the epithelium represents the principal cellular barrier between the environment and the internal milieu of the airways. However, after contact with exogenous stimuli, such as microbial infections, the epithelium is able to actively modulate the local inflammatory responses by releasing a variety of proinflammatory mediators and cytokines [16]. In humans, epithelial cells of the upper respiratory tract are the primary site of RSV infection and replication, with secondary
involvement of the lower respiratory tract mucosa [17]. Although few studies have investigated in vitro the nature of interaction between RSV and human bronchial and lung epithelial cell lines, information on the effect of RSV replication on primary cultures of upper respiratory epithelial cells is sparse [18–20].

In the present study, utilizing a model of primary cultures of human epithelial cells derived from nasal mucosa and adenoids, we have characterized the pattern of CXC and CC chemokines produced in response to RSV infection.

Materials and Methods

Nasal and adenoid epithelial cell culture. Specimens of nasal tissue were obtained from 11 patients (ages 15–65 years) undergoing polypectomy or conchotomy for nasal obstruction. Adenoid tissues were obtained from 6 patients (ages 7–12 years) undergoing adenoidectomy for adenoid hypertrophy. Epithelial cell monolayers were prepared, with minor modifications, according to the method described by Ohtoshi et al. [21]. Specimens were immersed in 1% iodine solution and subsequently washed extensively in Dulbecco's PBS (Life Technologies Gibco BRL, Gaithersburg, MD) supplemented with 200 U/mL penicillin, 200 μg/mL streptomycin, and 250 ng/mL amphotericin B. Epithelial cells were dissociated by gentle rocking for 16 h at 4°C in Ham's F12 medium (Life Technologies) containing 0.1% (vol/vol) protease (Sigma, St. Louis). After incubation, heat-inactivated fetal bovine serum (FBS, Life Technologies) was added to a final concentration of 15% (vol/vol) to neutralize protease activity. The cells were detached from the tissue by gentle agitation, filtered through a 60-μm nylon mesh (Spectrum, Houston), and washed twice with centrifugation at 200 g for 10 min. The cell pellet was finally resuspended in hormonally defined Ham's F12 medium, at 5 × 10^6 cells in 1 mL of medium were plated onto collagen (Vitrogen 100; Celtrix, Santa Clara, CA)-coated 24-well culture plates. The hormonally defined Ham's F12 medium contained 2% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 250 ng/mL amphotericin B, 2 mM glutamine, 5 μg/mL insulin (Life Technologies), 5 μg/mL transferrin (Life Technologies), 25 μg/mL epidermal growth factor (Life Technologies), 15 μg/mL endothelial cell growth supplement (Sigma), 6.5 ng/mL triiodothyronine (Sigma), and 0.5 μg/mL hydrocortisone (Life Technologies). Cells were incubated at 37°C in 5% CO2. The medium was changed at day 1 and subsequently every 2 days.

Characterization of cultured epithelial cells. Confluent monolayers of epithelial cells were harvested by trypsin-EDTA, and cytotoxic centrifuged cell smears were stained with anti-cytokeratin or anti-vimentin monoclonal antibody (MAB), using the Zymed streptavidin-biotin–alkaline phosphatase method (Histostain-SAP Kit; Zymed Laboratories, San Francisco). Briefly, cell smears were fixed in cold acetone for 10 min, blocked with 10% nonimmune goat serum for 30 min, and stained with mouse anti-cytokeratin MAB (C11, 1:100; Sigma) or with anti-vimentin MAB (V9, 1:20; Dako, Carpinteria, CA) or with isotype-matched control mouse IgG1 for 60 min at room temperature. After extensive rinsing, biotinylated goat anti-mouse IgG was applied for 30 min at room temperature. Streptavidin–alkaline phosphatase conjugate was applied and the reaction developed with the chromogen substrate. The fibroblast cell line NIH3T3 (ATCC, Rockville, MD) was used to control for the specificity of the staining.

Virus preparation. A human Long strain of RSV (A2) was grown on Hep-2 cells and purified by polyethylene glycol precipitation followed by centrifugation in a 35%–65% discontinuous sucrose gradient as previously described [22]. The purified RSV was aliquoted, quick-frozen in dry ice and alcohol, and stored at −70°C until use. The viral infectivity of RSV pools, as determined by a plaque-forming assay [23], was 7.5–8.5 log pfu/mL. To inactivate replicating virus, purified RSV was diluted in 1 mL of MEM containing 2% FBS and exposed for 3 min to a 254-nm UV light source at a 10-cm distance on ice [24]. Pools of purified RSV were tested by ELISA (R&D Systems, Minneapolis) and found negative for contaminating cytokines including IL-1α/β, tumor necrosis factor–α, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor.

Infection of epithelial cells with RSV. Monolayers of nose- and adenoid-derived epithelial cells grown to 80%–90% confluence in 24-well plates were infected with RSV at an MOI of 0.1–3. Twenty-four hours before infection, cell culture medium was switched to non–hormonally defined Dulbecco's MEM/F12 medium (Life Technologies) containing 2% FBS, glutamine, penicillin, streptomycin, and amphotericin B. To infect the cells, frozen RSV stock was rapidly thawed and diluted with Dulbecco's MEM/F12 medium containing 2% FBS. The virus (100 μL) was added immediately to the plates after removal of cultured medium. An equivalent amount of a 20% sucrose solution was added to control cell cultures, which did not receive RSV. The plates were rocked mechanically for 1 h at 37°C in 5% CO2, and then 0.9 mL of medium was added to the plates. The infection was continued for the indicated times in a 37°C incubator. Viral infection of RSV-exposed nasal and adenoid epithelial cells was confirmed by light-microscopy observation of typical cytopathic effect (CPE) and by indirect immunofluorescence. Briefly, acetone-fixed cytotoxic centrifuged smears of control or RSV-infected epithelial cells were stained for 45 min at 37°C with a MAb specific for RSV F glycoprotein [25] followed by a fluorescein isothiocyanate–conjugated anti-mouse F(ab')2. IgG antibody (1:200) (Tago, Camarillo, CA) for 45 min at 37°C. After an extensive wash, slides were counterstained and examined with a fluorescence microscope. Virus titers in nasal and adenoid epithelial cell supernatants collected at sequential times after RSV infection were determined in Hep-2 cells by the plaque-forming assay [23]. Virus titers are expressed as plaque-forming units/10^6 cells/mL, to correct for cell number at similar levels of monolayer confluences [26].
rabbit anti-human IL-8 antibody (2.5 μg/ml; Endogen, Cambridge, MA) was added for 1 h, followed by biotinylated donkey anti-rabbit IgG (1:20,000; Pierce Chemical, Rockford, IL) for 1 h and by streptavidin–horseradish peroxidase conjugate (1:4000; Zymed) for 30 min. After being washed, α-phenylenediamine dihydrochloride substrate (Sigma) was added, and the absorbance was read at 492 nm on a plate reader. RANTES was also analyzed by a similar ELISA, using MAb anti-human RANTES (2 μg/ml; R&D), polyclonal goat anti-human RANTES antibody (2 μg/ml; R&D), and biotinylated donkey anti-goat antibody (Pierce). IL-8 and RANTES were also measured in random samples by commercial ELISA kits (R&D). Excellent correlation was found between the concentrations measured by the in-house ELISA and those measured by commercial kits. GRO-α, MCP-1, and MIP-1α were analyzed by commercial ELISA kits (R&D).

Detection of RANTES mRNA by reverse transcription–polymerase chain reaction (RT-PCR). Confluent monolayers of nasal epithelial cells in 6-well plates were exposed to medium alone or were infected with RSV at an MOI of 1. After 24 h, cells were washed with PBS, and total RNA was extracted by the guanidinium thiocyanate method [27] with RNAzol B (BIOTECX, Houston), and RT-PCR was reacted by use of GenAmp RNA PCR Kit Components (Perkin-Elmer, Branchburg, NJ). In brief, 1 μg of total RNA was incubated at 42°C for 60 min in 20 μL of RT mixture (5 mM MgCl₂, 50 mM KCl, 10 mM TRIS/HCl [pH 8.3], 1 mM dNTPs, 1 U/μL RNase inhibitor, 2.5 U/μL murine leukemia virus reverse transcriptase, 2.5 μM random hexamers), denatured by heating at 99°C for 5 min, and rapidly cooled to 4°C. Next, PCR mixture consisting of 2 mM MgCl₂, 50 mM KCl, 10 mM TRIS/HCl, 2.5 U/100 μL AmpliTaq DNA polymerase, and 0.4 μM sense and antisense primers for RANTES or β-actin as internal control were added to the RT products. A RANTES cDNA probe was used as positive control. PCR reaction was carried out in a final volume of 100 μL in a DNA thermal cycler (Perkin Elmer) programmed as follows: denaturation cycle at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 2 min for a total of 35 cycles. To ascertain that the PCR products were analyzed during the linear increase of the product, the optimal cycle number of RANTES PCR was determined either using a constant amount of cDNA template [28] or by adding 5-fold serial dilutions of the target cDNA to the PCR reaction. Sequences of PCR primers used were the following: RANTES (sense, 5'-GCTGTCATCCTCTAGTCAGTCCTC-3'; antisense, 5'-CTCCATGCTAGTCATTAGTCCTC-3') [28]; β-actin (sense, 5'-ATCGTCCTCAACCCCTTCTACATGAGTGTCG-3'; antisense, 5'-CGTCTACACTCTCTGCTGCGCAATCATCTGC-3') (Clontech Laboratories, Palo Alto, CA). PCR products were resolved alongside pUC18 HaeIII DNA marker (Sigma) on an 8% polyacrylamide gel containing ethidium bromide, visualized under UV light, and photographed. The photographs were further analyzed by computer imaging (CorelDraw; Corel, Salinas, CA), and the ratio of RANTES/β-actin band intensity was calculated.

Statistical analysis. Chemokine concentrations were compared using analysis of variance for a two-factor experiment with repeated measures over time. The two factors were the in vitro treatment of polyp- and adenoid-derived epithelial cells (uninfected or RSV-infected) and the time. All tests were assessed at the .05 level of significance.

Results

Epithelial cell culture. Examination by inverted phase-contrast microscopy showed that nasal and adenoid epithelial cells grew as uniform monolayers of polygonal cells that were homogenous in shape and density with no apparent contamination of fibroblasts (figure 1). Nasal epithelial cells reached a confluent monolayer between 5 and 7 days and adenoid epithelial cells around 10 days after seeding. The success rate in reaching confluent monolayers was >90% for nasal epithelial cells but only ~50% for adenoid epithelial cells. Although we did not attempt

Figure 1. Confluent monolayers of epithelial cells from nasal mucosa (A) and adenoid (B) (phase contrast). Nasal-derived epithelial monolayer is at day 4 after seeding; adenoid-derived monolayer is at day 7. Cells have polygonal morphology characteristic of epithelial cells, and monolayers appear free of fibroblast contamination. Beating cilia, which could be appreciated by light microscopy observation, are not visible in photomicrographs. Bar = 20 μm (×100).
Table 1. Concentrations (mean ± SE, n = 3–11) of CXC and CC chemokines produced by nose- and adenoid-derived epithelial cells.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>CXC (ng/mL)</th>
<th>CC (pg/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IL-8</td>
<td>GRO-α</td>
</tr>
<tr>
<td>Nose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>29.6 ± 6.0</td>
<td>11.84 ± 0.6</td>
</tr>
<tr>
<td>RSV-infected</td>
<td>30.7 ± 6.3</td>
<td>11.06 ± 0.7</td>
</tr>
<tr>
<td>Adenoid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>22.6 ± 2.0</td>
<td>9.9 ± 2.4</td>
</tr>
<tr>
<td>RSV-infected</td>
<td>19.8 ± 1.3</td>
<td>10.1 ± 2.0</td>
</tr>
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NOTE. Monolayers of epithelial cells were infected with RSV or treated with medium for 48 h. IL, interleukin; GRO, growth-related peptide; MIP, macrophage inflammatory protein; MCP, monocyte chemotactic protein.

to determine contamination by adenovirus, others have reported a frequent recovery of adenovirus from human adenoids [29], which may in part explain the higher failure rate of adenoid epithelial cells in reaching confluence [30]. Ciliated cells could be seen either concentrated in localized areas or scattered throughout the monolayers. Ciliary beating, visible by direct microscopy observation [31], was present in both nasal and adenoid epithelial monolayers up to 1–2 weeks of culture, in agreement with recent findings by others [29]. The epithelial nature of cells and the high degree of purity in our culture system was confirmed by immunohistochemical staining with anti-cytokeratin and anti-vimentin antibodies. More than 98% of cultured cells in both nasal- and adenoid-derived monolayers were positive for cytokeratin. Once the monolayers reached 80% confluence, virtually all of the cells were found negative for vimentin. In comparison, the fibroblast cell line NIH3T3 was uniformly positive for vimentin and negative for cytokeratin.

Replication of RSV in nose- and adenoid-derived epithelial cells. Subconfluent monolayers of nasal and adenoid epithelial cells, which were exposed to RSV at an MOI of ~1, consistently showed presence of CPE, that is, syncytial-like formation 4–5 days after inoculation, as determined by inverted phase-contrast light microscopy. These observations were extended by performing indirect immunofluorescence studies using a MAb that recognizes the RSV F glycoprotein. Forty-eight hours after exposure to RSV, fluorescence staining was expressed by 42% ± 12% of epithelial cells (mean ± SD, n = 4). In two separate experiments, virus titration in supernatants of infected nasal- and adenoid-derived epithelial cells was also done by a plaque-forming assay. Increasing RSV titers were measured in supernatants collected 24, 48, and 72 h after infection (1.2, 2.2, and 5 × 10⁵ pfu/10⁶ cells/mL, respectively), using Hep-2 cells, a human cell line susceptible to RSV infection.

Effect of RSV infection on CXC and CC chemokine production. Concentration of chemokines was determined in the culture medium of confluent epithelial cell monolayers using specific ELISA (table 1). Nasal- and adenoid-derived epithelial cells constitutively released IL-8. At 24, 48, and 72 h, higher concentrations of IL-8 were released by 10⁶ uninfected nasal epithelial cells than by 10⁶ adenoid epithelial cells (figure 2). RSV infection at MOIs of 0.1, 1, or 3 did not significantly

![Figure 2](image-url)
alter IL-8 production by nasal and adenoid epithelial cells at all time points tested (data not shown). Similar results were observed for GRO-α, a chemokine that is also a member of the CXC family (table 1).

MIP-1α was markedly low in supernatants of uninfected nasal and adenoid epithelial cells, while high levels of MCP-1 were constitutively released by uninfected nasal and adenoid epithelial cells (table 1). Infection by RSV had no effect on MIP-1α or MCP-1 levels, even at a higher MOI (data not shown). RANTES was present at the low level of detection in uninfected cultures at all time points tested. On the other hand, RSV infection induced a time-dependent increase in RANTES production by nasal and adenoid epithelial cells (figure 3A, B). RANTES production by infected nasal epithelial cells reached a plateau at 48 h, while in adenoid epithelial cells, it continued to increase through 72 h. The mechanisms responsible for this different pattern have not been defined.

To examine the dose-effect of RSV on RANTES production, nasal epithelial cells were infected at MOIs of 0.3, 1, and 3, and the supernatants were tested by ELISA 48 h after inoculation (figure 3C). Infection with RSV at an MOI of as little as 0.3 was able to induce release of RANTES (616 ± 283 pg/mL). Comparable levels of RANTES were obtained when epithelial cells were infected with RSV at an MOI of 1 (595 ± 219 pg/mL), while >60% increase was obtained by infection at an MOI of 3 (990 ± 470 pg/mL). These differences, however, were not statistically significant. UV-inactivated RSV, on the other hand, failed to infect nasal epithelial cells and to induce RANTES production (figure 3C).

RANTES mRNA expression. To investigate the mechanism of RANTES induction by RSV infection, changes in the abundance of RANTES mRNA were determined using RT-PCR analysis. Total RNA was extracted from 3 separate specimens of nasal-derived epithelial cell monolayers, which were either treated with medium alone or infected with RSV for 24 h, and amplified using specific primers for RANTES. As shown in figure 4, uninfected epithelial cells expressed low levels of RANTES transcripts. On RSV infection, normalized RANTES mRNA signals (to internal control β-actin) increased in all 3 samples tested (figure 4, lower panel).

Discussion

Airway mucosa inflammation characterized by infiltration of mononuclear cells [32] and by activation of eosinophil and
basophil leukocytes [6-9, 33] has been shown in human RSV disease by postmortem histopathologic studies and by the recovery of cell-specific inflammatory mediators in nasopharyngeal secretions and tracheobronchial aspirates of infected children. Recent evidence has emerged to demonstrate that accumulation and activation of inflammatory cells in mucosal tissues is regulated by chemokines with discrete target cell selectivity that can be produced by epithelial cells [34, 35]. While the CXC chemokines have a potent activity on neutrophils, members of the CC chemokine family have been shown to be selective activators of eosinophil, basophil, and monocyte function. RANTES in particular is a powerful chemoattractant for eosinophils [36], basophils, [37] and memory CD4 T cells [38] and induces exocytosis of ECP [39]. The production of CC chemokines by virus-infected epithelial cells has not been reported before. It was reasonable to hypothesize that respiratory epithelial cells, which are a primary target of RSV, can express and release chemokines when infected by RSV. Furthermore, despite the current knowledge that the mucosa of the upper airway tract is the portal of entry for RSV [17], only one study to date has examined the replication of RSV in human nasal epithelial cells [40]. On the other hand, infection of adenoid epithelial cells by RSV has not been previously reported. Because of the strategic location and their lymphoepithelial nature, adenoids can function as antigen-presenting structures and an important source of mucosal B and T precursor cells for the respiratory tract [41]. Therefore, nasal- and adenoid-derived epithelial cells provide an ideal model to investigate the interaction between viruses and human respiratory mucosa, as recently shown by elegant studies with influenza virus [29].

To our knowledge, the present study demonstrates for the first time that infection of epithelial cells by a viral pathogen, RSV, is able to induce a dose- and time-dependent production of RANTES. The induction of RANTES in epithelial cells is dependent on the presence of replicating RSV, as is shown by the inability of UV-treated RSV to trigger RANTES production. Although constitutive expression of RANTES mRNA was found in the epithelial cells, low levels of RANTES protein were present in the uninfected cell supernatant. Following infection with RSV, RANTES gene transcripts and protein increased in both nasal- and adenoid-derived epithelial cells. At 24 h, the levels of RANTES protein and mRNA in RSV-infected nasal cells were increased by 3.2- and 2.3-fold, respectively, compared with uninfected nasal cultures. Studies to date indicate that RANTES gene expression may be controlled at several levels, including the transcriptional and posttranscriptional levels [42], and that a certain degree of uncoupling between mRNA expression and protein secretion is characteristic of chemokine production [43]. A cis-acting element has been identified in the human RANTES promoter containing a consensus binding site for the nuclear factor-IL-6 (NF-IL-6) transcriptional activator and regulating early RANTES gene transcription [44]. Our recent observation that infection by RSV induces the translation of NF-IL-6 in respiratory epithelial cells [45] suggests that virus-induced expression of NF-IL-6 may be involved in the activation of RANTES and perhaps other genes encoding inflammatory molecules in airway mucosa. Studies addressing this possibility are underway.

In the present study, nasal- and adenoid-derived epithelial cell monolayers constitutively produced high levels of IL-8. Similar findings have been reported in other studies in which comparable methods for nasal epithelial cell cultures were used [46]. On the other hand, IL-8 expression in human adenoid epithelial cells has not been previously reported. RSV infection of nasal and adenoid epithelial cells did not significantly alter the levels of IL-8 produced. In the only study thus far conducted in upper airway mucosa, increased expression of IL-8 transcripts were found in 2 of 3 samples of RSV-infected nasal mucosa scrapings that were analyzed [47]. The short-term cul-

![Figure 4.](image-url)
tures of the nasal mucosa (6–20 h), a higher degree of non-
epithelial cell contamination, and the use of nonpurified pools
of RSV in that study make any comparison with our current
results of difficult interpretation.

Increased levels of IL-8 have been recently measured in
nasal lavage fluids of children with clinical diagnosis of (viral)
upper respiratory tract infection [48]. Although biopsy cells
(90%–95% epithelial) that were taken during the acute illness
showed a small increase in IL-8 mRNA, the contribution of
other inflammatory and tissue resident cells to the overall IL-
8 production could not be determined. Furthermore, IL-8 con-
centrations in nasal lavage samples did not differ significantly
between children with RSV-positive and those with RSV-nega-
tive upper respiratory tract infection, suggesting that release
of neutrophil chemotactic factors may in fact represent a general
phenomenon in viral upper respiratory tract infection. No infor-
mation is available regarding the presence of CC chemokines
in the nasal lavage fluids of patients with RSV or other viral
upper respiratory tract infection. These studies will be neces-
sary to better understand the pathogenetic mechanisms of
wheezing associated with respiratory infections such as those
cauised, in addition to RSV, by rhinovirus and influenza and
parainfluenza viruses.

In conclusion, our data provide evidence that although sev-
eral chemokines are produced by epithelial cells of the upper
respiratory tract, RSV infection induces the selective produc-
tion of RANTES. We suggest that RANTES may regulate the
recruitment and activation of eosinophils and basophils in air-
way mucosa following infection with RSV, therefore playing
a central role in the pathogenetic mechanisms of RSV-induced
disease. In addition, the recent discovery that RANTES, MIP-
1α, and MIP-1β are the major human immunodeficiency virus—
suppressiv factors released by CDS T lymphocytes [49] raises
the intriguing question about the role of these molecules in the
context of a broader antiviral activity.

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