Mobilization of Plasmacytoid and Myeloid Dendritic Cells to Mucosal Sites in Children with Respiratory Syncytial Virus and Other Viral Respiratory Infections

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Background. Respiratory syncytial virus (RSV) is the principal etiologic agent of bronchiolitis and viral pneumonia in infants and young children. Yet, many aspects of its immunopathogenesis are not well understood.

Methods. We analyzed the immune cells that are mobilized by RSV and other respiratory viruses, by studying nasal wash samples from children hospitalized with acute viral respiratory infections.

Results. RSV mobilizes virtually all blood immune cells, including myeloid dendritic cells (DCs) and plasmacytoid DCs (pDCs), to the nasal mucosa. DCs were also mobilized to the nasal mucosa of children with other viral respiratory infections. The increased number of pDCs in the nasal compartment significantly correlates with RSV load (P < .022), and it is associated with a significant decrease in the number of pDCs in the blood (P = .007). The influx of DCs in the nasal mucosa is not transient, as even higher numbers of both DC subsets were found in respiratory secretions weeks after the acute symptoms of RSV infection had resolved. Immunochemistry analysis of respiratory samples has demonstrated the presence of the RSV fusion protein within HLA-DR–positive cells.

Conclusion. Infection with RSV and other respiratory viruses mobilizes DCs to the site of viral entry.

Control of viral infections represents a formidable challenge for the immune system. Mucosal viral infections are controlled by (1) preformed local and circulating antibodies [1, 2], (2) innate immunity mechanisms (such as local production of interferon [IFN] [3–5]), and (3) adaptive immunity mechanisms (such as cytotoxic responses [1] and antibody-secreting plasma cells [6, 7]).

Respiratory syncytial virus (RSV) is the leading viral respiratory pathogen in young children [8–11]. RSV infection does not lead to the development of protective immunity, and repeated infections are common. Additionally, there is a strong association between RSV bronchiolitis in infancy and recurrent wheezing [12–16].

The inflammatory response initiated by RSV infection likely contributes to the manifestations of RSV disease [17–21]. Little is known about the mobilization of the immune effectors and regulators at the site of viral entry (the nasal mucosa) in humans. Neutrophils were the predominant leukocyte found in both bronchoalveolar lavage and nasopharyngeal specimens from infants with RSV bronchiolitis, although lymphocytes and monocytes were also identified [22–24].

Despite considerable efforts, no RSV vaccine is currently available [25]. A better understanding of the interplay of RSV with immune cells may facilitate the development of an effective vaccine. Given the critical role of dendritic cells (DCs) in the initiation of immune responses, it is important to understand the relationship between RSV infection and DCs.

DCs constitute a complex system of cells that induce and control immune responses [26–30]. Two subsets of DC precursors circulate in the blood: lineage-neg-
Inclusion criteria for enrollment into the Patient population.

 PATIENTS, MATERIALS, AND METHODS

Viral respiratory infections. The present study demonstrates that subsets in children hospitalized with RSV infection and other RSV with DCs. Therefore, we have analyzed the mDC and pDC [43]. There is little information concerning the interplay of and tumor necrosis factor–related apoptosis-inducing ligand [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40].

Viruses employ many different strategies to escape the immune system, including targeting DCs and subverting their function [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40]. The measles virus, another paramyxovirus, modulates release of cytokines by DCs [41, 42] and renders function [39, 40].

\[ \text{viruses} \]

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RESULTS

Patient enrollment. Twenty-one patients with acute RSV infection, 8 with other acute viral respiratory infections, and 10

pellet was resuspended in PBS/2% fetal calf serum at 4°C (Sigma) and centrifuged again, as above. This was repeated until no visible mucus clumps remained in the cell/mucus solution. The sample was filtered over a Ficoll 100-µm filter (Dako). Visible cells were visualized by trypan blue exclusion and counted by use of a Reichert Bright Line hemocytometer.

Cell staining for flow-cytometric analysis. For nasal wash cell staining, 200,000 cells/tube were incubated with antibodies for 30 min, rinsed with PBS, centrifuged at 300 g for 10 min, and resuspended in 1% paraformaldehyde. For blood studies, whole blood was collected in tubes containing acid citrate dextrose. For staining, 100 µL of blood and 3–10 µL of each antibody were incubated for 30 min. The blood was then lysed with FACS Lysing Solution (BD Biosciences), rinsed with PBS, centrifuged at 300 g for 10 min, and resuspended in 1% paraformaldehyde. Samples were then acquired on a FACS Canto II flow cytometer and analyzed with CellQuest software (BD Biosciences).

The following fluorochrome-conjugated antihuman antibodies were used for both nasal wash and whole-blood stainings: LINEAGE–fluorescein isothiocyanate (FITC) cocktail (containing CD3, CD14, CD16, CD19, CD20, and CD56), CD123–phycoerythrin (PE), HLA-DR–peridin chlorophyll protein (PerCP), CD11c–allophtycocyanin (APC), CD4–FITC, CD8–PE, CD3–PerCP, and CD14–APC (BD Biosciences). In selected experiments, nasal wash cells were also stained with monoclonal antibody (MAb) directed against the fusion protein of RSV (Fitzgerald Industries International).

Quantification of RSV in nasal wash samples. Quantitative RSV cultures were performed using the standard plaque assay technique, as described elsewhere [23, 44].

Quantification of cytokines and chemokines in nasal wash samples. Concentrations of cytokines in nasal wash samples were measured by Luminex XMAP technology (Luminex), by use of commercially available kits (Upstate USA), following the manufacturers’ instructions.

Confocal microscopy. In several patients with RSV infection, cells from respiratory secretions were mounted onto slides and stained with MAb directed against HLA-DR–FITC and RSV fusion protein (conjugated to Texas Red). Cells were then visualized by confocal microscopy (Leica TCS SP confocal microscope with a Planapo 63/1.32X objective). Statistical analysis. The nonparametric Mann-Whitney U test or the parametric unpaired t test was used, as appropriate, to compare numbers of DCs and concentrations of cytokines between groups. The Wilcoxon signed rank test was used to compare numbers of DCs in patients with acute versus resolved RSV infection. Spearman’s coefficient was used for correlations.

RESULTS

Patient enrollment. Twenty-one patients with acute RSV infection, 8 with other acute viral respiratory infections, and 10

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Table 1. Patient demographics.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>Age, median (range), months</th>
<th>Duration of symptoms, median (range), days</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>21</td>
<td>3.0 (0.5–11)</td>
<td>5 (2–13)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>6.5 (0.3–11)</td>
<td>No symptoms</td>
</tr>
<tr>
<td>Viral control</td>
<td>8</td>
<td>6.0 (2–14)</td>
<td>5 (0–6)</td>
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NOTE. RSV, respiratory syncytial virus.

healthy control children were enrolled between December 2000 and February 2003 (table 1). All patients with acute RSV infection had symptoms of bronchiolitis requiring hospitalization. During the acute phase, they were treated symptomatically with oxygen, intravenous fluids, and β-adrenergic agents for variable periods of time, but they did not receive antiviral agents or corticosteroids. Age distribution was similar between the 3 groups. Among the patients with other acute viral respiratory infections, 4 had parainfluenza and 3 had influenza (2 influenza B and 1 influenza A) respiratory infections requiring hospitalization. These patients manifested respiratory symptoms including cough, nasal congestion, hypoxia, and bronchiolitis.

Another patient in this group was an asymptomatic infant initially enrolled in the control group whose nasopharyngeal viral culture later yielded cytomegalovirus (CMV). All patients with acute viral respiratory infections were enrolled during the same months of the year.

mDCs, pDCs, monocytes, T cells, and B cells in nasal wash samples from patients with viral respiratory infections.

Flow-cytometric analysis demonstrated that CD14+ monocytes, CD3+CD4+ T cells, CD3+CD4+ T cells, and CD20+ and CD19+ B cells can be identified in nasal wash samples from patients with acute RSV infection (figure 1A–1C). Neutrophils can also be found, as determined by morphological analysis of cytospins and Giemsa-stained cells (data not shown). This infiltrate was not due to bleeding, because (1) no bleeding was seen at the time samples were collected, (2) the red blood cell (RBC):white blood cell (WBC) ratio was considerably lower in nasal wash samples than in blood samples (the highest nasal wash RBC:WBC ratio was 25:1, but most were much lower), and (3) immune effectors were abundant in nasal wash samples that contained no RBCs.

Figure 1. Immune effectors in nasal wash samples from patients with respiratory syncytial virus (RSV) infection. Monocytes (A), T cells (B), and B cells (C) were identified in nasal wash samples by flow-cytometric analysis. D–G. Flow-cytometric analysis of dendritic cell (DC) subsets in nasal wash samples. D, Ungated view of DCs in nasal wash samples (5% of total events are shown). E, Events from the R1 gate (white blood cell), showing staining with lineage fluorescein isothiocyanate (FITC) cocktail (contains anti-CD3, -CD14, -CD16, -CD19, -CD20, and -CD56). A total of 20% of events are displayed in B, F, and G, The same analysis, gated on the lineage-negative (FITC cocktail–negative) populations. In both panels, 100% of the events are displayed. Anti-CD123 and anti-CD11c antibodies permit the detection of HLA-DR-positive CD123+ plasmacytoid DCs (red) and CD11c+ myeloid DCs (green), respectively. APC, allophycocyanin; FSC, forward scatter; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; SSC, side scatter.
DC subsets were defined by (1) lack of lineage markers, (2) expression of HLA-DR, and (3) expression of CD11c (mDCs) or CD123 (pDCs), as described elsewhere [45, 46]. As illustrated in figure 1D–1F, mDCs and pDCs were easily detected in nasal wash samples from patients with acute RSV infection.

Increase of mDCs in nasal wash samples and decrease in blood samples from children with acute viral respiratory infections. Using the parameters described above, we found that 27 of 29 evaluated patients with acute viral respiratory infections had mDCs in nasal wash samples (figure 2A), with a median of 2400 mDCs/nasal wash sample (range, 0–42,000 mDCs/nasal wash sample). In contrast, the healthy control children had very low numbers of mDCs in nasal wash samples (median, 45 mDCs/nasal wash sample; range, 0–1700 mDCs/nasal wash sample; \( P < .001 \), compared with all patients with viral infections). Of the 21 patients with acute RSV infection, 19 had mDCs in nasal wash samples (median, 1932 mDCs/nasal wash sample; range, 0–13,400 mDCs/nasal wash sample; \( P = .0041 \), compared with healthy control children). The 8 patients with other viral respiratory infections also had mDCs in nasal wash samples (median, 6665 mDCs/nasal wash sample; range, 578–42,000 mDCs/nasal wash sample). The numbers were significantly different from those in healthy control children (\( P = .0014 \)), but not from those in patients with RSV infection (\( P = .339 \)).

The frequency of mDCs in nasal wash samples from children with acute viral infections (median, 0.06%; range, 0.0%–0.6%) was similar to that in blood samples from the same patients (median, 0.07%; range, 0.01%–0.27%; \( P = .817 \)). However, figure 2B demonstrates that patients with viral respiratory in-
Infections had decreased numbers of mDCs in blood samples (median, 6600 mDCs/mL; range, 820–38,740 mDCs/mL), compared with those in healthy control children (median, 17,360 mDCs/mL; range, 1720–36,540 mDCs/mL) \( P = .033 \). The decrease in numbers of mDCs in blood samples was more pronounced in the RSV group (median, 6205 mDCs/mL; range, 820–15,000 mDCs/mL; \( P = .016 \), compared with healthy control children). Thus, patients with RSV infection displayed a mobilization of mDCs to the nasal mucosa.

**Increase of pDCs in nasal wash samples and decrease in blood samples from patients with acute viral respiratory infections.** Twenty-three of 29 patients with acute viral respiratory infections had pDCs in nasal wash samples (median, 760 pDCs/nasal wash sample; range, 0–32,200 pDCs/nasal wash sample) (figure 3A). Five of 10 healthy control children also had pDCs in nasal wash samples, although at much lower numbers (median, 6 pDCs/nasal wash sample; range, 0–680 pDCs/nasal wash sample; \( P = .0052 \), compared with patients with other viral respiratory infections). Fifteen of 21 patients with acute RSV infection had pDCs in nasal wash samples (median, 490 pDCs/nasal wash sample; range, 0–4880 pDCs/nasal wash sample; \( P = .043 \), compared with healthy control children). A positive correlation between the number of pDCs and the number of mDCs in nasal wash samples was identified \( (P < .01) \).

All 8 patients with other viral respiratory infections had pDCs in nasal wash samples (median, 1094 pDCs/nasal wash sample; range, 100–32,200 pDCs/nasal wash sample; \( P = .0003 \), compared with healthy control children).

Figure 3B demonstrates that patients with acute viral respiratory infections had decreased numbers of pDCs in blood samples (median, 5160 pDCs/mL; range, 0–32,480 pDCs/mL), compared with healthy control children (median, 16,210 pDCs/mL; range, 5600–59,450 pDCs/mL; \( P = .0014 \)). The reduction in numbers of pDCs in blood samples was greatest in patients with RSV infection (median, 4800 pDCs/mL; range, 0–23,760 pDCs/mL; \( P = .0007 \), compared with healthy control children). Thus, RSV-infected patients displayed a mobilization of pDCs to the nasal mucosa. No correlations between the severity of...
Figure 5. Increase in nos. of dendritic cells (DCs) in nasal wash and blood samples after resolution of respiratory syncytial virus (RSV) infection. A, Nos. of myeloid DCs (mDCs) in nasal wash samples from 7 patients with RSV infection during acute RSV infection and after resolution of acute RSV infection. B, Similar data, for plasmacytoid DCs (pDCs) in nasal wash samples. C and D, Increases in nos. of mDCs and pDCs in blood samples, respectively, after resolution of acute RSV infection.

illness and the numbers of mDCs or pDCs in blood or nasal wash samples were identified.

Increased concentrations of IL-6, IL-8, and macrophage inflammatory protein (MIP)–1α in nasal wash samples from patients with RSV infection. We next analyzed the concentrations of several cytokines and chemokines in the respiratory tract. Concentrations of IL-6, IL-8, and MIP-1α were significantly increased in nasal wash samples from patients with RSV infection, compared with those in nasal wash samples from healthy control children (figure 4A). Concentrations of IL-8 were also significantly increased in nasal wash samples from patients with RSV infection, compared with those in nasal wash samples from patients with other viral respiratory infections. Among the cytokines and chemokines measured, only MIP-1α was significantly increased in nasal wash samples from patients with other viral respiratory infections, but a trend for increased concentrations of IL-6 was also observed. No significant differences in the concentrations of IL-4, IL-5, or IFN-γ were observed (figure 4B).

Among the patients with RSV infection, there was a positive correlation between numbers of mDCs and concentrations of IL-6 in nasal wash samples \( (r = 0.5; P = .04) \). Although this correlation was not present in the control group, numbers of mDCs did strongly correlate with concentrations of MIP-1α \( (r = 0.77; P = .017) \). In patients with other acute viral respiratory infections, numbers of mDCs significantly correlated with concentrations of IL-8 \( (r = 0.88; P = .03) \), and there was a marked trend for increased concentrations of MIP-1α \( (r = 0.81; P = .058) \).

Increase in numbers of DCs in nasal wash and blood samples after resolution of acute RSV infection. Next, we determined whether the mobilization of DCs to the nasal mucosa was a transient or sustained event. We were able to analyze 7 of the RSV-infected patients 2–8 weeks after resolution of acute RSV infection. During the interval between acute illness and the follow-up collection of samples, 1 patient had persistent cough (RSV-04), another had an episode of rhinorrhea for several days that was resolved at her follow-up visit (RSV-16), and
a third had mild rhinorrhea at follow-up (RSV-17). All other patients were reportedly asymptomatic during the interval between their hospital admission and follow-up collection of samples. The results of viral respiratory cultures and DFA tests performed at follow-up were negative. Unexpectedly, their nasal wash samples contained even higher numbers of DCs. Data on each of the patients completing both acute and follow-up evaluations are displayed in figure 5A and 5B. The median number of mDCs was 10,032 mDCs/nasal wash sample, representing a 4-fold increase over the median number of 2,532 mDCs/nasal wash sample observed during acute RSV infection. Comparing paired measurements of mDCs in nasal wash samples in only the patients with RSV infection who returned for follow-up evaluations revealed a significant increase in numbers of mDCs in nasal wash samples after resolution of acute RSV infection (median, 0.01% after resolution of infection vs. 0.01% during acute infection; P = .14). There was a positive correlation between the increase in numbers of mDCs and pDCs in nasal wash samples after resolution of acute RSV infection (r = 0.93; P = .007). No evidence of RSV or other viruses was detected by routine viral cultures, DFA tests, or RSV plaque assays performed during the follow-up evaluations.

Although DC subsets in blood samples were decreased during acute RSV infection, compared with those in healthy control children, they subsequently increased after resolution of acute RSV infection. Figure 5C demonstrates the significant increase in the number of mDCs in blood samples after resolution of acute RSV infection (P = .031, Wilcoxon signed rank test [RSV-04 not included in analysis]). However, the increase in the number of pDCs in blood samples after resolution of acute RSV infection (figure 5D) did not reach statistical significance (P = .063, Wilcoxon signed rank test [RSV-04 not included in analysis]). Thus, acute RSV infection is associated with a continuous increase in DC subsets at the site of infection.

**Correlation of the number of pDCs with RSV load.** Because of the variable numbers of nasal pDCs identified in patients with RSV infection, we asked whether this was related to RSV load. RSV loads were measured by a quantitative plaque assay that used HEP-2 cells. Figure 6A shows the significant correlation between the number of nasal pDCs and RSV load (r = 0.585; P = .022). A correlation between the number of mDCs in nasal wash samples and RSV load was less apparent (r = 0.427; P = .077) (figure 6B).

**Expression of RSV proteins by HLA-DR-positive cells from the respiratory tract.** Next, we wondered whether nasal DCs...
Figure 7. Expression of respiratory syncytial virus (RSV) proteins by HLA-DR–positive cells from the respiratory tract wash samples from patients with RSV infection. Shown are photomicrographs of unsorted cells from the tracheal aspirate of a patient with RSV infection, representing a confocal acquisition with HLA-DR–fluorescein isothiocyanate (FITC) and RSV fusion protein–Texas Red staining. All photomicrographs were taken with a Leica TCS SP confocal microscope with a Planapo 63/1.32X objective. The upper panels represent HLA-DR–FITC staining (upper left panel), RSV fusion protein–Texas Red staining (upper middle panel), and an overlay of both stains (upper right panel). Both lower panels represent zoom images (×2) of the overlay images.

would carry RSV. To investigate this question, we stained unsorted nasal and tracheal wash cells from RSV-infected patients with antibodies directed against HLA-DR and RSV fusion protein. Respiratory samples from 2 patients demonstrated HLA-DR–positive cells that also expressed RSV fusion protein (figure 7). A few HLA-DR–negative cells were found to be positive for RSV fusion protein, and not all HLA-DR–positive cells expressed RSV fusion protein.

**DISCUSSION**

RSV infection is a major cause of morbidity in infants and children, indicating that it is not efficiently controlled by the immune system. Pathogens affect different components of the immune system for their survival and replication. In particular, DCs are targeted by many viruses, including HIV [47], dengue virus [48, 49], and CMV [50]. This prompted the present study, since very little is known about the interactions between RSV and the DC system, most particularly in humans, the primary host of RSV.

We initiated the present study by assessing what happens at the actual site of RSV entry/infection, the nasal cavity. We are naturally limited in the scope of our investigation by 2 parameters: (1) the young age of the patients (<15 months; median, 3 months in the RSV group); and (2) the small sample material to analyze (3-mL nasal wash samples), in which viscosity makes analysis of infiltrating cells challenging, and the limited volume of blood samples. Despite these limitations, several important conclusions can be made.

First, as reported elsewhere [22], in nasal wash samples, we found virtually all the immune effectors, including T cells, B cells, monocytes, neutrophils, and, more importantly, both pDCs and mDCs, the focus of the present study. The results demonstrate that RSV attracts both mDCs and pDCs to the site of viral infection. The number of pDCs, and, to a lesser extent, that of mDCs, positively correlates with RSV load. The number of mDCs at the site of RSV infection (median, 1932 mDCs) is significantly higher than the number of pDCs (median, 490 pDCs). This may be due to the considerable phenotypic and morphological alterations that pDCs undergo after maturation. Unfortunately, no reagents are currently available that allow the identification of mature pDCs, and these cells likely remain uncounted. No CD83+ cells could be detected, suggesting that mature DCs might remain associated with the tissue. The attraction of both DC subsets to the nasal mucosa is not specific to RSV infection, as the 8 patients with other viral respiratory infections also had similar findings.

With regard to mDCs, their increase in nasal wash samples was associated with a decrease in blood samples. This sug-
gests—it is difficult to formally demonstrate without controlled challenge studies—that the increase of mDCs in nasal mucosa results from their migration from the blood. The increased presence of mDCs in virus-infected nasal mucosa in humans is reported here for the first time. However, this finding is consistent with earlier studies performed in rats, in which *Moraxella catarrhalis* or Sendai virus recruited major histocompatibility complex (MHC) class II–positive cells (putative DCs) into the airway epithelium [51, 52].

In its consideration of pDCs, the present study is, to our knowledge, the first study to report their attraction to a mucosal site of viral replication. This could be viewed as an expected finding, since pDCs are considered to represent an early barrier to viral replication through their ability to secrete large amounts of IFN-α in response to viral challenge. Attraction of pDCs to the nasal mucosa in humans has been reported under a different pathological condition—that is, in allergic patients challenged with the relevant allergens [37]. Furthermore, studies of mice have reported the presence of cells with characteristics of pDCs in the bronchi of mice challenged with a leishmanial antigen [53]. These MHC class II–positive cells were long lasting, which is consistent with our observation of long-lasting infiltration of DCs into the nasal mucosa as late as 8 weeks after resolution of acute RSV infection. More-recent studies have confirmed that pDCs have a long half-life in vivo [54], a finding that contrasts with their extreme propensity to apoptosis after isolation [34]. In humans, pDCs have also been found to infiltrate cutaneous lesions caused by varicella-zoster virus [55]. Whether the infiltrating cells are long-lasting cells attracted at the time of acute infection or a set of new cells attracted by long-acting mechanisms initiated by viral infection represents a complex challenge to address in our patient population. Since we evaluated only patients with RSV infection after resolution of acute symptoms, we do not know whether the persistence of DCs is specific to RSV infection. It will be important to evaluate patients with other viral respiratory infections in future studies.

DC subsets in blood are reduced and impaired in patients with HIV infection [56–59]. The number of circulating pDCs correlated inversely with HIV load and the occurrence of opportunistic infections [58, 60]. In the present study, the number of pDCs in blood samples did not correlate with the RSV load measured in the respiratory tract. The decrease in pDCs in blood samples during acute RSV infection suggests that pDCs are recruited to the site of viral entry, the nasal mucosa. It is unknown whether RSV infects pDCs.

We have made significant attempts to determine whether the DCs present in the nasal wash samples were functional. Unfortunately, it is difficult to isolate sufficient cell numbers from the small viscous nasal wash samples, to complete such studies. Therefore, we will be limited to in vitro studies to determine how RSV might affect the functions of human DCs. This will be important to investigate, since RSV may interfere with DC-induced T cell activation and differentiation in a fashion that leads to characteristics of disease. Indeed, it has recently been shown that RSV induces human cord blood–derived mDCs to secrete IL-10, IL-11, and prostaglandin E2, whereas influenza primarily induces generation of IL-12 in mDCs [61].

We found significantly increased concentrations of IL-6, IL-8, and MIP-1α in nasal wash samples from patients with RSV infection. Despite the small number of patients in the group with other viral respiratory infections, the concentrations of MIP-1α in their nasal wash samples were also increased, and there was a clear trend for increased concentrations of IL-6. Concentrations of IL-8, however, were significantly greater in patients with RSV infection than in patients with other viral respiratory infections. In addition, the correlation between numbers of mDCs and concentrations of IL-6 was detected only in patients with RSV infection. Although it is difficult to draw conclusions from these correlations, we can speculate that the balance of cytokines/chemokines and DCs that exists in healthy control children is altered during RSV infection. Indeed, the correlation between numbers of mDCs in the mucosa and concentrations of MIP-1α observed in healthy control children was absent in patients with RSV infection.

Although we were unable to address the functional status of the DCs in nasal wash samples in patients with RSV infection, we have demonstrated that RSV antigen can be detected within HLA-DR–positive cells with the morphology of DCs. Further studies will be required to confirm that these cells are indeed DCs and to determine whether RSV replicates within these cells or whether RSV particles are simply captured from the mucosal microenvironment.

In conclusion, we have shown that RSV infection results in mobilization of DCs to the site of viral entry. How RSV affects the functions of these immune cells will be the focus of important future studies that may shed light on the pathogenesis of this common infection.

**Acknowledgments**

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**References**


