Gene Expression of Nucleic Acid-Sensing Pattern Recognition Receptors in Children Hospitalized for Respiratory Syncytial Virus-Associated Acute Bronchiolitis

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Given the critical role of pattern recognition receptors (PRRs) in acid nucleic recognition in the initiation of innate immunity and the orchestration of adaptive immunity, the aim of this study was to determine whether any heterogeneity of PRR expression in the airway tracts of infants with respiratory syncytial virus (RSV) infection might explain the broad clinical spectrum of RSV-associated bronchiolitis in infants. For this purpose, the levels of melanoma differentiation-associated protein-5 (MDA-5), retinoic acid inducible gene-1 (RIG-1), and Toll-like receptor 3 (TLR-3), TLR-7, TLR-8, and TLR-9 mRNAs were evaluated, using TaqMan quantitative reverse transcription-PCR, in cells from nasopharyngeal washes collected from 157 infants suffering from acute bronchiolitis whether or not they were associated with respiratory viruses. High interindividual variability was observed in both virus-positive and -negative infants; however, the relative gene expression levels of MDA-5, RIG-1, TLR-7, and TLR-8 were significantly higher in the virus-infected group, whereas the expression levels of TLR-3 and TLR-9 were not significantly different. The differences in the gene expression levels of MDA-5, RIG-1, TLR-7, and TLR-8 were more evident in infants with RSV infection than in those with bocavirus or rhinovirus infection. In RSV-infected infants, PRR-mRNA levels also were analyzed in relation to interferon protein levels, viral load, clinical severity, days of hospitalization, age, and body weight. A significant positive correlation was observed only between RSV viral load and RIG-1 mRNA levels. These findings provide the first direct evidence that, in infants with respiratory virus-associated bronchiolitis, especially RSV, there are substantial changes in PRR gene expression; this likely is an important determinant of the clinical outcome of bronchiolitis.

Respiratory syncytial virus (RSV) is one of the most common viral pathogens in infants causing severe lower respiratory tract infections, such as bronchiolitis and pneumonia (27). The clinical spectrum of RSV-associated bronchiolitis in infants is extremely variable, ranging from mild upper respiratory symptoms to severe respiratory distress and, occasionally, death.

There are several known risk factors associated with severe bronchiolitis, such as young age (<6 months), premature birth (<35 weeks of gestation), immunodeficiency or immunosuppression status, and congenital heart or chronic lung disease. However, most infants have no obvious risk factors (10, 30, 41). Thus, it is likely that other factors, such as the immune response and genetic heterogeneity of the host, together with well-known viral risk factors, contribute to RSV disease severity.

It is known that the innate immune system employs a set of receptors called pattern recognition receptors (PRRs) that recognize the evolutionarily conserved pathogen-associated molecular patterns (8). Two types of PRRs have been identified that recognize viral nucleic acids: Toll-like receptors (TLRs) and retinoic acid inducible gene 1 (RIG-1)-like RNA helicases (RLHs). TLRs patrol the extracellular and endosomal compartments, signaling results in a type I interferon (IFN) response and/or the production of proinflammatory cytokines. Of these, TLR-3 recognizes viral double-stranded RNA, TLR-7 and human TLR-8 identify viral single-stranded RNA, and TLR-9 binds viral DNA. TLRs are located in the endosomal compartments, whereas RLHs such as MDA-5 (melanoma differentiation-associated protein-5) and RIG-1 are cytoplasmic receptors for viral double-stranded RNA or single-stranded RNA, which play essential roles in the production of type I IFNs and cytokines in various cell types.

Substantial changes in PRR gene expression have been observed in airway epithelial cells infected with respiratory viruses (17, 19, 36). For RSV infections, it has been shown that RIG-1 mediates both an early antiviral response and TLR-3 expression in RSV-infected airway epithelial cells (25). In addition, it has been found that RSV induces both TLR-3 protein and protein kinase R, leading to an increased double-stranded RNA responsiveness in airway epithelial cells (15). The first evidence that RSV can counteract IFN production by human plasmacytoid dendritic cells in vitro through the inhibition of TLR-dependent and -independent responses was published in 2005 (38).

Despite the fact that some PRR family members have a role in RSV infection in vitro, PRR gene expression in the respi-
ratory tracts of infants with RSV-associated acute bronchiolitis has never been addressed.

Hence, considering the importance of PRRs in initiating innate immunity to virus infections, we hypothesized that the heterogeneity of PRR gene expression explains the broad clinical spectrum of RSV bronchiolitis. In light of this, we assessed the gene expression of PRRs involved in the recognition of nucleic acids in cells from nasopharyngeal washes (NPW) from 157 infants with a clinical diagnosis of acute bronchiolitis during two consecutive annual epidemic periods. In particular, transcript levels of MDA-5, RIG-1, TRLR-3, TRLR-7, TRLR-8, and TLR-9 were analyzed in infants with RSV and other respiratory infections. A similar analysis also was undertaken on uninfected infants. To further emphasize the importance of this issue, we also assessed the correlation between PRR expression and IFN protein levels, RSV load, physical characteristics (age and body weight), and markers of disease severity in infants with RSV infections.

MATERIALS AND METHODS

Patients. A total of 157 infants (mean and median [standard deviation] age, 65 and 46 [62] days, respectively; mean and median [standard deviation] weight, 3.09 and 3.1 [0.56] kg, respectively; gender, 80 male and 77 female; race, 79.5% Caucasian) with a clinical diagnosis of acute bronchiolitis, admitted during two successive winter seasons (2006 to 2008) to the Pediatric Department of Policlinico Umberto I Hospital, were enrolled in this study. The study was approved by the ethics committees and/or institutional review boards of the participating institutions, and informed consent was obtained from the children’s parents.

Bronchiolitis was diagnosed from the presence of a history of upper respiratory tract infection followed by the acute onset of respiratory distress with cough, tachypnea, retraction, and diffuse crackles on auscultation (wheezing alone was not considered sufficient cause for inclusion in the study). The exclusion criteria were underlying chronic disease (such as cystic fibrosis, chronic pulmonary disease, congenital heart disease, and immunodeficiency) and recurrent (more than one) wheezing episodes (44). Disease severity and clinical evolution were evaluated using the clinical score index described by Wainwright and colleagues (42). In particular, on admission to the hospital, a clinical severity score was assigned to each infant within the range of 0 to 8, based on respiratory rate (<45/min = 0, 45 to 60/min = 1, and >60/min = 2), arterial oxygen saturation in room air (>95% = 0, 95 to 90% = 1, and <90% = 2), the presence of retractions (none = 0, present = 1, and present plus nasal flare = 2), and the ability to feed (normal = 0, reduced = 1, and endoveneous = 2).

Specimen collection. NPWs were collected in the first 48 h after admission to the hospital from infants suffering from acute bronchiolitis, and an aliquot was tested for viruses as previously described (31). Subsequently, NPWs were centrifuged at 2,000 rpm for 10 min, and each cell pellet was resuspended in 1 ml of phenol and guanidine isothiocyanate reagent (Trizol, Gibco-BRL, NY) and frozen at −80°C for subsequent PRR gene expression analysis. Supernatants also were collected and frozen at −80°C for subsequent IFN type I protein level analysis.

PCR assays for respiratory viruses. A panel of reverse transcription-PCR (RT-PCR) or nested PCR assays, some in a multiplex format, were used for the detection of 13 respiratory viruses, including RSV; influenza viruses A and B; coronaviruses OC43, 229E, NL63, and HKU1; metapneumovirus; adenovirus; rhinovirus (RV); and parainfluenza virus types 1 to 3, as previously reported (31). The primers and probe set for RSV were obtained by cloning the 82 bp of viral N gene into the pCR2.1 plasmid using a TOPO TA cloning kit (Invitrogen Corporation, San Diego, CA). A linear distribution (r = 0.99) was obtained between 10^3 and 10^6 copies of RSV DNA. Data are expressed as the number of RSV copies/milliliter of NPW.

Statistical analysis. All results are expressed as means ± standard deviations (median). Differences between infants with or without viral infections, in terms of the level of PRR genes in cells from NPWs, were compared using the Mann–Whitney test. Spearman’s ρ coefficient was calculated in order to assess the correlation between the level of PRR genes in cells from NPWs and the clinical score index, day of hospitalization, RSV viral load, and physical factors (age and body weight). Significance was fixed at the 5% level. The analysis was performed using SPSS version 13.0 for Windows.

RESULTS

Detection of respiratory viruses in infants suffering from acute bronchiolitis. To determine the presence of respiratory viruses, 157 NPW washings from children with a clinical diagnosis of acute bronchiolitis were examined. Specifically, a panel of RT-PCR or nested PCR assays, some in a multiplex format, were used for the detection of 14 respiratory viruses, including RSV; influenza viruses A and B; coronaviruses OC43, 229E, NL63, and HKU1; metapneumovirus; adenovirus; RV; parainfluenza virus types 1 to 3; and HBoV. In 73/157 children (46.49%), at least one viral pathogen was identified; 65/157 (41.40%) had an infection with one of the viruses under investigation, and 8/157 (5.09%) had dual infections. The remaining 84 children (53.50%) tested negative for any respiratory virus. The results are summarized in Table 1. In children who tested positive for a single respiratory virus infection, the most common virus was RSV (29.29%), followed by HBoV (5.73%) and RV (5.09%). Of the 54 children who tested positive for RSV, 7 were coinfected with HBoV (4.45%). In addition, RV was found as a coinfection with RSV in only one patient (0.63%).

Gene expression of nucleic acid-sensing PRRs in infants suffering from acute bronchiolitis. The transcription levels of
PRRs involved in the recognition of nucleic acids (MDA-5, RIG-1, TLR-3, TLR-7, TLR-8, and TLR-9) were investigated using real-time RT-PCR in cells from NPWs from 157 infants with a clinical diagnosis of acute bronchiolitis during a period covering two consecutive annual epidemic seasons. The level of expression of all examined PRR genes showed high variability among infants with acute bronchiolitis (coefficient of variation, >100%).

The infants with clinical signs and symptoms of bronchiolitis were divided into two groups on the basis of the detection of viruses or the failure to detect viruses in their NPWs. Infants identified as being negative for any of the viruses tested for are referred to as the uninfected or virus-negative group. The level of expression of PRR genes in NPW cells was examined in both groups. The results indicated that transcript levels of RLHs (MDA-5 and RIG-1), TLR-7, and TLR-8 were higher in the virus-positive than in the virus-negative group (Fig. 1A, B, D, E) (P < 0.05). In contrast, there were no differences between groups for TLR-3 and TLR-9 levels (Fig. 1C, F) (P > 0.05).

Gene expression of nucleic acid-sensing PRRs in infants with RSV-associated acute bronchiolitis. The levels of PRR mRNA also were measured in NPW cells with single RSV infections. In RSV-infected infants, we found a trend toward higher gene expression levels of MDA-5, RIG-1, TLR-7, and TLR-8 than in infants with single RV or HBoV infections. The differences were more evident when RIG-1 was taken into consideration and less pronounced when TLR-7 was considered. The results did not reach statistical significance, probably because of the wide variability of PRR expression observed in the infants examined in this study and the small numbers of infants with single viral infections caused by HBoV (n = 9) or RV (n = 8). The gene expression levels of TLR-3 and TLR-9 were similar for infants with RSV and for those with HBoV or RV infection. In addition, we found no differences in expression levels of PRR mRNAs between infants in the RSV group and those with HBoV or RV coinfection (Table 2).

We also observed that in infants with single HBoV or RV infection, there was a trend toward a higher level of expression of MDA-5, RIG-1, and TLR-8, which was not seen in uninfected infants. Moreover, the gene expression levels of all PRRs examined in this study were similar for HBoV- or RV-infected infants, although a trend toward a higher expression of MDA-5, RIG-1, and TLR-8 was observed in infants with HBoV infection compared to that of infants with RV infection.

Factors influencing PRR mRNAs levels in infants with RSV-associated acute bronchiolitis. In an attempt to identify the factors influencing PRR gene expression in NPW cells from infants with RSV-associated acute bronchiolitis, the levels of expression of these molecules were examined for any significant correlation with IFN-α protein levels. ELISAs specific for IFN-α or IFN-β were performed on the NPW samples from those infants with RSV infections; however, in all of the samples tested, the amounts of IFN-α or IFN-β were below the levels of detection of the assays (see Materials and Methods).

We also investigated whether the viral load of RSV influences PRR gene expression in NPW cells from infants with RSV infections; however, no significant correlation was found between viral load and MDA-5, TLR-3, TLR-7, TLR-8, or TLR-9 expression (Fig. 2 and Table 3). In contrast, a significant positive correlation was observed between the viral load of RSV and mRNA levels of RIG-1 (Fig. 2 and Table 3).

To further address this issue, we attempted to assess whether the expression levels of PRR genes are related to disease severity. We observed that the clinical score index recorded at the time of hospital admission in infants with RSV infections did not correlate with RIG-1, TLR-3, TLR-7, TLR-8, and TLR-9 mRNAs or with viral load (Fig. 2 and Table 3).

Finally, the relationship between PRR mRNA production and other parameters, such as the number of days hospitalized or patient characteristics (age and body weight), was examined. No correlations between PRR mRNA levels and these parameters were found (Fig. 2 and Table 3).

**DISCUSSION**

The results of our study confirmed that RSV is the most frequently detected virus in infants with bronchiolitis; the same data also highlighted the potential significance of other viral pathogens, such as HBoV and RV, in this clinical setting (5, 7, 10, 20, 35). However, in this study we observed that a high percentage of infants with clinical signs and symptoms of bronchiolitis apparently had no respiratory viral infections. This finding is in contrast with the data from other studies (5, 7, 10, 20, 35) and with our group detection rates from previous years (31, 37). The reasons for these discrepancies are unclear. It does not appear to be related to the PCR assays used in respiratory virus detection, since in this study we used the same PCR assays as those used in our earlier studies (31, 37). In particular, in the 2005 to 2006 epidemic season, we observed that 87% of infants with bronchiolitis had respiratory virus infections (37). Moreover, the numbers of cells present in the NPW samples probably did not affect the rate of the detection of respiratory virus infections; indeed, although the numbers of cells recovered in NPW collected from infants with bronchiolitis were not measured, similar transcript levels of the beta-glucuronidase housekeeping gene were observed in both virus-positive and -negative infants (data not shown).

However, a variety of factors could influence either the viral survival or infectivity of respiratory viruses, such as meteorological factors, seasonal variations in social behavior, air pollution, and subgroup-specific immune responses throughout the year. The differences in expression levels of PRR genes could be related to the physiological factors, seasonal variations in social behavior, air pollution, and subgroup-specific immune responses throughout the year.
FIG. 1. Relative gene expression of MDA-5 (A), RIG-1 (B), TLR-3 (C), TLR-7 (D), TLR-8 (E), and TLR-9 (F) in infants with acute bronchiolitis in the presence or absence of respiratory virus infections. Infants with respiratory virus infections have higher levels of the expression of MDA-5, RIG-1, TLR-7, and TLR-8 than those of infants with no virus infections; $P < 0.05$ using a Mann–Whitney test.
the seasons (11, 13, 22, 39). Indirectly such factors are important in the rate of detection of respiratory viruses from infants with lower respiratory tract illnesses. In addition, the large number of virus-negative cases observed in this study could depend on the fact that bronchiolitis arises from other, as-yet unknown, pathogens. Further studies are required to identify risk factors that affect the prevalence of respiratory virus-related bronchiolitis.

Interestingly, the results of this study indicate, we believe for the first time, that infants with respiratory virus-associated bronchiolitis have, in their respiratory tracts, relatively high levels of the gene expression of several PRRs involved in viral recognition compared to levels for infants who are suffering from bronchiolitis but without, apparently, a respiratory virus infection. The differences in the levels of the gene expression of PRRs are more pronounced in infants with RSV infections than in those with HBoV or RV infection.

Since PRRs are important signal transducers that mediate inflammatory reactions that are induced by respiratory viruses through the pattern recognition of viral nucleic acids, the findings of this study could have implications in the understanding of the pathogenesis of RSV-associated bronchiolitis.

It is known that severe RSV infection involves the release of inflammatory mediators, epithelial cell necrosis, inflammation, and mucus plug production (26). The production of cytokines and chemokines is significantly increased in infants with RSV infections, and it generally is accepted that levels of these cell-signaling molecules correlate with the severity of illness (4, 43). Moreover, in infants, an imbalance in the Th1/Th2 cytokine immune response has been related to the pathogenesis of RSV bronchiolitis and to the severity of the infection (23, 33).

Previous studies have demonstrated that the induction of chemokines by RSV replication is mediated by TLR-3 signaling pathways (36). Moreover, TLRs have been identified as contributors to the diversity of the adaptive immune response by influencing the type 1/type 2 biasing found in specific immune responses (34).

Hence, it could be speculated that RSV infection directly triggers the activation of several PRR signaling pathways that drive the expression of cytokines and chemokines, which subsequently recruit and activate the cell populations responsible for airway inflammation.

Conversely, it also could be the case that the high level of expression of several PRR-sensing nucleic acids observed in infants with RSV infections could be related to the activation of IFN production in the airway tract during RSV infection. Indeed, IFN has been reported to mediate the expression of all of the PRRs investigated in this study (29, 40), and RLHs and

### TABLE 2. Relative gene expression of PRRs involved in nucleic acid recognition in infants

<table>
<thead>
<tr>
<th>PRR</th>
<th>RSV (n = 46) Mean ± SD</th>
<th>RSV + HBoV or RV (n = 8) Mean ± SD</th>
<th>HBoV (n = 9) Mean ± SD</th>
<th>RV (n = 8) Mean ± SD</th>
<th>No virus (n = 84) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-5</td>
<td>124.13 ± 272.62 (64.10)</td>
<td>121.79 ± 58.47 (57.63)</td>
<td>81.78 ± 100.14 (18.25)</td>
<td>68.48 ± 103.08 (33.68)</td>
<td>59.44 ± 133.07 (3.65)</td>
</tr>
<tr>
<td>RIG-1</td>
<td>406.24 ± 1,697.95 (73.96)</td>
<td>390.35 ± 530.34 (65.79)</td>
<td>154.03 ± 256.74 (25.45)</td>
<td>99.03 ± 142.23 (27.12)</td>
<td>28.30 ± 42.77 (4.46)</td>
</tr>
<tr>
<td>TLR-3</td>
<td>0.55 ± 0.86 (0.25)</td>
<td>0.73 ± 0.84 (0.40)</td>
<td>0.45 ± 0.21 (0.18)</td>
<td>0.52 ± 0.41 (0.11)</td>
<td>0.51 ± 0.97 (0.14)</td>
</tr>
<tr>
<td>TLR-7</td>
<td>2.76 ± 4.10 (1.62)</td>
<td>0.60 ± 0.24 (0.60)</td>
<td>1.24 ± 1.46 (0.63)</td>
<td>1.41 ± 1.35 (2.25)</td>
<td>1.10 ± 2.00 (0.15)</td>
</tr>
<tr>
<td>TLR-8</td>
<td>53.60 ± 94.01 (19.73)</td>
<td>38.99 ± 17.35 (18.50)</td>
<td>41.59 ± 77.67 (15.83)</td>
<td>31.53 ± 14.95 (30.06)</td>
<td>11.39 ± 25.55 (2.92)</td>
</tr>
<tr>
<td>TLR-9</td>
<td>2.12 ± 5.89 (0.50)</td>
<td>2.07 ± 6.05 (0.47)</td>
<td>2.81 ± 0.82 (0.44)</td>
<td>2.66 ± 0.44 (0.55)</td>
<td>2.30 ± 5.15 (0.28)</td>
</tr>
</tbody>
</table>

*a* Data are given as means ± standard deviations (median).

*b* RSV + HBoV (n = 7) and RSV + RV (n = 1) represent dual infections.

### FIG. 2. Viral load of RSV, clinical score index, number of days hospitalized, age, and body weight recorded for RSV-infected infants (n = 46).

Lanes: A, viral load of RSV (log copy numbers/milliliter); B, clinical score index; C, number of days hospitalized; D, age (months); and E, body weight (kilograms).
TLRs have been implicated in the viral induction of type I IFNs. In support of our hypothesis, we investigated whether IFN type I protein levels influence PRR expression in infants with RSV infections, but we found that the levels of IFN-α or IFN-β in NPW samples from all infants with RSV infections were below the sensitivity of the ELISAs used.

In addition, in contrast to our previous study, in which we demonstrated a significant inverse correlation between the severity of bronchiolitis and the expression of IFN-induced genes in the airway tract of infants with RSV infections (37), we found no significant relationship between the levels of RLH or TLR transcripts and the clinical severity score. This suggests that the gene expression of nucleic acid-sensing PRRs could not be involved directly in the pathogenesis of RSV bronchiolitis but could influence other aspects of the immune response that subsequently might be responsible for the clinical outcome of bronchiolitis.

One of the potential limitations of our study is that we did not evaluate the gene expression of type I IFNs as well as that of other cytokines related to the PRR-mediated signaling pathways in cells collected from NPV infants with RSV infections. Such an analysis may have allowed us to verify whether the high levels of several PRR-sensing nucleic acids observed in infants with RSV infections are related to the activation of IFN production in the airway tract during RSV infection or to some other activation pathway. Unfortunately, the sample volumes were limited, being only just sufficient for all of the measurements described in the present study; hence, the above-described issue could not be addressed.

Further studies are required to understand the mechanism by which RSV upregulates the expression of PRRs, as well as the effects of PRRs on IFN response during RSV bronchiolitis. The findings of this study could be consistent with the hypothesis that infection with respiratory viruses, especially RSV, sensitizes the airway epithelium to subsequent viral and bacterial exposures by upregulating some PRRs involved in nucleic acid recognition. Directly related to this, in vitro RSV infection has been shown to increase the expression of TLR-3 (15). However, in contrast to these observations, a similar expression of TLR-3 transcripts was found between uninfected and respiratory virus-infected infants, including those with RSV infections. In addition, a lower level of expression of TLR-3 and TLR-9 was observed compared to those of other PRRs investigated in this study. This suggests that PRRs other than TLR-3 could be activated during acute bronchiolitis.

Although previous studies have reported that TLRs are expressed in human bronchial epithelium cells (3), the collective evidence points toward a cell type specificity in the recognition of replicating viruses via TLRs or RLHs. In this regard, it has been reported that nonimmune cells, including fibroblasts, induce cytokines via RIG-1 and MDA-5, and this is independent of TLR-3, TLR-7, and TLR-9, whereas immune cells such as plasmacytoid dendritic cells rely on TLRs rather than RIG-1 or MDA-5 to produce antiviral cytokines (21). In confirmation of this, we found higher levels of expression for RLHs than TLRs in the airway tracts of infants suffering from acute bronchiolitis.

Interestingly, RSV-infected infants were observed to have both a higher level of expression of RIG-1 than other PRRs investigated in this study and a positive correlation between RIG-1 mRNA levels and the viral load of RSV. Our results, the first measurements of RIG-1 in infants with RSV infections, extend previous observation data, which were obtained from cultured RSV-infected cells, in which it was demonstrated that this RNA sensor is essential for the intracellular recognition of RSV RNA in airway epithelial cells (25). In addition, the authors demonstrated that RIG-1 also mediates RSV-induced early signaling events, leading to the activation of NF-κB and IRF-3, two key transcription factors controlling inflammatory cytokine and chemokine expression in airway epithelial cells. The importance of RIG-1 in the recognition of RSV also has been suggested by the recent observation that the NS2 protein of RSV inhibits RIG-1-mediated IFN promoter activation by binding to the N-terminal CARD domains of RIG-1 and inhibiting its interaction with the mitochondrial antiviral signaling protein (24). Further studies are needed to understand the processes that trigger and control RIG-1 signaling in infants with RSV-associated bronchiolitis.

As for the influence of the viral load of RSV on the severity of bronchiolitis, we found no significant relationship between viral load and the severity of bronchiolitis. This is in keeping with several other observations (4, 45) but is in contrast to the findings of other groups (12, 14, 16). The reasons for these discrepancies are unclear but could be related to the different methods employed in determining the viral load, such as plaque assay (12) or other molecular techniques (14, 16). In addition, it should be noted that, in our study, disease severity and the clinical evolution of RSV-associated bronchiolitis were performed using the clinical score index described by Wainwright and colleagues (42), whereas in other studies the calculation of disease severity was based mainly on the need for

<table>
<thead>
<tr>
<th>PRR</th>
<th>RSV RNA</th>
<th>Clinical score index</th>
<th>No. of days hospitalized</th>
<th>Age</th>
<th>Body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-5</td>
<td>0.20</td>
<td>−0.15</td>
<td>0.05</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>RIG-1</td>
<td>0.30</td>
<td>0.24</td>
<td>0.05</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>TLR-3</td>
<td>−0.03</td>
<td>−0.05</td>
<td>−0.20</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>TLR-7</td>
<td>0.01</td>
<td>−0.18</td>
<td>0.18</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>TLR-8</td>
<td>0.06</td>
<td>0.14</td>
<td>0.14</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td>TLR-9</td>
<td>−0.04</td>
<td>−0.16</td>
<td>0.15</td>
<td>0.15</td>
<td>−0.01</td>
</tr>
<tr>
<td>RSV RNA</td>
<td>NA</td>
<td>−0.25</td>
<td>0.01</td>
<td>0.07</td>
<td>0.08</td>
</tr>
</tbody>
</table>

\(a\) \(\rho\), Spearman’s correlation coefficient; significant correlations are highlighted in boldface.

\(b\) NA, not applicable.
...activation of RLHs and TLRs during acute bronchiolitis in clinical studies are required to gain a better understanding of the role of the viral load of RSV during bronchiolitis. Apart from RSV, we believe this study demonstrates that, in infants with HBoV infection, there is a relatively high activation of several nucleic acid-sensing PRR mRNAs compared to that of uninfected infants. The findings suggest that HBoV infection causes the activation of PRR expression in the airway tract, which could favor the development and maintenance of an airway immune response during bronchiolitis. A very recent study reported the activation of Th1 cytokine production during HBoV-associated bronchiolitis (9); this, together with our data, indirectly strengthens the hypothesis that HBoV is not an occasional replicating virus in children hospitalized for respiratory infections. Directly related to this, another recent report suggested that the presence of HBoV in samples from the respiratory tract of children is associated with recurrent wheezing and bronchiolitis, showing a clinical course different from that of other respiratory viruses in terms of diagnosis, fever, and age (6).

In conclusion, this study shows evidence of changes in the expression of several PRR genes that are implicated in the recognition of nucleic acid in the airway tracts of infants suffering from virus-associated bronchiolitis, mainly in infants with RSV infections. Given the critical role of PRRs in innate immunity and in the initiation of the appropriate adaptive response, the regulation of PRR expression might be an important determinant of the outcome of RSV infection. Longitudinal studies are required to gain a better understanding of the activation of RLHs and TLRs during acute bronchiolitis in early life.

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