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Thymic stromal lymphopoietin, IL-33, and periostin in hospitalized infants with viral bronchiolitis

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Abstract

Much attention has recently been focused on thymic stromal lymphopoietin (TSLP), IL-33, and periostin in allergic disease, but less is known about their role in viral bronchiolitis.

The aim of the study was to investigate whether infants exhibit enhanced nasal airway secretion of TSLP, IL-33, and periostin during natural respiratory viral bronchiolitis compared to healthy controls.

In total, 213 infants < 2 years of age, hospitalized with bronchiolitis from October/2013 to April/2016 were enrolled alongside 45 healthy infants. Nasopharyngeal aspirates (NPA) were screened for respiratory viruses by the polymerase chain reaction. TSLP, IL-33, and periostin were measured in NPAs. Clinical data were recorded.

At least 1 virus was detected in 186 (87.3%) hospitalized infants: 149 (70%) respiratory syncytial virus (RSV); 42 (19.7%) rhinovirus (HRV); 16 (7.5%) parainfluenza virus (PIV); 9 (4.2%) adenovirus; 10 (4.7%) bocavirus; and 7 (3.3%) metapneumovirus (hMPV). Infants with bronchiolitis had higher levels of TSLP ($P = .02$), IL-33 ($P < .001$), and periostin ($P = .003$) than healthy controls.

Detectable levels of TSLP and periostin were more frequent in virus-positive than in virus-negative patients ($P = .05$). TSLP and IL-33 were also more common in coinfections, mainly RSV and HRV, than in single-infections ($P < .05$). No patient with bronchiolitis but with negative viral detection had detectable levels of nasal TSLP or IL-33. Infants with hospital stay ≥5 days were more likely to have detectable levels of nasal TSLP and periostin after adjusting by age ($P = .01$).

Bronchiolitis by common respiratory viruses is associated with elevated nasal levels of TSLP, IL-33, and periostin, factors known to be important in the development of Th2-response. Respiratory viruses in early life might shift immune responses toward Th2, involving asthma, and allergic diseases.

Abbreviations: AEC = airway epithelial cells, CRP = C-reactive protein, HBoV = human bocavirus, hMPV = human metapneumovirus, HRV = human rhinovirus, ICU = intensive care unit, NPA = nasopharyngeal aspirates, PCR = polymerase chain reaction, PIV = parainfluenza virus, RSV = respiratory syncytial virus, TSLP = thymic stromal lymphopoietin, WBC = white blood cells.

Keywords: asthma, bronchiolitis, IL-33, infants, periostin, respiratory viruses, Th2, TSLP

1. Introduction

Bronchiolitis is the most common lower respiratory tract infection in infants and is the leading cause of hospitalization of children younger than 1 year of age. Respiratory syncytial virus (RSV) is the most frequent virus responsible for bronchiolitis worldwide, followed by rhinovirus (HRV).[1,2] New respiratory viruses such as human bocavirus (HBoV) and human metapneumovirus (hMPV) have also been detected in infants and children with lower respiratory tract diseases.[3,4]

Approximately 30% to 70% of infants develop bronchiolitis after primary RSV infection and 1% to 3% of them are hospitalized.[5] Even though risk factors for hospitalization have been identified, the mechanism of severe bronchiolitis is not completely understood.[6] Infants who are affected with RSV or HRV bronchiolitis during their first months of life, frequently develop recurrent wheezing and asthma.[7,8] Several mechanisms have been implicated to explain the different short and long term severity of bronchiolitis. Neutrophils can limit viral replication and spread, as well as stimulate an effective antiviral adaptive immune response.[9] However, one of the most widely accepted theories attributes severe RSV-bronchiolitis to an imbalance in the Th1/Th2 immune response.[10–12]

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The principal Th1-cytokine, IFN-γ, is likely to be the most important for cell-mediated immunity.[13] IFN-γ inhibits allergic responses but may also contribute to airway hyperresponsiveness, especially in non-atopic subjects.[14] Although it is considered an important molecule in antiviral host defense, few studies have investigated its expression by airway epithelial cells. It has been this suggested that IFN-γ may play an important role in eosinophilic inflammation in RSV bronchiolitis.[15]

Th2 immune response is characterized by low IFN-γ and IL-12 levels and higher IL-4, IL-10, and IL-13 levels, as observed in the airways and in peripheral blood mononuclear cells of infants with RSV bronchiolitis.[16] IL-10 can downregulate cytokine production by Th1-like T-cells and inhibit antigen presentation through downregulation of class II major histocompatibility complex antigens on monocytes.[17] It is frequently elevated in nasopharyngeal secretions of RSV infected children[18] and may be related to subsequent development of chronic airway morbidity.[19]

Recently, thymic stromal lymphopoietin (TSLP), an epithelium-derived cytokine, has been identified as a key initiator of allergic airway responses.[20] It is considered a master Th2-cytokine, as it promotes the differentiation of naive T0 cells into Th2 lymphocytes through the activation of antigen presenting cells.[21] Several studies have shown a strong link between TSLP expression and the production of Th2-associated effector cytokines IL-4, IL-5, IL-13, and TNFα.[22–24] Furthermore, TSLP seems to play a pivotal role in the pathobiology of allergic asthma and it is expressed at elevated levels in the lungs of humans with asthma.[24] Rhinovirus infection induces, in mice models, TSLP, and IL-33 expression as well as TSLP-dependent upregulation of OX40L, and all 3 of these molecules contribute to the development of an adaptive immune response that is characterized by an altered balance between suppressive peripherally induced regulatory T cell (pTreg cells) and Th2 effector cells.[25] These findings might be a common response of some other respiratory viruses associated with allergic disease, as in mice initially infected as neonates, TSLP expression induced by RSV infection is an important upstream event that controls OX40L expression, lung dendritic cells migration, and Th2 polarization.[26]

IL-33 is a member of the IL-1 family, which is a superfamily of cytokines mainly involved in early immune and inflammatory responses following infection. Unlike other interleukin 1 family members, IL-33 has been associated with the promotion of both systemic and localized Th2 cell responses.[27] Clinically, patients with mild to moderately severe asthma show increased nasal IL-33 levels following HRV inoculation and this increase correlates with symptom severity and viral load.[28]

Periostin is a matrix cellular protein, and its synthesis in airway epithelial cells and lung fibroblasts is induced by interleukin IL-4 and IL-13. Periostin has been shown to be involved in many aspects of allergic inflammation, such as eosinophil recruitment, airway remodeling, development of a Th2 phenotype, and increased expression of inflammatory mediators.[29] However, so far there are no published studies that assess the expression of periostin in infants with bronchiolitis.

The primary purpose of this cross-sectional study was to investigate whether infants exhibit enhanced nasal airway secretion of TSLP, IL-33 and periostin during natural respiratory viral bronchiolitis compared to healthy controls. Secondary analysis examined whether the nasal levels of TSLP, IL-33, and periostin could be related to clinical severity and to viral etiology of acute bronchiolitis, mainly RSV and HRV.

2. Patients and methods

2.1. Clinical assessment

This is a cross-sectional prospective study designed to investigate the nasal airway cytokine response during viral bronchiolitis in hospitalized infants. The study population comprised all infants less than 24 months of age with bronchiolitis admitted to the secondary public hospital Severo Ochoa (Leganés, Madrid), between October 2013 and April 2016 and a group of asymptomatic infants, with non-detectable virus by PCR testing, recruited during the same period. Informed consent was obtained from the children's parents and the study protocol was approved by the Ethics Committee of the Severo Ochoa Hospital, Alfonso X El Sabio University.

The classic criteria, an initial episode of acute onset expiratory dyspnea with previous signs of viral respiratory infection—whether this was associated with respiratory distress or pneumonia—were applied in diagnosing *bronchiolitis*.[30] Cases with focal infiltrates and consolidation in chest x-rays, in the absence of wheezing, were classified as *pneumonia*, and were excluded.

During the hospital stay, and as part of the study, a physician filled out a study-questionnaire with the following variables: age, sex, clinical diagnosis, history of prematurity and underlying chronic diseases, need for oxygen therapy assessed by transcutaneous oxygen saturation, axillary temperature ≥ 38°C, presence of infiltrates/atelectasis in radiographs, administration of antibiotic therapy, duration of hospital stay, total white blood cell (WBC) count, C-reactive protein (CRP) serum values, and result of blood culture if performed. Oxygen therapy was provided to achieve oxygen saturation > or equal to 94%.

For this study, patients with hospital stay ≥ 5 days or intensive care unit (ICU) admission were classified as having severe illness.

Healthy control infants of comparable age, sex, and race, with no history of respiratory illness in the preceding 2 weeks and without any detectable respiratory virus, were enrolled at the primary care physician office during well-child visits.

2.2. Virus detection

Samples of study were nasopharyngeal aspirates (NPA) that were collected at the onset of admission by a standard technique (both healthy and control infants) consisting of gently washing the nasal cavity with 1 mL of phosphate buffered saline in each nostril and collection into a standard mucus extractor. Each specimen was sent for virologic investigation to the Respiratory Virus and Influenza Unit at the National Microbiology Center (ISCIII, Madrid, Spain). NPA were processed within 24 hours after collection. Upon receipt, 3 aliquots were prepared and stored at –70°C. Both the reception and the NPA sample processing areas were separate from those defined as working areas.

A recent study by Lopez-Guisa et al[31] demonstrated good correlation between bronchial and nasal epithelial expression of pro-remodeling factors. NPA is a noninvasive method, especially useful in infants and young children.

2.3. Polymerase chain reactions (PCR) methods for detection of 16 respiratory viruses

Three RT-nested PCR assays were performed to detect a total of 16 respiratory viruses. In these assays, the reverse transcription (RT) and first amplification round were carried out in a single

tube using the Qiagen One Step RT-PCR kit (Qiagen). Influenza A, B, and C viruses were detected by using previously described primer sets only to amplify influenza viruses in a multiplex PCR assay.[32] A second multiplex PCR was used to detect parainfluenza viruses 1 to 4, human coronaviruses 229E and OC43, enteroviruses and rhinoviruses HRV.[33] The presence of respiratory syncityal virus (RSV) A and B types, human metapneumovirus (hMPV), human bocavirus (HBoV), and adenoviruses were investigated by a third multiplex RT-nested PCR-BRQ method.[34]

2.4. Detection of cytokines and proteins in nasal secretions

We analyzed IFN-γ, IL-10 using ELISA Ready Set Go (eBioscience, San Diego, CA) and TSLP, IL-33, and periostin by ELISA Kit (R&D Systems, Abingdon, UK), according to the manufacturers' instructions using provided standards and quality controls. The intra-assay and inter-assay coefficient of variation were: TSLP: 8.2% and 7.47%, respectively, IL-33: 4.7% and 6.9%, respectively. periostin: 2.19% and 9.99%, respectively, IL-10: 3.2% and 5.6%, respectively and IFNγ: 4.5% and 5.7%, respectively. The lower detection limit of these assays was 7.8 pg/mL (IFN-γ), 2.3 pg/mL (IL-10), 32.5 pg/mL (TSLP), 11.7 pg/mL (IL-33), and 62.5 pg/mL, (periostin).

Real-time quantitative PCR of cells from nasopharyngeal aspirates was conducted for several cytokine and protein genes. Total RNA was isolated from cells from NPA by Trizol reagent (Invitrogen, Carlsbad, CA) and it was treated with DNase I (Promega, WI). RNA was measured by spectrophotometry, and 1 μg of RNA was reverse-transcribed to cDNA using a high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Warrington, UK). Quantitative Real-Time PCR was performed on a 7500 Real-Time PCR system (Applied Biosystems, Warrington, UK). Taqman PCR was performed using a 20 μL final reaction volume containing 10 μL of TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, NJ), 1 μL of 20X Assays-on-Demand Gene Expression Assay Mix, and 9 μL of cDNA diluted in RNase-free water. Each assay was performed in triplicate. The PCR conditions used in all reactions were: 2 minutes at 50°C and 10 minutes at 95°C, repeated over 40 two-step cycles (95°C for 15 seconds and 60°C for 60 seconds). Assays-on-Demand Gene Expression primers specific for IFN-γ, IL-10, TSLP and periostin and rRNA 18S (used as an endogen) were obtained from Applied Biosystems (http://www.appliedbiosystems.com/).

Messenger RNA expression was evaluated for each sample using the cycle threshold (*Ct*) value. The number of amplification steps required to reach an arbitrary intensity *Ct* was computed. The relative gene expression was calculated as follows: Δ*Ct*, where $\Delta Ct = \Delta Ct_{\text{target}} - \Delta Ct_{18s}$ (housekeeping). The fold-change for the treatment was defined as the relative expression compared with the corresponding control, and was calculated as follows: 2^−ΔΔ*Ct*, where ΔΔ*Ct* = Δ*Ct*patient−Δ*Ct*healthy.

2.5. Statistical analysis

Values were expressed as percentages for discrete variables, or as mean and standard deviation or median and interquartile range for continuous variables. Comparisons used either X^2 or Fisher exact test (2-tailed) for categorical variables and Student's *t* test, Mann-Whitney *U* test, Kruskal-Wallis test, and analysis of variance (ANOVA) for continuous variables. Logistic regression analysis was performed to study the independent association between clinical variables and nasal cytokines responses. 95% confidence interval values were also computed. A probability of $<.05$ was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS), Version 23.0.

3. Results

3.1. Baseline characteristics

The study population consisted of 213 hospitalized infants younger than 2 years old, who were diagnosed with bronchiolitis and 45 healthy controls. There were not significant differences in the baseline demographic characteristics of the hospitalized and control groups, including gender and age.

A total of 186 (87.3%) infants with bronchiolitis had a positive respiratory viral identification, 19.7% as viral coinfections. RSV was detected in 149 (70%) patients, HRV in 42 (19.7%), PIV in 16 (7.5%), adenovirus in 9 (4.2%), HBoV in 10 (4.7%), and hMPV in 7 (3.3%).

The infants' median age at admission was 3 months (IQR 1.6–6.1); 25 (11.8%) were born prematurely and 15 (7%) had been diagnosed with neonatal distress. Hypoxia was common (71%) and 19 (4.49%) needed admission in the intensive care unit and/or high flow oxygen therapy.

Clinical parameters of single-RSV (N = 116) versus single-HRV (N = 18) bronchiolitis were compared. Infants with single-RSV infection presented mostly in winter months (97.7%), whereas HRV-cases were diagnosed mainly in March and October ($P < .001$). Mean length of hospital stay was similar in both groups. However, positive RSV-patients were 4 times more likely to have hypoxia than HRV-infants (76% vs 44.4%, $P = .006$, OR.3.929, 95%CI: 1.413–10.920). No other significant differences were observed among both viral groups (Table 1).

3.2. Nasal cytokine and protein determination

TSLP, IL-33, periostin, IL-10, and IFN- γ in bronchiolitis versus healthy control group

Unstimulated nasal TSLP, IL-33, periostin, IL-10, and IFN-γ frequency (Fig. 1A) and concentrations (Fig. 1B) were analyzed in NPA from bronchiolitis-hospitalized infants as well as in controls. We observed that TSLP, IL-33, and IL-10 were only detected in the bronchiolitis group. Infants with bronchiolitis had

Table 1

Clinical characteristics of single-RSV and single-HRV infants hospitalized with acute bronchiolitis.

	RSV (N = 116)	HRV (N = 18)	*P*
Male	77 (67%)	9 (50%)	.162
Prematurity	15 (13%)	1 (5.6%)	.364
Temperature > 37.9°C	47 (41%)	9 (50%)	.466
Hypoxia, SatO2 < 95%	87 (76%)	8 (44%)	.006
Abnormal chest radiograph	21 (39%)	2 (20%)	.253
Antibiotic treatment	10 (8.7%)	2 (11%)	.739
Passive smoking	24 (25%)	4 (33%)	.549
Breastfeeding	69 (67%)	5 (38.5%)	.06
Age, months*	4.2 ± 3.8	5.8 ± 4.2	.108
Hospital stay, days	4.8 ± 2.6	4.0 ± 2.1	.269
Hypoxia duration, days	3.4 ± 2.2	2.7 ± 0.9	.103

* mean ± standard deviation.

HRV = rhinovirus, RSV = respiratory syncytial virus.

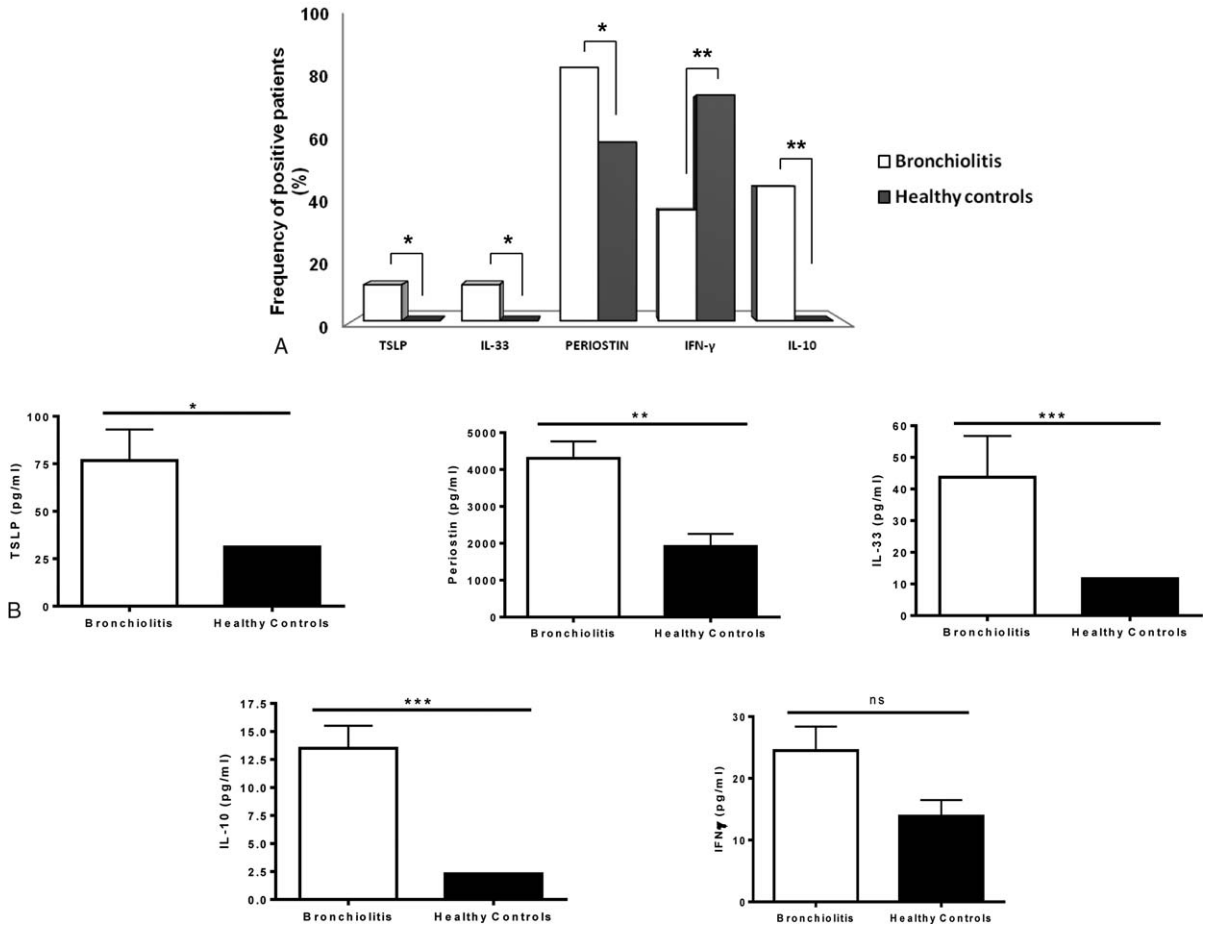

Figure 1. Frequency and soluble cytokine levels in supernatant of nasopharyngeal aspirates. Human TSLP, IL-33, periostin, IL-10, and IFN- γ levels in nasopharyngeal aspirates samples were measured by specific ELISA as described in Methods section. (A) Frequency distribution of several cytokines and proteins evaluated in supernatant of nasopharyngeal aspirates from healthy controls and bronchiolitis infants. (B) Quantitative evaluation of protein levels in supernatant. White bars represent the bronchiolitis group (mean ± SD, n = 213), and black bars the healthy group (mean ± SD, n = 45). Statistical analysis was performed by the Mann–Whitney *U* test. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. SD = standard deviation, TSLP = thymic stromal lymphopoietin.

significantly higher levels of TSLP, IL-33, periostin, and IL-10 than healthy controls (Fig. 1B). Although no differences regarding nasal concentrations of IFN-γ were found between both groups, its frequency expression is significantly higher in healthy infants than in bronchiolitis children ($P < .01$). In the rest of the proteins, the bronchiolitis group showed greater frequency than the healthy group reaching statistical significance (TSLP, IL-33 and, periostin: $P < .05$; IL-10: $P < .01$). No child in the control-group had detectable levels of nasal TSLP, IL-33, or IL-10.

To confirm these results, we evaluated transcripts for TSLP, periostin, IL-10, and IFN-γ in cells from nasal aspirates from bronchiolitis-hospitalized infants (n = 29) and in healthy infants (n = 7). We observed up-regulation of TSLP, periostin, IL-10, and IFN-γ mRNA levels in nasal cells from bronchiolitis infants when compared with the control group ($P < .05$, Fig. 2).

3.3. Comparison of nasal TSLP, IL-33, periostin, IL-10, and IFN- γ in the bronchiolitis group regarding virologic findings and clinical severity

Regarding viral etiology, patients with positive viral detection had more frequently detectable nasal TSLP (13.5% vs 0%, $P = .05$), IL-33 (11.7% vs 0%, $P = .032$), and periostin (85.1% vs 66.7%. $P = .034$) than patients without any identifiable virus. Also, nasal TSLP and IL-33 were detected more frequently in infants with viral coinfections than in those with single infections (26.3% vs 9.3%, $P = .006$ and 18.5% vs 7%, $P = .05$, respectively). No patient with bronchiolitis but with negative viral detection had detectable levels of nasal TSLP or IL-33.

Patients with RSV and HRV infection showed significantly higher concentrations of nasal TSLP compared to negative-virus infants ($P = .01$ and $P = .024$ respectively). When single-RSV, single-HRV, and RSV+HRV dual infections were compared, significant differences were found among the 3 groups ($P < .001$). The levels of TSLP in RSV+HRV coinfections were significantly higher than in RSV or HRV single infections separately ($P < .001$ and $P = .005$). All HRV infected infants with positive detection of TSLP presented viral coinfection, mainly with RSV, whereas no patient with single-HRV infection had detectable nasal TSLP.

Levels of periostin, IL-10, and IFN-γ were not different among these 3 groups (single RSV, single HRV, or dual RSV + HRV; Table 2).

Regarding IL-33, patients with HRV infection had more frequently detectable nasal IL-33 levels than those without identifiable HRV (18.4% vs 7%, $P = .05$) and showed higher concentrations of IL-33 (61.3 ± 241.3 pg/mL vs 36.0 ± 182.8 pg/mL,

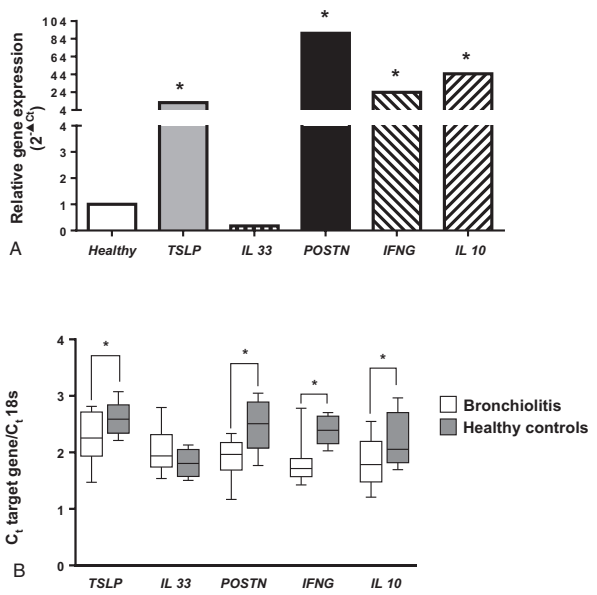

Figure 2. Nasal cytokine gene expression in cells of nasopharyngeal aspirates. Relative TSLP, periostin, IL-10, and IFN- γ mRNA levels from bronchiolitis and healthy control infants were determined by real-time semi-quantitative PCR. (A) Results show relative gene expression as determined by the ΔΔcycle threshold values (CT) method. (B) Results show the expression of target gene relative to the housekeeping gene expression (18s). High value correlates with fewer expressions. Statistical analysis was performed by the Mann–Whitney *U* test and significant differences in expression levels were obtained for the bronchiolitis group versus the control group, $^{*}P < .05$. PCR = polymerase chain reaction, TSLP = thymic stromal lymphopoietin.

P = .05). No other differences in IL-33 regarding virologic characteristics were found. No patient with bronchiolitis but with negative viral detection had detectable levels of nasal IL-33 (data not shown).

Regarding clinical severity, 8 (3.7%) infants needed ICU admission. The univariate analysis showed that infants who needed ICU admission presented more frequently detectable levels of TSLP than those with less severe disease (37.5% vs 11.3%, OR = 4.85, CI: 1.085–21.722, *P* = .028). Moreover, the concentrations of TSLP in these severe patients were significantly higher than in infants with milder symptoms (95.3 pg/mL ± 110.3 pg/mL vs 82.02 ± 298.7 pg/mL, *P* = .026). No differences in the levels of IL-33 or periostin were detected regarding ICU admission.

Ninety-nine (46.5%) infants needed hospital stay ≥ 5 days: 55 (56%) single- RSV infections; 8 (8.1%) single HRV, 10 (10.1%) dual RSV + HRV and less frequently PIV, HBoV, and hMPV. These patients were more likely to have detectable nasal periostin (49.5% vs 27.3%, OR = 2.635, CI: 1.155–6.009, *P* = .01) than those with shorter admissions. This difference remained significant after adjusting by age. However, levels of nasal periostin and IL-33 were not significantly higher in patients with a longer hospital stay (*P* = .388, *P* = .338, and *P* = .647, respectively).

Finally, a logistic regression analysis was used to test the association between detection of nasal TSLP with illness severity adjusting by viral etiology and age. Patients with hospital stay ≥ 5 days had a significantly greater probability of having nasal detectable TSLP during acute bronchiolitis (Adjusted OR = 4.124 [CI: 1.141–14.913], *P* = .029) as well as RSV+HRV coinfections (Adjusted OR = 9.330 [CI: 2.051–42.472], *P* = .004). However, ICU admissions were not significantly associated with nasal TSLP detection in the multivariable analysis.

Table 2

Nasopharyngeal aspirates proteins concentrations (pg/mL) from patients with single RSV, HRV, or dual RSV plus HRV infections.

	RSV	Rhinovirus	RSV + Rhinovirus	*P***
TSLP*	31.2 (31.2–31.2)	31.2 (31.2–31.2)	31.2 (31.2–102.4)	.050
Periostin*	2265 (1127–5494)	2116 (1350–7502)	2454 (422–6239)	.700
IL-10*	2.3 (2.3–12.0)	2.3 (2.3–8.3)	2.9 (2.3–17.5)	.660
IFN- γ*	7.8 (7.8–19.3)	7.8 (7.8–8.7)	7.8 (7.8–14.5)	.291

* Proteins concentrations that are expressed as median (IQR).
** *P*-value calculated with the Kruskall Wallis test.
HRV = rhinovirus, RSV = respiratory syncytial virus, TSLP = thymic stromal lymphopoietin.

4. Discussion

Our study is the first, to our knowledge, to demonstrate that naturally occurring severe infections by the most common respiratory viruses in hospitalized infants, and to induce nasal airway secretion of TSLP, IL-33, and periostin when compared with healthy controls. It is worth highlighting that any infant in the control group and any patient with bronchiolitis but without any identifiable virus had detectable levels of nasal TSLP or IL-33. Our results support the possible role of these 3 proteins in the inflammatory response triggered by the most common respiratory viruses and not only by RSV, rhinovirus or hMPV, as has been previously described, mostly in experimental studies.[22,26,35–37]

TSLP has been identified as a master switch for allergic inflammation in mouse models and it is an important cytokine in the development of allergic asthma.[38] Experimental studies comparing the TSLP response to RSV infection in bronchial epithelial cells from healthy and asthmatic children showed that cultures from asthmatic subjects displayed a significant increase in TSLP production, as compared to cultures from healthy subjects.[37] A recent report that showed how RSV induces up-regulation of TSLP receptor in human airway epithelial cells (AEC),[39] suggests a possible role for RSV-infected AECs in initiating an autocrine/paracrine TSLP signaling circuit, that could contribute to a TSLP-driven inflammatory response associated with virus infection.[40] Mehta et al[25] found that experimental infection by rhinovirus upregulates TSLP in human lung epithelial cells and TSLP seems to be central to the inflammatory activity of rhinovirus. Moreover, it has been reported, also in experimental studies, that TSLP is critical for the development of the immunopathology induced by RSV,[37] as well as by hMPV.[35]

In vivo studies, performed in young children with rhinovirus infection, have also found higher levels of nasal TSLP compared with subjects without any identifiable virus, in both term and preterm children.[41,42] The concentrations of nasal TSLP in our naturally acquired infections series, were significantly higher, not only in RSV and rhinovirus infections, but also in PIV and adenovirus-infants with bronchiolitis. The highest levels of TSLP were identified, in our patients, in infants with RSV + HRV coinfections. Multivariate logistic regression analysis found that infants with dual RSV + HRV infection were 9 times more likely to have detectable nasal TSLP and this association was independent of other factors such as age or illness severity. These findings suggest that the immunological response in acute bronchiolitis is partly dependent on virus-specific factors. Although other authors also found higher levels of nasal cytokines (IL-6, IL-1b, and IL-8) in infants infected with both RSV and HRV,[43] no previous study has evaluated the differential production of nasal TSLP in single versus dual

RSV and HRV infections. Furthermore, the role of coinfections in terms of clinical severity is still controversial. A recent study by our group in 1229 hospitalized children (599 RSV single infections, 510 single HRV infections and 120 RSV+HRV coinfections) found that patients with coinfections had more frequent fever ≥ 38°C and hypoxia than those with single RSV or single HRV infections.[44] Our results suggest that these clinical findings associated with severe RSV+HRV coinfections might be accompanied by a predominantly Th2 response, suggesting that these viral coinfections may lead to an altered mediator profile that biases towards a Th2 immune response. This hypothesis is also supported by the absence of significant differences in IFN-γ levels (principal Th1 cytokine) between bronchiolitis and healthy infants and between simple versus dual infections.

Another arising question is whether this stronger TSLP response in RSV+HRV coinfections might also be associated with higher incidence of recurrent wheezing later in life. Lukkarinen et al[45] recently reported that coinfection with HRV and HBoV1 resulted in a modified, non-Th2-type cytokine response, suggesting that HBoV1 may interfere with HRV-induced immune response. Furthermore, this immunological response was accompanied by the clinical finding that children with HRV+HBoV1 wheeze tended to develop recurrent wheezing later and less often than did those with HRV wheeze. Our results suggest that a virus–virus interaction may also occur in RSV and HRV coinfections, but in an opposite way, towards a predominant Th2 response. This shift in the immune response towards Th2 could favor the development of recurrent wheezing/asthma. However, as far as we know, no previous study has evaluated the frequency of recurrent wheezing after viral bronchiolitis in RSV+HRV coinfection versus the single ones.

On the other hand, it is hypothesized that periostin plays an important role in eosinophilic forms of asthma.[29,46] In murine models, periostin has been associated with severe asthmatic airway inflammation and hyperresponsiveness.[47] In humans, periostin has been found to prolong Th2/eosinophilic inflammation and to aggravate airway remodeling.[48] Levels of periostin reflect persistent eosinophilic airway inflammation in severe asthmatics despite a high dose of ICS.[46] In children, Lopez-Guisa et al[31] recently demonstrated significantly greater expression of periostin in bronchial and nasal epithelial cells from children with asthma compared to atopic nonasthmatic or healthy children. Prior to ours, no study has analyzed levels of periostin in infans with bronchiolitis. In our series, nasal concentrations of periostin were significantly higher in RSV, HRV, HBoV and hMPV-infected infants compared to healthy controls. Given the similarities in clinical manifestations between bronchiolitis and asthma, as well as the high frecuency of recurrent wheezing and asthma following an episode of bronchiolitis,[7,8] the high level of nasal periostin found in infants with viral bronchiolitis might suggest the presence of eosinophilic inflammation even at a young age. Further longitudinal studies are needed to elucidate if these infants with higher levels of periostin during acute bronchiolitis will develop recurrent wheezing/asthma more frequently later in life.

IL-33 is an inducer of type 2 inflammation in mouse models. The neutralization of IL-33 completely abolishes the severe RSV disease phenotype in neonatal mice, whereas the administration of the cytokine during infection in adult mice results in exacerbated Th2-inflammation, lung dysfunction, and airway mucus.[49] Saravia et al[49] detected IL-33 in nasal secretions of infants with RSV infection whose levels decreased during convalescence, although a limitation of their study is the lack of a control uninfected group. Recently, Jackson et al[50] demonstrated for the first time that IL-33 is induced by rhinovirus in the asthmatic adult airway in vivo. These observations have important implications for the understanding of virus-induced asthma exacerbations and offer a mechanism through which respiratory viruses, that are classic Th1 triggers, might promote type 2 inflammation in susceptible individuals.[50] Our study reports for the first time, that other respiratory viruses, besides RSV and HRV, also elicited secretion of IL-33 in the airways of infants with bronchiolitis compared to healthy control children. In our series, as was the case of TSLP, no infant in the healthy control group had detectable levels of IL-33, supporting the role of viral infections in IL-33 induction.

Previous studies have demonstrated that specific inflammatory mediators, as well as the imbalance in the immune response, can contribute to the severity of the viral disease.[43,51,52] The assessment of clinical severity in our study was based on the length of hospitalization ≥ 5 days and/or the need of ICU admission. Our results showed that the induction of nasal periostin and TSLP was more frequent in infants with longer hospitalizations even after adjusting by age as the main confounder. Nasal TSLP was also detected more often in infants who needed ICU admission, although the difference did not remain significant after logistic regression, probably because of the small sample size, as only 8 infants needed ICU admission. We are aware that more and wider studies are needed but our results suggest that a skewed pattern of immune response with a Th2 polarized response could contribute to more severe and prolonged clinical evolution in young hospitalized infants with bronchiolitis.

In contrast with Saravia et al,[49] our results found that other viruses besides RSV, as HRV, HBoV, hMPV, and PIV, can elicit the secretion of TSLP, periostin, and/or IL-33 in infants with bronchiolitis.

Limitations of the present study include the low frequency of bronchiolitis associated with the less common respiratory viruses such as hBoV or hMPV. Future studies are needed to elucidate the role of these respiratory viruses. There are also several strengths of this study. This is an in vivo study performed in naturally acquired viral infections in infants. Samples were prospectively collected, using rigorous methodology and identical collection procedures were employed in cases and controls.

5. Conclusion

Severe viral bronchiolitis by most common respiratory viruses is associated with elevated nasal airways levels of TSLP, IL-33, and periostin, factors known to be important in the development of Th2 response. These results suggest that respiratory viruses in early life might shift immune responses towards Th2 in early life which has been proven to be involved in asthma and allergic diseases development.

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