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Altered regulatory cytokine profiles in cases of pediatric respiratory syncytial virus infection

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ABSTRACT

Objectives: Regulatory cytokines are associated with viral infection. The objective of this study was to evaluate the relation between serum regulatory cytokines concentrations and respiratory syncytial virus (RSV) disease.

Methods: We enrolled 325 children aged < 24 months who were diagnosed with acute respiratory tract infection. Twenty age-matched healthy children were enrolled as controls. Nasopharyngeal swabs were analyzed to identify virus by reverse transcription polymerase chain reaction, and blood samples were taken to quantify the regulatory cytokine concentrations, including interleukin (IL)-35, IL-10 and transforming growth factor (TGF)-β1 using the Bio-Plex immunoassay or enzyme-linked immunosorbent assay.

Results: RSV disease was associated with a great regulatory cytokine response than healthy children, among 89 RSV-infected patients, serum IL-35 ($P = .0001$) and IL-10 ($P = .006$) was significantly elevated in comparison with healthy controls. Young children (0 < age ≤6 months) with RSV infection had significantly lower IL-35 and IL-10 expression but needed more oxygen therapy and more severe disease comparing with older children (12 < age < 24 months). Comparing with mild group, the expression levels of IL-10 were significantly lower in children with moderate and severe disease ($P = .012$ and $P = .005$, respectively). And levels of IL-10 was inversely associated with total duration of RSV infection symptoms ($r = -0.311$, $P = .019$).

Conclusion: Children with RSV infected had increased serum regulatory cytokine IL-10 and IL-35 concentrations. Elevated expression of IL-10 and IL-35 were contributed to protect hypoxia and reduce the severity of disease.

1. Introduction

Respiratory syncytial virus (RSV) is an enveloped, single-stranded, negative-sense RNA virus that belongs to the *Paramyxoviridae* family [1]. Infections with RSV are the leading cause of serious viral respiratory tract infections in children aged < 2 years [2]. RSV infections results in a spectrum of clinical presentations ranging from common cold symptoms to severe lower respiratory tract involvement requiring admission to pediatric intensive care units [3,4]. A growing body of evidence suggests that young children are more likely to develop life-threatening RSV infections and associated complications [5,6].

Most studies have demonstrated that RSV infections are typically correlated with a potent immune response in the lower respiratory tract due to T helper (Th)1 and Th2 cell imbalances and their associated pro-/anti-inflammatory cytokine responses [3,7,8]. Th2 cytokine responses are predominant in infants with RSV infection [9], and contribute to the pathogenesis of severe RSV disease, such as increased interleukin (IL)-4, IL-5 and IL-13 [3]. Some studies have indicated that Th1 cytokine responses such as interferon (IFN)-γ, IL-12 and tumor necrosis factor (TNF)-α decrease with severe RSV disease in infants [10,11]. All these data suggest that a balanced Th1/Th2 response is critical to mitigating RSV-mediated disease severity in pediatric patients.

The presence of precise combinations of cytokines can effectively regulate the development of polarized Th cell responses mounted in response to viral infections. It has been shown that IL-10, IL-35 and TGF-β were defined as regulatory cytokine due to the immunosuppressive roles in viral infections and autoimmunity disease [12–17]. For example, IL-10 inhibits acute inflammation and mitigates progression of an imbalanced Th1/Th2 response triggered by RSV infection [8,18,19]. IL-35 has recently been identified as a suppressive cytokine that contributes to the induction of type 1 regulatory T cells and modulates IL-10 production [12,20]. TGF-β is another immunosuppressive cytokine that interferes with the production of IFN-γ, IL-2, IL-12 and TNF-α in response to RSV infection [21]. All these data indicated that protective immune responses are regulated by regulatory

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cytokines and depend on the infectious agent. Conversely, other researches have demonstrated the IL-10 also induced strong Th2-dominant immune response and enhanced the RSV disease [19]. To evaluate the role of regulatory cytokines in RSV disease, we characterized changes in the expression levels of several regulatory cytokines including IL-10, IL-35 and TGF-β1 in the context of RSV infection in pediatric patients, and characterized the role in response to RSV infection.

2. Materials and methods

2.1. Patients and experimental design

This study was performed from January to December in 2014 at The First People Hospital of ChenZhou, China. Any child aged < 24 months who needed hospitalized with signs of acute upper respiratory tract infection (URTI) (defined as nasal congestion and or rhinorrhea) or lower respiratory tract infection (LRTI) (defined as bronchiolitis and/or pneumonia with fever, cough, sputum production, wheezing, tachypnea and radiological finding) were enrolled. Healthy controls consisted of children who met the following criteria: (i) age < 2 years old who before underwent surgery in the surgical department; (ii) without current and recent clinical symptoms of respiratory infection (i.e., cough, fever, wheezing, expectoration, anhelation, etc); (iii) without history of other system infection. Children with premature birth (< 37 weeks), underlying chronic diseases (e.g., chronic lung disease, congenital heart disease, or immunodeficiency), immune disease, asthma, or combined with other infection were excluded. When eligible children visited the hospital, a nasopharyngeal swab and blood were taken within 24 h of enrollment and tested for the presence of RSV and levels of cytokines. All patients have not treated.

The demographic and clinical information were also obtained by recording the clinical information of child everyday by asking the parents, filling out a form, physical examinations and a blood test. Clinical characteristics such as, fever, wheezing, tachypnea, respiratory frequency, heart rate, presence of retractions, oxygen saturation, need for oxygen, need intensive care, length of hospitalization, radiological finding and clinical diagnosis were individually assessed to describe disease severity using disease severity score (Table 1) [22–24].

RSV infection was confirmed from nasopharyngeal swabs using reverse transcription polymerase chain reaction (RT-PCR) as described previously [25]. Viral load was measured using real-time fluorescent quantitative PCR, according to a standardized protocol [26]. Respiratory virus (including adenovirus, influenza, parainfluenza, coronavirus, bocavirus and human metapneumovirus) detection and blood and sputum culture (only if indicated) were performed to exclude other viral and bacterial infections.

This study was approved by the Hospital Ethics Committee of The First People's Hospital of ChenZhou, China. Written informed consent was obtained from the parents or guardians of all participants.

Table 1
Disease severity score.

	Score			
	0	1	2	3
Respiratory frequency	Normal	N/A	Bradypnea/ tachypnea*	N/A
O2 saturation (%)	> 95	90–95	80–90	< 90
Presence of retractions	No	Present	Present + nasal flare	N/A
O2 supplementary (days)	0	1–2	3–4	> 5
Wheezing duration (days)	0	1–3	4–7	> 7
Heart rate	Normal	N/A	Bradycardia or tachycardia*	N/A
Radiological findings and clinical diagnosis	Normal	URTI	Bronchiolitis	Pneumonia
Length of fever (days)	0	1–2	2–3	> 4
Need intensive care	No	N/A	Yes	N/A
Length of hospitalization (days)	0	1–3	4–10	> 10

Note. Score value and classification of severity: lower than or equal 5: mild; from 6 to 9: moderate; and from 10 to 15: severe.
* Based on Pediatric Advanced Life Support (PALS) guidelines (2015).

2.2. Serum cytokine measurements

Serum samples were measured for the cytokine IL-35, IL-10 and TGF-β1. The concentrations of serum IL-35 and TGF-β1 were measured by enzyme-linked immunosorbent assay (ELISA) using the Human/Mouse TGF-β1 ELISA Ready-SET-Go (eBioscience, San Diego, CA, USA), and the Human Interleukin 35 (IL-35) ELISA Kit (CUSABIO, Wuhan, China). IL-10 was measured using the Bio-Plex Pro Assay Quick Guide 4 (Bio-Rad, Hercules, CA, USA). The lower limit of detection for all cytokines was 1 pg/ml.

2.3. Statistical analysis

The RSV-infected patients were divided into three groups as follows: 0 < age < 6 months, 6 ≤ age ≤ 12 months and 12 < age < 24 months as a means of assessing the role of age on disease severity, progression, and cytokine profiles.

For descriptive analysis, patient demographic and clinical characteristics were summarized as frequencies and percentages. Continuous variables were summarized as medians with interquartile ranges (IQRs). Data from different groups (RSV-infected patients, controls, and different age groups) were compared using χ^2 or Fisher's exact test for categorical variables or Mann–Whitney U test for continuous variables. Spearman's rank correlation coefficient was used for correlation analyses because most of data did not obey a normal distribution. $P < .05$ was considered to be statistically significant for all outcomes and relationships between different groups. All statistical analyses were performed using SPSS version 19.0 and graphs were generated using GraphPad version 6.0.

3. Results

3.1. Characteristics of study participants

From January to December in 2014, 325 patients with acute respiratory tract infection and 20 healthy controls were enrolled. And a total of 89 met the inclusion criteria, 62 (69.7%) male, and the average age (IQR) was 11 (7–18) months. Of the 20 healthy controls, there were 16 (80%) males, and the average age (IQR) was 12 (8–20) months (Table 2). RSV-infected children and healthy control were divided into 3 groups respectively as follows: 0 < age ≤ 6 months (43, 48.3% vs 2, 10%; $P = .002$), 6 < age ≤ 12 months (33, 37.1% vs 11, 55%; $P > .5$) and 12 < age < 24 months (13, 14.6% vs 7, 35%; $P > .5$). It was unbalanced for age group 0 < age ≤ 6 months. But there were no significant differences in age, sex and feeding patterns between the two groups. Children with RSV infection had a significantly higher percentage of lymphocytes and monocytes and lower percentage of neutrophils and eosinophils (Table 2).

The demographic data, laboratory, clinical characteristics, radiological findings and clinical diagnosis are summarized in Table 3. And the disease severity parameters were also compared in the three groups. In total of 89 cases, 43 (48.3%) was 0 < age ≤ 6 months old, 33 (37.1%) was 6 < age ≤ 12 and 13 (14.6%) was 12 < age < 24 months. Compared with children 0 < age ≤ 6 months old, Children 6 < age ≤ 12 months and 12 < age ≤ 24 months old were significantly more likely to have fever (P ≤ .001) and higher body temperature (P ≤ .0001) (Table 3). Children 0 < age ≤ 6 months and 6 < age

Table 2
Demographic and clinical characteristics of RSV-infected patients and healthy controls.

Variable	RSV-infected patients (n = 89)	Healthy controls (n = 20)	*P*
Demographics			
Age, months	11 (7–18)	12 (12–20)	0.082
0 < age ≤6 months, No. (%)	43 (48.3)	2 (10)	0.002
6 < age ≤12 months, No. (%)	33 (37.1)	11 (55)	0.207
12 < age < 24, No. (%)	13 (14.6)	7 (35)	0.052
Sex, No. (%) male	62 (69.7)	16 (80)	0.423
Breastfeeding, No. (%)	64 (71.9)	16 (80)	0.582
Laboratory characteristics			
WBCs/ul	8600 (6630–13,925)	8410 (7508–9650)	0.525
Neutrophils, %	35 (25–48.5)	44 (37.3–55)	0.015
Lymphocytes, %	53 (40.5–66)	45 (32.3 – 51.3)	0.018
Eosinophils, %	0.4 (0.2–0.8)	2.5 (1–4.3)	< 0.001
Monocytes, %	8.7 (7.9–11.3)	6.1 (3.7 – 10.1)	0.007

Note. Data are median values (IQRs [25th–75th percentile]), unless otherwise specified. $P \leq .05$ is considered to be significant.

≤12 months had more prolonged duration of symptoms ($P = .005$ and $P = .027$, respectively) and length of hospitalization ($P = .001$ and $P = .005$, respectively) than children 12 < age ≤24 months. In addition, children 0 < age ≤6 months were more likely to need supplemental oxygen than children 12 < age ≤24 months ($P = .003$), although the O2 saturation was no significant difference in three age group. Disease severity was also evaluated by disease severity score (Table 3). Compared with older children, more children 0 < age ≤6 months were classified as having severe disease. There was no significant difference in the three groups of symptoms such as cough, wheezing, sputum production, respiratory frequency, heart rate, tachypnea, need to the intensive care, requiring mechanical ventilation and clinical diagnosis. RSV loads, white blood cell count and the percentages of neutrophils, lymphocytes, eosinophils and monocytes did not differ significantly among the three groups.

3.2. RSV infection is associated with increased IL-10 and IL-35 expression

To understand the regulatory cytokines response in RSV disease, we examined the expression profiles of various regulatory cytokines including IL-35, IL-10 and TGF-β1 in RSV-infected patients and healthy controls (Fig. 1). The levels of IL-35 ($P = .0001$) and IL-10 ($P = .006$) (Fig. 1A,B) were significantly increased in RSV-infected patients compared to the levels in healthy controls. No significant difference was found in the levels of TGF-β1 between the two groups (Fig. 1C).

3.3. IL-10. And IL-35 were elevated in RSV-infected older children

To explain why more young children with RSV infection have severe disease, it was important to assess the cytokine profiles in relation to age. We compared the levels of IL-10, IL-35 and TGF-β1 in patients in different age groups (Table 3). Our data show that the expression levels of IL-10 and IL-35 were significantly lower in children 0 < age ≤6 months than 12 < age < 24 months ($P = .043$ and $P = .029$, respectively). No changes in the levels of serum TGF-β1 were observed in any group. To exclude the influence of increasing age on the level of cytokines, we analyzed the correlation between age and cytokine levels in RSV-infected patients and healthy controls (data not shown). These results did not show any correlation between age and the levels of IL-10, IL-35 or TGF-β1 in the two groups.

3.4. The level of IL-10 correlated with disease severity and duration of symptoms

The impact that cytokine levels have on the severity of disease were investigated (Fig. 2). Comparing with mild group, the expression levels of IL-10 were significantly lower in children with moderate and severe disease ($P = .012$ and $P = .005$, respectively). We also investigated the correlation between the level of the cytokines (IL-10, IL-35 and TGF-β1) with the total duration of symptoms. We found that the concentrations of IL-10 was inversely correlated with the total duration of symptoms ($r = -0.311$, $P = .091$) (Table 4). Similar differences and associations for the levels of IL-35 and TGF-β1 were not observed. To evaluate the relation between viral infection and the cytokine responses, we also analyzed correlations among the RSV load, cytokine concentrations and WBCs, but we have not found any correlation among these parameters.

4. Discussion

This study indicated that IL-10 and IL-35 elicited by RSV infection stimulated regulate T cell immune responses in young children. IL-10, IL-35 and TGF-β were generated in periphery from regulate T cells and mediated immunosuppression. IL-35 is produced by $CD4^+FoxP3^+$ regulatory T cells and IL-10 and TGF-β are mainly generated in the periphery from conventional $CD4^+FoxP3^-$ induced Treg cells [19,27]. Previous studies have demonstrated how immunosuppressive cytokines such as IL-10 and IL-35 inhibit acute inflammation and regulate the balance between Th1 and Th2 responses [8,18,19,27,28]. Induced IL-10 and IL-35 expression could benefit the host response to limit the exaggerated inflammatory response to RSV infection, such as Th1/Th2 cytokine responses. Although TGF-β1 possesses anti-inflammatory properties with the potential of inhibiting both Th1 and Th2 responses elicited in response to bacterial and viral infection [29–31], we did not find that TGF-β1 expression profiles differed between RSV-infected patients and healthy controls.

IL-10 is a multifunctional cytokine. Some studies have demonstrated that IL-10 produced by effector T cells and monocytes played a pivotal role in controlling disease severity by suppressing the production of Th1 cytokines (IFN-γ and IL-12) and pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) [18,32]. In addition, IL-10 also can suppress Th2 cell generation and further suppress airway hyper-responsiveness and tissue remodeling in RSV-infected patients [19]. But IL-4, IL-5, IL-13 and IL-10 also were excess produced by type 2 T cells, which can enhance the RSV disease [7,33]. In this study, we found the expression levels of IL-10 were significantly lower in children with moderate and severe disease and inversely associated with total duration of symptoms. These data indicate that IL-10 was plays a protective role. Lower level of IL-10 was associated with more-severe RSV disease. IL-10 producing by $CD4^+FoxP3^+$ and $CD4^+FoxP3^-$ T cell were plays a critical role in regulating the immune response in RSV infection by restricting T-cell-mediated pulmonary inflammation and injury [34]. IL-35 has been shown to have potential suppressive activities through the expansion of Treg cells and IL-10 production to inhibit the CD4+ effector cells inducing Th1 and Th17 cells [27]. Although our results demonstrated increased expression of IL-35 in RSV-infected pediatric patients, expression levels did not correlate with the severity of disease. However, we cannot exclude the possibility that the enhanced levels of IL-35 by affecting the IL-10 expression and indirectly contribute to amelioration of disease in RSV-infected patients. Novel mechanisms indicated that B lymphocytes exert a suppressive function in viral infection in an IL-10-independent manner through production of IL-35 [35]. IL-10, IL-35 and from inducible T regulatory cells exhibits the potential suppressive function and plays a critical role maintaining immune balance [20]. Future studies will be required to evaluate the correlation between IL-10 and IL-35 in RSV disease.

It has been demonstrated that older age is inversely associated with susceptibility to RSV infection, in addition to decreasing the risk of

Table 3
Demographic and clinical characteristics of RSV-infected patients.

Variable	RSV-infected patients			
	0 < age ≤6 months (n = 43)	6 < age ≤12 months (n = 33)	12 < age < 24 months (n = 13)	*P* value
Demographic				
Age, months	3 (2–5)	9 (8–11.5)	20 (14–22)	
Male, No. (%)	35 (81.40)	26 (78.79)	10 (76.92)	NS
Clinical Characteristics				
Fever, any, No. (%)	10 (23.26)	26 (78.79)	9 (69.23)	≤.001 #,*
Body temperature, mean (SD),°C	36.9 (0.5)	38.9 (0.4)	39.0 (0.5)	≤.0001 #,*
Cough, No. (%)	43 (100)	33 (100)	13 (100)	NS
Respiratory frequency, mean (SD), respirations/min	41.4 (12.3)	38.7 (10.5)	36.8 (9.8)	NS
Heart rate, mean (SD), beats/min	152.4 (24.1)	148.6 (21.5)	140.3 (19.7)	NS
Wheezing, No. (%)	30 (69.77)	22 (66.67)	8 (61.53)	NS
Wheezing duration [a] (days)	4 (2–6)	3 (2–5)	3 (1–5)	0.04 #
Sputum production, No. (%)	34 (79.07)	25 (75.76)	8 (61.5)	NS
Tachypnea, No. (%)	12 (27.91%)	6 (18.18%)	1 (7.69)	NS
Radiological finding and clinical diagnosis, No. (%)				
URTI	2 (4.65%)	4 (12.12%)	5 (38.46%)	NS
Bronchiolitis	24 (55.81%)	18 (54.54%)	5 (38.46%)	NS
Pneumonia	17 (39.53%)	11 (33.33%)	3 (23.08%)	NS
Total duration of symptoms (days)	13 (11–20)	12 (10–18)	10 (9–12)	0.005 *, 0.027 ±
Length of hospitalization (days)	8 (7–9)	7 (6–8)	6 (4–7)	0.001 *, 0.005 ±
O2 saturation, mean (SD), %	93.6 (8.7)	94.1 (8.5)	97.6 (7.9)	NS
Need for oxygen, No. (%)	31 (72.09)	18 (54.55)	3 (23.08)	0.003 *
Total duration of O2 (days)	4.1 (2.2–6.5)	3.8 (1.2–6.0)	1.5 (0.5–3.0)	NS
Need to the intensive care, No. (%)	7 (16.2)	4 (12.1)	0	NS
Requiring mechanical ventilation, No. (%)	3 (6.9)	1 (3)	0	NS
Disease severity score, No. (%)				
Mild (0–5)	13 (30.23)	16 (48.48)	9 (69.23)	0.021 *
Moderate (6–9)	22 (51.16)	14 (42.42)	3 (23.08)	NS
Severe (10–15)	8 (16.28)	3 (9.1)	1 (7.69)	NS
Laboratory characteristics				
WBCs/ul	8280 (6540–11,800)	8740 (7065–13,345)	8310 (6570–10,820)	NS
Neutrophils, %	27 (18–40)	38 (28–55)	39 (31–60)	NS
Lymphocytes, %	62 (47–67)	51 (36.5–64)	50 (27–59)	NS
Eosinophils,%	0.5 (0.2–1.2)	0.2 (0.1–0.9)	0.3 (0.2–0.8)	NS
Monocytes, %	9.2 (6.9–13.6)	8.2 (7.2–9.7)	8.7 (7.3–11.5)	NS
RSV load, log10 copies/ml	1.14×10^4 (1.14×10^3 –1.06×10^5)	2.21×10^4 (7.6×10^3 –5.87×10^4)	2.17×10^4 (3.88×10^3 –1.04×10^4)	NS
Cytokines concentrations, pg/ml				
IL-35	40.56 (23.83–59.67)	56.93 (23.78–115.63)	65.23 (27.83–133.20)	0.043 *
IL-10	8.34 (5.32–10.94)	9.56 (3.87–12.80)	11.13 (7.84–22.26)	0.029 *
TGF-β1	1066 (464–1581)	931 (357–1560)	1413 (1070–1701)	NS

Note. Data are median values (IQRs [25th–75th percentile]), unless otherwise specified. $P \leq .05$ is considered to be significant.
WBC, white blood cell count.
[#] Defined as age groups 0 < age ≤6 months versus 6 < age ≤12 months.
[*] Defined as age groups 0 < age ≤6 months versus 12 < age < 24 months.
[±] Defined as age groups 6 < age ≤12 months versus 12 < age < 24 months.
[a] Only for patients with confirmed wheezing.

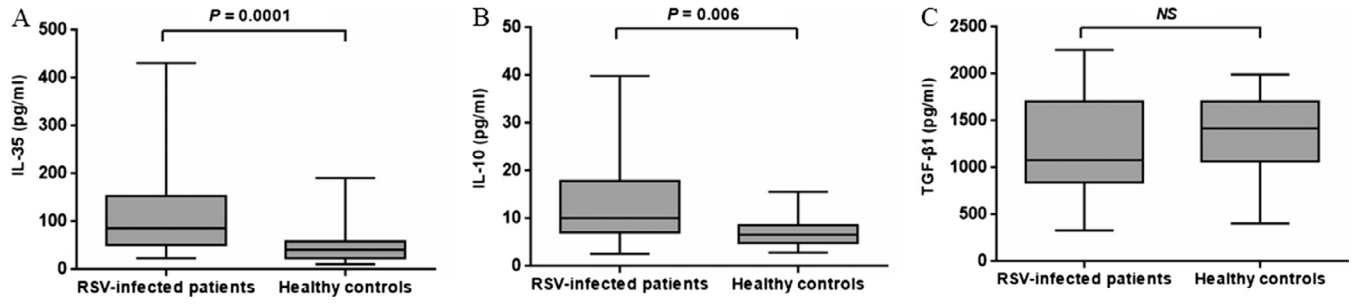

Fig. 1. Regulatory cytokine concentrations in serum. Levels of IL-10, IL-35 and TGF-β1 were determined in serum of RSV-infected patients ($n = 89$) and Healthy controls ($n = 20$). Mann–Whitney *U* tests were used to determine differences between the two groups. $P \leq .05$ was considered to be significant. NS, not significant.

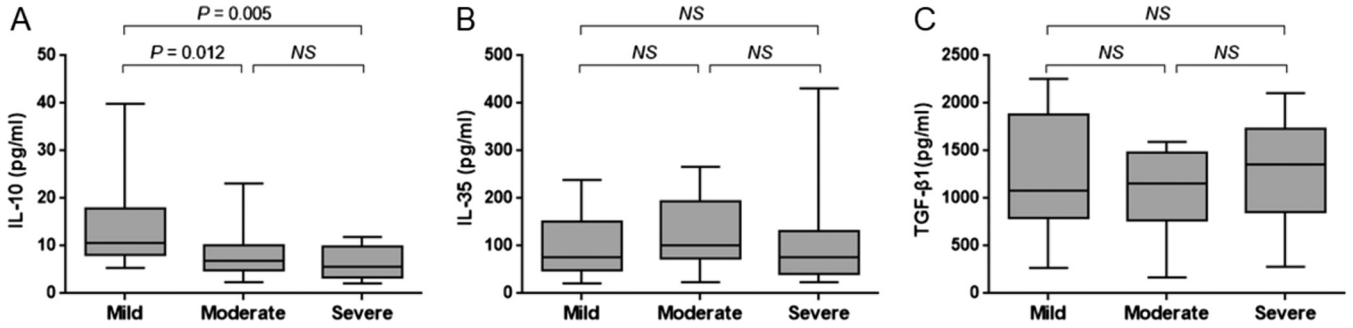

Fig. 2. The impact that cytokine levels on the severity of disease. The levels of IL-10, IL-35 and TGF-β1 in the different disease severity groups: Mild (n = 38), Moderate (n = 39) and Severe (n = 12)) were analyzed. Mann–Whitney U tests were used to determine differences among the three groups. P ≤ .05 was considered to be significant. NS, not significant.

Table 4
Correlation between inhibitory cytokine concentrations (pg/ml) and the total duration of symptoms (days) in RSV-infected patients.

Cytokine	Correlation coefficient	*P* value
IL-10	−0.311	0.019
IL-35	0.046	0.723
TGF-β1	0.192	0.137

Note. $P \leq .05$ is considered to be significant.

hospitalization and disease severity [5,36]. It is interestingly that the children less than 6months had significantly lower IL-10 and IL-35 compared to children 12 < age < 24 months old, but a correlation between age and the regulatory cytokines was not observed. As we known, the infant less than 6months has an immature immune system, including innate immune response and adaptive response, but due to the residual maternal antibody that may prevent induction of a good immune response [1,37]. Other study had revealed that the cytokines/chemokines including IL-1β, IL-2, IL-6, IFN-γ, TNF-α were not significantly different between the age group in children with RSV infection [38]. The results of the present study have shown that the levels of IL-10 and IL-35 were significant lower in children 0 < age ≤6 months who were more likely to need supplemental oxygen. These data indicated that IL-10 and IL-35 expression is RSV specific, and play a protective role in RSV disease. In accordance with other investigators, the study has shown that IL-10 was increased in response to RSV infection, and lower concentration of IL-10 was associated with more severe RSV disease [16,38]. We had not found any study about IL-35 in RSV disease. Others have demonstrated that IL-10 inhibits viral replication [16], but we had not found any relationships between IL-10 and RSV load. Disease presentation has for a long time been used as an indicator of the severity of disease resulting from RSV infection. Our results suggested that children 0 < age ≤6 months were significantly more likely to present with a longer total duration of symptoms. This observation was in agreement with previous studies demonstrating that younger children are at a greater risk of infection and more likely to develop severe disease symptoms following infection with RSV [39,40]. Serum IL-10 level was inversely associated with total duration of symptoms. It also indicated that high IL-10 level was associated with a favorable outcome and contributed to the recovery and attenuation of disease progression in RSV-infected children.

Our study had some limitations. First, compared to serum samples, respiratory secretions are a more distant source for studies on cytokines in children with RSV infection. Second, we measured regulatory cytokines only at a single time point from each child, so it is not clear whether they persisted or were attenuated with time. Third, we only had a small number of children over a wide age range, and we may have included primary and reinfection with RSV. Therefore, according to the present data, we can't evaluate the significance of therapeutic of each cytokine.

In summary, we showed that RSV infection is associated with altered levels of regulatory cytokines IL-10 and IL-35. The elevated serum regulatory cytokine IL-10 and IL-35 concentrations may be protective hypoxia and contributed to reduce the severity of disease. Future studies will be required for the characterization of the regulatory cytokine profiles associated with RSV infection, and to elucidate the role of regulatory cytokines in the RSV disease.

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