Cytokine 103 (2018) 57–62

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

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

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ARTICLE INFO

Keywords:
Respiratory syncytial virus
Regulatory cytokines
Interleukin-35
Interleukin-10

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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https://doi.org/10.1016/j.cyto.2017.12.028
Received 30 August 2017; Received in revised form 25 December 2017; Accepted 27 December 2017

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