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The expression of a novel anti-inflammatory cytokine IL-35 and its possible significance in childhood asthma

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ABSTRACT

Interleukin-35 (IL-35) is a novel anti-inflammatory cytokine and has been shown to play an important role in maintaining immune homeostasis. However, the effect of IL-35 on human asthma remains unclear. The present study is to investigate the expression and significance of IL-35 in childhood asthma. Forty-one asthmatic children and forty-two healthy controls were recruited in Qilu Children's Hospital of Shandong University. Serum total immunoglobulin E level was measured by radioimmunosorbent test. Peripheral blood eosinophils were counted using BC-5800 Automatic Blood Cell Analyzer. IL-35 mRNA in peripheral blood mononuclear cells was detected by quantitative real-time polymerase chain reaction. Serum IL-35, IL-4 and interferon-γ levels were measured using enzyme-linked immunosorbent assay. The correlations among the above indexes were also analyzed using Pearson's method. Our results showed that serum total IgE, eosinophil count and serum IL-4 were significantly increased in asthmatic children compared with control children, and serum IFN-γ level in asthmatic patients was obviously lower than that in healthy controls. We also found that there was an obviously positive correlation between serum IgE and IL-4 levels in asthmatic patients. In addition, significantly negative correlation was found between serum total IgE and IFN-γ levels. More importantly, we found that the expression of IL-35 mRNA and protein was both down-regulated in asthmatic children, and serum IL-35 level was inversely related to serum IL-4 level. Moreover, significantly positive correlation was also found between serum IL-35 and IFN-γ levels. The results suggest that the decreased expression of IL-35 could be involved in the pathogenesis of childhood asthma.

1. Introduction

Childhood asthma is a chronic inflammatory disease of the airways in children and has become increasingly prevalent in the world [1]. About one-third to half of cases of moderate to severe childhood asthma may persist to adulthood [2]. Therefore, childhood asthma becomes a serious global health problem that is of great concern among medical and health professionals. Childhood asthma is induced by bronchial hyper-responsiveness to specific allergen [3,4], and it is mainly characterized by the infiltration of mast cells, basophils, eosinophils, mononuclear cells and the increase of serum total immunoglobulin E (IgE) [5,6]. In the pathogenesis of childhood asthma, CD4+ Th2 cells play an important role both in the initiation and challenge phases of asthma by producing IL-4 and IL-13 which are the critical stimuli inducing the production of IgE, as well as producing IL-5 which regulates the growth and differentiation of mast cells and eosinophils. Apart from Th2 cells, other CD4+ Th effector subsets, such as Th1, Th17 and CD4+CD25+Foxp3+ regulatory T (Treg) cells also participate in disease pathogenesis [7–10].

Abbreviations: BALF, bronchial alveolar lavage fluid; EBI3, Epstein–Barr virus induced 3; ECP, eosinophil cationic protein; EO, eosinophils; ICOS, inducible costimulator; IFN-γ, interferon-γ; IgE, immunoglobulin E; IL-4, interleukin-4; IL-35, interleukin-35; PBMCs, peripheral blood mononuclear cells; qRT-PCR, quantitative real-time polymerase chain reaction; Treg, regulatory T.

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Interleukin-35 (IL-35), which acts as a novel anti-inflammatory cytokine, is a member of the interleukin-12 (IL-12) cytokine family [11,12]. The IL-12 cytokine family contains IL-12, IL-23, IL-27 and IL-35, and they are all composed of α chain (P19, P28 or P35) and β chain (P40 or Epstein–Barr virus induced 3, EBI3) [13]. IL-35 is a dimeric protein with two subunits, P35 and EBI3 [14]. The structure of IL-35 is homology to that of IL-12 because they have same α chain P35, and also similar to that of IL-27 due to their same β chain EBI3 [15,16]. However, unlike the other IL-12 family members, IL-35 is highly expressed on non-stimulated mouse Treg cells and stimulated human Treg cells but not detected in non-stimulated human Treg cells [11,17]. Recently, Guttek and Reinhold reported that the production of IL-35 is strongly increased in cell culture supernatants of stimulated human peripheral pan T cells or CD4+, CD8+ and CD4+CD25− T subpopulations. More interestingly, high concentrations of IL-35 could be measured both in cell culture supernatants of resting and stimulated CD4+CD25+ T cells [18]. Moreover, IL-35 can be also upregulated in human non-T cells including microvascular endothelial cells, aortic smooth muscle cells, and epithelial cells by the stimulations with tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) and IL-1β [19]. It has been reported that IL-35 promoted the proliferation of CD4+CD25+ T cells, inhibited the proliferation of CD4+CD25− T cells and the differentiation of Th17 cells in vitro. In vivo, IL-35 suppressed established collagen-induced arthritis in mice by decreasing IL-17 production and enhancing IFN-γ synthesis [20,21]. In addition, IL-35 effectively attenuated dust mite allergen-specific murine CD4+ memory/effector Th2 cell-mediated airway inflammation and IL-35 production by inducible costimulator (ICOS)-positive Treg cells reversed established IL-17-dependent allergic airways disease in mice [22,23]. However, the expression and significance of IL-35 in human asthma patients remain unclear.

In the present study, we measured the expression of IL-35 mRNA in PBMC and IL-35 protein in serum from asthmatic children and healthy controls, and explore the significance of IL-35 expression in childhood asthma. We found that the expression of IL-35 mRNA and protein was decreased in asthmatic patients compared with normal controls, and serum IL-35 levels were negatively related to serum IL-4 levels in the children with asthma. Moreover, significantly positive correlation was also found between serum IL-35 and IFN-γ levels. These results suggested that IL-35 could be involved in the pathogenesis of childhood asthma.

2. Materials and methods

2.1. Subject selection and recruitment

Forty-one asthmatic subjects were recruited from the children who visited a doctor or were hospitalized in Qilu Children's Hospital of Shandong University (Jinan, China) between March 2011 and November 2013. They had no use of oral or inhaled corticosteroids within 2 month before blood sampling. Among these patients, 31 cases were mild persistent, 6 cases were moderate persistent, and 4 cases were severe persistent. The diagnosis of asthma and classification of asthma severity were made according to the modified criteria for childhood asthma established by Subspecialty Group of Respiratory Diseases Society of Pediatrics, Chinese Medical Association and Chinese Journal of Pediatrics Editorial Board [24]. Forty-two age and gender-matched normal controls were recruited from the children undergone physical examination in Children Health & Care Center of Qilu Children's Hospital of Shandong University between March 2011 and November 2013. None of them had previously suffered from allergic diseases or experienced asthma-like syndromes, and they had also no any respiratory diseases. The study protocol was approved by the ethical committee affiliated to Qilu Children's Hospital of Shandong University, and informed consent was obtained from all parents of qualified study subjects. All clinical characteristics of asthmatic children and normal controls are shown in Table 1.

Table 1
Characteristics of the studied subjects.

Characteristics	Asthma patients ($n=41$)	Healthy controls ($n=42$)
Female (%)	24%	33%
Male (%)	76%	67%
Age (years)	4.35 ± 0.35	3.75 ± 0.21
Serum total IgE (IU/ml)	443.2 ± 62.91	19.52 ± 0.61
Blood eosinophils (10^9/L)	0.31 ± 0.04	0.17 ± 0.01
WBC[a] (10^9/L)	9.18 ± 0.41	7.49 ± 0.3
Basophils (10^9/L)	0.026 ± 0.006	0.03 ± 0.003
CRP[b] (mg/L)	9.12 ± 1.18	3.73 ± 0.19
FEV1[c] (% predicted)	76.53 ± 15.52	
Family history of asthma/atopy (%)	33%	
Eczema/rhinitis history (%)	40%	

Data shown as mean ± SEM.
[a] White blood cells.
[b] C-reactive protein.
[c] Forced expiratory volume in 1 s.

2.2. Detection of serum total IgE level and eosinophil count

Serum total IgE levels were measured by radioimmunosorbent test using fully automatic specific protein analyzer (BNP, Marburg, Germany). Eosinophils (EO) were counted using BC-5800 Automatic Blood Cell Analyzer (Mairui, Shenzhen, China).

2.3. RNA extraction and quantitative real-time PCR

Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation from peripheral blood of asthmatic patients and healthy controls. Total mRNA was isolated using a modified TRIzol one step extraction method. cDNA was synthesized using the Rever Tra Ace qPCR RT Kit (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) for P35, EBI3, P28, P40 and GAPDH was respectively performed in 20 μL volume containing 0.2 μL of cDNA, 10 μL of UltraSYBR Mixture (CWBIO, Beijing, China) and 1 μL of each primer (Table 2) according to the following programs: the samples were denatured at 95 °C for 10 min, and then followed by 39 cycles of 95 °C for 15 s, 60 °C for 1 min and 65 °C for 5 s to stop the reaction. Each sample was conducted in triplicate. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method. All samples were normalized to GAPDH, which was served as an endogenous control.

Table 2
Primers sequence for quantitative real-time PCR.

Primer name	Accession no.[a]	Primer sequence (5′–3′)
EBI3	NM_005755.2	TCCCAGAGATCTTCTCACTGAAGTA
		GCACAGCCCTGAGGATGAA
P35	NM_000882.3	AGGAATGTTCCCATGCCTTCA
		CCAATGGTAAACAGGCCTCCAC
P28	NM_145659.3	ACCGCTTTGCGGAATCTCA
		AGGTCAGGGAAACATCAGGGA
P40	NM_002187.2	TGTCACCAGCAGTTGGTCATCTC
		CTCACTGCTCTGGTCCAAGGTC
GADPH	NM_001256799.1	AACGGATTTGGTCGTATTGGG
		CCTGGAAGATGGTGATGGGAT

[a] Accession numbers are for *Homo sapiens*.