



Interleukin-33 promotes helper T cell type-2/17 inflammation in children with allergic rhinitis

Yuanming Wang¹ · Chuling Li¹ · Xianglin Luo¹ · Deyu Xu¹ · Yaxiong Xu¹ · Guanquan Li¹ · Fang Liao¹ · Shujuan Tan¹

Received: 3 October 2017 / Accepted: 7 November 2017
© Springer-Verlag GmbH Germany, part of Springer Nature 2017

Abstract

Objectives Interleukin-33 is a recently described cytokine which involved in helper T cell type-2 (Th2) mediated diseases. However, its regulatory effect in the pathogenesis of children allergic rhinitis (AR) needs to be further characterized. This study aims to evaluate the expression and role of IL-33 in children with AR.

Methods Forty children with AR and twenty normal controls were included. IL-33 and Th cytokine production in serum and nasal lavage was examined by immunosorbent assay (ELISA), respectively. Peripheral blood mononuclear cells (PBMCs) were purified for in vitro regulation experiment of IL-33.

Results The IL-33 protein levels in both serum and nasal lavage were significantly enhanced in AR compared with normal controls, especially in children with asthma. The IL-33 protein expression was correlated to severity of disease. In vitro study showed that IL-33 promotes Th2/17 cytokine expression and thus amplified Th2/17 inflammation.

Conclusions Our results demonstrate that IL-33 expression in AR aggravated and amplified Th2/17 inflammation, and provide a possible explanation for IL-33's regulatory role in the pathogenesis of AR.

Keywords Interleukin-33 · Allergic rhinitis · Children

Introduction

Allergic rhinitis (AR) is a common allergic disease in ENT and affects 10–40% of children with increasing prevalence in China mainland [1, 2]. The histological characteristics of AR include enhanced helper T cell type-2 (Th2) inflammation, infiltration with eosinophils, allergen-specific IgE production and thickening of the nasal basement membrane [3, 4]. Activation of Th2 cells and further released cytokines induced accumulation of various immune cells, which contributed to the occurrence of AR [5]. Recent studies also suggested that Th17 cells may enhance Th2 cell-mediated eosinophilic airway inflammation in mouse models of asthma [6, 7]. Besides, Serum IL-17 levels are increased in AR patients and are associated with clinical symptoms, peripheral eosinophil counts, and the need for medication [8,

9]. Despite involvement of Th2/17 inflammation in AR was clarified, its regulation was not fully understood.

Interleukin (IL)-33 is a member of the IL-1 superfamily and identified as a ligand for the orphan IL-1 family receptor ST2 [10]. Interaction between IL-33 and its receptor ST2 promotes systemic Th2 response of different cell types such as mast cells, Th2 lymphocytes, eosinophils, basophils, and structural cells such as airway epithelium [10, 11]. In AR, IL-33 has been reported to correlate with disease severity [12, 13]. In an experimental mouse model of allergic rhinitis, allergen exposure induce IL-33 production from nasal epithelial cells, and is essential for the accumulation of eosinophils and basophils in the nasal mucosa by increasing histamine release from the mast cells and inducing production of chemoattractants from the basophils [14].

Increasing evidences suggest that IL-33 may play positive roles in Th2/17-linked AR. In this study, we aimed to explore the expression and role of IL-33 in Th2/17 inflammation in children AR.

✉ Chuling Li
chulinglidg@163.com

¹ Department of Otolaryngology, Dongguan Women and Children's hospital, No 99, Zhenxing Road, Dongguan 523120, China

Methods

Patients

Forty children (< 18 years old) with perennial AR were enrolled in this study. The diagnosis of AR and asthma was made according to disease history, physical examination, skin prick test, and specific IgE measurement as described in Allergic Rhinitis and its Impact on Asthma (ARIA) guideline (2010) and the global initiative for asthma (GINA) criteria [15, 16]. Twenty healthy control children with comparable age and gender were recruited as the control group. Those with chronic diseases (e.g., malnutrition, anatomic malformation of the respiratory system, chronic lung disease, heart disease, gastro-oesophageal reflux disease, cystic fibrosis) and those with drug use history within 1 month (e.g., oral or nasal corticosteroids, antiepileptics, immune suppressives) were excluded from our study. This study was approved by the Dongguan Women and Children's hospital Ethical Committee and informed consent was signed by the children's parents.

Blood and lavage sample preparation and analysis

Venous blood samples were collected into vacuette tubes and centrifuged at 3000g for 15 min at 4 °C. The whole blood cell counts were performed using LH-785 system (Beckman Coulter, Galway, Ireland). Total serum IgE was measured by electrochemiluminescence (ECLIA) method using an ELX-800 system.

Nasal lavage was performed using saline warmed to 37 °C. The process was performed as previously described [17]. The samples were centrifuged to remove cellular debris and aliquots of the supernatants were stored at −20 °C in eppendorff tubes for analysis. Total protein concentrations were determined with Bio-Rad protein assays according to Bradford.

Enzyme-linked immunosorbent assay (ELISA) for protein expression and ECLIA for ECP protein expression

Enzyme-linked immunosorbent assay (ELISA) kits were used for measuring serum and nasal lavage IL-33, IL-4, IL-5, IL-17 and ECP levels (R&D system), according to the manufacturer's protocols. The detection limits of the assays were as follows: IL-33, 125 pg/ml, IL-4, 1.56 pg/ml, IL-5, 7.8 pg/ml, IL-17, 5 pg/ml.

Peripheral blood mononuclear cell (PBMCs) preparation

PBMCs from AR and control children were isolated by Lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway) density-gradient centrifugation from heparinized leucocyte-enriched buffy coats. PBMCs were then cultured at 2×10^6 /ml in 24-well plates in culture medium: RPMI 1640 supplemented with 5% human AB serum, 5 mmol/l of glutamine, and penicillin and streptomycin solution. Stimulation was given through addition of rhIL-33, HDM with or without other cytokines or stimulators.

Statistical analysis

All data were expressed as mean $\pm$ SD except additional note. Analysis of variance was performed and ad hoc post test was done for multiple comparisons. The Spearman's rank correlation test was used to analyze the correlation among the expression of biomarkers. $P < 0.05$ was considered as significant difference.

Results

Demographic and laboratory characteristics of the study population

This study was conducted with 60 children, 40 of whom suffered from AR, with ages ranging between 60 and 144 months (mean age 98.7 ± 39.0 months, 22 males), and 20 of whom were healthy controls with ages ranging between 60 and 144 months (mean age 95.0 ± 37.1 months, 11 males). The demographic features and laboratory parameters of the population are summarized in Table 1. The basophil count, the eosinophil count, total serum IgE, and eosinophil cationic protein (ECP) levels were significantly higher in the AR group compared with control group, especially in children with asthma.

Increased serum and local IL-33 protein levels as well as Th2/17 cytokine protein in AR

The serum and local IL-33 protein expression in AR group were significantly higher than those in the control group, especially in those children with asthma (Table 2). Our results showed that enhanced protein expression of IL-4, IL-5, IL-17 in both serum and nasal lavage of AR children compared with control (Table 2), especially in children with asthma. Serum and nasal IL-4, IL-5, IL-17 were positively correlated with serum and nasal IL-33 expression,

Table 1 Demographic characteristic of AR children and normal controls

Groups	AR group	AR with asthma group	Control
Number	40	10	20
Sex (male:female)	22:18	6:8	12:8
Age (months)	98.7 ± 39.0	94.3 ± 38.3	95.0 ± 37.1
History of asthma, <i>n</i> (%)	–	10 (100)	–
Eosinophil ^a (count/mm ³)	155 (510–1560)*	191 (112–1750)*, **	47 (5–221)
Neutrophil ^a (count/mm ³)	7300 (2300–16,600)	12,100 (7100–18,800)*, **	7600 (3100–14,900)
Basophil ^a (count/mm ³)	43 (10–120)*	31 (5–110)*	8 (3–12)
Monocytes ^a (count/mm ³)	244 (70–2710)	257 (80–2610)	249 (80–2370)
Lymphocyte ^a (count/mm ³)	6600 (3000–14,800)	7500 (2900–16,500)	8000 (2800–14,400)
ECP ^a (ng/ml)	39.8 (4.5–178.0)*	55.6 (24.0–155.0)*, **	12.1 (3.9–101.0)
IgE ^a (IU/ml)	89.5 (2.5–1675.0)*	101.2 (11.3–1870.0)*, **	25.0 (7.1–81.0)

^aData presented as median values (minimum – maximum)*Compared with control group, *p* < 0.05**Compared with AR group, *p* < 0.05**Table 2** IL-33 and Th2/17 cytokine protein expression in serum and nasal lavage of AR and control children

	AR group	AR with asthma group	Control group
Serum			
IL-33(ng/ml)	85.2 ± 23.6*	113.6 ± 33.8*, **	34.2 ± 17.6
IL-4 (pg/ml)	2.68 ± 0.51*	3.96 ± 0.76*, **	1.47 ± 0.22
IL-5 (pg/ml)	17.21 ± 5.1*	35.42 ± 6.1*, **	8.21 ± 1.99
IL-17 (pg/ml)	26.4 ± 11.6*	33.5 ± 12.3*, **	8.11 ± 1.25
Nasal			
IL-33(ng/ml)	9.1 ± 3.3*	17.2 ± 6.8*, **	2.2 ± 0.8
IL-4 (pg/ml)	3.7 ± 1.6*	7.5 ± 2.5*, **	1.52 ± 0.33
IL-5 (pg/ml)	81.2 ± 23.7*	123.6 ± 33.8*, **	31.6 ± 13.1
IL-17 (pg/ml)	31.5 ± 12.1*	38.1 ± 16.1*, **	13.6 ± 2.34

*Compared with control group, *p* < 0.05**Compared with AR group, *p* < 0.05**Table 3** Relationship between nasal IL-33 and Th cytokines

	<i>r</i>	<i>P</i>
Nasal IL-33		
Nasal IL-4	0.62	0.01
Nasal IL-5	0.68	0.01
Nasal IL-17	0.59	0.02
Serum IL-33		
Serum IL-4	0.56	0.05
Serum IL-5	0.61	0.02
Serum IL-17	0.55	0.03
Serum ECP	0.63	0.01
HDM-specific IgE	0.74	0.02
Eosinophil counts	0.56	0.03

respectively (Table 3). The serum ECP and IgE levels as well as eosinophil counts were found positively correlated with serum IL-33 expression (Table 3).

IL-33 promotes Th2/17 inflammation of peripheral blood mononuclear cells (PBMCs)

After being stimulated with HDM and rhIL-33, we found significant upregulation of Th2 cytokines (IL-4, IL-5) and IL-17 level in PBMCs from AR donor in a dose-dependent manner (Fig. 1). In PBMCs from controls, these changes were not found. Interestingly, when we used combination of IL-33 and IL-4/IL-5/IL-17 to stimulate PBMCs, the Th2/17 cytokine production was not changed compared with stimulation by single IL-33 (Fig. 1). Besides, anti-IL-33 inhibited HDM-induced Th2/17 cytokine production, which further confirmed the positive role of IL-33 on Th2/17 cells (Fig. 1). To investigate the effect of Th2/17 cytokines on IL-33 expression, we used rhIL-4/IL-5/IL-17 to stimulated PBMCs and found no changes of IL-33 expression (data not shown), suggesting that involvement of IL-33 in AR occurrence was a single-way manner.

Discussion

Our present study demonstrates for the first time that elevated IL-33 expression in AR was closely related to the development of Th2/17 inflammation. In vitro study also revealed direct stimulation of IL-33 on PBMCs in AR.

IL-33 is a member of the IL-1 family of cytokines, such as IL-1β and IL-18. IL-33 is believed to be involved in Th2-mediated inflammatory responses in allergic diseases such as asthma, anaphylaxis, and atopic dermatitis, and in host defense against parasites [18]. In experimental models of asthma, blocking of the IL-33/ ST2 signaling cascade reduces the severity of disease [19–23]. Anti-IL-33 antibody significantly reduces Th2 cytokine production in vivo, eosinophilic inflammation, and airway hyperreactivity [24].

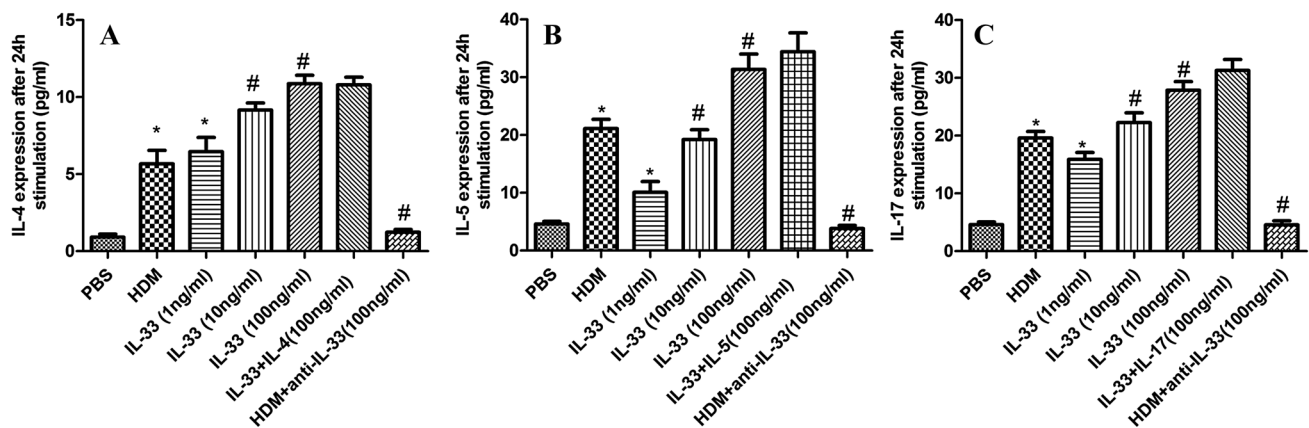


Fig. 1 After being stimulated with rhIL-33, significant up-regulation of Th2 cytokines (IL-4, IL-5, IL-17) level in PBMCs from AR donor in a dose dependent manner (a–c). * $P < 0.05$, comparison with PBS group. # Comparison with IL-33 (1 ng/ml) group

Our study showed that both the systematic and local expression of IL-33 cells and Th2 cytokines were significantly increased in AR. The correlation between Th2 cytokines (IL-4, IL-5) and IL-33 expression were found in both serum and nasal lavage, suggesting that IL-33 promote Th2 inflammation locally and systemically.

On allergen sensitization, Th17 cells enhance not only neutrophilic airway inflammation but also Th2 cell-mediated eosinophilic airway inflammation in mouse models of asthma [7, 25]. IL-17 is a key cytokine produced by Th17 cells. Serum IL-17 levels are increased in AR patients and are associated with clinical symptoms, peripheral eosinophil counts, and the need for medication [8, 9]. In our study, we found that elevated IL-17 expression was correlated with IL-33 expression, suggesting that IL-33 may be involved in Th17 inflammation.

To characterize IL-33's direct role in Th response, we purified PBMCs from both AR and control donors. Our results showed that IL-33 induced significant upregulation of Th2/17 cytokine level in PBMCs from AR donor in a dose-dependent manner (no differences were found in children with and without asthma). To investigate the effect of Th2 cytokines on IL-33 expression, we further used rhIL-4/IL-5/IL-17 to stimulated PBMCs and found no changes of IL-33 expression. However, block of IL-33 can reduce poly I:C-induced Th2/17 cytokine production, suggesting that IL-33 plays important roles in Th2/17 inflammation. These results suggested that involvement of IL-33 in AR occurrence was a single-way manner and IL-4/IL-5/IL-17 had no effect on Th2/17 cytokine production.

Collectively, our finding showed that IL-33 may be a critical cytokine which mediates and amplifies Th2/17 inflammation and a potential marker for predicting disease severity in AR. Our research may provide a new target for AR prevention and treatment in future.

Compliance with ethical standards

Conflict of interest All authors declare that he/she has no conflict of interest.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Strachan D, Sibbald B, Weiland S et al (1997) Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the international study of asthma and allergies in childhood (ISAAC). *Pediatr Allergy Immunol* 8: 161–76
2. Ait-Khaled N, Anderson HR, Asher MI et al (2007). Worldwide time trends for symptoms of rhinitis and conjunctivitis: phase III of the International Study of Asthma and Allergies in Childhood. *Pediatr Allergy Immunol* 19: 110–24
3. Bochner BS, Schleimer RP (2001) Mast cells, basophils and eosinophils: distinct but overlapping pathways for recruitment. *Immunol Rev* 179:5–15
4. Hansen I, Klimek L, Mösges R, Hörmann K (2004) Mediators of inflammation in the early and late phase of allergic rhinitis. *Curr Opin Allergy Clin Immunol* 4:159–63
5. Ling EM, Smith T, Nguyen XD et al (2004) Relation of CD41CD251 regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 363:608–15
6. Wakashin H, Hirose K, Maezawa Y et al (2008) Nakajima H. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 178:1023–1032
7. Wilson RH, Whitehead GS, Nakano H et al (2009) Allergic sensitization through the airway primes Th17-dependent neutrophilia

- and airway hyperresponsiveness. *Am J Respir Crit Care Med* 180:720–730
8. Ciprandi G, De Amici M, Murdaca G et al (2009) Serum interleukin-17 levels are related to clinical severity in allergic rhinitis. *Allergy* 64:1375–1378
 9. Moon IJ, Hong SL, Kim DY et al (2012) Blocking interleukin-17 attenuates enhanced inflammation by staphylococcal enterotoxin B in murine allergic rhinitis model. *Acta Otolaryngol* 132(Suppl 1):S6–S12
 10. Kurowska-Stolarska M, Hueber A, Stolarski B et al (2011) Interleukin-33: a novel mediator with a role in distinct disease pathologies. *J Intern Med* 269:29–35
 11. Lloyd CM (2010) IL-33 family members and asthma-bridging innate and adaptive immune responses. *Curr Opin Immunol* 22:800–806
 12. Asaka D, Yoshikawa M, Nakayama T et al (2012) Elevated levels of interleukin-33 in the nasal secretions of patients with allergic rhinitis. *Int Arch Allergy Immunol* 158:47–50
 13. Glück J, Rymarczyk B, Rogala B (2012) Serum IL-33 but not ST2 level is elevated in intermittent allergic rhinitis and is a marker of the disease severity. *Inflamm Res* 61:547–550
 14. Haenuki Y, Matsushita K, Futatsugi-Yumikura S et al. A critical role of IL-33 in experimental allergic rhinitis. *J Allergy Clin Immunol* 130:184–194
 15. Brozek Jan L, Bousquet J, Baena-Cagnani Carlos E et al (2010) Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. *J Allergy Clin Immunol* 126:466–476
 16. Pedersen SE, Hurd SS, Lemanske RF Jr et al (2011) Global strategy for the diagnosis and management of asthma in children 5 years and younger. *Pediatr Pulmonol* 46:1–17
 17. Kristina I, Jörgen PP, Magnus PB et al (2012) Clara cell protein in nasal lavage fluid and nasal nitric oxide-biomarkers with anti-inflammatory properties in allergic rhinitis. *Clin Mol Allergy* 10:4
 18. Miller A (2011) Role of IL-33 in inflammation and disease. *J Inflammation* 8:22
 19. Kurowska-Stolarska M, Kewin P, Murphy G et al (2008) IL-33 induces antigen-specific IL-5 + T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 181:4780–4790
 20. Kearley J, Buckland KF, Mathie SA et al (2009) Resolution of allergic inflammation and airway hyperreactivity is dependent upon disruption of the T1/ST2-IL-33 pathway. *Am J Respir Crit Care Med* 179:772–781
 21. Liu X, Li M, Wu Y et al (2009) Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma. *Biochem Biophys Res Commun* 386:181–185
 22. Oshikawa K, Yanagisawa K, Tominaga S et al (2002) Expression and function of the ST2 gene in a murine model of allergic airway inflammation. *Clin Exp Allergy* 32:1520–1526
 23. Pushparaj PN, Tay HK, H'ng SC et al (2009) The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci USA* 106:9773–9778
 24. Coyle AJ, Lloyd C, Tian J et al (1999) Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J Exp Med* 190:895–902
 25. Wakashin H, Hirose K, Maezawa Y et al (2008) IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 178:1023–1032



RETRACTED ARTICLE: Interleukin-33 promotes helper T cell type-2/17 inflammation in children with allergic rhinitis

Yuanming Wang¹ · Chuling Li¹ · Xianglin Luo¹ · Deyu Xu¹ · Yaxiong Xu¹ · Guanquan Li¹ · Fang Liao¹ · Shujuan Tan¹

Received: 3 October 2017 / Accepted: 7 November 2017 / Published online: 17 November 2017
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

The authors have retracted this article because of significant overlap with a previously published article [1]. All authors agree with this retraction. The online version of this article contains the full text of the retracted article as electronic supplementary material.

[1] Wenlong Liu et al. Interleukin-31 promotes helper T cell type-2 inflammation in children with allergic rhinitis *Pediatric Research* (2015) 77:20–28. <https://doi.org/10.1038/pr.2014.151>

The online version of this article <https://doi.org/10.1007/s00405-017-4807-1> contains supplementary material, which is available to authorized users.

✉ Chuling Li
chulinglidg@163.com

¹ Department of Otolaryngology, Dongguan Women and Children's hospital, No 99. Zhenxing Road, Dongguan 523120, China