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Original Article

IL-31, IL-33, and TSLP expression and relation to severity of asthma and rhinitis in Chinese allergic patients

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Abstract: The aim of this study was to investigate the clinical and pathological significance of interleukin 31 (IL-31), interleukin 33 (IL-33), and thymic stromal lymphopoietin (TSLP) in serum of patients with asthma and/or rhinitis. The levels of IL-31, IL-33, and TSLP were measured by enzyme-linked immunoadsorbent assays in serum samples from 64 cases of asthma patients, 64 cases of rhinitis patients, 64 cases of asthmatic patients with allergic rhinitis, and 32 cases of healthy controls. Human specific immunoglobulin E (sIgE) was measured by EUROALLER system. Compared with healthy controls, serum levels of IL-31, IL-33, and TSLP in patients with rhinitis, asthma, or rhinitis and asthma were significantly increased. The level of TSLP in serum was changed with statistical difference in patients with asthma, and obviously increased in both sIgE positive patients with asthma and sIgE positive patients with rhinitis, among which, the serum levels of IL-31, IL-33, and TSLP were the highest in both sIgE positive patients with rhinitis caused by mites and sIgE positive patients with rhinitis caused by mugwort. IL-31, IL-33, and TSLP might be involved in the pathogenesis of asthma and rhinitis. Besides, both mites and mugwort were the major allergens in the induction of asthma and rhinitis.

Keywords: Asthma, rhinitis, interleukin-31, interleukin-33, thymic stromal lymphopoietin

Introduction

Allergic respiratory diseases, especially asthma and rhinitis, are considered as widespread epidemic diseases all around the world. There are up to 300 million patients with asthma and 500 million patients with rhinitis according to the World Health Organization [1]. Asthma is a chronic inflammatory disease with histologic lesions in bronchial wall. It affects all ages, characterized by infiltration of mast cells, reversible airflow obstruction, airway hyperreactivity, and airway remodeling. In spite of the fact that there is a decline in the number of asthma-associated hospitalizations and deaths, it is still widely recognized as an unbearable public health burden because of its uncontrolled capacities of escaping previous therapeutic strategies [2]. It is generally agreed with that allergic rhinitis is sustained by an inflammatory process hallmarked with intense eosinophil infiltration, mucus hypersecretion, and airway remodeling. Persistent allergic rhinitis is usually accompanied by asthma, which is a common pathological feature especially in pedia. Although several preventive and therapeutic strategies are available, the prevalence and incidence of asthma and rhinitis still unexpectedly increase. Therefore, it is urgent to elaborate the pathogenic mechanisms.

Thymic stromal lymphopoietin (TSLP) originally discovered as a growth factor for lymphocyte progenitors, is now well-known as a secreted protein mainly from activated epithelial cells. TSLP expression triggers an excessive and constant inflammatory response driven by type 2 helper T (Th2) cells. Th2 cells and corresponding cytokines are main regulators of the initiation and development of allergic diseases. Several murine studies have established a potential role of TSLP in allergic asthma. Epithelia-specific over-expression of pulmonary TSLP [3], or epithelium stimulated with TSLP and antigen [4], caused mice with asthma-like symptoms, which was closely related to activation of Th2 cells and excessive production of antigen-specific IgE. Symptoms of allergic asthma

were yet slightly ameliorated in TSLP receptor (TSLPR)-deficient mice, suggesting that TSLP might not be a good potential candidate for pathogenic treatment [3, 5]. These conflict experimental results suggested that clinical trials were urgently needed to uncover the correlation between TSLP and asthma. A recent clinical trial demonstrated that an anti-TSLP mAb attenuated early and late asthmatic responses caused by allergen challenge in patients with asthma [6]. These findings suggested that further clinical investigation should be explored and improved to thoroughly explain the role of TSLP.

Interleukin 33 (IL-33) belongs to the IL-1 cytokine family and plays an important role in both allergic inflammation and transcriptional regulation. IL-33, produced in a fully active form by airway epithelial cells, fibroblasts, and smooth muscle cells, serves as a signal molecule in different cell compartments to modulate the adaptive immune response. IL-33 drives Th2-mediated inflammation in both human and mice with allergic airway diseases. IL-33-responsive innate immune cells also induce other cytokines, such as IL-5 and IL-13 [7]. Moreover, IL-33 stimulates the production of TSLP from epithelial cells [8]. All of these discoveries have revealed a potential role of IL-33 in allergic inflammation. Recently, IL-33 has emerged as a key regulator of asthma pathogenesis [9]. A clinical trial showed that IL-33 expression was increased in severe asthma patients [10]. However, up to date few clinical trials have comprehensively explained the functional relationships between IL-33 and asthma and rhinitis.

Interleukin 31 (IL-31), a member of IL-6 cytokine family, is widely expressed in human tissues. IL-31 is mainly secreted from activated $CD4^+$ T cells, especially Th2 cells [11]. IL-31 stimulates the secretion of proinflammatory cytokines and the expression matrix metalloproteinases [12]. Recent finding has shown that IL-31 might be participated in allergic asthma by triggering allergic inflammation and airway epithelial cell response [13, 14]. In asthmatics, single nucleotide polymorphisms (SNPs) of IL-31 were significantly correlated with total levels of IgE in serum [15].

This study was aimed to illuminate the role of IL-31, IL-33, and TSLP in patients with asthma and/or rhinitis by detecting the levels of these cytokines in serum. Further, we summarized several major environmental allergens of asthma and rhinitis.

Materials and methods

Study population

One hundred and ninety-two patients with asthma and/or rhinitis were recruited from allergy department of our hospital between July 2015 and March 2016. Patients were divided into sixty-four asthmatic patients, sixty-four rhinitis patients, and sixty-four asthmatic patients with rhinitis. Thirty-two age-matched healthy controls without history of any systemic diseases and chronic diseases associated with endocrine, cardiovascular, pulmonary, and hepatorenal system, local and systemic infectious diseases, atopic dermatitis and any other allergic diseases. The atopic status of patients and control subjects was determined by serum sIgE using EUROALLER system (**Table 1**). The diagnosis was based on the criteria of the Global Initiative for Asthma. All subjects had no history of family heredity, and were free from allergic diseases, autoimmune diseases, tumor and other serious systemic diseases. It was also guaranteed that neither antihistamine agents were taken in three days nor glucocorticoids were taken in a month prior to recruitment to the study.

Sample collection

Peripheral venous blood samples were collected from each patient and healthy volunteer. Serum was separated from blood after centrifugation at 2000 r/min for 10 minutes. Serum was collected and stored at -20°C for further biological analyses.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-31, IL-33, and TSLP in human serum were measured by corresponding ELISA kits according to the protocols from the manufacturer (Sigma-Aldrich, St Louis, MO, USA). The absorbance was read at 450 nm with reference wavelength at 570 nm using a SpectraMax™ microplate spectrophotometer. The concentrations of cytokines were calculated according to the instructions of protocols.

Table 1. The atopic status of patients and control subjects

Characteristic	Healthy controls (*n=32*)	Asthma patients		Rhinitis patients		Asthma combined with rhinitis patients	
		Atopic asthmatics (n=32)	*Nonatopic asthmatics (n=32)*	*Atopic rhinitis patients (n=32)*	*Nonatopic rhinitis patients (n=32)*	*Atopic patients (n=32)*	*Nonatopic patients (n=32)*
Sex (M:F)		18:14	16:16	15:17	14:18	16:16	13:19
Age (years)		11-59	14-52	13-50	11-51	10-48	10-54
		31.9 ± 15.0	32.3 ± 11.0	30.1 ± 12.6	31.7 ± 11.6	30.1 ± 8.8	31.4 ± 12
Disease duration (months)	N/A	3-60	3-60	3-60	3-60	3-60	3-60
		15.2 ± 3.4	16.5 ± 5.2	16.2 ± 4.6	17.5 ± 5.2	17.5 ± 5.1	18.5 ± 7.2
Allergens	N/A	*Single allergen (n=6)*: Acarid (n=3), Mugwort (n=1), Freshwater fish (n=1), Penicillium (n=1), Soybean (n=1)	N/A	*Single allergen (n=9)*: Mugwort (n=5), Acarid (n=3), Cockraoch (n=1)	N/A	*Single allergen (n=11)*: Mugwort (n=4), Acarid (n=3), Freshwater fish (n=2), Cockraoch (n=1), Penicillium (n=1)	N/A
		Multiple allergens (n=26): Acarid-related (n=21), Food-related (n=14), Mugwort-related (n=9)		*Multiple allergens (n=23)*: Mugwort-related (n=23), Acarid-related (n=18), Food-related (n=10)		*Multiple allergens (n=21)*: Acarid-related (n=20), Mugwort-related (n=13), Food-related (n=10)	
Serum sIgE	Negative	Positive	Negative	Positive	Negative	Positive	Negative

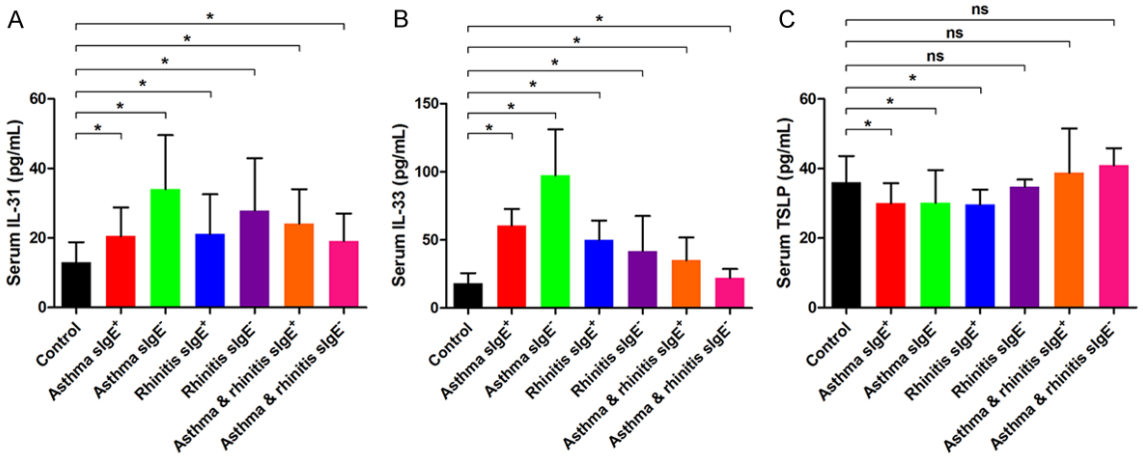

Figure 1. IL-31 and IL-33 expression are increased in asthma and rhinitis patients. The levels of serum IL-31 (A), IL-33 (B), and TSLP (C) were assessed in patients with atopic asthma (n=32), nonatopic asthma (n=32), atopic rhinitis (n=32), nonatopic rhinitis (n=32), atopic asthma combined with rhinitis (n=32), nonatopic asthma combined with rhinitis (n=32), and controls (n=32) as measured by ELISA. Data are expressed as mean ± SD, *P < 0.05 compared with the control.

Statistical analysis

Data were expressed as mean ± SD, and the results were analyzed using SPSS 13.0 statistical software. The statistical significance of difference was determined by two sample *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results

Serum IL-31 and IL-33 expression are increased in patients with asthma and/or rhinitis

To identify the role of IL-31, IL-33, and TSLP in triggering allergic airway diseases, levels of IL-31, IL-33, and TSLP in human serum were firstly measured by ELISA assays. Results showed that compared with the healthy controls, the serum level of IL-31 was clearly increased in asthmatic patients, rhinitis patients, and asthmatic patients with rhinitis (**Figure 1A**). Variations of serum IL-33 levels between each group represented a similar trend with that of IL-31 (**Figure 1B**). Noteworthily, results showed an impressive increase in the serum level of TSLP in patients with asthma and patients with atopic rhinitis but not in patients with non-atopic rhinitis or asthmatic patients with rhinitis, compared with the healthy controls (**Figure 1C**). Altogether, these results suggested that IL-31 and IL-33 expression were increased in patients with asthma and/or rhinitis.

IL-31 and IL-33 expression in patients with asthma are clinically correlated with the categories of allergens

Allergic asthma is triggered by allergens from different sources. Our prior results have established a role of IL-31, IL-33, and TSLP in atopic asthma and rhinitis, while the correlation between all of the cytokines and allergens were not completely understood. Herein, serum levels of IL-31, IL-33, and TSLP in different subgroups, categorized by types of allergens, of asthma group were accurately quantified. We found that serum levels of IL-31 and IL-33 in atopic asthmatics caused by mite, dust mite, and mugwort were abnormally increased. The levels of IL-31 and IL-33 in serum were raised in different degrees in patients with allergic asthma caused by crab, cockroach, and cat (**Figure 2A** and **2B**). However, compared with the control, statistical significance of difference in the level of TSLP in serum was not existed in each group (**Figure 2C**). Collectively, the expression of IL-31 and IL-33 in patients with asthma was clinically related to the categories of allergens.

IL-31, IL-33, and TSLP expression in patients with rhinitis are clinically correlated with categories of allergens

Allergic rhinitis shares common pathological mechanisms with asthma. Serum levels of IL-31, IL-33, and TSLP in different subgroups of rhinitis group were also determined. It was

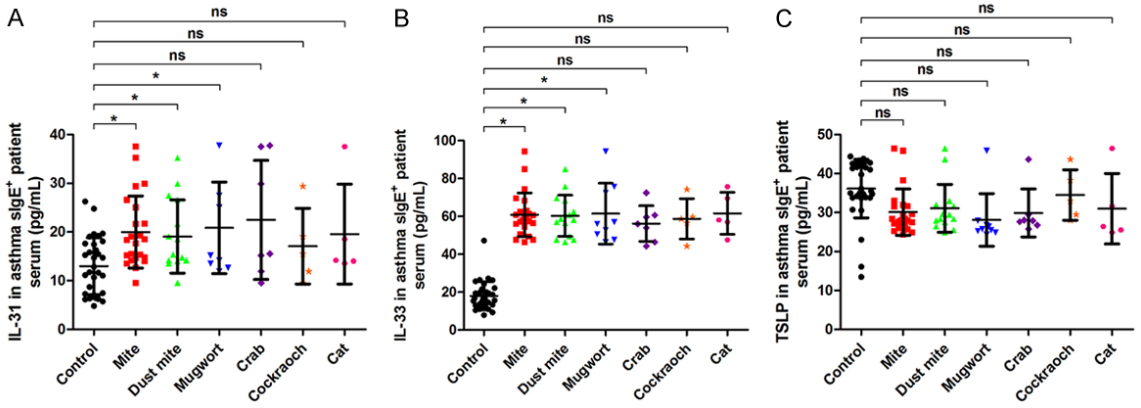

Figure 2. IL-31 and IL-33 expression in asthma are clinically correlated with categories of allergens. The levels of serum IL-31 (A), IL-33 (B), and TSLP (C) were assessed in controls (n=32) and patients with atopic asthma (n=32) triggered by mite, dust mite, mugwort, crab, cockroach, and cat as measured by ELISA. Data are expressed as mean ± SD, *P < 0.05 compared with the control.

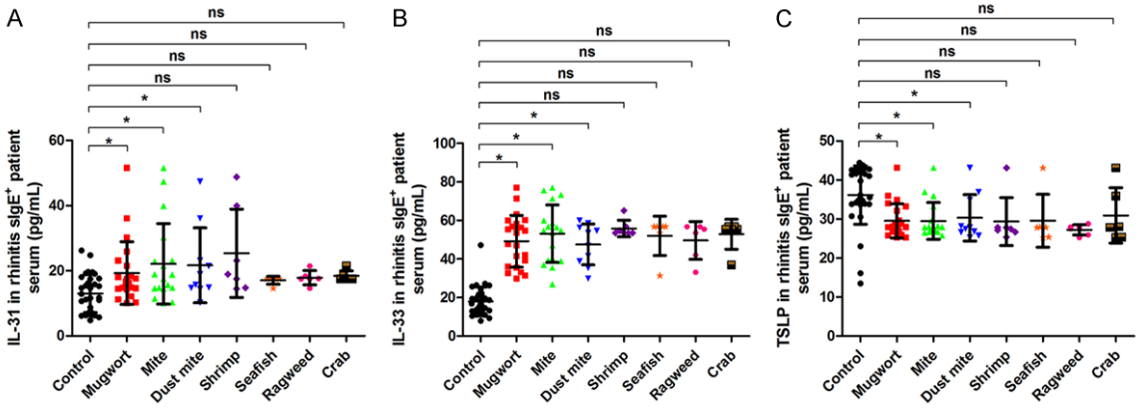

Figure 3. IL-31, IL-33, and TSLP expression in rhinitis are clinically correlated with categories of allergens. The levels of serum IL-31 (A), IL-33 (B), and TSLP (C) were assessed in controls (n=32) and patients with atopic rhinitis (n=32) triggered by mugwort, mite, dust mite, shrimp, seafish, ragweed, and crab as measured by ELISA. Data are expressed as mean ± SD, *P < 0.05 compared with the control.

found that IL-31, IL-33, and TSLP levels in atopic rhinitis induced by mugwort, mites, and dust mites were dramatically higher than those in control group, while no significant increase in serum levels of these cytokines was observed in atopic rhinitis induced by shrimp, seafish, ragweed, and crab (**Figure 3**). Taken together, the expression of IL-31, IL-33, and TSLP in rhinitis was clinically associated with categories of allergens, among which mugwort, mites, and dust mites induced obvious increase in the expression of these cytokines.

Discussion

Over the past decades, the prevalence of rhinitis and asthma has been rapidly increasing worldwide. Nasal airway and paranasal sinus are organic parts of respiratory tract [16, 17]. As is known to all, nasal airway and bronchial airway share many similarities in the histologic structure. Of note, nose and lung have functional complementation [18]. Numerous contemporary studies, guided by the concept “one airway - one disease”, have revealed the epidemiological, pathophysiological, and clinical correlation of asthma and rhinitis. Rhinitis usually aggravates the serious symptom of asthma, while it is not exact that rhinitis patients will certainly suffer from asthma, implying that differences exist between rhinitis and asthma [19, 20]. In spite of the fact that asthma is a chronic inflammatory disease of airway, the exact mecha-

nisms remain unclear, especially the mechanism behind inflammation initiation. Abundant studies have indicated that there existed crosstalk between Th1 and Th2 cells. In physical condition, Th1 and Th2 keep a dynamic balance in their quantities, which when broken would serve as an inducer of asthma [21]. Recent studies demonstrated that asthma is attributed to differentiation of Th1 and Th2 cells induced by genetic and environmental allergens [22].

IL-33 is a newly discovered cytokine produced by activated Th2 cells. Its functional receptor exists as the heterologous dimers, composed of IL-31R subunit and oncostatin M receptor. Both of the subunits are constitutively expressed in epithelial cells and keratinocyte cells, or expressed in activated monocytes. IL-31 can activate JAK/STAT, MAPK, and PI3K/Akt signaling pathways by acting on its receptor, which plays an important role in the physiopathology of asthma, rhinitis, and dermatitis. In addition, it elicits a broad spectrum of biological responses, including antigen presentation, hemopoiesis, cell proliferation and migration, and induction of chemokine expression [23]. Previous study has revealed that the expression of IL-31 receptor was dramatically increased in the pathological tissues of model animals with airway allergy [24]. In the present study, compared with healthy controls, serum IL-31 level was impressively increased in asthmatic patients, patients with rhinitis, and asthmatic patients with rhinitis, suggesting that serum IL-31 might be a principal trigger of rhinitis and asthma. Noteworthily, IL-31 serum level was the highest in sIgE positive patients with asthma caused by mites, followed by mugwort. These data strongly implied that allergy could strengthen the induction of asthma on IL-31 in serum. Our new findings provided a novel insight into the pathological mechanisms underlying allergic asthma.

IL-33, ubiquitously expressed in murine and human epithelial cells, is related to various immunological diseases, including natural immune response and adaptive immune response. It mainly regulates immune response dominated by Th2 cells, mast cells, and the balance between Th1 and Th2 cells. Previous studies have revealed the role of IL-33 in inflammation and autoimmune diseases [25-27], showing that the expression of IL-33, ST2, and sST2 was apparently increased in pulmonary tissues from allergic asthmatic mice with ovalbumin-induced airway inflammation. IL-33 can cause a series of autoimmune diseases, such as bronchial asthma, systemic lupus erythematosus, and allergic shock, leading to enhanced production of inflammatory factors from Th2 cells. In mast cells and natural killer cells, IL-33 also promotes the production of inflammatory factors and chemotactic factors [28]. In our study, IL-33 was induced in patients with rhinitis, patients with asthma, and asthmatic patients with rhinitis. This could be explained by the previous study that IL-33, when combined with its ligand, could enhance the production of IL-4, IL-5, and IL-13, resulting in the progression of allergic inflammation-mediated asthma [29]. IL-33 when combining with ST2 receptor functions well in regulating mucosal and allergic immune in various diseases [30, 31]. In the present study, for the first time we confirmed that IL-33 expression was closely associated with both asthma and rhinitis, which could be a helpful clinical guideline for early diagnosis of asthma and rhinitis. Further, aeroallergen, mites and mugwort, from environment were the major inducers.

TSLP is a cytokine belonging to IL-7 family, which was initially discovered in the supernate of culture medium of murine thymic stromal cells [32]. And it is widely expressed in lung, skin, and intestine [33]. TSLP is significantly induced in mast cells activated by IgE receptor, suggesting that an additional type of cells might be involved in triggering allergic inflammation. *In-situ* hybridization result indicated that TSLP expression was apparently induced in asthma airway, which was associated with the expression of inflammatory chemokines in Th2 cells and the severity of disease [34]. Recent study showed that TSLP might be an inductor of allergic diseases since it induced Th2-mediated allergic inflammation and promoted the secretion of IL-4, IL-5, IL-13, and TNF-α at the early stage of allergic inflammation [35]. In the present study, TSLP was increased in asthma patients and rhinitis sIgE positive patients. However, compared with healthy controls, there was no statistical significance in both rhinitis sIgE negative patients

and rhinitis combined with asthma patients, which suggested that TSLP was involved in allergic diseases. It was also reported that there was a relationship between the allergy-positive test and severity of asthma, which emphasized the critical role of TSLP in allergic diseases [36]. In addition, TSLP, secreted from epithelial cells, can directly activate macrophages. These findings collectively supported the hypothesis that TSLP could induce allergic inflammation in partial organs while no specific systematic symptoms were observed.

Mite-caused allergic responses are attributed to the mite polypide, secretion, and excreta. When inhaled, they will cause anaphylaxis and threaten human health. Therefore, acarus killing has important significance for prevention of mite-caused allergic diseases [37]. Pollen could deposit in nostril and eyes to cause or aggravate rhinitis and asthma [38, 39]. Specific immunotherapy has gained increasing attention in recent years, which has been recognized as an effective and safe strategy for rhinitis and asthma treatment [40].

In conclusion, we demonstrated that IL-31, IL-33, and TSLP were involved in the pathogenic process of asthma and rhinitis while concrete mechanisms underlying remained to be unclear. Among different kinds of allergens, mite and mugwort were of great importance for their role in triggering asthma and rhinitis. Our study provided valuable clinical basis for further research on the mechanisms of asthma and rhinitis and brought a novel insight into the clinical evaluation and treatment for asthma and rhinitis.

Disclosure of conflict of interest

None.

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