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Levels of Interleukin-33 and other cytokines in nasal fluid in patients with respiratory syncytial virus infections

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Abstract

INTRODUCTION : Respiratory syncytial virus (RSV) infections are one of the most common viral infections in children. Almost all children are infected with RSV by the age of 2 years, and reinfection is common. It has long been speculated that acute RSV infection during infancy is associated with a greater risk of allergic asthma later in life. Interleukin-33 (IL-33) is a member of the IL-1 family, which induces Th2 cytokines. The IL-33 receptor, ST2, is found as either a membrane or circulating soluble protein. A high concentration of IL-33 is strongly associated with atopic asthma, allergic disease, rheumatic disease, and viral infections, including RSV.

METHODS : The levels of IL-33, 6 chemokines, and 11 other cytokines were determined in nasal fluid or nasopharyngeal aspirate samples obtained from 30 patients with RSV infection to elucidate the association between IL-33 and recurrent wheezing or asthma. The levels of chemokines and cytokines were measured using a Bio-Plex suspension array (Bio-Rad Laboratories). Concentrations of IL-33 were quantified using a human IL-33 Quantikine ELISA kit (R&D Systems).

RESULTS : A markedly high IL-33 concentration was observed in 3 out of 30 patients (2 in nasal fluid and 1 in nasopharyngeal aspirate). These patients developed recurrent wheezing or asthma later in life.

CONCLUSION : A high IL-33 concentration as a result of RSV infection might be associated with airway hypersensitivity, which occurs in secondary reactive airway diseases, such as recurrent wheezing and asthma.

Introduction

Respiratory syncytial virus (RSV) infection is a very common viral infection, with almost all children being infected by 2 years of age, and reinfection is common. It is the most common cause of lower respiratory tract infection, and it has long been speculated that acute RSV infection during infancy is associated with a greater risk of allergic asthma and recurrent wheezing later in life[1)2)]. Many studies reported that children who experienced RSV infection in early life subsequently had a higher incidence of asthma and recurrent wheezing[3)]. Interleukin (IL)-33 is a member of the IL-1 family, which was found by Schmitz in 2005[4)5)]. It induces Th2 cytokines, and the IL-33 receptor, ST2, is found either as a membrane or circulating soluble protein[6)7)]. Interleukin-33 also regulates the functions of immune cells, including T-cells, B-cells, dendritic cells, macrophages, mast cells, and innate lymphoid cells[8)]. Its signaling affects various immune cells during differentiation, immune responses, and homeostasis, and is strongly associated with atopic asthma, allergic disease, rheumatic disease, and viral infections, including RSV[10-13)]. The role of IL-33 in asthma is shown in Figure 1. Epithelial cells and macrophages release IL-33 when they detect allergens via their cell surface receptors, which then activates ST (+) CD4 T-cells, which produce the Th2 cytokines IL-5 and IL-13[14)]. These cytokines induce hyperproduction of mucus, fibrotic changes in epithelial cells, and smooth muscle hyperplasia, which are all causes of

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asthma and recurrent wheezing. An increase in IL-33 levels in the lungs of asthma patients, and the association between IL-33 and asthma has been reported[15]. One genome-wide association study (GWAS) has identified associations between IL-33 and IL1RL1 and asthma[16-18]. An association between hospitalization owing to RSV infection and recurrent wheezing or asthma later in life has been reported[1-3]. However, there are no substantial lines of evidence on an association between increased IL-33 expression during RSV infection in infancy and recurrent wheezing or asthma later in life.

Objective

The goals of this study were to determine whether there were high levels of IL-33 and other chemokines and cytokines during RSV infection in infants and, if so, investigate whether this was associated with recurrent wheezing or asthma later in life.

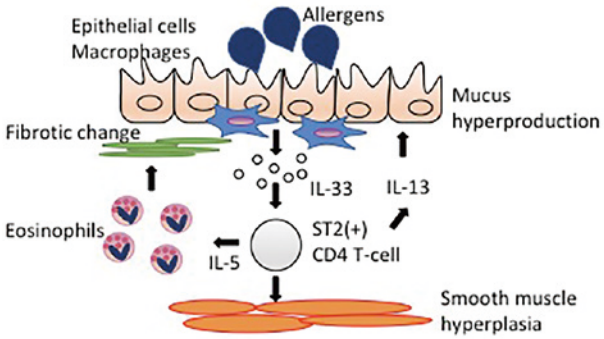

Fig. 1. Involvement of IL-33 in pathogenesis of asthma
Epithelial cells and macrophages release IL-33 upon binding of allergens to their cell-surface receptors. IL-33 acts on ST2 (+) CD4 T-cells, which subsequently releases IL-5, IL-13, which then act on eosinophils. Eosinophils subsequently induce fibrotic changes, smooth muscle hyperplasia, and mucus hyper-production.

Materials and methods

Figure 2 shows a flowchart of the study. A total of 40 male and female patients with a clinical diagnosis of acute respiratory infection, and who underwent a medical check-up or were hospitalized at the Department of Pediatrics at Tokyo Medical University between August 1, 2008 and December 31, 2015, were enrolled in the study. Nasal fluid, nasopharyngeal aspirate, or endothelial tube aspirate were collected from these patients. The inclusion criteria were as follows : a runny nose or sneezing ; and suspected viral infection with a subsequent diagnosis of acute respiratory infection due to RSV. The patients were followed up for 2 years after the diagnosis of RSV infection. The exclusion criteria were as follows : a diagnosis of chromosomal abnormalities ; no RSV infection ; a pre-existing diagnosis of asthma or recurrent wheezing ; and lack of availability to follow-up. Nasal fluid or nasopharyngeal aspirates and endothelial tube aspirates were obtained from outpatients or hospitalized patients. Nasal fluid samples were obtained when the patients had a medical check-up, or when they were admitted to hospital. Endothelial tube aspirates were obtained when the patients were intubated. The samples obtained were stored at −70°C until use. The diagnosis was obtained by performing a rapid assay for RSV infection (CheckRSV ; Alfresa, Japan) on nasal fluid and real-time polymerase chain reaction (PCR). Whether real-time PCR was performed or not depended on the physician's judgement. The concentrations of IL-33, and 6 chemokines, 11 other cytokines (IL-

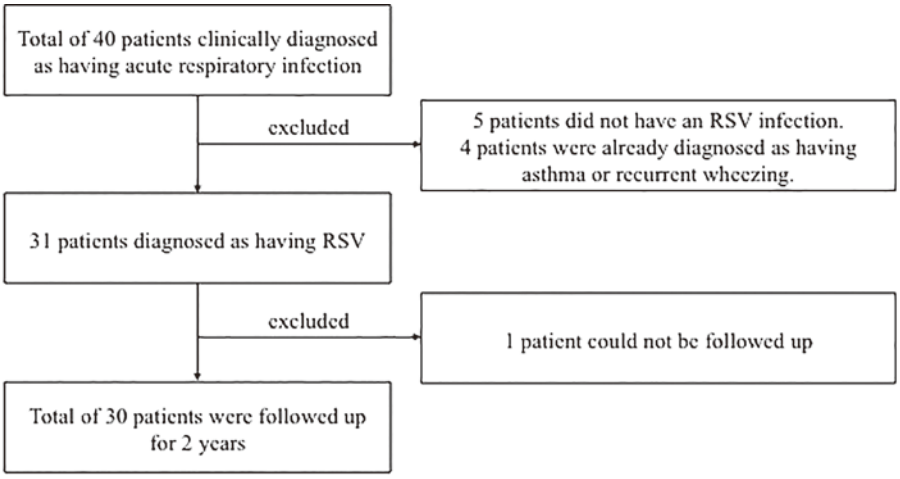

Fig. 2. Study flowchart
Total of 40 patients clinically diagnosed as having acute respiratory infection were enrolled in study. Five patients were excluded as they did not have an RSV infection ; 4 patients were excluded as they were already diagnosed as having asthma or recurrent wheezing. Therefore, total of 30 patients were followed up for 2 years.

1β/2/4/5/6/7/8/10/12/13/17, granulocyte-colony stimulating factor, granulocyte macrophage colony-stimulating factor, interferon-gamma, chemokine (CC motif) ligand 2, chemokine (CC motif) ligand 4, and tumor necrosis factor-α) were analyzed in nasal fluid or nasopharyngeal aspirate samples to elucidate the association between IL-33 and recurrent wheezing or asthma. Concentrations of cytokines and chemokines in endothelial tube aspirate samples obtained from intubated patients were also determined. Concentrations of 5 chemokines and 11 other cytokines were measured using the Bio-Plex suspension array (Bio-Rad Laboratories, Japan) or 17-Plex Panel (Bio-Rad Laboratories). Concentrations of IL-33 were quantified using the human IL-33 Quantikine ELISA kit (R & D Systems). Baseline levels of IL-33 in nasal fluid or nasopharyngeal aspirates and endothelial tube aspirates were not determined. The following levels were established : IL-33 < = 4 pg/mL as low, and > 4 pg/mL as high. To analyze the clinical severity of RSV infection, Nariai's RSV infection clinical scoring system (shown in Table 1) was used[19]. In this scoring system, 0 points indicates mild ; 1-3 points indicates moderate ; and more than 4 points indicates severe infection. Patients were also checked for whether they had asthma or atopic dermatitis, and whether they were taking leukotriene receptor antagonists or inhaled corticosteroids 2 years after RSV infection based on clinical records. Patients who did not undergo a medical check-up after 2 years were interviewed by telephone. A comparison of the 2 groups was performed using the Mann-Whitney U test, and categorical data were analyzed by the χ^2 or Fisher exact test. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference between the 2 groups. The Ethics Committee of Tokyo Medical University approved this study (study approval no : 3577), and written informed consent was obtained from all patients.

Results

A total of 40 patients were initially enrolled in this study. Of these, 10 were subsequently excluded because they had already been diagnosed as having asthma or recurrent wheezing ; the absence of an RSV infection ; or lack of availability to follow-up. Therefore, the final total number was 30 patients. Nasal fluid or nasopharyngeal aspirate samples were collected from the 26 non-intubated patients (15 outpatients and 11 hospitalized patients), and endothelial tube aspirates were obtained from the 4 intubated patients. The intubated patients were younger, had a greater number of days of hospitalization, and had more severe RSV infection (Table 3). Figure 3 shows the chemokine and cytokine concentrations observed I these patients. A marked high IL-33 concentration was observed in 2 of the 26 nasal fluid and nasopharyngeal aspirate samples. A markedly high IL-33 concentration was observed in 1 of the 4 endothelial tube aspirate samples. Although the number of samples was small and statistical processing could not be performed, it seems there was no difference in the concentration of the other chemokines and cytokines between patients showing high IL-33 concentrations and those who did not.—None of the patients showed high concentrations of IL-33 in blood serum. Table 4 shows the characteristics of the patients showing high IL-33 concentrations at 2 years after RSV infection. One of the 3 patients had developed asthma. Regarding those

Table 1. Nariai's scoring system for RSV infection severity.

Score	SpO_2 (%)	Respiratory frequency (/min)	Wheezing	Retractive breathing
0	≥95	<40	(−)	(−)
1	90-94	40-59	(+)	Slight
2	<90	≥60	(+)	Marked

SpO_2, Percutaneous arterial oxygen saturation

Table 2. Comparison of patients with and without high IL-33 concentrations.

	Patients with high IL-33	Patients without high IL-33	*p*-value
	Number (%)/ median (range)		
Number	3	27	
Men, *n* (%)	1 (33.3)	11 (40.7)	0.15
Age (months), median (range)	8 (2-60)	14 (1-15)	0.56
Outpatients, *n* (%)	1 (33.3)	14 (51.9)	0.14
Days of hospitalization, *n* (%)	18 (8-28)	9 (2-33)	0.65
Nariai's score, median (range)	4 (0-7)	0 (0-5)	0.26

Table 3. Comparison of characteristics between intubated and non-intubated patients.

	Non-intubated patients	Intubated patients	*p*-value
	Number (%)/ median (range)		
Number	26	4	
Men, *n* (%)	10 (38.5)	1 (25)	0.20
Age (months), median (range)	9 (0-39)	3 (1-6)	0.22
Outpatients, *n* (%)	15 (51.2)	0 (0)	0.01
Days of hospitalization, median (range)	8 (2-19)	22.5 (12-33)	0.01
Nariai's score, median (range)	0 (0-4)	4.5 (3-7)	0.01
High IL-33 concentration, *n* (%)	2 (7.7)	1 (25.0)	0.06

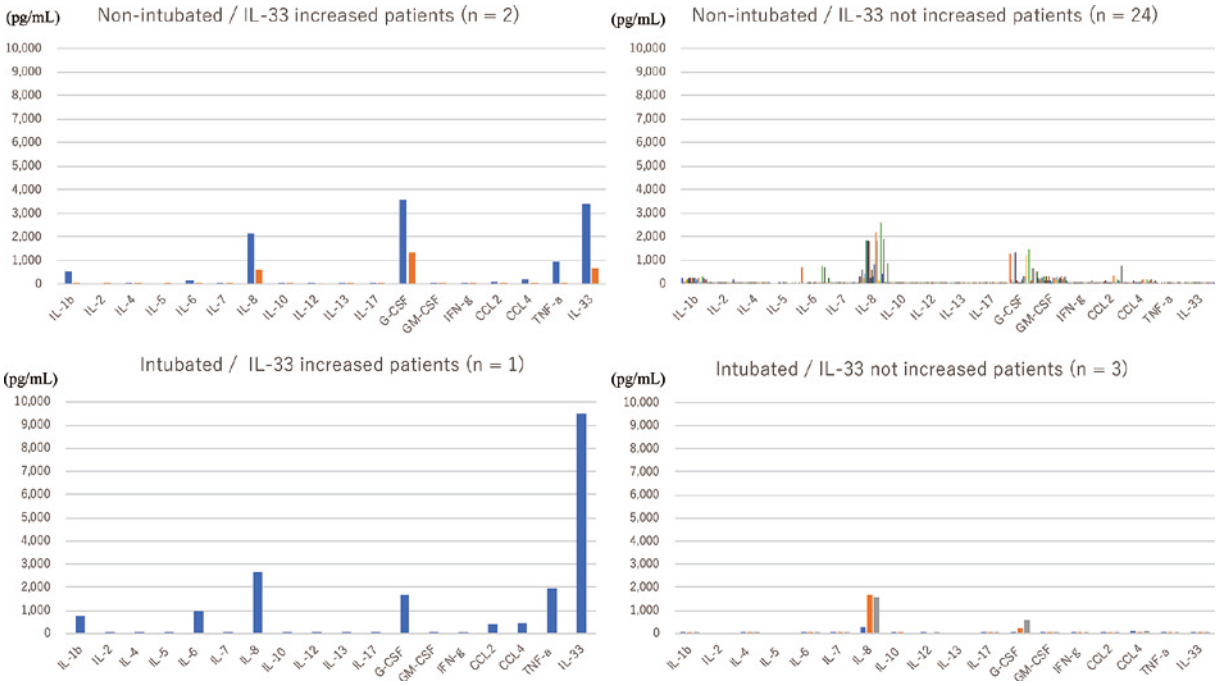

Fig. 3. Chemokine and cytokine profiling of patients
Patients with increased IL-33 levels showed high levels of other cytokines.
Th2 cytokines IL-5 and IL-13 showed no increase.
IL, Interleukin ; G-CSF, granulocyte-colony stimulating factor ; GM-CSF ; granulocyte macrophage colony-stimulating factor ; INF-g, interferon-gamma ; CCL2, chemokine (CC motif) ligand 2 ; CCL4, chemokine (CC motif) ligand 4 ; TNF-a, tumor necrosis factor-α.

Table 4. Characteristics of patients who showed high IL-33 concentration, 2 years after RSV infection.

	1	2	3
Sample	Nasal fluid	Nasal fluid	Endothelial tube aspirate
IL-33 (pg/mL)	3,375	638	9,502
Asthma	(+)	(−)	(−)
LTRA	(+)	(−)	(−)
ICS	(+)	(−)	(−)
Atopic dermatitis	(−)	(−)	(+)

LTRA, leukotriene receptor antagonist ; ICS, inhaled corticosteroids

patients that did not show high IL-33 concentrations, 6 of the 27 patients had developed asthma or recurrent wheezing at 2 years after infection.

Conclusion

One patient (nasal fluid sample) and 2 patients (nasopharyngeal aspirate samples) showed high IL-33 concentrations, and one of them had asthma at 2 years after RSV infection.

Therefore, the high concentrations of IL-33 observed in patients with RSV infection might be associated with airway hypersensitivity and secondary reactive airway disease, such as recurrent wheezing and asthma, but statistical processing could not be performed. Further studies are required to determine whether acute RSV infection during infancy is associated with a greater risk of allergic asthma later in life.

Discussion

The results of this study revealed a tendency for high IL-33 levels to be associated with secondary airway disease. It should be noted, however, that the sample was small, which meant that statistical processing could not be performed. Furthermore, whether these high IL-33 levels were a result of secondary reactive airway disease, or whether this was the other way around could not be clarified. Susceptibility to asthma is affected by both genetic and environmental factors. The GWAS study suggested an association between some single-nucleotide polymorphisms (SNPs) and asthma[16-18]. In that study, IL-33 was reported to be associated with rs1342326 on chromosome 9. This suggests that a genetic association between IL-33 and asthma could be elucidated by SNPs. In the present study, patients who showed high IL-33 levels also showed high concentrations of other chemokines and cytokines. This suggests that patients with high levels of IL-33 will show stronger inflammation. Although concentrations of the Th2 cytokines IL-5 and IL-13 were expected to be high, no such elevation was observed. These cytokines were reported to show an increase at 10-14 days after an increase in IL-33 levels[14]. Therefore, we expected an increase in levels of IL-5 and IL-13 if we performed the analysis at a later time. Further study is needed to analyze time-dependent changes in cytokine concentrations by cytokine profiling.

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RS ウィルス感染症における上気道の IL-33 とその他のサイトカイン

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【要旨】【諸言】 Respiratory syncytial virus（RSV）は 2 歳までにはほぼ 100% の児が感染を受け、細気管支炎や肺炎を併発し、乳児期の感染では、その後の気管支喘息罹患率を増やすことが知られている。近年、気管支喘息の重症度に関連があると注目されている Interleukin-33（IL-33）は IL-1 ファミリーに属するサイトカインであり、受容体である ST2 に作用し即座に炎症反応を起こす。2 型免疫応答を誘導し、花粉症をはじめとした各種アレルギー疾患、好酸球性副鼻腔炎、気管支喘息、好酸球性肺炎、肺組織の繊維化、RSV をはじめとした各種のウィルス感染症に関連する。上気道、下気道でも発現し、気管支喘息患者では気道肺胞洗浄液中および、肺組織中に IL-33 の発現が上昇することが報告されており、また、RSV 罹患時にも上気道で上昇することが知られている。

【目的及び方法】 乳幼児期の RSV 罹患とその後の気管支喘息発症において IL-33 の関連についての検討を行うことを目的とした。当科で経験した RS ウイルス患者 30 例において、鼻汁中と気管洗浄液の IL-33 測定とサイトカインプロファイリングを行った。IL-33 は ELISA 法にて測定し、サイトカイン・ケモカイン・血管由来因子を BioRad の multiple cytokine assay にて測定した。

【結果】 30 例中の 3 例で上気道中の IL-33 上昇を認めた。また、高値例のサイトカインプロファイリングでは IL-8 と IL-1β が高い値を示していたが、Th2 型サイトカインの上昇はなかった。臨床的に IL-33 が高値であった例ではその後も喘鳴を伴い受診を繰り返していた例があった。

【結論】 幼少期の RS ウィルス罹患時における上気道での IL-33 の上昇がその後の気管支喘息発症に関連がある可能性が示唆されるが、IL-33 上昇例が少なく、今後より多くの研究を重ねる必要があると考えられた。

〈キーワード〉 インターロイキン-33、気管支喘息、反復性喘鳴、RS ウィルス、サイトカインプロファイリング