

Clinical Significance of Interleukin 33 (IL-33) in Patients with Eosinophilic Pneumonia

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ABSTRACT

Background: Interleukin 33 (IL-33) works as a functional mediator in allergic disease by enhancing the activity of eosinophils and inducing expression of T helper 2 (Th2)-associated cytokines. However, the role of IL-33 in pulmonary eosinophilia has not been elucidated. We investigated the levels of IL-33 in eosinophilic pneumonia (EP) together with associated cytokines, and discussed the clinical significance of IL-33 in EP.

Methods: Sera and bronchoalveolar lavage fluid (BALF) were obtained from 16 patients with EP, including acute eosinophilic pneumonia (AEP) and chronic eosinophilic pneumonia (CEP). Twelve patients with acute respiratory distress syndrome (ARDS) were also included for comparison. The concentration of IL-33 and Th2 cytokines (IL-4, IL-5, IL-13) were measured by enzyme-linked immunosorbent assay (ELISA).

Results: The concentration of serum IL-33 was significantly higher in patients with AEP than in CEP. In CEP, only patients with atopic factors showed mild increase of serum IL-33. The concentration of BALF IL-33 was also significantly elevated in AEP, however, it remained quite low in CEP. Among Th2 cytokines, IL-5 was significantly increased in both serum and BALF in AEP, and the level of IL-5 was positively correlated with that of IL-33. ARDS showed no increase of serum and BALF IL-33.

Conclusions: The remarkable increase of BALF IL-33 in AEP indicated the local production of IL-33 in lungs. IL-33 is considered to be a local key molecule for triggering pulmonary eosinophilia, together with IL-5. BALF IL-33 appears to be a useful marker for discriminating AEP from CEP and ARDS.

KEY WORDS

eosinophil, eosinophilic pneumonia, IL-33, IL-5, Th2 cytokine

INTRODUCTION

IL-33 is a new functional cytokine belonging to the IL-1 cytokine family,¹ and was first identified as a ligand of ST2L, which is the molecule related to various allergic and inflammatory reactions.²⁻⁴ The main functions of IL-33 identified so far by *in vitro* studies are the induction of Th2-associated cytokine expression in Th2-polarized naïve T cells¹ and the activation of eosinophils as well as IL-5.^{5,6} In animal models, Schmitz *et al.* first demonstrated that the administration of IL-33 induced an increase of eosinophils and mononuclear cells in blood, and infiltration of eosinophils around pulmonary vessels.¹ Kondo *et al.* and Oboki *et al.* also showed that administration of IL-33

directly induced airway eosinophilia and hyper-responsiveness like bronchial asthma, even in RAG 2 α/α mice lacking T cells and B cells.^{7,8} In humans, there are several clinical studies focusing on IL-33; Sakashita *et al.* reported an increase of serum IL-33 level and polymorphism of IL-33 gene were found in patients with Japanese cedar pollinosis,⁹ Prefontaine *et al.* showed that the expression of IL-33 in airway smooth muscle cells was increased in association with the severity of bronchial asthma,¹⁰ and the present authors previously published data describing an increase of serum IL-33 levels in severe atopic asthma.¹¹ From these evidences, it has been established that IL-33 works as an important mediator in allergic airway disease. On the other hand, thus far, the

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clinical behavior and implications of IL-33 in pulmonary eosinophilia have not been elucidated. Therefore, we focused our attention on eosinophilic pneumonia (EP) and investigated the level of IL-33.

EP is clinically subdivided into acute eosinophilic pneumonia (AEP) and chronic eosinophilic pneumonia (CEP).¹²⁻¹⁴ Although both EPs show remarkable eosinophilia in the lung tissue, they are quite different in their clinical course and pathological findings.^{15,16} There have been several basic studies to compare AEP with CEP; the numbers of lymphocytes and IL-5 and eotaxin levels in BALF were significantly increased in AEP.^{15,17-19} However, at present, their etiologies are still unknown and the biological markers to distinguish them have not been established. Therefore, in this study, we compared the levels of IL-33 and related cytokines in AEP and CEP, and discussed the difference between them.

Pathological findings of AEP have traditionally been characterized as diffuse alveolar damage, similar to those of acute lung injury/acute respiratory distress syndrome (ALI/ARDS).¹² In addition, because the radiological findings of AEP also resemble the initial phase of ARDS by reflecting pulmonary interstitial edema, and peripheral eosinophilia is generally lacking in the initial phase of AEP, distinguishing AEP from ARDS is sometimes clinically difficult. Therefore, we also had an interest in the IL-33 levels in patients with ARDS and investigated as well as EP.

METHODS

SUBJECTS

Sixteen patients with EP who had been admitted and treated at Jichi Medical University Hospital between 2000 and 2011 were selected. These 16 patients included 4 patients with AEP and 12 patients with CEP. Twelve healthy non-atopic adult volunteers were involved as controls. Twelve patients with ARDS were also analyzed. Each patient was treated at Jichi Medical University Hospital between 2000 and 2009. AEP patients were selected according to the criteria proposed by Allen *et al.*¹³ and Philit *et al.*²⁰ CEP was diagnosed according to Carrington's original criteria¹⁴ and the recent criteria by Jederlinic *et al.* and Mochimaru *et al.*^{16,21} ARDS was diagnosed according to the North American-European Consensus Conference definition for ARDS²²: namely, acute onset of illness, bilateral opacities on chest radiograph, a PaO₂/FIO₂ ratio (P/F ratio) of less than 200, and no clinical evidence of left heart failure. ARDS was caused by multiple etiologies; the underlying disease was severe pneumonia in 6 patients, sepsis in 5, and rickettsia infection in 1. All patients were examined by chest X-ray and high-resolution CT scan at admission. The radiological findings in this study were reviewed by 2 pulmonologists and 1 radiologist in order to confirm diagnoses. This study was conducted according to the principles expressed in the Declaration of Hel-

sinki, and the protocol of study was approved by the Ethical Committee of Jichi Medical University. Informed consent for participation was obtained from each patient.

COLLECTION OF BLOOD SAMPLE

Blood samples were obtained at admission, and the numbers of leukocytes and eosinophils were counted by a COULTER LH750 analyzer (Beckman Coulter, USA). Then, the sera of the patients were separated after centrifugation at 1500 × g at 4°C for 10 min and stored at -80°C until use.

BRONCHOALVEOLAR LAVAGE AND PREPARATION OF BALF

Bronchoalveolar lavage (BAL) was collected in the involved lung segment of patients with EP. Fifty-ml aliquots of saline solution were instilled three times and recovered. The total number of cells was immediately counted, and for the determination of cell types, cell components obtained after centrifugation in a cyto-spin at 120 × g for 10 min were stained with Diff-Quick (Sysmax, Kobe, Japan). The remaining BALF was centrifuged at 400 × g for 10 min at 4°C and the supernatant was collected and frozen at -80°C until the analysis.

MEASUREMENT OF CYTOKINES AND IgE

Cytokines (IL-4, IL-5, IL-13, IL-33) were measured using enzyme-linked immunosorbent assay (ELISA) kits (IL-33; R&D Systems, MN, USA, IL-4 and IL-5; Biosource, CA, USA, IL-13; Invitrogen, CA, USA). The minimum detectable level of each ELISA was as follows: IL-4 < 2 pg/ml, IL-5 < 4 pg/ml, IL-13 < 4 pg/ml, and IL-33 < 3.9 pg/ml. IgE was analyzed by fluorescence enzyme immunoassay.

STATISTICAL ANALYSIS

Data are presented as means ± standard deviations. Statistical analysis was performed using Mann-Whitney's U test for comparing two groups or by ANOVA followed by Turkey's test for comparing multiple groups. The *r* correlation coefficient test was determined by Pearson's correlation test. SPSS 11.0J (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All statistical tests were 2-sided and *p* values < 0.05 were considered statistically significant.

RESULTS

CLINICAL FEATURES OF EP PATIENTS

The sex and the mean ages, duration of symptoms (duration from first symptom to diagnosis), titers of PaO₂ and serum IgE, numbers of leukocytes and eosinophils in the peripheral blood and total cell counts and numbers of eosinophils in BALF are listed in Table 1. As described in the Table 1, the duration from the first symptom to diagnosis was significantly longer in CEP than in AEP. In AEP, the numbers of

Table 1 Clinical features of subjects

	healthy (n = 12)	AEP (n = 4)	CEP (n = 12)
Age (years old)	47.5 ± 23.3	22.0 ± 1.7	51.8 ± 22.8
M/F	9/3	4/0	6/6
Duration of symptoms (days)	N.A.	2.7 ± 0.5	27.8 ± 13.0*
PaO ₂ (Torr)	N.A.	45.3 ± 8.4*	74.5 ± 14.0
WBC (×10 ³ /μl)	5.1 ± 1.0	18.8 ± 6.8 [†]	11.7 ± 3.7
Eosinophil (×10 ³ /μl)	0.7 ± 0.6	0.3 ± 0.1	3.7 ± 2.3 ^{†*}
IgE (IU/ml)	22.2 ± 22.3	799.8 ± 874.1	987.8 ± 1164.1
CRP (mg/dl)	0.03 ± 0.03	16.2 ± 7.2 ^{†*}	6.1 ± 6.8
BALF Total Cell (×10 ⁵ /ml)	N.A.	10.2 ± 4.2	29.3 ± 27.3
BALF Eosinophil (×10 ⁵ /ml)	N.A.	4.2 ± 1.0	18.1 ± 26.2

N.A., not assessment. [†]*p* < 0.05, compared with healthy, **p* < 0.05, compared with CEP.

leukocytes and CRP were markedly elevated and respiratory failure was more severe. On the other hand, CEP showed eosinophilia in both peripheral blood and BALF, whereas in AEP eosinophilia was shown in BALF only. There were no significant differences in IgE levels between AEP and CEP.

CONCENTRATION OF IL-33 IN THE PATIENTS WITH EP

We first compared the concentrations of serum IL-33 in healthy controls and all EP patients. The serum IL-33 levels in EP patients were 447.7 ± 745.6 pg/ml, much higher than those in controls (27.1 ± 39.9 pg/ml) (Fig. 1a). However, the values of IL-33 in EP were widely distributed, so that the difference in IL-33 levels between EP and controls was not statistically significant.

Then, EP patients were divided into AEP and CEP, and separately reanalyzed. The AEP group showed a significant elevation of serum IL-33 (1344.0 ± 1095.0 pg/ml) compared with controls and CEP (148.9 ± 203.9 pg/ml in CEP) (Fig. 1b). In addition, AEP also showed significant elevation of BALF IL-33 (394.7 ± 318.8 pg/ml). By contrast, the levels of BALF IL-33 in CEP were very low and almost undetectable (0.09 ± 0.24 pg/ml) (Fig. 1c).

In the present study, each one case of AEP and CEP was accompanied with pleuritis. We obtained pleural effusion by thoracocentesis and measured the level of IL-33; it was 681.7 pg/ml in the AEP patient but undetectable in the CEP patient.

CONCENTRATION OF Th2 CYTOKINES IN EP

IL-4, IL-5 and IL-13 are known as representative Th2 cytokines induced by IL-33.¹ Here, we examined the levels of these cytokines in both serum and BALF.

In all healthy controls and CEP patients, each serum IL-4 level was under the detectable level in the present assay system; in contrast, only serum IL-4 in AEP was detectable (3.68 ± 3.78 pg/ml) (Fig. 2a). On the other hand, BALF IL-4 was detected in both

groups, but there were no significant differences between these levels in AEP and CEP (AEP; 3.73 ± 3.24 pg/ml, CEP; 1.67 ± 0.64 pg/ml) (Fig. 2b).

In contrast, serum IL-5 was significantly higher in AEP (AEP, 1000.6 ± 356.8 pg/ml) than in other subjects (healthy, 6.39 ± 8.92 pg/ml; CEP, 19.7 ± 25.7 pg/ml) (Fig. 3a). In BALF analysis, AEP also showed a significant increase of IL-5 (AEP, 717.0 ± 698.9 pg/ml; CEP, 38.4 ± 38.8 pg/ml) (Fig. 3b).

Serum and BALF IL-13 were detectable in both AEP and CEP; however, only serum IL-13 in AEP showed a statistically significant increase compared with other groups (Fig. 4a, b). Titers of serum IL-13 were 3.7 ± 5.5 pg/ml in healthy controls; 34.2 ± 55.9 pg/ml in AEP, and 3.9 ± 6.9 pg/ml in CEP; BALF IL-13 values were 24.0 ± 34.2 in AEP and 4.2 ± 4.0 pg/ml in CEP.

In correlation analyses between Th2 cytokines and IL-33 in patients with EP, only the concentration of IL-5 was significantly correlated with that of IL-33 in both serum (*r* = 0.673, *p* = 0.003) and BALF (*r* = 0.729, *p* = 0.032) (Fig. 3c, d).

CONCENTRATION OF IL-33 IN PATIENTS WITH ARDS

We also examined the levels of IL-33 in patients with ARDS in this study. The clinical features and the level of IL-33 in ARDS are listed and compared with that of AEP in Table 2. Every patient with ARDS showed an increase of the number of leukocytes and CRP as well as AEP. Their respiratory failure was more severe and P/F ratios were consistent with the international criteria of ARDS (91.4 ± 23.8). However, their serum IL-33 levels were not elevated (1.0 ± 2.9 pg/ml), a value equivalent to those of healthy controls. BALF IL-33 was also difficult to detect (2.4 ± 6.2 pg/ml).

UNDERLYING ATOPIC DISEASE AND SERUM IL-33 LEVEL IN CEP

In CEP, the high standard deviation of serum IL-33

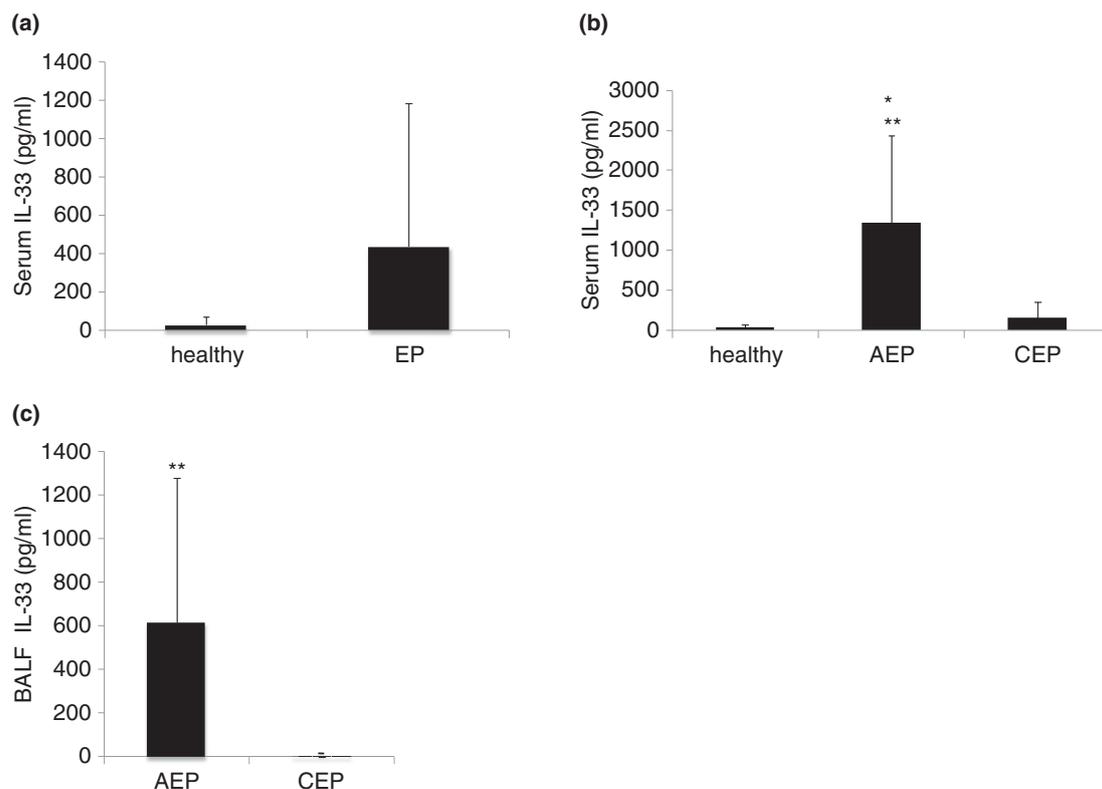


Fig. 1 (a) Concentration of IL-33 in serum. Samples were obtained from healthy volunteers (healthy), patients with eosinophilic pneumonia (EP). Data are presented as the mean \pm SD. healthy: $n = 12$, EP: $n = 16$. (b) Concentration of IL-33 in serum. Samples were obtained from healthy volunteers (healthy), patients with acute eosinophilic pneumonia (AEP) and chronic eosinophilic pneumonia (CEP). Data are presented as mean \pm SD. healthy: $n = 12$, AEP: $n = 4$, CEP: $n = 12$. ** $p < 0.01$, compared with healthy. * $p < 0.05$, compared with CEP. (c) Concentration of IL-33 in BALF. Samples were obtained from patients with AEP and CEP. Data are presented as the mean \pm SD. AEP: $n = 4$, CEP: $n = 12$. ** $p < 0.01$, compared with CEP.

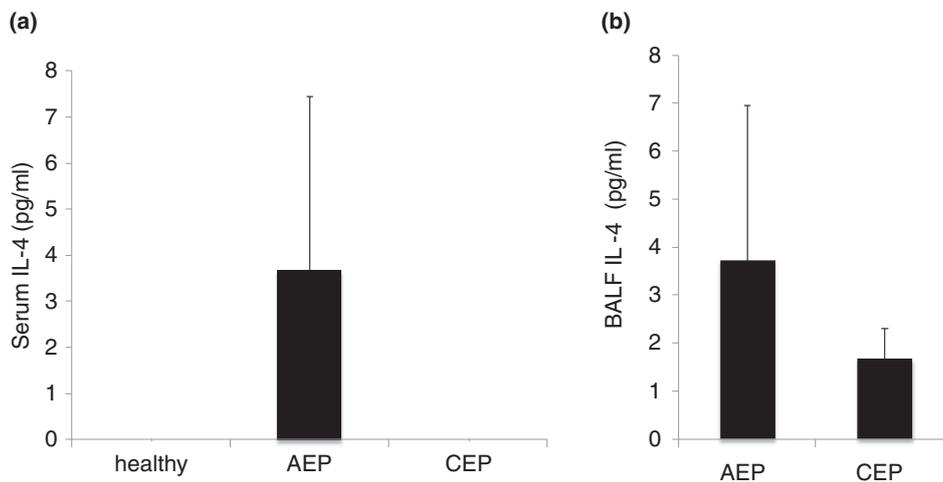


Fig. 2 (a) Concentration of IL-4 in serum. Samples were obtained from healthy volunteers (healthy), AEP and CEP. Data are presented as the mean \pm SD. healthy: $n = 12$, AEP: $n = 4$, CEP: $n = 12$. * $p < 0.05$, compared with healthy and CEP. (b) Concentration of IL-4 in BALF. Samples were obtained from patients with AEP and CEP. Data are presented as the mean \pm SD. AEP: $n = 4$, CEP: $n = 12$.

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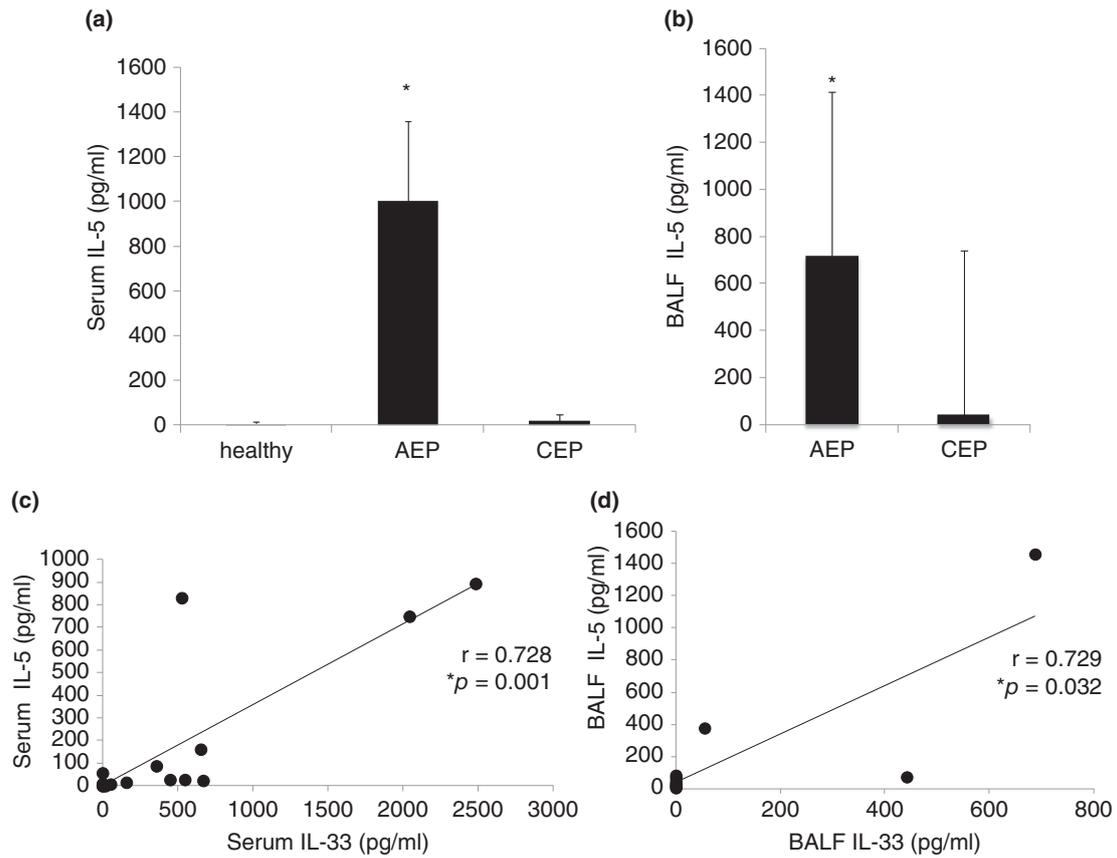


Fig. 3 (a) Concentration of IL-5 in serum. Samples were obtained from healthy volunteers (healthy), AEP and CEP. Data are presented as the mean \pm SD. healthy: $n = 12$, AEP: $n = 4$, CEP: $n = 12$. $*p < 0.05$, compared with CEP. (b) Concentration of IL-5 in BALF. Samples were obtained from patients with AEP and CEP. Data are presented as the mean \pm SD. AEP: $n = 4$, CEP: $n = 12$. $*p < 0.05$, compared with CEP. (c) Correlation analysis was determined by Pearson's correlation test. The correlations between serum IL-33 and IL-5 levels in EP were analyzed ($n = 16$). (d) Correlation analysis was determined by Pearson's correlation test. The correlations between BALF IL-33 and IL-5 levels in EP were analyzed ($n = 16$).

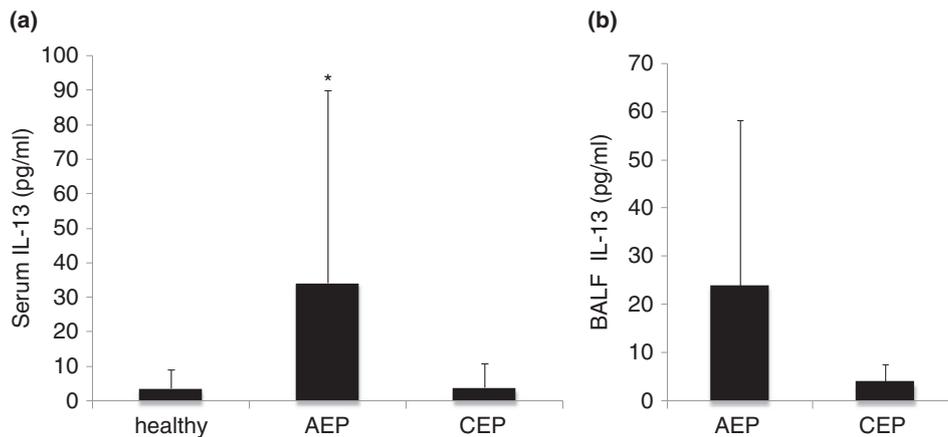


Fig. 4 (a) Concentration of IL-13 in serum. Samples were obtained from healthy volunteers (healthy), patients with AEP and CEP. Data are presented as the mean \pm SD. healthy: $n = 12$, AEP: $n = 4$, CEP: $n = 12$. (b) Concentration of IL-13 in BALF. Samples were obtained from patients with AEP and CEP. Data are presented as the mean \pm SD. AEP: $n = 4$, CEP: $n = 12$. $*p < 0.05$, compared with healthy and CEP.

Table 2 Clinical features and concentrations of IL-33 in ARDS and AEP

	Age	M/F	WBC ($\times 10^3/\mu\text{l}$)	P/F ratio	CRP (mg/dl)	Serum IL-33 (pg/ml)	BALF IL-33 (pg/ml)
ARDS ($n = 12$)	69.7 \pm 8.6*	8/4	7.5 \pm 5.4	91.4 \pm 23.8*	17.6 \pm 7.1	1.0 \pm 2.9	2.4 \pm 6.2
AEP ($n = 4$)	22.0 \pm 1.7	4/0	18.8 \pm 6.8	215.6 \pm 48.9	16.2 \pm 7.2	1344.0 \pm 1095.0*	616.1 \pm 665.7*

* $p < 0.05$, compared between ARDS and AEP.

Table 3 Concentration of serum IL-33 in CEP with or without atopic disease

	Age	M/F	WBC ($\times 10^3/\mu\text{l}$)	Eosinophil ($\times 10^3/\mu\text{l}$)	IgE (IU/ml)	Serum IL-33 (pg/ml)
CEP with atopic disease ($n = 7$)	44.0 \pm 22.5	3/4	11.6 \pm 4.7	4.1 \pm 2.8	1357.9 \pm 1486.5*	255.3 \pm 215.2
CEP without atopic disease ($n = 5$)	60.8 \pm 26.0	3/2	11.9 \pm 2.3	2.9 \pm 1.0	206.5 \pm 93.4	undetectable

* $p < 0.05$, compared between CEP with or without atopic disease.

indicated a wide range of titers of IL-33 (148.9 \pm 203.9 pg/ml). In this study, fifty-eight percent of CEP patients (7 patients) had a history of atopic disease such as bronchial asthma or allergic conjunctivitis or rhinitis; therefore, we focused our attention on atopic disease and divided CEP patients into two groups according to those with or without atopic disease and compared serum IL-33 levels (Table 3). CEP patients with atopic disease had significantly elevated serum levels of IgE and IL-33 (255.3 \pm 215.2 pg/ml). In contrast, IgE was almost within the normal range and IL-33 was undetectable in non-atopic CEP patients.

DISCUSSION

Several experiments employing rodents have demonstrated that IL-33 played important roles in allergic disease^{1,7,8}; however, studies focusing on the clinical significance of IL-33 in humans have been few.⁹⁻¹¹ In this study, we investigated the IL-33 level in EP patients and demonstrated that IL-33 was especially increased in both serum and BALF in AEP. This is the first report to evaluate the level and significance of IL-33 in EP.

In the present study, the level of BALF IL-33 was significantly increased in patients with AEP; it was 394.7 \pm 318.8 pg/ml, which was estimated to be about 20% of that of serum levels (1344.0 \pm 1095.0 pg/ml). Allen *et al.* previously pointed out that a high concentration of BALF IL-5 in patients with AEP suggested the local production of IL-5 in lungs.¹⁹ BAL is generally performed by a large volume of saline, and BALF normally contains approximately 1/100 of the usual plasma protein concentration.²³ Therefore, the high concentration of BALF IL-33 in AEP suggests that IL-33 is also locally produced in the lungs and released into the airway. Although histological analysis could not be performed in this study, several reports have demonstrated the increase of IL-33 expression in epithelial cells of the lung.^{1,24} Considering the high val-

ues in BALF, airway epithelial cells are regarded as a main candidate for the IL-33-producing cells in AEP.

In the *in vitro* experiment, it was elucidated that intracellular IL-33 was cleaved by caspase-3 or -7 and became biologically inactive in apoptotic cells²⁵; meanwhile, IL-33 was released as an active full-length form from necrotic cells.^{25,26} Pathological findings of AEP are traditionally characterized as diffuse alveolar damage; therefore, we initially expected that local increase of IL-33 in AEP was derived from cell necrosis by severe lung injury. Then, to confirm whether the release of IL-33 actually depended on the tissue damage, we also analyzed IL-33 levels in patients with ARDS, which was a representative disorder with acute lung injury. To our surprise, we found no increase of IL-33 in serum and BALF, despite severe respiratory failure and pulmonary parenchymal damage. From this result, we know that release of IL-33 is not simply dependent on tissue injury; it is likely that specific mechanisms to induce IL-33 are activated in AEP. Cigarette smoking is regarded as a main trigger of AEP, and several impressive studies have documented how cigarette smoking elicited oxidative stress and induced the release of lactate dehydrogenase and cell necrosis in airway epithelial cells.^{27,28} The precise mechanism of the release of IL-33 could not be explored here; however, specific acute stimuli like cigarette smoking are considered to be associated with cell necrosis and production of IL-33 in epithelial cells in AEP. By contrast, it was reported that serum IL-33 was decreased in patients with emphysema who had long smoking histories.¹¹ Further *in vivo* and *in vitro* basic study is required to explore the secretion pathway of IL-33 by cigarette smoking. On the other hand, several studies have demonstrated that apoptosis of epithelial cells is implicated in the pathogenesis of ARDS^{29,30}; namely, DNA fragmentation and increase of caspase-3 have been observed in alveolar cells in ARDS. Together with the evidence

that IL-33 was cleaved by caspase and inactivated during apoptosis,²⁵ we speculate that inactivation of IL-33 in apoptotic epithelial cells would contribute to the very low values of IL-33 in ARDS.

Th2 cytokines, such as IL-4, IL-5, and IL-13 were also examined in this study. Although these cytokines of sera were significantly elevated in AEP, only IL-5 was significantly higher in both serum and BALF in AEP, and the level of IL-5 was positively correlated with that of IL-33. It was established that IL-33 induces a Th2-immuno response via IL-4, IL-5, and IL-13,^{1,5,6} but Kurowska-Stolarska *et al.* demonstrated that IL-33 polarized naïve CD4 (+) cells into IL-5-producing T cells predominantly and exacerbated airway inflammation independent of IL-4.³¹ Allakhverdi *et al.* also showed that IL-33 stimulated hematopoietic progenitor cells to release IL-5 and IL-13, but not IL-4.³² In addition, Cherry *et al.* found that IL-33 has a synergistic effect with IL-5 in the activation of eosinophils.⁵ Our study was consistent with previous studies in the strong correlation between IL-33 and IL-5; it is likely that these two cytokines have a synergistic effect in inducing pulmonary eosinophilia.

It was recently demonstrated that circulating hematopoietic progenitor cells had IL-33 receptors, and when directly stimulated by IL-33, matured to be eosinophils in an IL-5-dependent manner.^{32,33} In addition, under allergic conditions, hematopoietic progenitors are distributed in pulmonary tissue and differentiate to eosinophils locally, as well as in bone marrow.³⁴ In this study, IL-33 and IL-5 were strongly induced in AEP compared with CEP, and based on the function of IL-33 for the differentiation of eosinophils, it is speculated that IL-33 and IL-5 might contribute not only the chemotaxis of mature eosinophils from bone marrow, but also the local differentiation of eosinophils from progenitors in the pulmonary tissue in AEP. In addition, various cytotoxic proteins from accumulated eosinophils, such as major basic protein and eosinophil cationic protein, are able to damage airway epithelial cells and possibly contribute to further necrosis and production of IL-33. Thus, eosinophilia could be accelerated by IL-33, like a paracrine loop, in the injured tissue of AEP.

On the other hand, IL-33 was hardly detected in BALF of all patients with CEP, and only the seven CEP patients with atopic disease showed a mild increase of serum IL-33 (255.3 ± 215.2 pg/ml pg/ml), equivalent to the levels previously reported in patients with atopic disease.^{9,11} Based on this result, we speculate that the serum IL-33 level in CEP is dependent on underlying atopic disease. Furthermore, because eosinophilia is mildly pre-existing under the influence of atopic disease or proceeds slowly in CEP, rapid chemotaxis and differentiation of eosinophils by IL-33 seems not to be a pivotal process in CEP.

Because of the few opportunities to encounter patients with EP, obtaining sufficient numbers of sub-

jects for analyses was difficult in the present study. In the future, more large-scale studies are needed to explore the clinical implication of IL-33. In addition, in this study, we selected ELISA as a method to detect IL-33 protein. We could quantify the level of IL-33 by ELISA; however, this procedure could not visualize the molecular weight of IL-33. It has been believed that full-length IL-33 is the only biologically active form,^{25,26,35} however, Lefrançois *et al.* recently demonstrated that IL-33 cleaved by neutrophil elastase or cathepsin G showed stronger biological activities than full-length IL-33.³⁶ This evidence suggests the possibility that highly active IL-33 derived from inflammatory tissue contains not only full-length IL-33 but also IL-33 variously cleaved by several proteases released from inflammatory cells. In future, we will investigate the varieties of IL-33 in patients with EP by western blotting.

Regardless of this limitation, and in spite of the small scale of this study, we believe our findings provide a basis to develop future investigations of IL-33 and pulmonary eosinophilia.

In conclusion, IL-33 is considered to be a local key molecule for triggering pulmonary eosinophilia in AEP, together with IL-5. BALF IL-33 appears to be a very useful marker for discriminating AEP from CEP and ARDS.

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