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Original Article

Interleukin-18 is associated with the severity of atopic dermatitis

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ABSTRACT

Background: Interleukin (IL)-18 acts as both a Th1 and Th2 cytokine, but its association with allergic diseases remains unclear. The aim of the present study was to measure plasma IL-18 and serum IgE levels in atopic children to evaluate how IL-18 is associated with allergic diseases.

Methods: The plasma IL-18 and serum IgE levels in 51 atopic children, 28 healthy control children and 14 healthy control adults were measured by enzyme-linked immunosorbent assay (ELISA). The 5' end of the IL-18 gene of 48 atopic children and 20 healthy control children was sequenced.

Results: The plasma IL-18 level was significantly elevated in children with bronchial asthma and/or atopic dermatitis. Plasma IL-18 levels in the moderate or severe atopic dermatitis group were significantly higher than those in either the control group or the mild atopic dermatitis group. There was a positive correlation between plasma IL-18 and serum IgE levels. Three allelic combinations of polymorphisms in the IL-18 gene promoter region were observed. There was no significant difference in the plasma IL-18 levels between groups carrying these genotypes. However, bronchial asthma patients had significantly higher frequencies of the -137 G/G genotype than did control children.

Conclusions: The plasma IL-18 level was elevated, particularly in patients with atopic dermatitis. As the clinical severity of atopic dermatitis increased, the

plasma IL-18 level also tended to increase. These findings suggest that IL-18 may be associated with the severity of atopic dermatitis.

Key words: atopic dermatitis, bronchial asthma, IgE, interferon- γ , interleukin-18, interleukin-18 promoter.

INTRODUCTION

Interleukin (IL)-18, originally known as an interferon (IFN)- γ -inducing factor (IGIF), is a recently cloned cytokine secreted by Kupffer cells of the liver and activated macrophages.¹ Interleukin-18 strongly enhances IFN- γ production by T cells, natural killer cell cytotoxicity and T cell proliferation. Moreover, recent studies have demonstrated that IL-12, which is also known as an IFN- γ -inducing factor, and IL-18 exert a synergistic effect on IFN- γ production by T cells.^{2,3}

Interleukin-18 is a proinflammatory cytokine. Its increased production was observed in the acute phase of experimental autoimmune encephalomyelitis (EAE)⁴ and antibodies to IL-18 could prevent EAE in Lewis rats.⁵ In humans, IL-18 expression has been observed in Th1-mediated chronic inflammatory diseases, such as Crohn's disease,⁶ rheumatoid arthritis⁷ and acute infectious mononucleosis.⁸ Increased serum or plasma IL-18 levels have been observed in severe melioidosis,⁹ hemophagocytic lymphohistiocytosis¹⁰ and multiple sclerosis.¹¹ Increased IL-18 levels in cerebral spinal fluid have been observed in bacterial meningitis¹² and multiple sclerosis.¹¹

However, how IL-18 is associated with allergic diseases remains unclear. We have reported previously that the serum IgE level was negatively correlated with IFN- γ production by phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC).¹³ The IFN- γ

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production by IL-12-stimulated PBMC in the atopic group was lower than that in the control group and the serum IgE level was negatively correlated with IFN- γ production by IL-12-stimulated PBMC.¹⁴ One of the reasons for this was that reduced IFN- γ production following IL-12 stimulation was associated with heterozygous IL-12R β 2 mutations in atopic patients.¹⁵ The presence of abnormalities not only in the IL-12, but also in the IL-18, signal transduction pathway could be assumed in atopic patients. However, recently there have been some reports that IL-18 is also associated with Th2 reactions.¹⁶⁻¹⁹ Nakanishi *et al.* reviewed the dual regulatory roles of IL-18 in the immune system; that is, IL-18 regulates not only the Th1 pathway, but also the Th2 pathway.²⁰

In the present study, we found a correlation between the plasma IL-18 level and the severity of atopic dermatitis in children.

METHODS

Patients and control subjects

All patients and healthy volunteers were recruited from the Department of Pediatrics, Gifu University School of Medicine, and informed consent was obtained from all subjects or their parents. Fifty-one children with allergic diseases, particularly atopic dermatitis and bronchial asthma, 28 healthy control children and 14 healthy control adults were studied. Seventeen patients had bronchial asthma only (eight patients were in the no asthma attack phase, nine patients were in the asthma attack phase), 18 patients had atopic dermatitis only and 16 patients had both bronchial asthma and atopic dermatitis. The diagnosis of atopic dermatitis was made according to the criteria of Hanifin,²¹ whereas that of bronchial asthma was made according to the criteria of the American Thoracic Society.²² The severity of atopic dermatitis was evaluated based on the criteria of Rajka and Langeland.²³ The serum IgE levels, age and sex of the study subjects are listed in Table 1. The

healthy controls did not have a history of atopic diseases and their serum IgE levels were within normal limits for their age. They were healthy and free of acute infection at the time of testing.

Plasma and cell preparation

Plasma and leukocytes were separated from the heparinized blood of control donors and patients. All plasma samples were stored at -30°C until assay.

Interleukin-18 assay

Plasma IL-18 levels were measured with a human IL-18 enzyme-linked immunosorbent assay (ELISA) kit (Medical & Biological Laboratories, Nagoya, Japan); the lower detection limit was 12.5 pg/mL.

IgE assay

Plasma or serum IgE levels were determined by chemiluminescent enzyme immunoassay. Plasma IgE values were regarded as serum IgE levels. Specific IgE antibodies for house dust, *Dermatophagoides*, hen egg and cows' milk were measured with a fluoroenzyme immunoassay by means of a Uni-Cap assay kit (Pharmacia, Uppsala, Sweden). Scores of 3+ to 6+ were considered positive.

Sequencing of the 5' end of the IL-18 gene

Genomic DNA was extracted from leukocytes using a SepaGene (Sanko Junyaku, Tokyo, Japan). A 1492 bp fragment of the IL-18 gene was amplified by polymerase chain reaction (PCR) using primers 5'-TTGATCCCACT-TCGTGCTTTCA-3' and 5'-CCTTTCCTCTCCCGAA-GCTGT-3'. Conditions for the PCR were 40 cycles of 94°C for 1 min, 64°C for 1 min and 72°C for 2 min. DNA was purified using geneclean II (BIO 101, Carlsbad, CA, USA) and used for big dye terminator bidirectional sequencing (Applied Biosystems, Foster

Table 1 Subject characteristics

	Control		Bronchial asthma	Atopic dermatitis	Atopic dermatitis + bronchial asthma
	Adults	Children			
No. subjects	14	28	17	18	16
Age (years)	33.9 \pm 9.4	4.8 \pm 4.4	8.1 \pm 4.1	4.9 \pm 4.6	5.1 \pm 3.8
Sex (males/females)	5/9	16/12	14/3	13/5	10/6
IgE (IU/mL)	8-140	5-110	24-2400	11-11 000	18-5400

City, CA, USA). Primers 5'-CCCTCCTAGCAAAG-TAATAC-3', 3'-GAATAATCAGTCCTATTGGGG-5', 5'-CCAATAGGACTGATTATTCCGCA-3' and 3'-AGGAG-GGCAAATGCACTGG-5 were used for sequencing an approximate 700 bp fragment upstream of the known IL-18 cDNA sequence. For the position -607 specific PCR, a common reverse primer 5'-TAACCTCATTGAG-GACTTCC-3' and two sequence-specific forward primers 5'-GTTGCAGAAAAGTGAAAAATTATTAC-3' and 5'-GTTGCAGAAAAGTGAAAAATTATTAA-3' were used. For the position -137 specific PCR, a common reverse primer 5'-AGGAGGGCAAATGCACTGG-3' and two sequence-specific forward primers 5'-CCCCAACTTT-TACGGAAGAAAAG-3' and 5'-CCCCAACTTTTACG-GAAGAAAAC-3' were used. Each PCR was performed with rTaq (Takara Shuzo, Shiga, Japan) and the conditions for PCR were 40 cycles of 94°C for 1 min, 64°C for 1 min and 72°C for 1 min. Products separated by 2% agarose gel electrophoresis were visualized by staining with ethidium bromide.

Statistical analyses

The significance of differences between two groups was analyzed by the Mann-Whitney *U*-test. The significance of differences between multiple groups was evaluated by the Kruskal-Wallis test and further analysis was performed by the Bonferroni/Dunn test. Spearman's correlation coefficient (*R*) was used to calculate the correlation between two variables. The frequencies of alleles were compared through the use of χ^2 statistics. Statistical significance was assumed for $P < 0.05$.

RESULTS

Mean (\pm SD) plasma IL-18 levels in patients with allergic diseases (394 ± 20 pg/mL), healthy control adults (201 ± 79 pg/mL) and control children (174 ± 87 pg/mL) are shown in Fig. 1a. We found significantly higher levels of plasma IL-18 in patients with allergic diseases ($P < 0.0001$) than in healthy control children. There was no significant difference in plasma IL-18 levels between healthy control adults and healthy control children.

In the present study, we particularly investigated bronchial asthma and atopic dermatitis as representative allergic diseases in children. Mean (\pm SD) plasma IL-18 levels in patients with bronchial asthma (324 ± 122 pg/mL), atopic dermatitis (508 ± 238 pg/mL) and atopic dermatitis and bronchial asthma (338 ± 236 pg/mL)

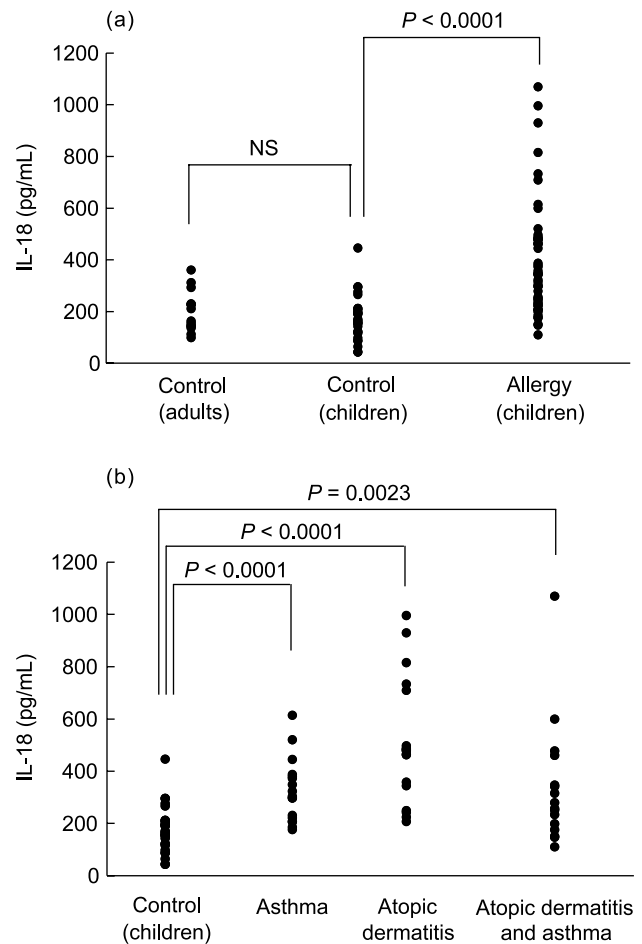


Fig. 1 (a) Comparison of plasma interleukin (IL)-18 levels in control adults, control children and children with allergic disease. Plasma IL-18 levels in children with allergic disease were significantly higher than those in healthy control children. (b) Comparison of plasma IL-18 levels in control children and children with bronchial asthma, atopic dermatitis and atopic dermatitis plus bronchial asthma. Plasma IL-18 levels in children with bronchial asthma, atopic dermatitis and atopic dermatitis plus bronchial asthma were significantly higher than those of healthy control children.

are shown in Fig. 1b. We found significantly higher levels of plasma IL-18 in the bronchial asthma group ($P < 0.0001$), the atopic dermatitis group ($P < 0.0001$) and the atopic dermatitis and bronchial asthma group ($P = 0.0023$) compared with healthy control children. Furthermore, the plasma IL-18 level of the atopic dermatitis group was particularly high. The atopic dermatitis group has significantly higher levels of plasma IL-18 than the bronchial asthma group ($P = 0.001$). There was no significant difference in plasma IL-18 levels between the

acute phase (323 ± 153 pg/mL) and non-acute phase of asthma (325 ± 85 pg/mL).

Figure 2a,b shows the plasma IL-18 and serum IgE levels of each group classified according to the severity of atopic dermatitis with or without bronchial asthma.²³ Using the Kruskal–Wallis test, we found significant differences in levels of plasma IL-18 ($P < 0.0001$) and serum IgE ($P < 0.0001$) between groups of patients classified

according to the severity of atopic dermatitis. Further analysis using the Bonferroni/Dunn test was performed for comparisons among groups. Plasma IL-18 levels in the moderate or severe atopic dermatitis group were significantly higher than those in either the control group or the mild atopic dermatitis group. Serum IgE levels in the severe atopic dermatitis group were significantly higher than those in the other groups and serum IgE levels in the mild and moderate atopic dermatitis groups were significantly higher than those in the control group. Figure 2c shows the relationship between plasma IL-18 and serum IgE levels in healthy controls and patients. It was reported that serum IL-18 levels in Nc/Nga mice tended to be negatively correlated with serum IgE levels²⁴ but, in the present study, there was a positive correlation between plasma IL-18 and serum IgE levels ($R = 0.472$; $P < 0.0001$). Three patients (P1, P2, P3) had higher plasma IL-18 levels more than the control group + 2SD, but their serum IgE levels were within normal limits for their age. These three patients were also negative for specific IgE.

We analyzed the 5' end of the IL-18 gene sequence in 48 allergic patients and 20 healthy control children. Five single-nucleotide polymorphisms were detected. Two are located in the 5'-untranslated region of the IL-18 gene and three are located in the promoter region. Three allelic combinations of the polymorphisms observed in the Japanese populations were the same as those found in the Swedish population²⁵ (Table 2). Figure 3 shows the

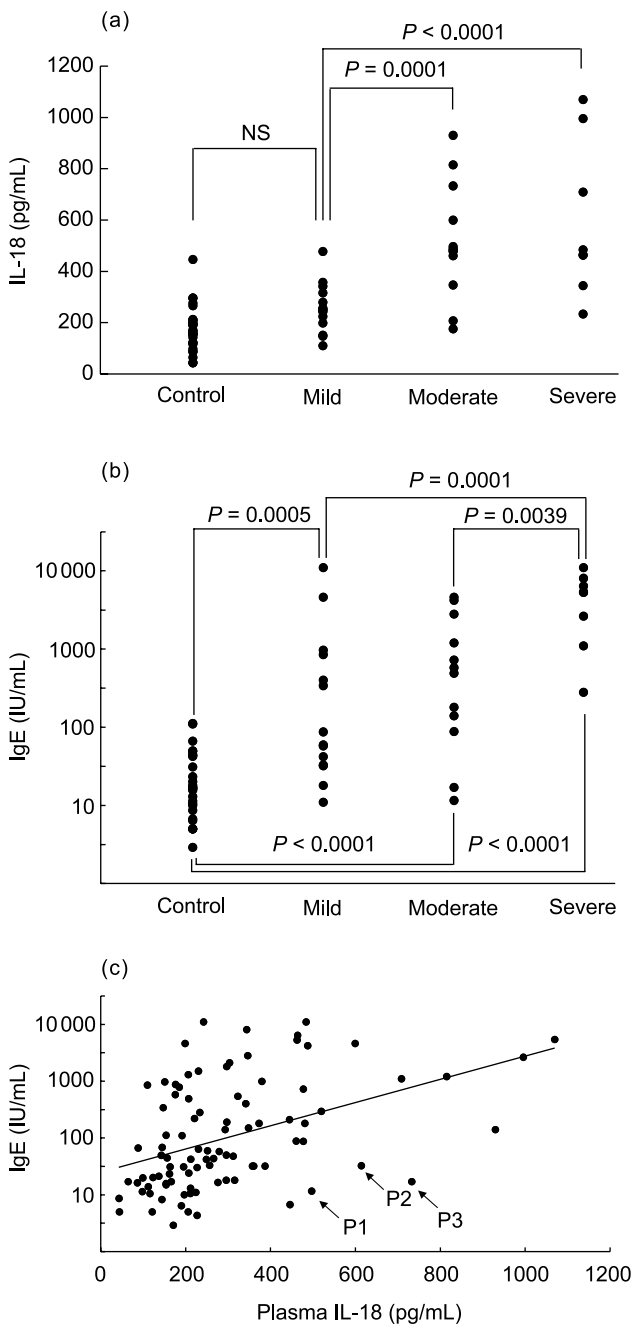
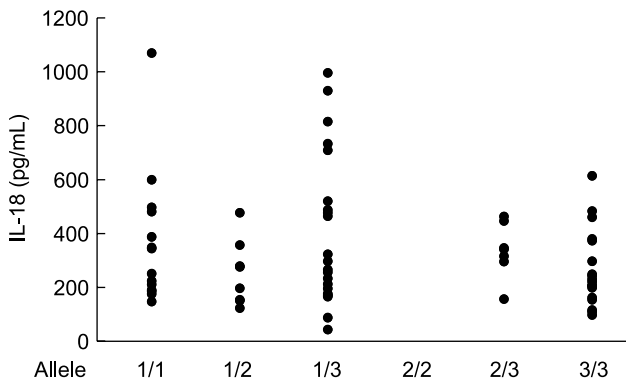


Fig. 2 (a) Relationship between plasma interleukin (IL)-18 levels and severity of atopic dermatitis. Plasma IL-18 levels in the moderate or severe atopic dermatitis group were significantly higher than those in the control group or mild atopic dermatitis group (mild ($n = 14$) 257 ± 95.9 pg/mL; moderate ($n = 12$) 549 ± 226 pg/mL; severe ($n = 8$) 595 ± 302 pg/mL). (b) Relationship between serum IgE levels and the severity of atopic dermatitis. Serum IgE levels in the severe atopic dermatitis group were significantly higher than those in the other groups and serum IgE levels in the mild and moderate atopic dermatitis groups were significantly higher than those in the control group (mild ($n = 14$) 1320 ± 3030 IU/mL; moderate ($n = 12$) 1250 ± 1660 IU/mL; severe ($n = 8$) 5020 ± 3600 IU/mL). (c) Relationship between plasma IL-18 levels and serum IgE levels in healthy controls and patients. Plasma IL-18 levels were significantly and positively correlated with serum IgE levels ($R = 0.472$; $P < 0.0001$). Arrows indicate patients with high plasma IL-18 levels, but low serum total IgE levels; they were also negative for specific IgE (patient 1, patient 2, patient 3).

Table 2 Polymorphisms at the 5' end of the interleukin-18 gene

	Position				
	-656	-607	-137	+113	+127
Allele 1	G	C	G	T	C
Allele 2	T	A	C	G	T
Allele 3	T	A	G	T	C

**Fig. 3** Relationship between levels of plasma interleukin (IL)-18 and genotypes of the 5' end of IL-18 gene polymorphisms in atopic patients and healthy control children. There was no significant difference ($P = 0.3596$) between these groups (1/1 ($n = 14$) 365 ± 246 pg/mL; 1/2 ($n = 8$) 252 ± 121 pg/mL; 1/3 ($n = 21$) 408 ± 282 pg/mL; 2/2 ($n = 0$); 2/3 ($n = 7$) 267 ± 143 pg/mL; 3/3 ($n = 18$) 336 ± 217 pg/mL).**Table 3** Genotype frequencies in 48 allergic patients and 20 healthy control children

Genotype	Control	Allergic patients
1/1	2	12
1/2	4	4
1/3	7	14
2/2	0	0
2/3	3	4
3/3	4	14
<i>P</i>		0.3559

Table 4 Frequencies of single nucleotide polymorphism -137 G/C of the interleukin-18 promoter region in patients and healthy control children

Genotype	Control ($n = 20$)	Allergy ($n = 48$)	Bronchial asthma ($n = 14$)	Atopic dermatitis ($n = 18$)	Atopic dermatitis + bronchial asthma ($n = 16$)
G/G	13	40	14	15	11
G/C	7	8	0	3	5
C	/ 0	C 0	0	0	0
<i>P</i>		0.0967	0.0130	0.2000	0.8125

plasma IL-18 levels of children with different combinations of alleles. Children with the genotype 1/1 and 1/3 showed a relatively higher mean level of plasma IL-18 (1/1, 365 ± 246 pg/mL; 1/3, 408 ± 282 pg/mL). However, there was no significant difference between these groups ($P = 0.3596$). Table 3 shows genotype frequencies of the IL-18 promoter region in allergic patients and healthy control children. There were no significant differences. Further analysis was performed for comparisons among allergic patients and healthy control children at position -137 of the IL-18 promoter region. Table 4 shows the frequencies of single nucleotide polymorphism -137 G/C of the IL-18 promoter region in allergic patients and healthy control children. Bronchial asthma patients had significantly higher frequencies of the -137 G/G genotype than did control children.

DISCUSSION

Interleukin-18 was initially identified as a strong inducer of a Th1-mediated cytokine, IFN- γ , and this function is synergistically performed with IL-12.²⁶ Moreover, IgE suppression had been thought as the result of IFN- γ induction by IL-18 because the serum IgE level was negatively correlated with the amount of IFN- γ secreted by the PBMC of atopic patients.¹³ However, it was reported that IL-18 levels in the sera of adult patients and in atopic dermatitis model mice (Nc/Nga) were elevated.²⁴ In the present study, we showed that the plasma IL-18 levels increased significantly in children with allergic diseases, particularly atopic dermatitis. Tanaka *et al.* reported that the serum IL-18 level may reflect asthma disease activity,²⁷ but we found no significant difference in plasma IL-18 levels between the acute and non-acute phases of asthma in the present study. In contrast with patients with bronchial asthma, the plasma IL-18 levels of patients with atopic dermatitis tended to increase as the severity of the disease increased.

Why is the IL-18 level elevated particularly in patients with bronchial asthma and severe and moderate atopic dermatitis? One of the possible reasons is the association with lipopolysaccharide (LPS) or endotoxin. Jorgensen *et al.* reported that, in leukocytes from patients with atopic dermatitis, bacteria and endotoxin induce the release of basophil histamine.²⁸ Recently, it was reported that endotoxemia was associated with elevations in the plasma IL-18 level in infections after acute lung injury.²⁹ Seki *et al.* reported that IL-18 secretion is mediated by activation of endogenous caspase-1 without *de novo* protein synthesis after stimulation with LPS.³⁰ El-Mezzein *et al.* reported an increase in the secretion level of IL-18 by LPS-stimulated PBMC of patients with bronchial asthma or atopic dermatitis.³¹ Therefore, LPS and endotoxin could be associated with caspase-1 activity and IL-18 secretion in atopic dermatitis.

The second possible reason is differences in IL-18 promoter activity among IL-18-producing cells in the patients. Vilmantas *et al.* reported that alleles 1 and 3 of IL-18 promoter region polymorphisms showed a higher transcriptional activity than allele 2, as determined by luciferase assay using transfected human HeLa 229 cells that were stimulated with phorbol myristate acetate and ionomycin.²⁵ A change from C to A at position -607 disrupts a potential cAMP-responsive element binding protein binding site. A change from G to C at position -137 changes the H4TF-1 nuclear factor binding site to a binding site for an unknown factor found in the granulocyte-macrophage colony stimulating factor promoter.²⁵ In the present study, children with the genotype 1/1 and 1/3 showed a relatively higher average level of plasma IL-18, but the association of plasma IL-18 level with polymorphisms of IL-18 promoter alleles and their frequencies was not significant. However, the -137 G/G genotype had a significantly higher frequency in bronchial asthma patients than in control children. Therefore although the number of subjects is too small to conclusively determine the association between plasma IL-18 and these polymorphisms, we can speculate that alleles 1 and 3, which had G at position -137 of the IL-18 promoter region, may affect the allergy state through IL-18.

Another possibility is the existence of abnormalities in the signal transduction pathway of IL-18. Matsui *et al.* reported that IL-12R β 2 mutations resulted in a decrease of IFN- γ production.¹⁵ Similarly, Shikano *et al.* reported that, in several atopic patients, IFN- γ production was not induced sufficiently by IL-18, but it was induced sufficiently by phytohemagglutinin or IL-12.³² In our recent

study on the IL-18 receptor, 950delCAG in the IL-18R α chain cDNA was found to be associated with reduced IFN- γ production and a high serum IgE level in atopic patients.³³ Mühl *et al.* reported that IFN- γ upregulated the expression of IL-18 binding protein, which is an inhibitor of IL-18 activity, suggesting a negative feedback mechanism between IL-18 and IFN- γ .³⁴ Therefore, abnormalities of IL-18 signal transduction to IFN- γ production and the impairment of the negative feedback mechanism may induce the increase in plasma IL-18 levels.

How is IL-18 associated with exacerbation of atopic dermatitis? Atopic dermatitis is a chronic inflammatory skin disease, with remissions and exacerbations. It is generally known that serum IgE levels are elevated in 80% of patients with atopic dermatitis^{35,36} and the severity of atopic dermatitis highly correlates with the levels of serum IgE.³⁵ In the present study, as the clinical severity of atopic dermatitis increased, the serum IgE and plasma IL-18 levels tended to increase. Moreover, the plasma IL-18 levels were significantly correlated with serum IgE levels. Yoshimoto *et al.* showed that, in the presence of IL-3, IL-18 induces basophils and mast cells to release large amounts of Th2 cytokines both *in vitro* and *in vivo*.¹⁸ Moreover, IL-18 alone has the capacity to induce IgE accumulation *in vivo*.^{18,19} Therefore, IL-18 may be associated with allergic diseases, particularly atopic dermatitis exacerbation through IgE production.

It is interesting that three patients (P1, P2, P3) had high plasma IL-18 levels, but low serum total IgE levels; they were also negative for specific IgE. In fact, many allergic patients have low serum total IgE levels and specific IgE levels. It has been reported that 20% of atopic dermatitis patients with typical eczema have normal serum IgE levels.^{21,37} Yoshimoto *et al.* reported that IL-18 could directly stimulate histamine release by basophils.¹⁸ Recently, Tsutsui *et al.* also reported that keratinocyte-caspase-1 transgenic/signal transducers and activators of transcription (STAT) 6-deficient mice developed atopic dermatitis without IgE production and their serum IL-18 levels were high.³⁸ Moreover, Yagi *et al.* recently reported that STAT6-deficient NC/Nga mice showed development of atopic dermatitis, although these mice failed to produce IgE and IL-18 had been highly expressed at the skin lesions.³⁹ Interleukin-18 may participate in atopic dermatitis exacerbation by histamine production from basophils or mast cells without IgE production.

In the present study, we found that the plasma IL-18 level was elevated in children with bronchial asthma and atopic dermatitis. The plasma IL-18 level showed a

positive correlation with serum IgE levels. Furthermore, we showed that the plasma IL-18 level correlated with the clinical severity of atopic dermatitis. Interleukin-18 has dual roles in the Th1/Th2 system depending on conditions, such as the existence of antigens or other cytokines, including IL-10.⁴⁰ Further studies of cytokine profiles in allergic diseases and analysis of gene polymorphisms of IL-18 and IL-18-related molecules (IL-18R α , IL-18R β , MyD88, IL-1 receptor-associated kinase (IRAK), TNF receptor-associated factor (TRAF) 6) are important in order to clarify the association of IL-18 with atopic diseases.^{41–43}

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