

Relationship between serum levels of interleukin-18, IgE and disease severity in patients with atopic dermatitis

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Summary

Background. Interleukin (IL)-18 is a pleiotropic cytokine. Synergistically with IL-12, IL-18 promotes immune responses of the T helper type, by enhancing synthesis of interferon- γ and inhibiting IgE production. IL-18 can also enhance production of IL-4 and IL-13 production, and stimulate synthesis of IgE. Moreover, in the presence of IL-3, IL-18 can directly stimulate basophils and mast cells to produce their mediators in an IgE-independent manner. These results indicate a role for IL-18 in the pathogenesis of atopic dermatitis (AD).

Aim. To examine the association of serum IL-18 with IgE levels and disease severity in patients with AD.

Methods. ELISA was used to measure IL-18 and total IgE levels in the sera of 67 patients with AD and 50 healthy volunteers. The SCORing Atopic Dermatitis (SCORAD) tool was used to determine the severity of this disease.

Results. The mean serum level of IL-18 in study group (155.08 pg/mL) was significantly higher than that of controls. IL-18 was also significantly higher in the sera of the patients with severe AD than in those with milder disease. There was a correlation with IgE and IL-18 levels, as patients who had high IgE levels also had high IL-18 levels, compared with controls.

Conclusion. IL-18 seems to play an important role in the pathogenesis of AD, but this requires further study. IL-18 could be a useful clinical marker of disease severity in AD.

Introduction

Atopic dermatitis (AD) is a chronic, clinically variable, inflammatory skin disease.^{1,2} It is associated with severe itch, which can have a detrimental effect on the quality of life of patients and their families.^{3–5} Although many studies have been carried out investigating the immunological factors affecting the disease, the genetic basis and the pathogenesis underlying the skin-barrier

dysfunction remain unclear. Recently, interleukin (IL)-18 has been implicated in the development of atopic disease.^{6–14}

IL-18 was first described in 1989, and cloned in 1995 as a single peptide chain with a molecular weight of around 18 kDa.^{15,16} It is produced by a variety of cells, including keratinocytes, macrophages, monocytes, dendritic cells, osteoblastic stromal cells, epithelial cells, microglia and ependymal cells.^{14,15,17,18} IL-18 is synthesized as an inactive precursor pro-IL-18 (24 kDa), which is subsequently cleaved and activated by caspase-1.^{8,15,17} The IL-18 receptor is present on T lymphocytes, dendritic cells, mast cells, basophils, macrophages, neutrophils and natural killer (NK) cells.^{15,19} It is a heterodimer composed of two units: the IL-18 receptor (IL-18R) α , responsible for extracellular binding of

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IL-18; and IL-18R β , responsible for signal transduction.¹⁶ Binding of IL-18 to its receptor leads to activation of IL-1 receptor-associated kinase, which in turn activates tumour necrosis factor receptor-associated factor-6. Thereafter, the pathway leads through the mitogen-activated protein kinases, and then to the c-Jun N-terminal kinase. Finally, activator protein-1 and nuclear factor (NF) κ B are activated.^{6,7,15,16,20} NF κ B upregulates cytokine gene expression, and elevates transcription of various cytokines, including IL-4, IL-13, and interferon (IFN) γ .⁷

IL-18 was shown to be a member of the IL-1 superfamily,²¹ and was originally thought to be an IFN γ -inducing factor because of its action on T and NK cells.^{9,12,14,19–21} A synergistic effect of IL-12 and IL-18 causes the T helper (Th)1 immune response to become predominant, and also inhibits IgE synthesis and exhibits antiallergy properties.^{14,17} IL-18 can also have an influence on Th2 cytokines by enhancing production of IL-4 and IL-13, inducing IgE synthesis.^{8,19} In the presence of IL-3, IL-18 directly stimulates basophils and mast cells to produce mediators such as histamine, serotonin, IL-4 and IL-13 in an IgE-independent manner.^{19,22} Thus, it is possible that IL-18 rather than IgE is responsible for the development of the inflammatory dermatitis by activation of mast and T cells.²² Moreover, it was shown in a mouse model of AD that IL-18 contributes to the spontaneous development of AD-like inflammatory skin lesions independently of IgE levels.^{13,22} IL-18 is a pleiotropic cytokine, which can influence either the Th1 or Th2 response, depending on the cytokine milieu and genetic background,^{7–9,14,17,22} and it plays a crucial role in the control of balance between Th1 and Th2. IL-18 also influences IgE production.^{7–9,14,17} Recently, a study on the neuro-immunomodulatory action of IL-18¹⁸ was reported. Additionally, the gene of IL-18 is located on chromosome 11q22.2–22.3, which has been designated a candidate region for atopy.^{1,6,7} Consequently, the importance of IL-18 in atopic disease has come under scrutiny.^{6–14}

The aim of this study was to investigate the association between serum IL-18 and IgE levels in sera from patients with AD, and determine the influence of IL-18 on the development of AD, by examining disease severity in patients with normal and raised IgE levels. We investigated the correlation of IL-18 and disease onset, disease severity, itch, eosinophilia, specific sensitization, sleep disturbances, age and gender. We also compared the IL-18 levels in the sera of patients with AD and those with AD plus other allergic diseases.

Methods

The study was approved by the local ethics committee (Medical University of Gdańsk), and informed consent was obtained from all participants.

Patients

In total, 117 people were enrolled in the study. The patient group comprised all patients with AD aged > 5 years of age who attending the Dermatological Department, Medical University of Gdańsk ($n = 67$; 28 male, 39 female; male : female ratio 0.7 : 1; mean $\pm$ SD age 21.1 ± 11.5 years, range 5–54). Mean $\pm$ SD age at disease onset was 2.8 ± 6.01 years, and children made up 46.2% of the study group. AD was defined according to the criteria of Hanifin and Rajika: 28 patients (41.8%) had severe AD, 25 (37.3%) had moderate AD and 14 (20.9%) had mild AD. Exclusion criteria included previous use of ultraviolet therapy or immunotherapy at any time; and oral treatment with corticosteroids and ciclosporin A within 1 year before study start; topical treatment with tacrolimus or pimecrolimus within 1 year before study start; and presence of autoimmune, inflammatory or infectious disease, or any malignancies.

The control group comprised 50 healthy volunteers with no history of allergy (21 male, 29 female; male : female ratio 0.7 : 1; mean $\pm$ SD age 22.2 ± 6.9 years, range 5–51).

Sampling and scoring

Blood samples were taken from all participants and separated by centrifugation at 400 *g* for 10 min. Serum samples were stored at -70°C until used. Total IgE levels were measured using fluorimmunoassay (Unicap 100; Phadia, Sweden), following the manufacturer's instructions. The cut-off point for serum IgE was 100 kU/L. An ELISA was used for quantitative detection of IL-18, in accordance with the manufacturer's instructions (Bender MedSystems GmbH, Vienna, Austria). The tool SCORing Atopic Dermatitis (SCORAD) was used to determine the severity of the disease course, and rated as severe, moderate or mild.

Statistical analysis

Data were tabulated using Excel 2003 (Microsoft Corp., Redmond, WA, USA) using STATISTICA (Version 8.0; StatSoft, Tulsa, OK, USA). The Mann–Whitney *U*-test, Kruskal–Wallis test and Spearman *r* test were used. $P < 0.05$ was considered significant.

Results

Serum IL-18 in the patients was 155.68 ± 192.92 g/mL, compared with (65.73 ± 61.66 pg/mL) in the control group, a highly significant difference ($P < 0.001$) (Fig. 1a). IL-18 was also significantly higher in the sera of the patients with severe AD than in those with mild AD (206.66 vs. 76.68 , respectively; $P < 0.03$) (Fig. 1b).

For IgE, the mean $\pm$ SD level was 1.80 ± 2.03 MU/L (range 6.18 kU/L to 5 MU/L), significantly ($P < 0.001$) higher than that of the control group (36.42 ± 39.27 kU/L; range 2–167). In the patient group, 50 patients had raised IgE and 17 had normal IgE, compared with 4 and 46 people, respectively, in the control group.

Furthermore, there was a correlation between IgE and IL-18. In the group of patients with raised IgE ($n = 50$) and SCORAD score of 55, IL-18 was also higher compared with the control group ($P < 0.001$). (Fig. 1c).

SCORAD score was 33 for patients with normal IgE ($n = 17$), and the IL-18 concentration for this group was twice as high (112 pg/mL) as that for the control group (65.73 pg/mL), but this was not significant ($P = 0.46$) (Fig. 1c).

There was no correlation of IL-18 levels with patient age ($P = 0.36$) or gender ($P = 0.8$), onset of AD ($P = 0.27$), sleep problems ($P = 0.1$), itching severity ($P = 0.15$), presence of eosinophilia ($P = 0.33$), or specific sensitization to grass pollens ($P = 0.7$) or mite allergens ($P = 0.57$). There were no differences in IL-18 serum level between patients with AD and patients with AD plus other allergic diseases (conjunctivitis $P = 0.39$, asthma $P = 0.85$, rhinitis $P = 0.54$) even though the group of 67 patients included 10 with conjunctivitis, 14 with asthma and 21 with rhinitis (some patients had more than one condition). The other 35 patients had no concomitant allergic disease.

For IgE, there were significant differences between patients with AD and those with AD and other allergic diseases (conjunctivitis $P = 0.03$, asthma $P = 0.01$, rhinitis $P = 0.04$). There was also a significant correlation between the IgE level eosinophilia ($P < 0.001$), SCORAD ($P < 0.001$), itching severity ($P < 0.001$) and sleep disturbances ($P \leq 0.001$).

The mean $\pm$ SD SCORAD score was 49.6 ± 24.5 (range 11.5–85). Sleep problems were reported by 52.2% of the patients. There was also a correlation between SCORAD score and IgE levels ($P < 0.001$),

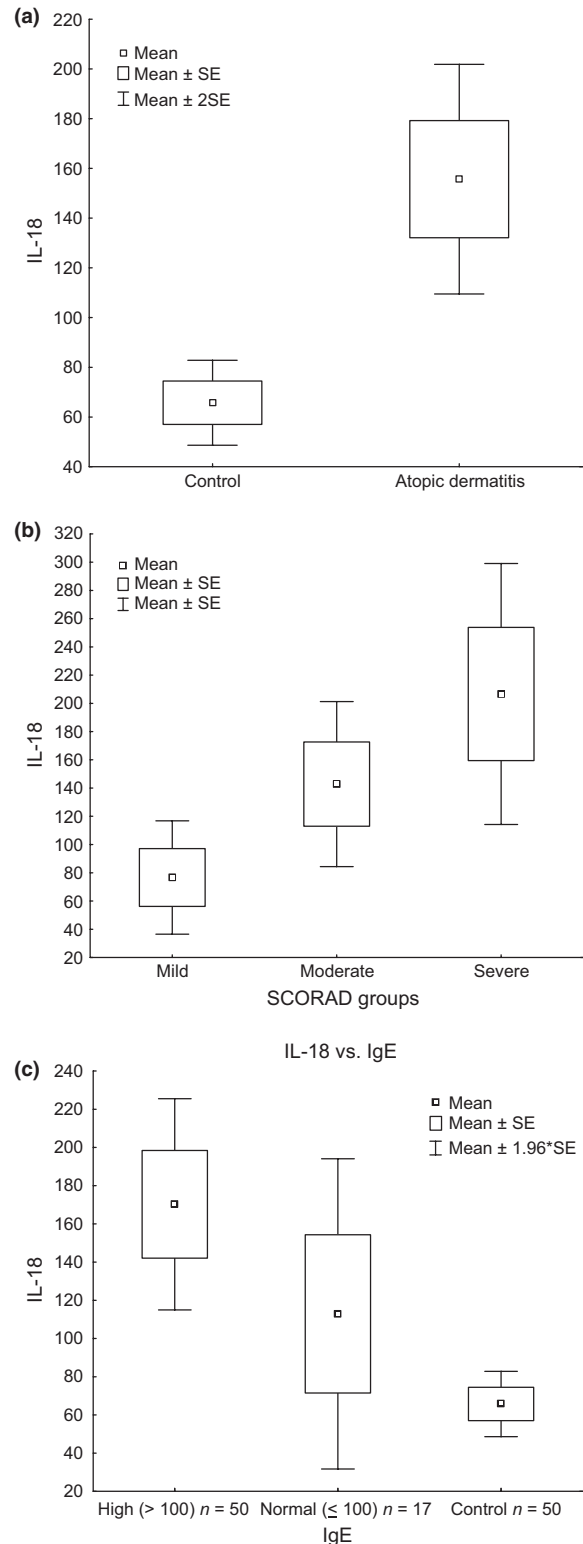


Figure 1 (a) Level of interleukin (IL)-18 concentration in the sera of patients with atopic dermatitis (a) compared with control group; (b) correlated with disease severity; (c) correlated with level of IgE. SCORAD SCORing Atopic Dermatitis.

Table 1 Serum concentration of IL-18 in the patients with AD and the controls group coming from different country.

Country	Controls			Patients			P (test)
	n	IL-18 level, pg/mL		n	IL-18 level, pg/mL		
		Mean	Range		Mean	Range	
Korea ⁸	24	213.5	86.2–1112.6	50	259.15	43.8–908.9	0.05 (K–W)
Egypt ²³	10	125.8	104–140	17	401.2	209–615	NS
Turkey ⁹	19	12.5	12.5–73	20	68	23–81	< 0.001 (M–W)
Japan ¹¹	10	144	116–178	26	207	172–242	0.026 (M–W)
China ²⁰	–	–	–	19	394	204–612	< 0.05 (M–W)
Japan ²¹	–	122	–	18	200	50–500	0.05 (S–t)
Korea ²⁴	–	151	–	–	332	–	0.05 (S–t)
Poland, current study	50	65.73	1.5–295.2	67	155.68	2.3–1207.6	< 0.001 (M–W)

IL, interleukin; M–W, Mann–Whitney *U*-test; K–W, Kruskal–Wallis two sample rank sum test; NS, not stated; S–t, Student *t*-test.

eosinophilia ($P < 0.001$), sleep disturbances ($P < 0.001$) and itching severity ($P < 0.001$).

Discussion

An association between SCORAD score and IL-18 levels has been reported previously in studies on Turkish,⁹ Japanese,¹¹ Chinese²⁰ and Korean²⁴ populations. IL-18 level was found to be higher in the sera of patients with AD than of controls in Turkish,⁹ Chinese,²⁰ Japanese^{11,21} African²³ and Korean^{8,24} populations. We found similar results with our study, which is the largest to date and on a predominantly white population (Table 1).

There have been conflicting results regarding the correlation between IL-18 and IgE levels. Similar to our study, Kim *et al.*⁸ and Ando *et al.*¹⁰ found a correlation between IL-18 and IgE levels, whereas Shida *et al.*²¹ and Hon *et al.*²⁰ did not, but their studies had smaller numbers of patients (18 and 19, respectively).

Many patients with AD (75–90%) have raised serum IgE levels, but around 30% of patients with AD have normal IgE levels.^{9,13} A study from Japan,²² using transgenic mice with depletion of IL-18 or of the gene encoding signal transducer and activator of transcription (STAT)6, showed that IL-18 is principally responsible for inducing the skin alterations associated with itching and for inducing skin lesions (such as those in AD), independently of IgE/IgG1 elevation. Those authors concluded that IL-18 contributes to the spontaneous development of AD-like inflammatory skin lesions independently of IgE/STAT6, under specific pathogen-free conditions²². Ando *et al.*¹⁰ examined 370 schoolchildren with AD, and concluded that serum

IL-18 levels are associated with allergic symptoms independent on IgE levels. In our study, IL-18 was higher in the group of patients with AD with normal IgE compared with the control group, but this was not significant. We could not confirm that IL-18 is responsible for AD development in the group of patients with AD with normal IgE. This warrants further studies, particularly as this group was relatively small in our study.

Our results confirm that IL-18 may be a clinical marker of disease severity, consistent with some of the previous studies;^{10,20,24} however, other studies^{8,23} found no correlation between SCORAD score and IL-18 levels. Our data also confirmed that SCORAD score correlates with IgE level, again consistent with previous observations.^{8,11,12,21} Our observation of a correlation between SCORAD score and eosinophilia is also similar to previous findings^{8,11,24}. Unlike some previous authors,^{9,11} we found no correlation between IL-18 concentration and eosinophilia, similar to the findings of Kim *et al.*⁸ We did find an association between IgE levels and eosinophilia in the sera of patients with AD, contrary to the findings of Yoshizawa *et al.*¹¹ We did not find any correlation between IL-18 and sleep disturbances, itching severity or positive results on skin-prick tests, similar to the findings of Hon *et al.*²⁰

Conclusions

Our data indicated that IL-18 plays an important role in the pathogenesis of AD, and could be a useful clinical marker for disease severity. The influence of IL-18 on the IgE-independent development of AD needs further study.

What's already known about this topic?

- IL-18 is a pleiotropic cytokine that can have an influence on the development of allergic disease.
- Only a few studies have been published on IL-18 in atopic dermatitis, which mainly consist of small groups of patients and mainly come from Asia.
- The correlation results between IL-18 and IgE and SCORAD in these studies conflict.

What does this study add?

- IL-18 also plays a crucial role in the development of AD in the Polish population.
- IL-18 is increased in the sera of AD patients in Poland.
- IL-18 can be a useful clinical marker for the severity of AD.

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