

Interleukin-18 is elevated in the horny layer in patients with atopic dermatitis and is associated with *Staphylococcus aureus* colonization

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Summary

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Conflicts of interest

None declared.

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Background An increase in interleukin (IL)-18 production from epidermal cells has been reported in an atopic dermatitis (AD) mouse model, and subsequent topical application of *Staphylococcus aureus* results in severe dermatitis.

Objectives To reveal the relationship between *S. aureus* colonization of skin lesions and keratinocyte IL-18 production, particularly in AD with relatively low serum IgE levels. We also aimed to establish a simple and noninvasive method of assaying IL-18 produced by epidermal keratinocytes to evaluate local skin inflammation and therapeutic effects in patients with AD.

Methods IL-18 in the horny layer of the skin was collected via a tape-stripping method and measured in 95 patients with AD and 40 healthy controls by enzyme-linked immunosorbent assay (ELISA). Clinical severity, blood data and *S. aureus* skin colonization were evaluated before and after treatment.

Results IL-18 levels in the horny layer were significantly higher in the skin lesions of patients with AD than in healthy controls and correlated with SCORAD, levels of serum IL-18, IgE, lactate dehydrogenase, thymus and activation-regulated chemokine, blood eosinophils and transepidermal water loss. In the AD group with serum IgE < 1500 IU mL⁻¹, significantly higher IL-18 levels were observed in the horny layer of patients colonized with *S. aureus* compared with those who were not.

Conclusions Epidermal IL-18 production was associated with the severity of AD. *Staphylococcus aureus* colonization seems to contribute to this IL-18 production, especially in the AD group with relatively low IgE production. Tape stripping provides an easy and noninvasive method to assess epidermal IL-18 production by ELISA.

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease accompanied with allergic and immunological abnormalities, including increased serum IgE levels. Various environmental factors contribute to the development of AD. The pathogenesis of AD is mediated partly by type 2 helper T cells (Th2 cells) expressing interleukin (IL)-4 and IL-13 which induce IgE production. However, while AD with high IgE production accounts for about 80% of AD, AD with low or mild IgE production exists,¹ indicating a highly complicated immunological pathogenesis in AD.

IL-18 was first identified in mice as an interferon (IFN)- γ inducer.² However, recent studies have revealed a pleiotropic role for IL-18. It was reported that IL-18 induces AD-like lesions independently of Th2 or IgE responses in mice producing abundant IL-18.^{3,4} Sugimoto et al.⁵ and Hata

et al.⁶ demonstrated that IL-18 induces both IFN- γ and IL-13 in antigen-stimulated type 1 helper T cells (Th1 cells). As the cells simultaneously produce both Th1 and Th2 cytokines, Terada et al.⁷ proposed to designate them 'super Th1 cells'. They also showed that when *Staphylococcus aureus* protein A (SpA) was applied to the skin of NC/Nga mice, an atopic mouse model, after destruction of the skin barrier, IL-18 was secreted by keratinocytes.⁷ IL-18 directly stimulated mast cells and basophils with IL-3 and induced production of cytokines such as IL-4 and IL-13, and histamine.⁸

Taken together, we speculated that the IL-18 production from keratinocytes and *S. aureus* colonization on the skin are relevant to the pathogenesis of human AD with relatively low serum IgE levels. The aim of our study was to clarify the

relationship between colonization of the skin lesion with *S. aureus* and keratinocyte IL-18 production especially in AD with relatively low serum IgE levels. We also aimed to establish a simple and noninvasive method of repeatedly assaying IL-18 produced by epidermal keratinocytes to assess local skin inflammation and therapeutic effects in patients with AD.

Materials and methods

Subjects

Patients were diagnosed with AD by a dermatologist according to the criteria of Hanifin and Rajka.⁹ Ninety-five patients with AD (45 men and 50 women; mean age 34.2 years) and 40 healthy controls (HCs) (19 men and 21 women; mean age 31.1 years) were included in this study. Clinical characteristics of the patients are listed in Table 1. All patients were treated with topical corticosteroids and oral antihistamines according to the criteria in the 'Therapeutic guidelines for atopic dermatitis 2002'.¹⁰ Corticosteroid treatment ceased about 12 h before the examination day, and the body was cleansed the day before tape stripping.

Written informed consent was obtained from all patients and healthy volunteers. This study was approved by the ethical committee of Yokohama City University School of Medicine (approval no. 180).

Assessments of the clinical conditions

Patients were evaluated for the general severity of their AD using the SCORing AD (SCORAD) index.¹¹ They were also evaluated for the local severity of the lesions used for IL-18 measurement on the cubital fossa and abdomen using local skin scores defined as the sum of individual scores of erythema, oedema, erosion/crust, scratch, lichenification and scale/xerosis at three points for each parameter. At the same time the levels of serum thymus and activation-regulated chemokine (TARC), serum IgE, serum lactate dehydrogenase

(LDH), blood eosinophils and transepidermal water loss (TEWL) were measured before and after 4 or 6 weeks treatment.

Tape-stripping methods and assay for horny layer interleukin-18

The tape (17 × 45 mm Cellotape®; Nichiban, Tokyo, Japan) was applied to the skin of patients with AD and to HCs, removed immediately, cut finely, and soaked in 700 µL phosphate-buffered saline (PBS). After ultrasonication (Sonifer®; Branson Ultrasonics Corporation, Danbury, CT, U.S.A.) for 1 min at 4 °C, it was centrifuged for 15 min (11 755 g) and the supernatant was collected. IL-18 and total protein were measured using a human IL-18 enzyme-linked immunosorbent assay (ELISA) kit (MBL, Nagoya, Japan) and via the Bradford method (Bio-Rad Protein Assay; Bio-Rad, Hercules, CA, U.S.A.) according to the manufacturer's instructions, and the horny layer IL-18 value was calculated (pg µg⁻¹ protein).

Immunohistochemistry

Skin tissue biopsies were immediately fixed with 10% formalin and embedded in paraffin. For immunohistochemical staining, tissue sections were deparaffinized and hydrated using xylene and a graded alcohol series. The sections were incubated in normal goat serum and overlaid with rabbit antihuman IL-18 polyclonal antibody (diluted 1 : 800; MBL, Woburn, MA, U.S.A.). Slides were washed in PBS, and IL-18 was detected with the antirabbit Envision+ kit (Dako, Glostrup, Denmark) for 30 min. After further washing, immunoperoxidase staining was developed using a diaminobenzidine chromogen (Dako) followed by counterstaining with haematoxylin.

Measurement of *Staphylococcus aureus* colonization

Colonization of *S. aureus* on the skin used for IL-18 measurements was assessed by the stamp method using the Mannitol

Table 1 Patient characteristics

	Patients with atopic dermatitis	Healthy controls
Number	95	40
Female/male	50/45	21/19
Age (years), mean ± SD		
Women	33.7 ± 13.9	30.0 ± 8.8
Men	34.8 ± 19.8	32.4 ± 7.0
SCORAD, mean ± SD	44.1 ± 20.5	
Peripheral blood eosinophils (number µL ⁻¹), mean ± SD	693 ± 587	
Serum lactate dehydrogenase (IU L ⁻¹), mean ± SD	251 ± 100	
Serum IgE (IU mL ⁻¹), mean ± SD	9010 ± 17 383	< 300
Serum thymus and activation-regulated chemokine (pg mL ⁻¹), mean ± SD	4716 ± 11 290	< 450
Serum interleukin-18 (pg mL ⁻¹), mean ± SD	178 ± 97.7	81.6 ± 23.8

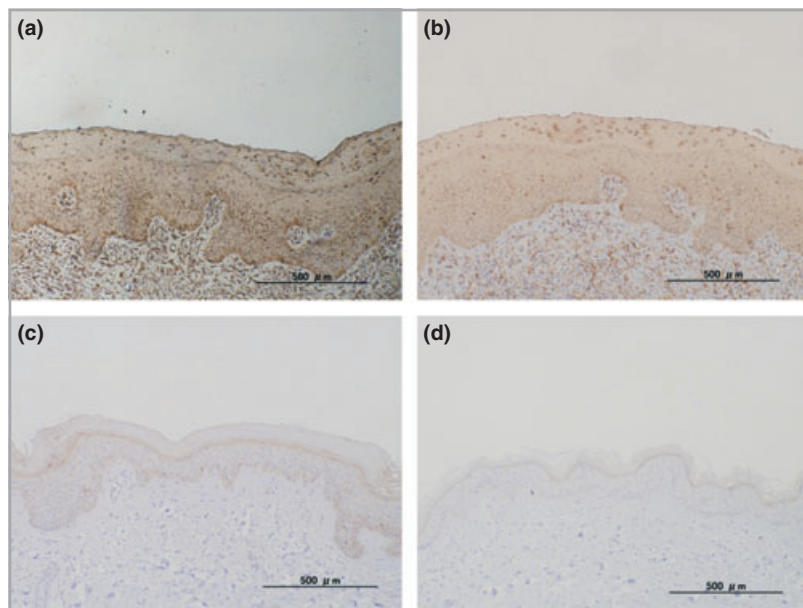


Fig 1. Interleukin (IL)-18 immunohistochemistry of the skin from patients with atopic dermatitis (AD) and healthy controls (HCs). Samples obtained from nine patients with AD [three severe (a), three moderate (b), three mild (c)] and three HCs (d) were embedded in paraffin, sectioned, and stained with anti-IL-18 antibody. Representative staining of tissue sections from patients with AD and HCs is shown. As acanthosis and cell infiltration dermatitis became severe, IL-18 protein expression was gradually increased in the epidermis of AD. IL-18 was diffusely expressed in the horny layer and in the cytoplasm of epidermal cells. Immunohistochemical staining of IL-18 was performed as described in Materials and methods. Original magnification $\times 100$. Scale bar represents 500 μm .

Salt Agar with Egg Yolk kit (Direct Detect Checker[®]; Kyokutouseiyaku, Tokyo, Japan). The medium was gently pressed against the surface of the skin for about 5 s and then incubated at 35 °C for 24–48 h.¹² If the skin was colonized with *S. aureus*, round yellow/cream colonies formed with a yellow surround around the colonies. This test was performed with patients with AD both on the cubital fossa and on the abdomen before and after treatment.

Statistical analysis

Statistical analysis was performed using the SPSS program version 17.0 (SPSS Inc., Chicago, IL, U.S.A.). The Mann–Whitney U-test, Wilcoxon signed-rank test, Spearman rank-order correlation test and Jonckheere–Terpstra rank test were used to evaluate the statistical significance of the results. Results are shown as mean $\pm$ SD. $P < 0.05$ was considered statistically significant.

Results

Expression of interleukin-18 in the horny layer of atopic skin and in healthy controls

We performed immunohistochemical analysis of lesional skin from patients with AD to determine IL-18 expression. Typical histological IL-18 expression in mild, moderate and severe AD is shown in Figure 1. IL-18 protein was expressed at higher levels in the epidermis of AD skin lesions than in the skin of

HCs, and expression increased with the severity of dermatitis. We then compared IL-18 levels in the horny layer, both on the cubital fossa and on the abdomen, between patients with AD ($n = 95$) and HCs ($n = 40$). Horny layer IL-18 levels were significantly higher in patients with AD than in HCs, both on

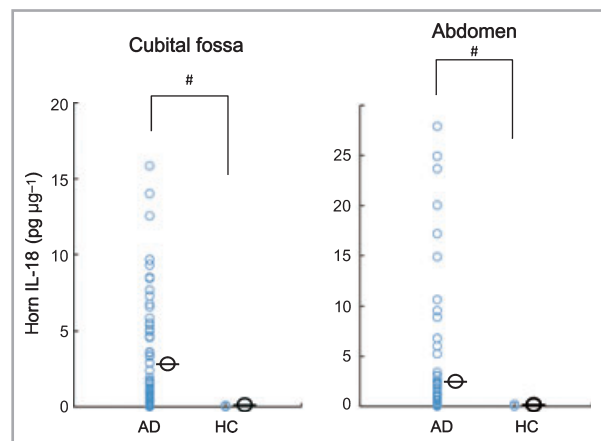


Fig 2. Horny layer interleukin (IL)-18 levels in patients with atopic dermatitis (AD) and healthy controls (HCs). IL-18 was measured by enzyme-linked immunosorbent assay using samples collected by the tape-stripping method as described in Materials and methods. The horny layer IL-18 levels were significantly higher in patients with AD ($n = 95$) than in HCs ($n = 40$) both on the cubital fossa and on the abdomen. Horizontal bars indicate mean horny layer IL-18 values. $\#P < 0.001$, AD vs. HCs.

the cubital fossa (2.29 ± 3.50 and 0.03 ± 0.017 pg μg^{-1}) and on the abdomen (2.40 ± 5.55 and 0.07 ± 0.04 pg μg^{-1}) (Mann–Whitney U-test, $P < 0.001$) (Fig. 2).

Correlation between horny layer interleukin-18 levels and the clinical condition of patients with atopic dermatitis

We reviewed the relationship between the horny layer IL-18 levels and the local lesions on the cubital fossa and abdomen ($n = 95$). The horny layer IL-18 levels correlated with local skin scores (sum of individual scores of symptoms) both on the cubital fossa and on the abdomen (Fig. 3a, b) (Spearman's rank correlation test, $P < 0.01$). Horny layer IL-18 levels also correlated with the general severity of AD (SCORAD) (Fig. 3c, d) (Spearman's rank correlation, $P < 0.05$).

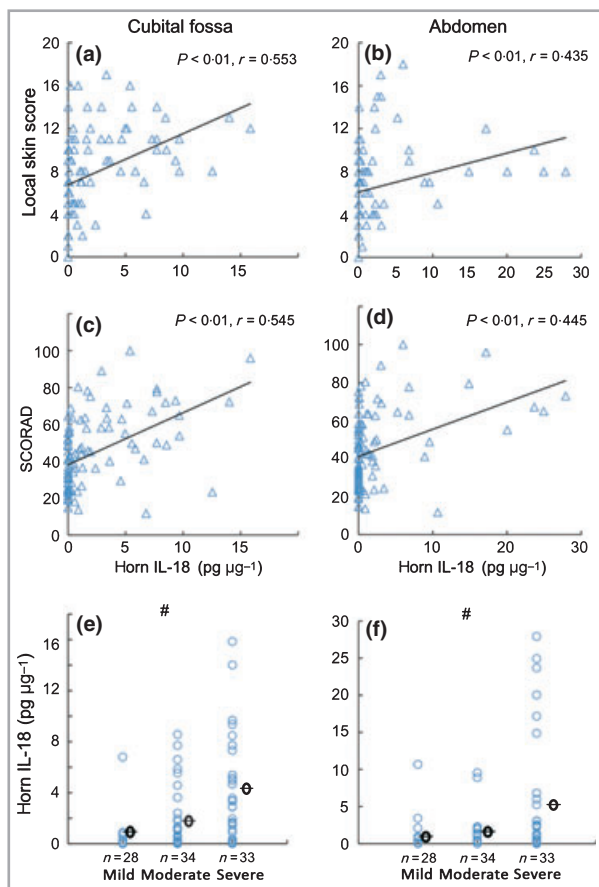


Fig 3. Correlation between horny layer interleukin (IL)-18 levels and clinical parameters in patients with atopic dermatitis (AD). The horny layer IL-18 level was correlated with local severity (a, b) and general severity (SCORAD) (c, d) of AD ($n = 95$). Local severity was expressed as the sum of individual scores of erythema, oedema, erosion/crust, scratch, lichenification and scale/xerosis at three points for each parameter. The general severity of AD was divided into mild (SCORAD < 25), moderate (SCORAD 25–50) and severe (SCORAD > 50) and examined with the Jonckheere–Terpstra rank test (e, f) ($\#P < 0.001$). Horizontal bars indicate mean horny layer IL-18 values.

When the general severity was divided into three groups [mild (SCORAD < 25), moderate (SCORAD 25–50) and severe (SCORAD > 50)], a stronger correlation between horny layer IL-18 levels, both on the cubital fossa and on the abdomen, and disease severity was found (Jonckheere–Terpstra rank test, $P < 0.001$) (Fig. 3e, f). The mean $\pm$ SD horny layer IL-18 levels in mild, moderate and severe AD were 0.86 ± 2.63 , 1.71 ± 2.49 and 4.11 ± 4.24 pg μg^{-1} , respectively, on the cubital fossa, and 0.69 ± 2.10 , 1.09 ± 2.25 and 5.16 ± 8.28 pg μg^{-1} , respectively, on the abdomen.

Horny layer interleukin-18 levels in relation to laboratory data and transepidermal water loss

We examined the relationship between horny layer IL-18 levels and laboratory data that reflect AD disease status (Fig. 4). Horny layer IL-18 levels correlated with both serum LDH and TARC levels (Spearman's rank correlation test, $P < 0.01$, $r > 0.4$). There was a mild correlation with total serum IgE levels and blood eosinophil counts ($P < 0.01$, $r < 0.4$). Serum IL-18 and TEWL correlated with SCORAD (data not shown) and with horny layer IL-18 on the cubital fossa (Spearman's

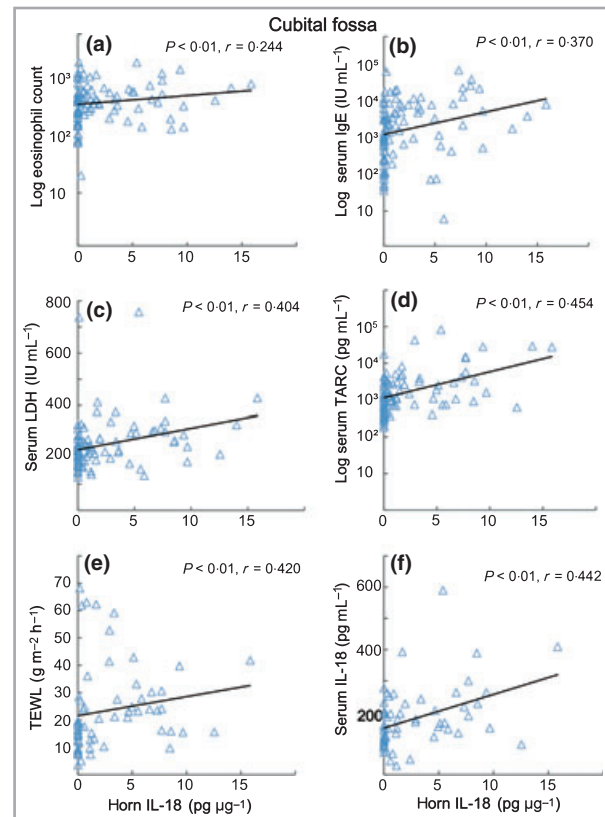


Fig 4. Correlation between horny layer interleukin (IL)-18 levels and laboratory parameters/serum IL-18/transepidermal water loss (TEWL) in patients with atopic dermatitis. Correlations with blood eosinophil counts (a), serum IgE (b), serum lactate dehydrogenase (LDH) level (c), serum thymus and activation-regulated chemokine (TARC) level (d), TEWL (e) and serum IL-18 level (f) are shown ($n = 95$).

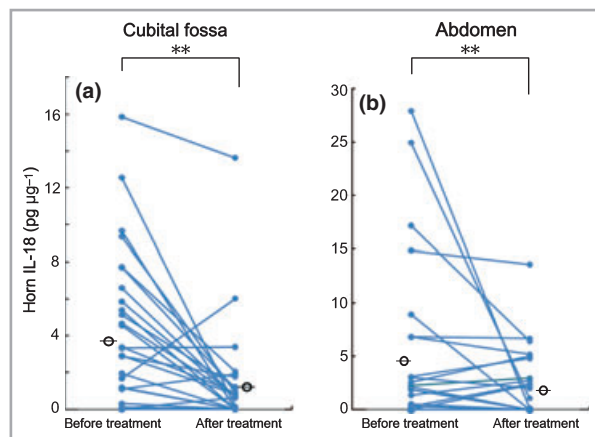


Fig 5. Changes in horny layer interleukin (IL)-18 levels after treatment in patients with atopic dermatitis (AD). The horny layer IL-18 level of patients with AD ($n = 30$) was evaluated before and after treatments with topical corticosteroids and oral antihistamines both on the cubital fossa (a) and on the abdomen (b). Horizontal bars indicate mean horny layer IL-18 values. The horny layer IL-18 levels decreased significantly after treatment both in the cubital fossa (** $P < 0.01$) and in the abdomen (** $P < 0.01$).

rank correlation test, $P < 0.01$, $r > 0.4$) (Fig. 4). These data showed that tape stripping can be used to evaluate epidermal IL-18 production for assessment of severity of AD.

Changes in the interleukin-18 levels in the horny layer after treatment

Horny layer IL-18 levels were compared on the cubital fossa and abdomen ($n = 30$) before and after treatment. The mean $\pm$ SD horny layer IL-18 level on the cubital fossa of patients with AD before treatment was $3.83 \pm 4.12 \text{ pg } \mu\text{g}^{-1}$, decreasing to $1.41 \pm 2.92 \text{ pg } \mu\text{g}^{-1}$ after treatment. The mean $\pm$ SD horny layer IL-18 level on the abdomen also decreased, from $4.43 \pm 3.11 \text{ pg } \mu\text{g}^{-1}$ to $2.61 \pm 5.64 \text{ pg } \mu\text{g}^{-1}$. These changes in the horny layer IL-18 level were significant, both on the cubital fossa (Wilcoxon signed-rank test, $P < 0.01$) and on the abdomen ($P < 0.01$) (Fig. 5). These results demonstrate that the serial measurement of IL-18 levels is useful for evaluating the effects of treatment in patients with AD.

Patient horny layer interleukin-18 levels in association with *Staphylococcus aureus* colonization

To clarify the role of *S. aureus* colonization on epidermal IL-18 production, we examined horny layer IL-18 in association with *S. aureus* colonization on the skin surface in patients with AD. *Staphylococcus aureus* was detected at a higher rate in patients with AD compared with HCs: on the cubital fossa in 62/95 (65%) of patients with AD and 5/40 (15%) of HCs, and on the abdomen in 59/95 (62%) of patients with AD and 8/40 (20%) of HCs. In patients with AD, we assessed horny layer

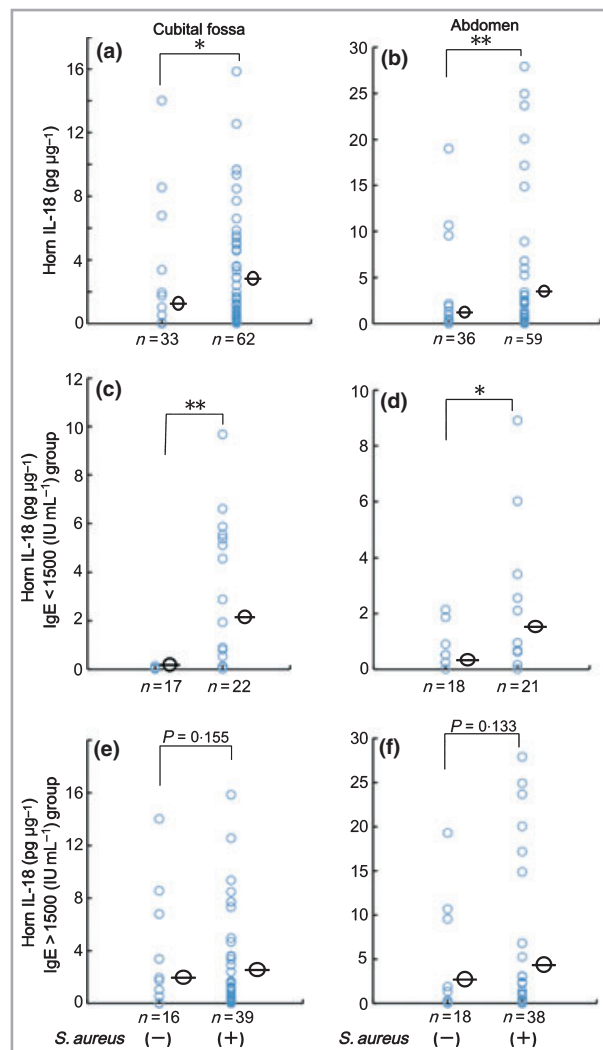


Fig 6. Correlation of the horny layer interleukin (IL)-18 level of the patients with atopic dermatitis (AD) associated with *Staphylococcus aureus* colonization. The horny layer IL-18 level was significantly higher both on the cubital fossa (a) and on the abdomen (b) in the patients from whom *S. aureus* was detected compared with patients from whom *S. aureus* was not detected ($n = 95$). The detection of *S. aureus* colonization was performed as described in Materials and methods. Correlation of the horny layer IL-18 level in association with *S. aureus* colonization in patients with AD divided into two groups as determined by serum IgE levels, < 1500 and $> 1500 \text{ IU mL}^{-1}$, and assessed for horny layer IL-18 levels on the cubital fossa (c, e) and abdomen (d, f). In the group with IgE $< 1500 \text{ IU mL}^{-1}$, the horny layer IL-18 level on the cubital fossa was significantly higher in the patients with *S. aureus* colonization. Horizontal bars indicate mean horny layer IL-18 values. * $P < 0.05$, ** $P < 0.01$.

IL-18 levels according to whether or not *S. aureus* was detected on the skin. The mean $\pm$ SD horny layer IL-18 level on the cubital fossa was $2.71 \pm 3.55 \text{ pg } \mu\text{g}^{-1}$ in colonized patients and $1.16 \pm 3.01 \text{ pg } \mu\text{g}^{-1}$ in noncolonized patients. Similarly, levels on the abdomen were 3.29 ± 6.83 and $1.53 \pm 3.76 \text{ pg } \mu\text{g}^{-1}$, respectively (Fig. 6a, b). Horny layer IL-18 levels were significantly higher on both sites in patients

colonized with *S. aureus* than in noncolonized patients (Mann–Whitney U-test, $P < 0.05$). These results suggest that *S. aureus* colonization is relevant to IL-18 production in human epidermis.

We next investigated whether the relationship between horny layer IL-18 and *S. aureus* was dependent on IgE. In the IgE < 1500 IU mL⁻¹ group, horny layer IL-18 levels on the cubital fossa and abdomen were significantly higher in patients with *S. aureus* colonization (mean $\pm$ SD horny layer IL-18 = 2.39 ± 2.94 pg μ g⁻¹ and 1.59 ± 2.58 pg μ g⁻¹, respectively) than in noncolonized patients (mean $\pm$ SD horny layer IL-18 = 0.19 ± 0.03 pg μ g⁻¹ and 0.34 ± 0.68 pg μ g⁻¹, respectively) (Mann–Whitney U-test, $P < 0.01$, $P = 0.036$) (Fig. 6c, d). This was not observed on the cubital fossa and abdomen in the AD group with IgE > 1500 IU mL⁻¹ (Mann–Whitney U-test, $P = 0.155$, $P = 0.133$, respectively) (Fig. 6e, f).

Discussion

In the present study, we demonstrated that horny layer IL-18 levels, derived from activated keratinocytes, could be measured by ELISA using tape-stripping horny layer samples. Horny layer IL-18 levels were significantly higher in atopic skin than in healthy skin. Levels correlated with local and general severity of AD, skin barrier dysfunction assessed with TEWL and laboratory data (serum LDH, total serum IgE and serum TARC), which reflects the severity of disease in patients with AD.^{13–16} This is the first report on the quantification of epidermal IL-18 production in patients with AD.

It has been reported that serum IL-18 was significantly higher in patients with AD than in HCs and reflects the severity of AD.^{17,18} Here, we also observed significantly higher serum IL-18 levels in patients with AD than in HCs ($r = 0.506$, $P < 0.01$). However, there have been no reports about IL-18 expression in the epidermis of patients with AD. Therefore, we used immunohistochemistry to investigate IL-18 protein expression in the epidermis in patients with AD and HCs. We found that as dermatitis severity increased, IL-18 protein expression also increased in the lesions (Fig. 1). The horny layer IL-18 levels, which are likely to represent the level of keratinocyte IL-18 production, correlated with the severity of AD and decreased significantly after treatment compared with pretreatment levels, although they were not related to the severity of pruritus (data not shown). These data showed that the measurement of IL-18 in the horny layer is useful for evaluating IL-18 production of epidermal keratinocytes as well as the effects of therapies in patients with AD.

IL-18, a proinflammatory cytokine that belongs to the IL-1 cytokine family, was originally identified as a cytokine that induces T cell IFN- γ production.² IL-18 is produced as a biologically inactive precursor, pro-IL-18, by macrophages, respiratory and intestinal epithelial cells, and keratinocytes.¹⁹ Pro-IL-18 is activated by caspase-1 and released extracellularly.^{20,21} It was recently suggested that IL-18 processing might also occur extracellularly via mast cell chymase.²² In

mice, IL-18 induces both IFN- γ and IL-13 production in antigen-stimulated Th1 cells.^{5,6}

It has been reported that keratinocyte-specific IL-18 transgenic mice and skin-specific caspase-1 transgenic mice, which specifically overexpress IL-18 or caspase-1 in the skin, spontaneously develop relapsing dermatitis and later show gradual increases in their serum IgE levels.^{3,4,23} In the skin lesions of the transgenic mice, lymphocytes, mast cells and neutrophils infiltrate the epidermis.⁴ Konishi *et al.*⁴ pointed out that IL-18 production is essential for the development of AD-like dermatitis in these transgenic mice. In these mice, local accumulation of IL-18 triggers systemic IgE responses without exposure to antigen.²⁴ A similar mechanism might be relevant to human AD with relatively low serum IgE levels. Figure 6 (a, b) shows that IL-18 production from keratinocytes may be facilitated via *S. aureus* colonization. Particularly in the IgE < 1500 IU mL⁻¹ group, *S. aureus* colonization on the skin was related to epidermal IL-18 production by keratinocytes (Fig. 6c, d). However, whether increased IL-18 is exclusively associated with *S. aureus* colonization in patients with AD with relatively low IgE levels (but not those with high IgE) requires further study. For example, Omoto *et al.*²² showed that the scratching of AD lesions markedly promotes the release of mast cell chymase which, in turn, may increase the activation of IL-18 in the skin. However, our preliminary results suggest a specific association between IL-18 and *S. aureus* colonization of patients with AD with relatively low IgE.

As for other diseases, expression of IL-18 in the epidermis is enhanced in cutaneous lymphoma,²⁵ cutaneous lupus erythematosus²⁶ and psoriasis.²⁷ However, no study investigated *S. aureus* colonization in these diseases. We showed that horny layer IL-18 levels in psoriasis are high, but do not correlate with *S. aureus* colonization of skin lesions (data not shown).

DS-Nh mice, a mouse model of AD, spontaneously develop dermatitis when housed under conventional conditions, but not when housed under specific pathogen-free conditions.^{28,29} In these mice, a remarkable increase in *S. aureus* colonization on the skin surface is observed under conventional conditions before the development of skin lesions and serum IgE elevation.²⁹ Application of *S. aureus* to the skin of the mice accelerates the onset of skin eruptions.²⁹ In DS-Nh mice, increases in IL-13 and IFN- γ , but not in IL-4, production by splenocytes were observed in association with an increase in *S. aureus* colonization when skin lesions were aggravated with repeated application of trinitrochlorobenzene.¹⁰ This suggests that *S. aureus* colonization influences skin lesions by affecting cytokine production in these mice. Indeed, lymphocytes from patients with AD with low serum IgE produce greater amounts of IL-13 and IFN- γ than those from healthy individuals,³⁰ thus suggesting a possible mechanism for developing and/or accelerating human AD.

The mechanisms underlying IL-18 secretion by epithelial cells and keratinocytes have not been elucidated. It has been reported that stimulation with various agents, including pathogenic agents, induces keratinocytes, tracheal epithelial cells and intestinal epithelial cells to produce IL-18.^{24,31,32} *Staphylococcus*

aureus stimulates epidermal keratinocyte IL-18 production and might trigger dermatitis.²⁴ In NC/Nga mice and Balb/c mice, IL-18 secretion from epidermal keratinocytes was induced by the application of both SpA and detergents to the skin.^{7,24} Depending on the IL-18 levels, these mice developed dermatitis with epidermal hyperplasia and an infiltrate of mast cells, eosinophils, neutrophils and T cells without hyperproduction of IgE.⁷ Lymphocytes from these treated mice produced Th1 (IFN- γ) and Th2 (IL-13) type cytokines as well as IL-3. As blockade of IL-18 prevented the development of dermatitis in the mice, the authors suggested that IL-18 is the pathological inducer.

Taken together, these data suggest that it is possible that AD with relatively low IgE production in humans might be induced by *S. aureus* colonization on the skin through IL-18 production from epidermal keratinocytes. Typical AD lesions have been observed even in patients with agammaglobulinemia, who are usually susceptible to repeated infections.³³ This supports the idea that IgE is not always required for the development of human AD.

Quantification of cytokines in the horny layer using tape stripping is both simple and safe.^{34–36} It was shown that horny layer nerve growth factor levels measured by ELISA using tape stripping reflected the severity of pruritus and eruptions in AD.³⁴ In addition to these reports, the present data showed that quantitative analysis using tape stripping is useful for assessing keratinocyte cytokine production.

In conclusion, *S. aureus* colonization may contribute to epidermal IL-18 production, especially in patients with AD with relatively low IgE production. Measurement of horny layer IL-18 levels can be used to assess epidermal IL-18 production, and treatments that target IL-18 might be beneficial for patients with AD with high IL-18 production, although further studies are required.

What's already known about this topic?

- Atopic dermatitis (AD) may show high IgE production or low or mild IgE production. Epidermal cell interleukin (IL)-18 production induced by *Staphylococcus aureus* protein A causes severe dermatitis, and serum IgE levels are gradually elevated later in AD model mice. The mechanism behind this dermatitis is independent of Th2 or IgE responses and is thus a model of intrinsic AD.

What does this study add?

- *Staphylococcus aureus* colonization may contribute to epidermal IL-18 production, especially in the AD group with relatively low IgE production. This is the first report on the quantification of epidermal IL-18 production in patients with AD. Measurement of IL-18 in the horny layer is useful for evaluating the IL-18 production of epidermal keratinocytes as well as the effects of therapies in patients with AD.

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