

Interleukin-8 Content in the Stratum Corneum as an Indicator of the Severity of Inflammation in the Lesions of Atopic Dermatitis

Tseveendorj Amarbayasgalan Hitoshi Takahashi Itaru Dekio Eishin Morita

Clinic for Atopic Dermatitis, Department of Dermatology, Faculty of Medicine, Shimane University, Izumo, Japan

Key Words

Atopic dermatitis · Stratum corneum · Tape-stripping · Interleukin-8 · Interleukin-18 · Vascular endothelial growth factor · Transforming growth factor- α

Abstract

Background: Atopic dermatitis (AD) is an inflammatory skin disease characterized by both acute and chronic eczema. Various markers are used to clinically evaluate the severity of AD. In order to identify a marker of local severity of AD, we measured IL-8, IL-18, vascular endothelial growth factor (VEGF), and transforming growth factor- α (TGF- α) levels in the stratum corneum (sclL-8, sclL-18, scVEGF and scTGF- α) and evaluated the correlation between the levels of these cytokines and the clinical severity scores of localized skin lesions. **Methods:** Stratum corneum samples were collected from the skin lesions of 50 patients with AD using the tape-stripping technique, and the sclL-8, sclL-18, scVEGF and scTGF- α levels were evaluated using the ELISA method. The trans-epidermal water loss and skin water content of the lesions were also measured prior to tape stripping. **Results:** The levels of sclL-8, sclL-18, scVEGF and scTGF- α were significantly higher in patients with AD than in healthy controls. Additionally, the levels of sclL-8, sclL-18 and scVEGF signifi-

cantly correlated with the severity of AD. **Conclusions:** Among these cytokines, sclL-8 showed the highest correlation with the severity scores of lesions in AD as well as other parameters. Our results also suggest that measuring cytokines in the stratum corneum by using ELISA combined with tape stripping is a convenient method to evaluate the severity of skin lesions in AD.

Copyright © 2012 S. Karger AG, Basel

Introduction

Atopic dermatitis (AD) is a relapsing or chronic skin disease that is characterized by highly pruritic eczema. The prevalence of AD has increased in the last half century, but the mechanisms involved in the initiation and maintenance of skin inflammation in AD are not fully understood [1]. Recent studies have provided insights into the underlying immunologic mechanisms, suggesting an amplification cycle of atopic skin inflammation [2]. Investigations of AD in patients and animal models suggest that this disease is initiated, maintained and perpetuated by the actions of various inflammatory cytokines and growth factors secreted by keratinocytes, antigen-presenting dendritic cells, T cells and other inflam-

matory cells, although there is also evidence of a skin barrier defect [3]. These cytokines and growth factors regulate not only the immune and inflammatory responses but also the proliferation and differentiation of skin components [4], thus these components can reflect inflammatory conditions of lesional skin in AD. The skin biopsies taken at acute and chronic skin lesions of AD patients indicate that acute skin lesions are associated with increased numbers of CD4⁺ cells that infiltrate the epithelium and increased number of Th2 cells producing interleukin (IL)-4, IL-5 and IL-13 compared with chronic lesions [5]. In chronic lesions, cells expressing IL-4 and IL-13 were significantly decreased, but cells expressing IL-5, granulocyte macrophage colony stimulating factor (CSF), IL-12 and IFN- γ were increased [5, 6]. CTACK is known to attract a mixture of CCR10⁺ Th1/Th2 cells that are characteristics of chronic AD lesions [7].

Currently, blood level of thymus- and activation-regulated chemokine (TARC) are measured to evaluate the severity of AD. We previously reported a method for assessing the severity of individual lesions to measure TARC levels in lesional stratum corneum obtained by tape-stripping [8]. However, this method is not fully quantitative because the TARC was visualized by fluorescence-conjugated anti-TARC antibodies under a fluorescence microscope. In our next study, we measured the amount of vascular endothelial growth factor (VEGF) in the stratum corneum obtained by tape-stripping with specific ELISA directly, and found that the amount of VEGF is well correlated with the severity of the acute phase of inflammation, such as erythema and edema, but not correlated with the chronic phase of inflammation of lesional skin of AD [9]. In the following preliminary study we evaluated the amount of several cytokines and growth factors, including tumor necrosis factor- α , IL-1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 17, 18, 22 and 31, regulated upon activation, normal T cells expressed and presumably expressed (RANTES), eotaxin, monocyte chemoattractant protein-1, macrophage inflammatory protein-1 α , macrophage inflammatory protein-1 β , granulocyte-CSF, macrophage-CSF, granulocyte macrophage CSF, nerve growth factor, stem cell factor, transforming growth factor (TGF)- α , and TGF- β , which might have proinflammatory ability, in the stratum corneum by using the tape-stripping technique and specific ELISA. We found that IL-8, IL-18 and TGF- α can be detected in the lesional stratum corneum of AD in addition to VEGF. IL-18 has already been reported to elevate in the stratum corneum in patients with AD and is associated with *Staphylococcus aureus* colonization [10], and

Table 1. Characteristics of AD patients

Severity	n	Age	Gender (M/F)	SCORAD
Severe	7	28.0 $\pm$ 5.7	5/2	61.5 $\pm$ 16.9
Moderate	11	26.2 $\pm$ 3.4	10/1	45.7 $\pm$ 12.9
Mild	10	27.6 $\pm$ 2.6	5/5	23.0 $\pm$ 7.6
Slight	22	29.6 $\pm$ 2.5	9/13	6.7 $\pm$ 5.1
Total	50	28.2 $\pm$ 11.3	29/21	26.2 $\pm$ 3.2

IL-8 was speculated to be involved in the pathogenesis of AD [11]. TGF- α promotes proliferation of keratinocytes and regulates their function, thus it is likely to play some roles in the pathogenesis of AD [12–14]. In this study, in order to establish a means of evaluating the severity of individual lesions, the levels of IL-8, IL-18, VEGF and TGF- α in the stratum corneum (scIL-8, scIL-18, scVEGF and scTGF- α) obtained from various skin sites in patients with AD were evaluated and examined, including the correlation between the levels of lesional cytokines and the clinical severity scores of the corresponding lesions.

Subjects and Methods

Subjects

Fifty patients with AD (aged 9–58 years, mean 28.2) who fulfilled the diagnostic criteria for AD established by the Japanese Dermatological Association were enrolled in the study [15]. The diagnostic criteria for AD consists of three points: (i) pruritus, (ii) typical morphology and distribution, and (iii) chronic and chronically relapsing course [15]. Of the 50 patients, 29 were men (aged 14–58 years, mean 26.8) and 21 were women (aged 9–55 years, mean 30.1). A total of 12 healthy subjects (aged 22–50 years, mean 28.8) including 8 men (aged 24–32 years, mean 26.9) and 4 women (aged 22–50 years, mean 31.6) formed the control group. The inclusion criteria for healthy subjects were as follows: (i) no inflammatory skin diseases, (ii) normal physical findings and (iii) no past history or family history of allergies. Prior to initiating this study, the aims and methodology were fully explained to each subject or his/her guardian and written informed consent was obtained. This study was approved by the Ethical Committee of Shimane University, Faculty of Medicine (Approval No. 459).

Clinical Severity of AD

The clinical severity of AD was evaluated as slight, mild, moderate or severe according to the diagnostic criteria for the severity scoring of atopic dermatitis (SCORAD) index [16] (table 1). The SCORAD index was calculated by the equation $A/5 + 3.5B + C$, where A is the percentage of body area with a skin rash; B is the total score of erythema, edema/papules, excoriation, oozing/crust, lichenification and xerosis, each of which was evaluated on a 4-point scale (0: none, 1: mild, 2: moderate or 3: severe), and C

Table 2. Cytokine levels in the stratum corneum of AD patients and healthy controls

	scIL-8, pg/mg		scIL-18, pg/mg		scVEGF, pg/mg		scTGF- α , pg/mg	
	AD (n = 50)	healthy (n = 12)	AD (n = 50)	healthy (n = 12)	AD (n = 50)	healthy (n = 12)	AD (n = 50)	healthy (n = 12)
Arm	238.1 $\pm$ 754.7**	0.7 $\pm$ 1.5	63.6 $\pm$ 144.1**	0.0 $\pm$ 0.0	119.1 $\pm$ 114.1**	30.0 $\pm$ 35.2	52.5 $\pm$ 98.1**	4.2 $\pm$ 6.8
Neck	204.4 $\pm$ 444.6**	0.9 $\pm$ 3.1	236.4 $\pm$ 579.7**	0.0 $\pm$ 0.0	127.5 $\pm$ 103.3**	43.4 $\pm$ 44.1	31.6 $\pm$ 73.1**	2.4 $\pm$ 5.7
Back	122.6 $\pm$ 206.8**	0.1 $\pm$ 0.2	157.3 $\pm$ 362.9**	0.0 $\pm$ 0.0	134.6 $\pm$ 138.5**	29.3 $\pm$ 24.0	25.1 $\pm$ 38.6*	10.5 $\pm$ 18.4

* p < 0.05; ** p < 0.01.

is the sum of the visual analog scale (a scale ranging from 0 to 10), which was used in the subjective assessment of both itching and sleep loss (average for the last 3 days) [16]. The severity of the disease in the 50 patients with AD who participated in this study was as follows: 22 patients had slight AD, 10 patients had mild AD, 11 patients had moderate AD and 7 patients had severe AD.

Collection of Stratum Corneum Samples and Evaluation of Skin Sites

The stratum corneum was collected from the healthy subjects and patients with AD by using the tape-stripping technique on the skin of the forearm, neck and back [8]. Plastic tape (24 mm $\times$ 5 cm; Cellotape, Nichiban, Tokyo, Japan) was applied and removed from the skin lesions 5 times and stored at -20°C until required. The appearance of AD at the skin sites was evaluated on a 4-point scale (0: none, 1: mild, 2: moderate or 3: severe) for each parameter of skin symptoms described in B scores of SCORAD: erythema, edema/papules, excoriation, oozing/crust, lichenification and xerosis [16].

Trans-epidermal water loss (TEWL) and skin water content (SWC) at the skin sites were measured using a Corneometer CM825 and a Tewameter TM300 MPA5 (Courage and Khazako, Cologne, Germany) in an ordinary air-conditioned room at the clinic.

Laboratory Tests

Among the 50 patients with AD who were enrolled in the study, 47 (aged 9–58 years, mean 27.7) consented to the blood tests. Of these, 27 were men (aged 14–58 years, mean 26.9) and 20 were women (aged 9–55 years, mean 29.0). Serum levels of total IgE, lactate dehydrogenase (LDH) and peripheral blood eosinophil count were measured by laboratory tests. Serum TARC was measured by ELISA and the normal range of the serum TARC value was different depending on the ages: 6–12 months (below 1367 pg/ml); 1–2 years (below 998 pg/ml); 2–18 years (below 743 pg/ml), and adult (below 450 pg/ml). The results were as follows: serum TARC $3,605 \pm 7,716$ pg/ml; serum IgE $3,607 \pm 6,268$ IU/ml; serum LDH 232 ± 80 IU/l, and eosinophil count 629 ± 646 cells/ml.

Measurement of scIL-8, scIL-18, scVEGF and scTGF- α

The collected tapes were cut finely into 5-cm sections and immersed in 5 ml of hexane. After collecting the horny layer by centrifugation, proteins were extracted in 1 ml of extraction buffer [0.1 M Tris-HCl (pH 8.0) and 0.5% Triton X-100] under ultrasound

sonication (Branson Sonifier 450; Emerson Japan, Atsugi, Japan) for 3 min. The supernatant was purified using a 4-mm filter (Millex Filter Units; Nihon Millipore, Tokyo, Japan) and centrifuged at 15,000 rpm for 15 min at 4°C . The resulting purified supernatant contained the stratum corneum. The amounts of IL-8, IL-18, VEGF and TGF- α in the extracts were measured using specific ELISA kits. For IL-8, VEGF, TGF- α (R&D Systems, Minneapolis, Minn., USA) SuperSignal ELISA Femto Maximum Sensitivity Substrate (Meridian, Rockford, Ill., USA) was used. For IL-18 (Medical & Biological Laboratories, Nagoya, Japan) chemiluminescent peroxidase substrate (St. Louis, Mo., USA) was used. The minimum detectable dose of IL-8 was 3.5 pg/ml, of IL-18 was 12.5 pg/ml, of VEGF was 5.0 pg/ml and of TGF- α was 2.27 pg/ml. Total protein levels of the samples were measured using the DC protein assay (Bio-Rad, Hercules, Calif., USA).

Data Analysis

For statistical analysis, Mann-Whitney's U test was used for comparison between 2 groups, and Spearman's rank test was used to analyze correlation. Results are expressed as mean $\pm$ SD and were considered to be significant or correlated with p < 0.01 or p < 0.05, as indicated.

Results

Correlation between scIL-8, scIL-18, scVEGF, scTGF- α and Clinical Severity in AD Patients

Mean levels of scIL-8, scIL-18, scVEGF and scTGF- α in the patients with AD and in healthy controls are shown in table 2. The levels of all measured cytokines from the arm, neck and back region were significantly higher in the AD patients as compared to those of healthy controls. Figure 1 shows a comparison of the levels of scIL-8, scIL-18, scVEGF and scTGF- α from the arm, neck and back among unaffected and affected areas of AD patients and the healthy controls. The cytokine levels of both the unaffected and the affected areas of AD patients were significantly higher than those of healthy controls. Additionally, the levels of scIL-8 and scIL-18, but not scVEGF

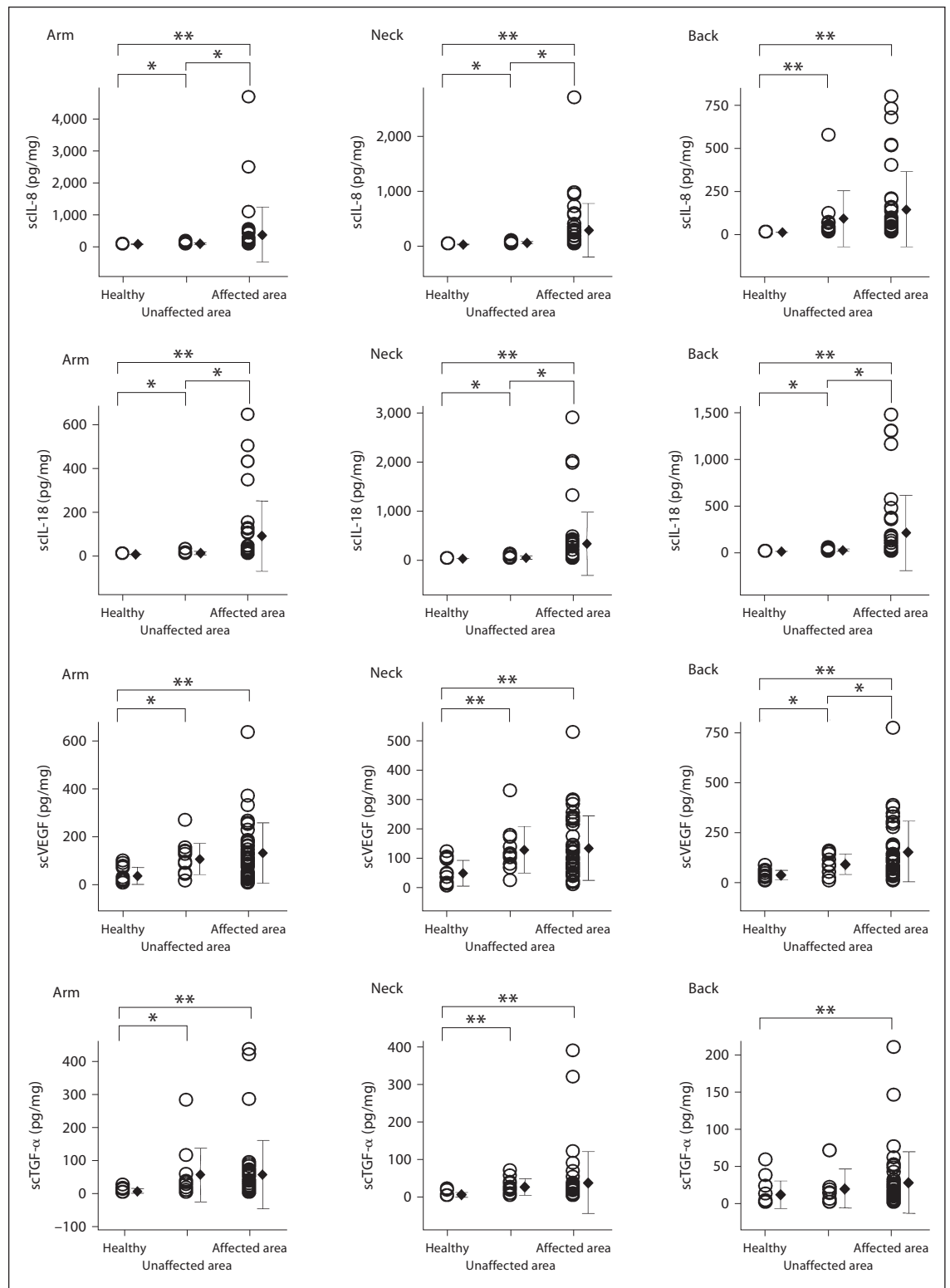


Fig. 1. sclL-8, sclL-18, scVEGF and scTGF-α of arm, neck and back in patients with AD and healthy subjects. In the patients with AD, the values were compared between unaffected area (B score of SCORAD = 0) and affected area (B score of SCORAD > 1). The

number of healthy subjects was 12 in the arm, neck and back; the number of affected areas studied in the arm was 38, in the neck 39 and in the back 39; the number of unaffected studied areas in the arm was 12, in the neck 11 and in the back 11. * p < 0.05; ** p < 0.01.

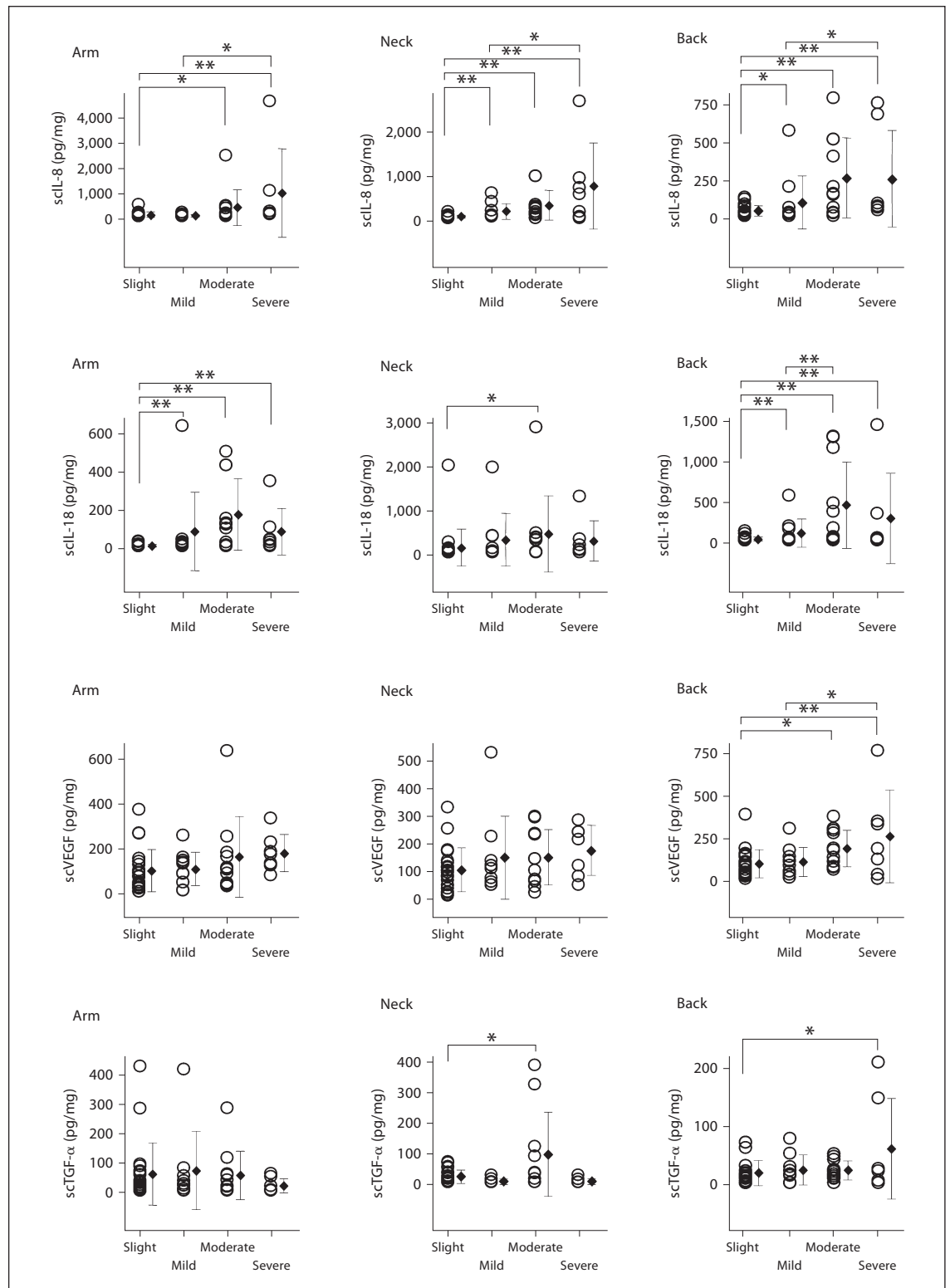


Fig. 2. Correlation between sIL-8, sIL-18, scVEGF, scTGF-α and severity of AD. Clinical severity of AD was determined by the SCORAD criteria [16]. * p < 0.05; ** p < 0.01.

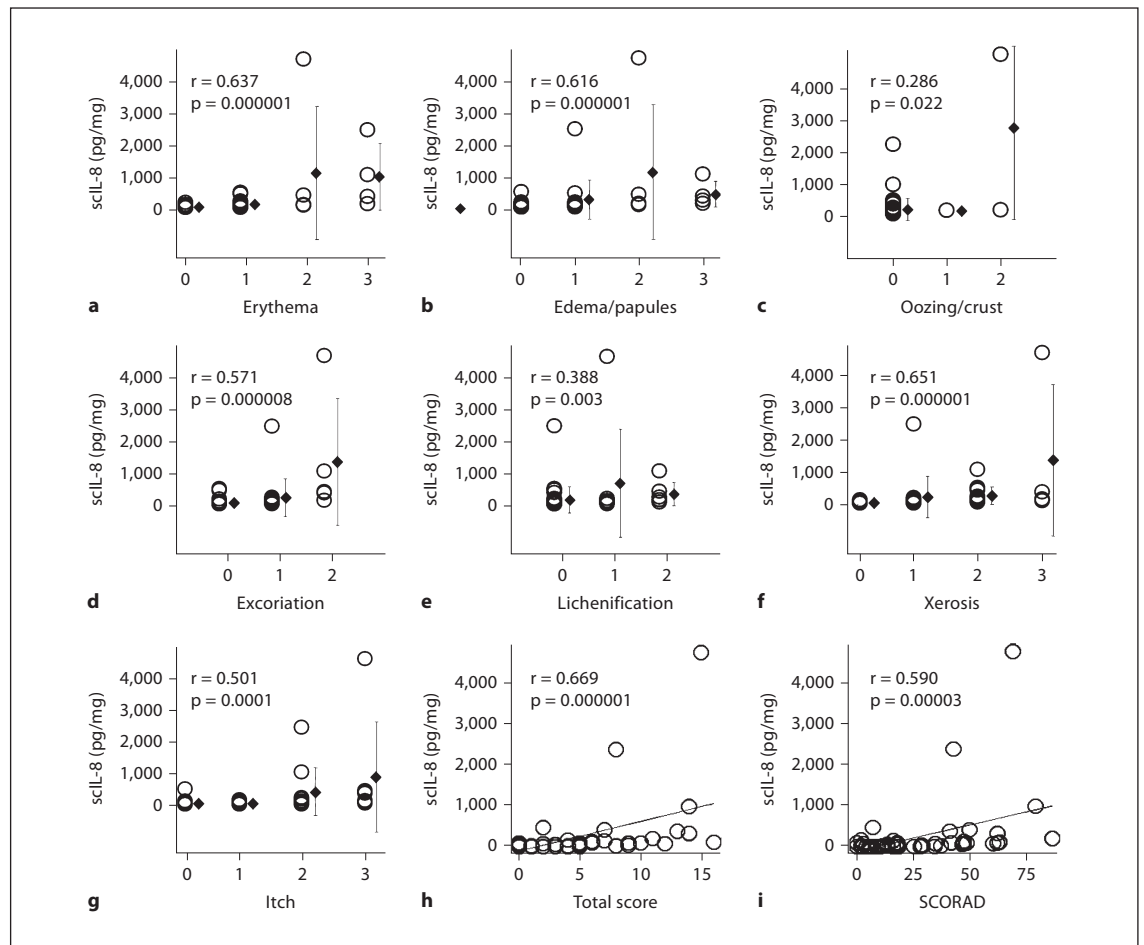


Fig. 3. Correlation between scIL-8 and manifestation score of skin lesions in the arm of patients with AD. Erythema (a), edema/papules (b), oozing/crust (c), excoriation (d), lichenification (e), xerosis (f), itch (g), total score (h) and SCORAD (i) are shown.

and scTGF- α , were significantly higher in the affected versus the unaffected areas. When the levels of the cytokines were compared among the patient groups divided according to disease severity, the increase in scIL-8 was shown to be directly proportional to the severity of AD, with a significant increase observed in the arm, neck and back (fig. 2). A significant increase in scIL-18 proportional to the severity of AD was also seen in the arm and back, but not the neck. A significant increase in scVEGF was seen only in the back, and no significant increase was observed in scTGF- α in any site tested.

Correlation between scIL-8, scIL-18, scVEGF, scTGF- α and Each Parameter of the Skin Symptoms

The correlation of scIL-8, scIL-18, scVEGF and TGF- α with the severity of local inflammation was assessed us-

ing the manifestation scores of skin lesions (erythema, edema/papules, oozing/crust, excoriation, lichenification, xerosis, itch, total score and SCORAD) in the affected skin of AD patients. The scIL-8 level correlated highly with the scores for erythema (fig. 3a), edema/papules (fig. 3b), excoriation (fig. 3d), lichenification (fig. 3e), xerosis (fig. 3f) and itch (fig. 3g). A weak but significant correlation was observed with the oozing/crust scores (fig. 3c). A significant correlation was also seen between scIL-8 level and total score (fig. 3h) as well as SCORAD (fig. 3i). The correlation scores of scIL-8, scIL-18, scVEGF and scTGF- α are summarized in table 3. A significant correlation was observed between the scIL-18 level and erythema, edema/papules, excoriation, xerosis, itch, total score of clinical conditions and SCORAD in the arm, neck and back of patients with AD. A significant correla-

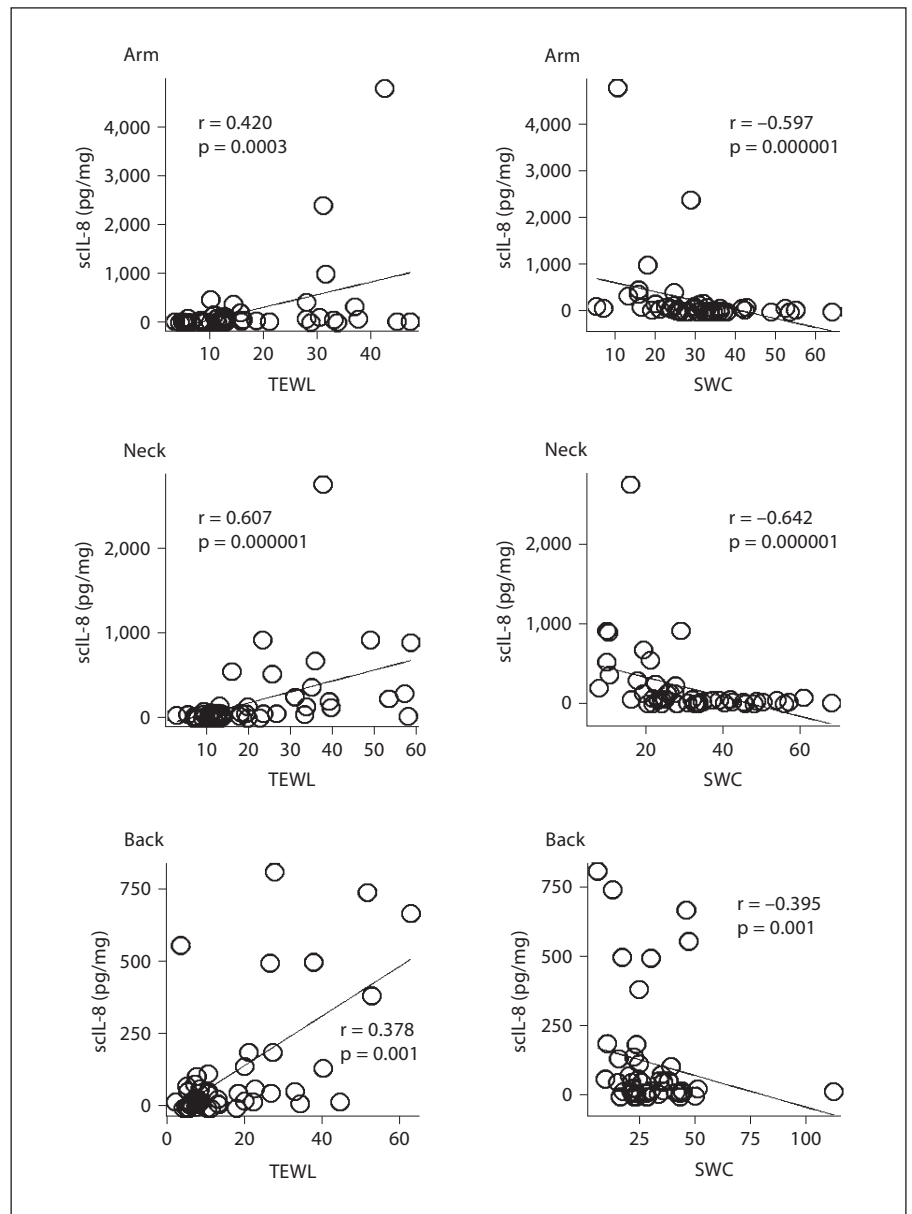


Fig. 4. Correlation between scIL-8 and skin barrier parameters (TEWL and SWC).

tion was also observed between the scVEGF level and erythema, edema/papules, excoriation, itch and total score. No correlation was seen between scTGF- α and the manifestation scores of the lesions in AD.

Correlation between scIL-8, scIL-18, scVEGF, scTGF- α and Skin-Barrier Parameters

Correlations between the level of scIL-8 in the skin (arm, neck and back) and the skin-barrier parameters including TEWL and SWC are shown in figure 4. The levels of scIL-8 and scIL-18 in all the sites correlated pos-

itively with TEWL and negatively with SWC (table 3). The correlation between scVEGF and the skin-barrier parameters was found to be variable in the different sites, and there was no significant correlation between the levels of scTGF- α and skin-barrier parameters at any site (table 3).

Correlation between scIL-8, scIL-18, scVEGF, scTGF- α and Laboratory Data

The scIL-8 level in the arm of AD patients was compared with the serum levels of total IgE, LDH and TARC,

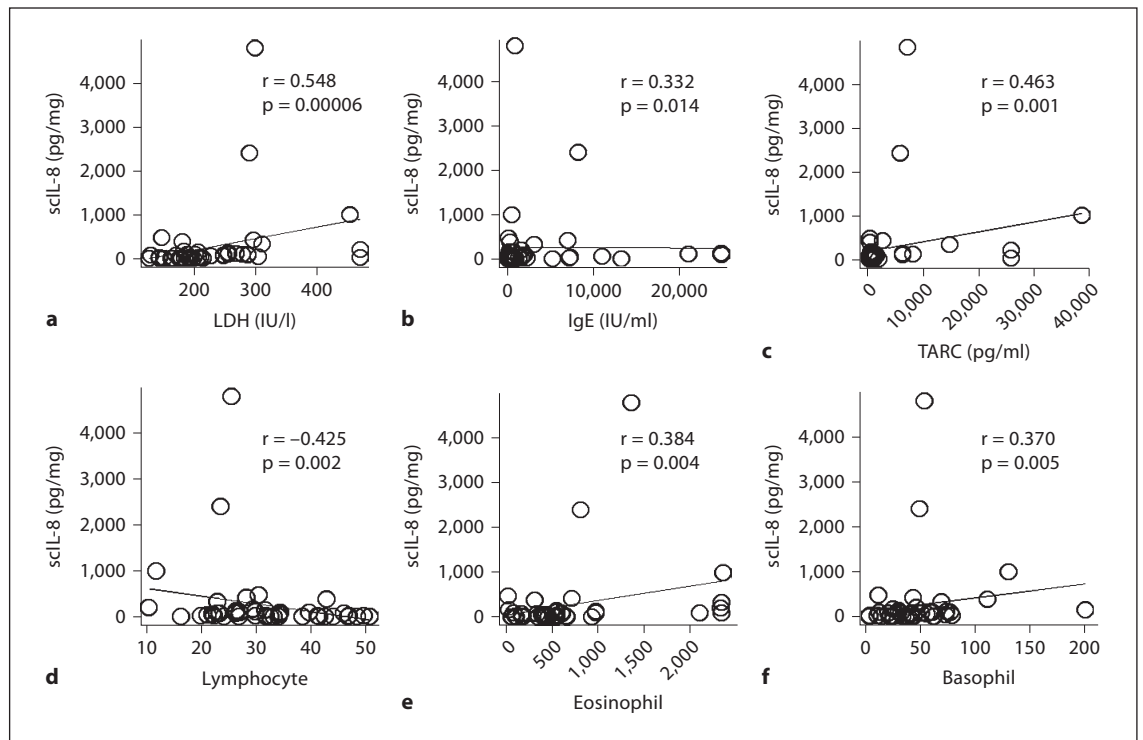


Fig. 5. Correlation between scIL-8 in the arm and clinical severity parameters: serum LDH, IgE and TARC, blood lymphocyte, eosinophil and basophil counts.

and blood lymphocyte, eosinophil and basophil counts (fig. 5). We found scIL-8 level to have a positive correlation with serum LDH (fig. 5a), serum total IgE (fig. 5b), serum TARC (fig. 5c), eosinophil count (fig. 5e) and basophil count (fig. 5f), and a significant negative correlation with lymphocyte count (fig. 5d). A similar but weak correlation was seen between the scIL-18 level in the arm and these laboratory parameters. The scVEGF level in the arm correlated with serum LDH, serum TARC, blood eosinophil count and basophil count, but not with serum IgE level and blood lymphocyte count (table 3). In contrast, scTGF- α level did not correlate with serum levels of any of the laboratory parameters at any site (table 3).

Discussion

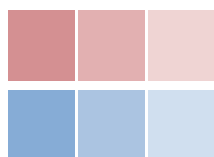
We found the highest correlation of scIL-8 in 18 of the 27 parameters of clinical scores obtained from the arm, neck and back as compared with the other cytokines tested (IL-18, VEGF and TGF- α ; table 3), indicating that scIL-8 is a superior indicator of local severity in both

acute and chronic inflammation in AD lesions. The present study also revealed a strong correlation between scIL-8 and skin-barrier dysfunctions (fig. 4), as well as laboratory parameters that are known to be severity markers of AD (fig. 5). Increased IL-18 protein expression has been demonstrated recently in the lesions of AD by immunohistochemistry and the level of IL-18 in the horny layer was found to be useful for evaluating the production of IL-18 in epidermal keratinocytes as well as the effects of therapy in patients with AD [10]. We have previously demonstrated a good correlation between scVEGF and the parameters of acute inflammation such as erythema and edema [9]. In this study, we established that the scIL-8 level in the lesions of AD is best correlated with the severity of lesions among a variety of cytokines and growth factors.

In our previous study, the TARC level detected with an immunofluorescence technique was found to be well correlated with the acute inflammation parameters erythema and edema, but not with chronic inflammation parameters, lichenification or xerosis [8]. The IL-8 level detected in the present study was not directly compared

Table 3. Summary of correlation coefficient between cytokine levels and clinical scores, physiological measurement and laboratory data

	scIL-8			scIL-18			scVEGF			scTGF- α		
	Arm	Neck	Back	Arm	Neck	Back	Arm	Neck	Back	Arm	Neck	Back
Clinical scores												
Erythema	0.63	0.63	0.59	0.63	0.56	0.49	0.28	0.35	0.52	-0.21	-0.15	-0.10
Edema/papules	0.61	0.64	0.60	0.64	0.63	0.49	0.32	0.45	0.50	-0.17	-0.13	-0.13
Oozing/crust	0.28	0.28	0.22	0.35	0.15	0.18	0.19	0.14	0.10	-0.14	-0.10	-0.01
Excoriation	0.57	0.57	0.38	0.60	0.52	0.45	0.42	0.38	0.31	-0.20	-0.23	0.23
Lichenification	0.38	0.38	0.30	0.46	0.19	0.31	0.21	0.16	0.22	-0.12	-0.26	-0.03
Xerosis	0.65	0.58	0.39	0.59	0.50	0.26	0.18	0.21	0.24	-0.01	-0.34	0.03
Itch	0.50	0.60	0.34	0.52	0.49	0.45	0.20	0.24	0.30	0.11	-0.09	0.02
Total score	0.65	0.67	0.47	0.65	0.60	0.46	0.27	0.29	0.36	-0.11	-0.24	0.11
SCORAD	0.59	0.62	0.50	0.61	0.52	0.46	0.24	0.19	0.26	-0.02	-0.22	0.22
Physiological measurement												
TEWL	0.42	0.60	0.37	0.43	0.48	0.33	0.13	0.26	0.41	0.03	-0.07	0.13
SWC	-0.59	-0.64	-0.39	-0.57	-0.59	-0.33	-0.25	-0.34	-0.24	-0.16	0.06	-0.09
Laboratory data												
LDH	0.53	0.53	0.61	0.33	0.29	0.33	0.46	0.27	0.36	0.05	-0.21	0.05
IgE	0.32	0.58	0.40	0.16	0.42	0.30	0.07	0.11	0.18	-0.06	0.09	-0.10
TARC	0.44	0.51	0.53	0.32	0.35	0.40	0.32	0.17	0.34	0.03	-0.03	-0.03
Lymphocyte	-0.40	-0.44	-0.35	-0.24	-0.40	-0.31	-0.05	-0.20	-0.31	0.16	0.02	0.09
Eosinophil	0.37	0.62	0.50	0.21	0.35	0.38	0.30	0.32	0.41	-0.18	0.03	-0.16
Basophil	0.24	0.34	0.19	0.32	0.43	0.13	0.34	0.24	0.18	-0.04	-0.16	-0.15



Positive correlations: $p < 0.001$, $p < 0.01$, $p < 0.05$.

Negative correlations: $p < 0.001$, $p < 0.01$, $p < 0.05$.

Table 4. Summary of correlation coefficient among each cytokine level

		scIL-18	scVEGF	scTGF- α
Arm	scIL-8	0.49	0.33	-0.03
	scIL-18		0.32	-0.10
	scVEGF			0.09
Neck	scIL-8	0.60	0.38	-0.28
	scIL-18		0.25	-0.30
	scVEGF			0.08
Back	scIL-8	0.45	0.38	0.04
	scIL-18		0.53	0.10
	scVEGF			0.09

			Positive correlations: $p < 0.001$, $p < 0.01$, $p < 0.05$.
			Negative correlations: $p < 0.001$, $p < 0.01$, $p < 0.05$.

with our previous results of TARC because the TARC levels were determined by the intensity of immunofluorescence, whereas the IL-8 level was determined with ELISA directly. When the contents of IL-8 and TARC were measured in the extraction of collected stratum corneum of AD patients, the mean level of IL-8 was approximately 1,200 (range 900–2,850) pg/mg scale, and the mean level of TARC was 37 (range 3–185) pg/mg scale, indicating that the IL-8 content was more than 30 times higher than that of TARC content.

IL-8 was identified to be a potent chemotactic factor for neutrophils but not for eosinophils [17]. IL-8 has been extracted from the scales obtained from psoriasis lesions and is found to be produced by epidermal cells, and thus considered to play an important role in inflammatory processes of the skin with sterile neutrophil infiltration, such as psoriasis [18, 19]. In contrast to the role of IL-8 in the pathogenesis of psoriasis, its involvement in the pathogenesis of AD has not been well delineated. Our study suggests that IL-8 production in the epidermal cells is a ubiquitous process in the initiation of skin inflamma-

tion, including that in AD. Although IL-8 detected in tape-stripped stratum corneum is considered to derive from activated keratinocytes, it is also conceivable that a part of IL-8 is derived from activated leukocytes, especially in cases of spongiosis or excoriated skin. IL-8 isolated from stratum corneum is still biologically active [20], thus may activate multiple intracellular signaling pathways downstream of two cell surface receptors, CXCR1 and CXCR2, which are expressed on human neutrophils, CD8⁺ T cells and NK cells [21]. Immunohistochemical studies using anti-IL-8 antibodies have demonstrated intracellular IL-8 staining in the lesion of AD, indicating that IL-8 plays an important role in the pathogenesis of AD by recruiting inflammatory cells [22].

scIL-8 was found to be well correlated with scIL-18 (table 4). IL-18 is a unique cytokine with a capacity to induce either Th1 or Th2 polarization, depending on the immunologic context. IL-18 induces IFN- γ production from Th1 cells but induces Th2 cells by the induction of IL-4 production from naïve T cells [23]. IL-18 is an im-

portant regulator of both innate and acquired immunity, and IL-18 may play a role in chronic inflammatory skin diseases by its capacity to activate keratinocytes [24, 25]. Although scIL-8 is well correlated with scIL-18, the induction mechanism of IL-8 in AD lesions is unclear. Because IL-8 is produced by cultured keratinocytes stimulated with *S. aureus*, it is likely that both IL-8 and IL-18 are induced by *S. aureus* colonization through a common induction mechanism [12, 26].

scVEGF is also weakly but significantly correlated with scIL-8, suggesting a common trigger in the expression of these cytokines. We have demonstrated that VEGF produced in the lesions of AD is a subtype of VEGF, which has the ability to exclusively enhance microvascular permeability [27, 28], suggesting that this may be a potent mediator of erythema and edema. No correlation was found between the level of scTGF- α and the severity of AD, suggesting a regulatory rather than an inflammatory role of TGF- α in the pathogenesis of AD.

Besides IL-8, a series of chemokines has been involved in the pathogenesis of AD, including CCL11 (eotaxin) and RANTES [30–33]. Dermal CCL11 plays a role in tissue eosinophilia observed in AD lesions and plasma eotaxin level is increased in the chronic eczematous lesions

of AD [29, 30]. RANTES is a potent chemoattractant for eosinophils, lymphocytes, monocytes and basophils, and its promoter gene polymorphism is known as a risk factor for AD susceptibility [31, 32]. CC chemokines (RANTES, MCP-1, MIP-1 α and MIP-1 β) are also members of cytokines involved in the pathogenesis of AD [33]. Amounts of these chemokines are negligible compared with that of IL-8 in the stratum corneum obtained in the lesions of AD.

In conclusion, scIL-8 levels are strongly correlated with the severity of individual lesions, particularly the degree of erythema, edema/papules and excoriation. Additionally, a tape-stripping method is useful to evaluate the scIL-8 in combination with the IL-8-specific ELISA. Thus scIL-8 might be utilized to evaluate precisely the effect of new treatment tools for topical use.

Acknowledgment

This work was partly supported by Grant-in-Aid 22791073 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

References

- De Benedetto A, Agnihotri R, McGirt LY, et al: Atopic dermatitis: a disease caused by innate immune defects? *J Invest Dermatol* 2009;129:14–30.
- Homey B, Steinhoff M, Ruzicko T, Leung DY: Cytokines and chemokines orchestrate atopic skin inflammation. *J Allergy Clin Immunol* 2006;118:178–189.
- Chan LS: Atopic dermatitis in 2008. *Curr Dir Autoimmun* 2008;10:76–118.
- Uchi H, Terao H, Koga T, Furue M: Cytokines and chemokines in the epidermis. *J Dermatol Sci* 2000;24:S29–S38.
- Hamid Q, Boguniewicz M, Leung DY: Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870–876.
- Fiset PO, Leung DY, Hamid Q: Immunopathology of atopic dermatitis. *J Allergy Clin Immunol* 2006;118:287–290.
- Ong PY, Leung DY: Immune dysregulation in atopic dermatitis. *Curr Allergy Asthma Rep* 2006;6:384–389.
- Morita E, Takahashi H, Niihara H, Dekio I, et al: Stratum corneum TARC level is a new indicator of lesional skin inflammation in atopic dermatitis. *Allergy* 2010;65:1166–1172.
- Amarbayasgalan T, Takahashi H, Dekio I, Morita E: Content of vascular endothelial growth factor in stratum corneum well correlate to local severity of acute inflammation in patients with atopic dermatitis. *Int Arch Allergy Immunol* 2011;157:251–258.
- Inoue Y, Aihara M, Kirino M, et al: Interleukin-18 is elevated in the horny layer in patients with atopic dermatitis and is associated with *Staphylococcus aureus* colonization. *Br J Dermatol* 2011;164:560–567.
- Kimata H, Lindley I: Detection of plasma interleukin-8 in atopic dermatitis. *Arch Dis Child* 1994;70:119–122.
- Miller LS, Sørensen OE, Liu PT, et al: TGF- α regulates TLR expression and function on epidermal keratinocytes. *J Immunol* 2005;174:6137–6143.
- Mascia F, Cataisson C, Lee TC, Threadgill D, et al: EGFR regulates the expression of keratinocyte-derived granulocyte/macrophage colony-stimulating factor in vitro and in vivo. *J Invest Dermatol* 2010;130:682–693.
- Mascia F, Mariana V, Girolomoni G, Postore S: Blockade of the EGF receptor induces a deranged chemokine expression in keratinocytes leading to enhanced skin inflammation. *Arn J Pathol* 2003;163:303–312.
- Saeki H, Furue M, Furukawa F, Hide M, et al: Guidelines for management of atopic dermatitis. *J Dermatol* 2009;36:563–577.
- Kunz B, Oranje AP, Labrèze L, et al: Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;195:10–19.
- Schröder JM, Mrowietz U, Morita E, et al: Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin-1 activity. *J Immunol* 1987;139:3474–3483.
- Schröder JM, Christophers E: Identification of C5a_{des arg} and an anionic neutrophil-activating peptide in psoriatic scales. *J Invest Dermatol* 1986;87:53–58.
- Ferran M, Galvan AB, Gemenez-Arnau A, et al: Production of interleukin-8 by circulating CLA⁺ T cells with skin tropism in patients with psoriasis and in healthy controls (in Spanish). *Actas Dermosifiliogr* 2010;101:151–155.
- Schröder JM: Identification and structural characterization of chemokines in lesional skin material of patients with inflammatory skin disease. *Methods Enzymol* 1997;288:266–297.

- 21 Lai Y, Shen Y, Lui XH, Zhang Y, et al: Interleukin-8 induces the endothelial cell migration through the activation of phosphoinositide 3-kinase-Rac 1/RhoA pathway. *Int J Boil Sci* 2011;7:782–791.
- 22 Sticherling M, Bornscheuer E, Schröder JM, Christophers E: Immunohistochemical studies on NAP-1/IL-8 in contact eczema and atopic dermatitis. *Arch Dermatol Res* 1992;284:82–85.
- 23 Nakanishi K, Yoshimoto T, Tsutsui H, et al: Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 2001;19:423–474.
- 24 Terada M, Tsutsui H, Imai Y, Yasuda K, et al: Contribution of IL-18 to atopic-dermatitis-like skin inflammation induced by *Staphylococcus aureus* product in mice. *Proc Natl Acad Sci USA* 2006;103:8816–8821.
- 25 Wittmann M, Purwar R, Hartmann C, Gutzmer R, et al: Human keratinocytes respond to interleukin-18: implication for the course of chronic inflammatory skin diseases. *J Invest Dermatol* 2005;124:1225–1233.
- 26 Oлару F and Jensen LE: *Staphylococcus aureus* stimulates neutrophil targeting chemokine expression in keratinocytes through an autocrine IL-1 α signaling loop. *J Invest Dermatol* 2010;130:1866–1876.
- 27 Zang Y, Matsuo H, Morita E: Increased production of vascular endothelial growth factor in the lesions of atopic dermatitis. *Arch Dermatol Res* 2006;297:425–429.
- 28 Zhang Y, Furumura M, Morita E: Distinct signaling pathways confer different vascular responses to VEGF 121 and VEGF 165. *Growth factors* 2008;26:125–131.
- 29 Gahr N, Fölster-Holst R, Weichenthal M, Christophers E, et al: Dermal fibroblasts from acute inflamed atopic dermatitis lesions display increased eotaxin/CCL11 responsiveness to interleukin-4 stimulation. *Br J Dermatol* 2011;164:586–592.
- 30 Hossny E, Aboul-Magd M, Bakr S: Increased plasma eotaxin in atopic dermatitis and acute urticaria in infants and children. *Allergy* 2001;56:996–1002.
- 31 Tanaka K, Poberts MH, Yamamoto N, Sugura H, et al: Upregulating promoter polymorphisms of RANTES relate to atopic dermatitis. *Int J Immunogenet* 2006;33:423–428.
- 32 Nickel RG, Casolaro V, Wahn U, Beyer K, et al: Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokines RANTES. *J Immunol* 2000;164:1612–1616.
- 33 Kaburagi Y, Shimada Y, Nagaoka T, Hasegawa M, et al: Enhanced production of CC-chemokines (RANTES, MCP-1, MIP-1 α , MIP-1 β , and eotaxin) in patients with atopic dermatitis. *Arch Dermatol Res* 2001;293:350–355.