

Increased serum cutaneous T cell-attracting chemokine (CCL27) levels in patients with atopic dermatitis and psoriasis vulgaris

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Background: Both atopic dermatitis (AD) and psoriasis vulgaris (PsV) are characterized as chronic and relapsing inflammatory skin diseases associated with various immunologic abnormalities. Cutaneous T cell-attracting chemokine (CTACK; CCL27) is a member of the CC chemokine family and a functional ligand for CC chemokine receptor 10. It is selectively expressed in skin and attracts CC chemokine receptor 10-expressing skin-homing memory T cells. The epidermal keratinocyte is a main source of CTACK, suggesting the involvement of various inflammatory skin diseases.

Objective: The purpose of this investigation was to clarify whether CTACK produced by keratinocytes is detected in the sera of patients with AD and PsV and to examine the correlation between the serum CTACK levels and disease activity of patients with AD and PsV.

Methods: We measured the serum CTACK levels in 50 patients with AD, 30 patients with PsV, and 22 healthy control subjects. We also divided 50 patients with AD into 3 groups (ie, those with mild, moderate, and severe disease) and compared them among 3 categories. Moreover, we compared the serum CTACK levels of patients with AD and PsV with clinical or laboratory data. Immunohistochemical staining of CTACK and IFN-induced protein of 10 kd (IP-10; CXCL10) was performed on the lesional skin of patients with AD and PsV.

Results: The serum CTACK levels in patients with AD and PsV were significantly higher than those in healthy control subjects. The serum CTACK levels in patients with AD significantly correlated with scoring atopic dermatitis (SCORAD) scores, serum soluble IL-2 receptor levels, serum soluble E-selectin levels, serum thymus and activation-regulated chemokine levels, and serum macrophage-derived chemokine levels. Serum CTACK levels in patients with PsV significantly correlated with the serum IP-10 levels but not with the Psoriasis Area and Severity Index score. Immunohistochemical stain-

ing showed CTACK was strongly expressed in lesional keratinocytes of patients with AD and PsV, whereas IP-10 was strongly expressed in lesional keratinocytes of patients with PsV and focally in those with AD.

Conclusion: These results suggest that CTACK might be one of the important chemokines for the pathogenesis of AD and PsV. (*J Allergy Clin Immunol* 2003;111:592-7.)

Key words: Cutaneous T cell-attracting chemokine, CCL27, atopic dermatitis, psoriasis vulgaris, disease activity

Atopic dermatitis (AD) is an inflammatory skin disease that is characterized by pruritic and eczematous lesions persisting chronically. It is often associated with increased serum IgE levels and peripheral blood eosinophilia. Histopathologically, the skin lesions in AD show infiltration of T lymphocytes, especially cutaneous lymphocyte antigen-positive memory T cells, as well as eosinophils and macrophages.^{1,2} Previous studies revealed that serum soluble IL-2 receptor (sIL-2R) levels,³ serum soluble E-selectin levels,⁴ serum thymus and activation-regulated chemokine (TARC) levels,⁵ and serum macrophage-derived chemokine (MDC) levels⁶ significantly correlate with the disease activity of AD, suggesting involvement in the pathogenesis of AD.

Psoriasis vulgaris (PsV) is also a chronic and relapsing inflammatory skin disease characterized as a T cell-mediated autoimmune disorder.⁷ It has been proposed that interaction between T cells and epidermal keratinocytes plays a central role in the pathogenesis of PsV.⁸

Cutaneous T cell-attracting chemokine (CTACK; CCL27) belongs to the CC chemokine family and is a ligand for CC chemokine receptor 10.⁹⁻¹¹ It selectively chemoattracts cutaneous lymphocyte antigen-positive, CC chemokine receptor 10-positive memory T cells into the inflammatory sites.^{9,12} It is reported that CTACK is expressed only in the skin but not in other organs and is especially expressed constitutively in epidermal keratinocytes.⁹ In addition, previous *in vitro* data revealed that the expression and production of CTACK in keratinocytes is upregulated by stimulation with both IL-1 β and TNF- α .^{9,13,14} Although the quantitative PCR analysis of CTACK showed no significant differences between the lesional skin of patients with AD and PsV and normal skin,¹³ on the basis of these results, CTACK might be

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Abbreviations used

AD:	Atopic dermatitis
CTACK:	Cutaneous T cell-attracting chemokine
CCR10:	CC chemokine receptor 10
IP-10:	IFN-induced protein of 10 kd
MDC:	Macrophage-derived chemokine
PASI:	Psoriasis Area and Severity Index
PsV:	Psoriasis vulgaris
SCORAD:	Scoring atopic dermatitis
sIL-2R:	Soluble IL-2 receptor
TARC:	Thymus and activation-regulated chemokine

associated with various inflammatory skin diseases, such as AD and PsV. Indeed, very recently, Homey et al¹⁴ reported that CTACK is strongly expressed in the lesional keratinocytes of patients with AD compared with in the normal keratinocytes of healthy control subjects. However, it has not yet been clarified whether CTACK secreted from keratinocytes is found in the sera of patients with inflammatory skin diseases, such as AD or PsV.

In this study we measured the serum CTACK levels in patients with AD and PsV and compared them with those in healthy control subjects. We also compared the serum CTACK levels in patients with AD before and after treatment. Moreover, we examined the correlation between the serum CTACK levels in patients with AD and other laboratory data for disease activity of AD, such as serum sIL-2R, soluble E-selectin, serum TARC, and serum MDC levels and eosinophil numbers in peripheral blood. In patients with PsV, we also examined the correlation among the serum CTACK level, the Psoriasis Area and Severity Index (PASI) score, and the serum IFN-induced protein of 10 kd (IP-10; CXCL10) level. Furthermore, we performed immunohistochemical staining of CTACK in the lesional skin of patients with AD and PsV and compared the results with IP-10 expression in the lesional skin of patients with these diseases.

METHODS

Samples and reagents

The sera of 50 patients with AD (mean $\pm$ SEM age, 28.5 $\pm$ 6.8 years), 22 healthy control subjects (mean $\pm$ SEM age, 36.4 $\pm$ 8.5 years), and 30 patients with PsV (mean $\pm$ SEM age, 51.2 $\pm$ 8.0 years) were used in this study. All patients with AD were given a diagnosis according to the criteria of Hanifin and Rajka¹ and were treated with topical corticosteroids in combination with oral antihistamines. Twenty-six of 50 patients with AD had personal histories of respiratory allergy. Disease activity of AD was determined by using the modified scoring atopic dermatitis (SCORAD) index system.^{15,16} In this study we divided the patients with AD into 3 groups (ie, those with mild, moderate, and severe disease) according to a proposal for severity grading of AD by using only objective criteria (mild disease, SCORAD score = <15; moderate disease, SCORAD score = 15-40; and severe disease, SCORAD score = >40).¹⁶ Twenty-two healthy control subjects and 30 patients with PsV had no histories of allergy, and their total serum IgE levels were within the normal range. In addition, we estimated the clinical severity of symptoms in patients with PsV by using the PASI score. After venous blood was drawn, serum was separated and stored at -20°C until use.

For immunohistochemical staining, mouse anti-human CTACK mAb (R&D Systems), mouse IgG (R&D Systems), rabbit anti-human IP-10 antibody (Santa Cruz Biotechnology Inc), and rabbit IgG (Jackson ImmunoResearch) were used.

ELISA protocol for CTACK

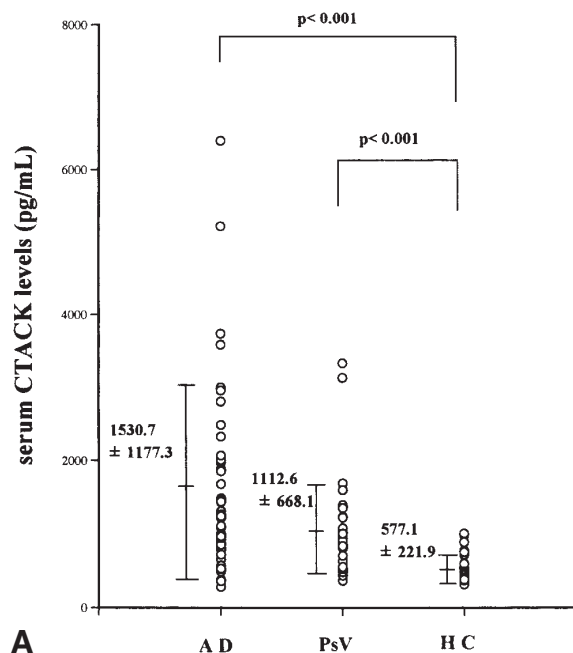
In this study recombinant human CTACK (R&D Systems), anti-human CTACK mAb (clone 124308, R&D Systems) and biotinylated anti-human CTACK polyclonal antibody (R&D Systems) were used. Two nanograms of anti-human CTACK mAb was applied to each well of a 96-well microplate (Costar Co) and coated overnight at room temperature. Wells were then washed 3 times and blocked for at least 1 hour with 300 μ L of PBS containing 1% BSA (Interger Co), 5% sucrose, and 0.05% NaN₃. Subsequently, standard dilutions of recombinant CTACK (2000, 1000, 500, 250, 125, 62.5, 31.2, and 0 pg/mL) and samples were added and incubated for 2 hours at room temperature. After washing as above, each well was incubated for 2 hours with 100 μ L of biotinylated anti-human CTACK antibody diluted 200-fold in TBS containing 0.1% PBS, 0.05% Tween-20, and 2% normal goat serum (Sigma chemical Co). After washing as above and staining with streptavidin-horseradish peroxidase (R&D Systems) diluted 200-fold in PBS containing 1% BSA for 20 minutes, 100 μ L of substrate solutions A and B (R&D Systems) mixed 1:1 was added to each well. Fifteen minutes after substrate addition, the reaction was stopped by the addition of 50 μ L of 2N H₂SO₄, and the OD₄₅₀ was measured with a microplate reader (Model 550, Bio-Rad). Recombinant human CTACK was used for standard curves. Data of CTACK levels obtained from the ELISA are presented as means $\pm$ SD.

ELISA for sIL-2R, soluble E-selectin, TARC, MDC, and IP-10

The serum levels of sIL-2R, soluble E-selectin, TARC, MDC, and IP-10 were measured by using a commercially available ELISA system. A 96-well microplate coated with either mAb against human sIL-2R (R&D Systems), soluble E-selectin (MedSystems), TARC (Techne Corp), MDC (R&D Systems), or IP-10 (R&D Systems) was used. Each sample was added and incubated. After the removal of unbound material by means of aspiration and washing, a conjugate with sIL-2R, soluble E-selectin, TARC, MDC, or IP-10 was detected on the basis of its reaction with a substrate solution, which yielded a colored product proportional to the amount of conjugate. The OD₄₅₀ was measured with a microplate reader. Data obtained from the ELISA are presented as means $\pm$ SD.

Immunohistochemical staining of CTACK and IP-10 in the lesional skin of patients with AD and PsV and the normal skin of healthy control subjects

Biopsy samples were taken from the lesional skin of patients with AD (n = 3) and PsV (n = 3) and the normal skin of healthy control subjects (n = 2). The obtained skin samples were embedded in Tissue-Tek OCT compound (Miles Inc) for at least 24 hours at -80°C. These frozen sections were cut with a cryostat set at 6 μ m of thickness and fixed in 4% paraformaldehyde. They were incubated overnight with mouse anti-human CTACK mAb, mouse IgG, rabbit anti-human IP-10 antibody, or rabbit IgG at 4°C after the endogenous peroxidase activity. The samples were then incubated with biotin-conjugated anti-mouse IgG (DAKO Co) or biotin-conjugated anti-rabbit IgG (DAKO Co) for 30 minutes. Subsequently, the samples were washed with PBS, incubated with ABC complex followed by diaminobenzidine solution until brown staining was visible, and counterstained with Mayer hematoxylin, according to the manufacturer's instructions.

**A****Statistical analysis**

The data were analyzed by using the Mann-Whitney *U* test. Correlation coefficients were determined by using the Spearman rank correlation test. *P* values of less than .05 were considered to be statistically significant.

RESULTS**Serum CTACK levels in patients with AD and PsV**

The mean $\pm$ SD serum CTACK levels in patients with AD and PsV were 1530.7 ± 1177.3 and 1112.6 ± 668.1 pg/mL, respectively, whereas the level in healthy control subjects was 577.1 ± 221.9 pg/mL. The serum CTACK levels in patients with AD and PsV were significantly higher than those in healthy control subjects ($P < .001$, respectively; Fig 1, A). In addition, we measured the serum CTACK levels in 10 patients with AD before and after treatment with a topical corticosteroid in combination with oral antihistaminic drugs. Before treatment, the serum CTACK levels were 2680.4 ± 851.3 pg/mL, whereas they decreased to 860.5 ± 352.1 pg/mL after treatment. There was a significant difference between levels before and after treatment ($P < .01$; Fig 1, B).

Serum IP-10 levels in patients with AD and PsV

The mean $\pm$ SD serum IP-10 levels in patients with AD and PsV were 124.55 ± 41.95 and 199.50 ± 129.78 pg/mL, respectively. In addition, the level in healthy control subjects was 87.70 ± 27.76 pg/mL. The serum IP-10 levels in patients with PsV were significantly higher than those in healthy control subjects ($P < .05$), whereas the serum IP-10 levels in patients with AD were not significantly higher than those of healthy control subjects. Moreover, there was no significant difference in the serum IP-10 levels between patients with AD and those with PsV.

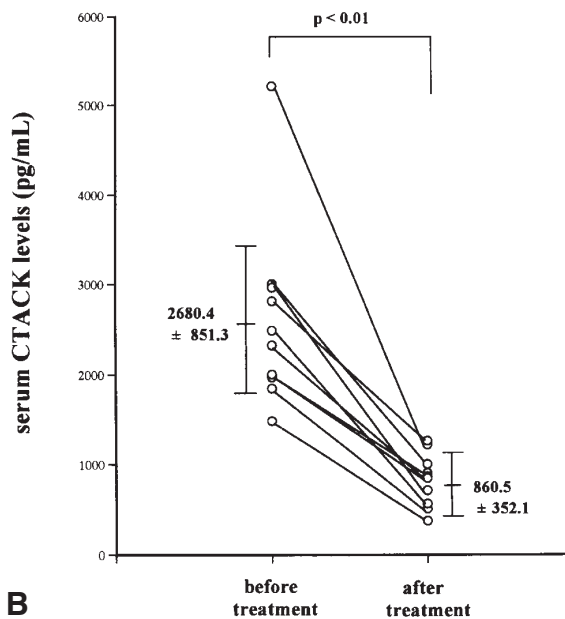
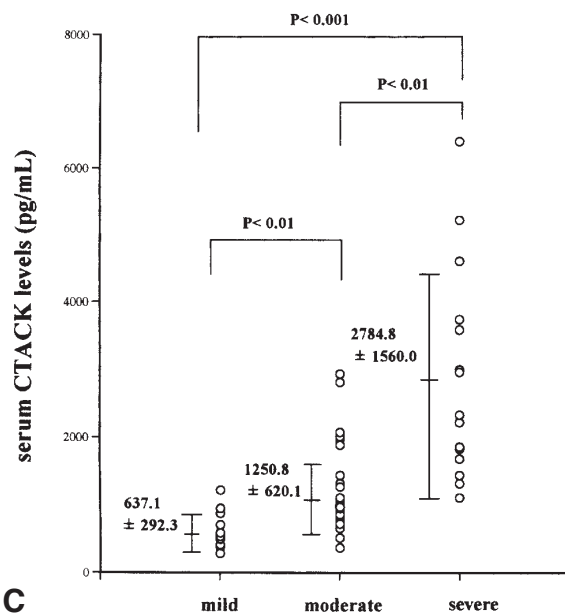
**B****C**

FIG 1. A, Serum CTACK levels in patients with AD ($n = 50$), patients with PsV ($n = 30$), and healthy control subjects (HC; $n = 22$). B, Serum CTACK levels in patients with AD ($n = 10$) were evaluated before and after treatment. C, Comparison of serum CTACK levels among healthy control subjects and 3 groups of patients with AD: those with mild ($n = 10$), moderate ($n = 24$), and severe disease ($n = 16$).

Serum CTACK and IP-10 levels in patients with AD and PsV and disease activity

The mean $\pm$ SD CTACK levels in the mild, moderate, and severe groups of patients with AD were 637.1 ± 292.3 , 1250.8 ± 620.1 , and 2784.8 ± 1560.0 pg/mL, respectively. The serum CTACK levels in the severe group were significantly higher than those in the mild group ($P < .001$) or the moderate group ($P < .01$), indi-

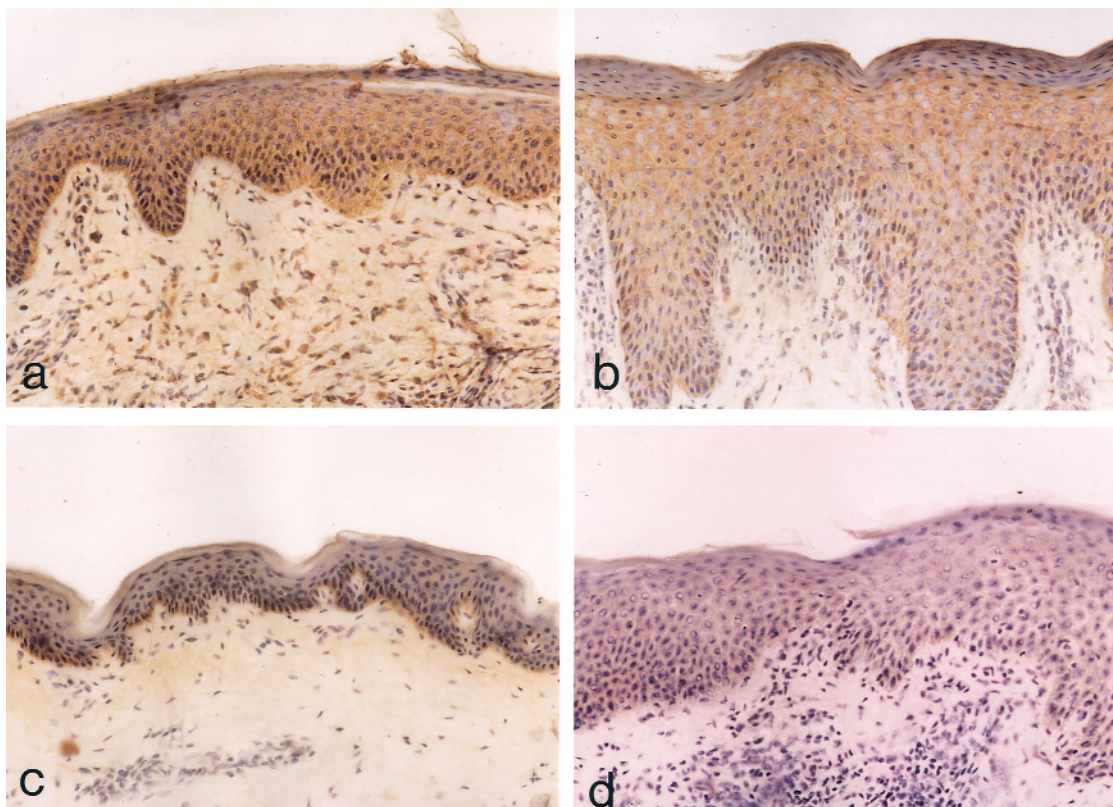


FIG 2. CTACK was strongly expressed in the lesional keratinocytes of patients with AD (a) and PsV (b). In contrast, it was moderately expressed in the basal layer of the normal skin of healthy control subjects (c). When an isotype-matched control was used, no reactivity was observed (d; original magnification: 200 $\times$).

cating that they increased in accordance with the disease severity (Fig 1, C). Moreover, we compared the serum CTACK levels with the clinical and laboratory data that were previously reported to indicate the disease activity of AD. The serum CTACK levels significantly correlated with the SCORAD score ($r = 0.70$, $P < .001$), serum sIL-2R levels ($r = 0.74$, $P < .001$), serum MDC levels ($r = 0.85$, $P < .001$), serum TARC levels ($r = 0.58$, $P < .001$), serum soluble E-selectin levels ($r = 0.56$, $P < .002$), and eosinophil numbers in peripheral blood ($r = 0.62$, $P < .001$). However, there was no significant correlation between the serum CTACK levels and the serum IP-10 levels because the average levels of serum IP-10 in patients with AD were the same as those in healthy control subjects. We also compared the serum CTACK levels in patients with PsV with the serum IP-10 levels and the PASI score. The serum CTACK levels significantly correlated with the serum IP-10 levels ($r = 0.54$, $P < .002$) but not with the PASI score. The results between the serum CTACK levels and other data are summarized in Table I.

Immunoreactive CTACK and IP-10 in the lesional skin of patients with AD and PsV

In the lesional skin of patients with AD and PsV, immunohistochemical staining revealed that CTACK was strongly expressed almost completely in the keratinocytes (Fig 2, a and b). By contrast, in the normal

skin of healthy control subjects, CTACK was moderately expressed in the basal cells but not in the squamous cells (Fig 2, c). When an isotype-matched control was used, no positive staining was observed (Fig 2, d). IP-10 was strongly detected in all layers of lesional keratinocytes of patients with PsV and focally in the lesional keratinocytes of patients with AD (Fig 3). No reactivity was observed when the isotype-matched control was used (data not shown).

DISCUSSION

CTACK is produced in keratinocytes,^{9-11,13} especially after stimulation with TNF- α and IL-1 β in vitro. In this study we investigated whether CTACK is detected in the sera of patients with AD and PsV and found that the serum CTACK levels in patients with AD and PsV were significantly greater than those in healthy control subjects. This result indicates that CTACK secreted by lesional keratinocytes can be detectable in the sera of patients with these inflammatory skin diseases. We also showed that in patients with AD, the serum CTACK levels increased in accordance with the severity of AD. Previous studies reported that the serum sIL-2R,³ E-selectin,⁴ TARC,⁵ and MDC⁶ levels correlated with the disease activity of AD. Our study further clarified that the serum CTACK levels significantly correlated with these

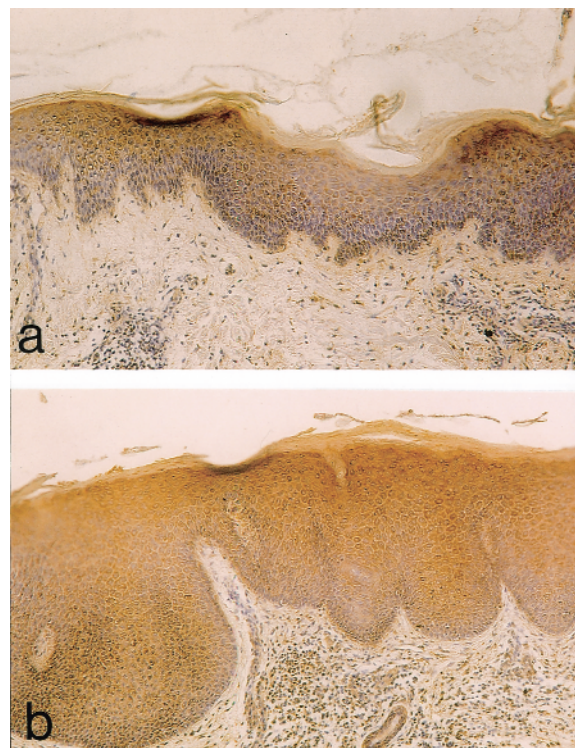
TABLE I. Correlation coefficients between the serum CTACK levels and other clinical and laboratory data in patients with AD and PsV

	CTACK	
	<i>r</i>	<i>P</i> value
AD		
MDC	0.85	<.001
sIL-2R	0.74	<.001
SCORAD	0.70	<.001
Eosinophils	0.61	<.001
TARC	0.58	<.001
Soluble E-selectin	0.56	<.002
PsV		
IP-10	0.58	<.009
PASI	0.07	.17

Eosinophils, Numbers of eosinophils in peripheral blood.

markers, as well as with the SCORAD score, suggesting that the serum CTACK levels also correlate with the disease activity of AD. This is the first report describing a correlation between serum CTACK levels and the disease activity of AD, and we suggest that it might be useful to measure serum CTACK levels when estimating the severity of other inflammatory skin diseases. Immunohistochemical staining of CTACK in the lesional skin of patients with AD and PsV was consistent with the findings of a previous report that lesional keratinocytes abundantly produce CTACK in patients with inflammatory skin disease.¹⁴ Thus the high serum levels and the strong expression of CTACK in lesional keratinocytes suggest that CTACK might play an important role in the pathogenesis of AD and PsV.

Previously, Giustizieri et al¹⁷ showed that IP-10 was highly detected in the lesional keratinocytes of patients with PsV, whereas it was weakly detected in the lesional keratinocytes of those with AD. In this study we confirmed that IP-10 was strongly expressed in the lesional keratinocytes of patients with PsV, whereas it was focally expressed in those with AD. Moreover, the serum IP-10 levels in patients with PsV, but not those with AD, were significantly higher than those in healthy control subjects and correlated with the serum CTACK levels. However, there was no significant difference in serum IP-10 levels between patients with AD and those with PsV. Accumulated evidence revealed that IP-10 was secreted from not only keratinocytes but also from various other cell types¹⁸ and was also detected in the supernatants of PBMCs in vitro.¹⁹ Thus we assume that the serum IP-10 levels reflect the total amount of secretion from not only skin cells but also peripheral blood cells. This is why data on the serum IP-10 levels did not correspond to those of immunohistochemistry of the skin in contrast to CTACK. In addition, both the serum IP-10 levels and the serum CTACK levels did not correlate with the PASI score, which is considered to be available for quantifying the extent of the clinical symptoms of PsV.²⁰ Because the number of patients with PsV was smaller than the num-

**FIG 3.** IP-10 was focally expressed in the lesional keratinocytes of patients with AD (a), whereas it was strongly expressed in those with PsV (b). When an isotype-matched control was used, no reactivity was observed (data not shown; original magnification: 100 $\times$).

ber of patients with AD, increased numbers will be required to make a conclusion concerning the correlation between serum CTACK levels, serum IP-10 levels, and disease severity.

In conclusion, serum CTACK levels were significantly higher in patients with AD and PsV than in healthy control subjects and significantly correlated with the disease activity of AD but not PsV. In addition, immunoreactive CTACK was strongly detected in the lesional keratinocytes of patients with AD and PsV. These results strongly suggest that CTACK might be one of the important chemokines in the lesional formation of AD and PsV but might have a distinct role in AD and PsV.

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