

Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity

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Background: Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease characterized by the predominant infiltration of T_H2-type cells in lesional skin. Thymus and activation-regulated chemokine (TARC/CCL17) is a chemokine that attracts CC chemokine receptor 4-positive (CCR4⁺) or CCR8⁺ cells.

Objective: The purpose of this study was to investigate the participation of TARC in AD.

Methods: We measured serum TARC levels in 40 patients with AD, 20 healthy control subjects, and 20 patients with psoriasis. We also examined disease activity by using SCORAD score; serum soluble E-selectin, soluble IL-2 receptor, IgE, and GM-CSF levels; and eosinophil numbers in peripheral blood, as well as correlations between TARC levels and these factors. The positivity of CCR4 of CD4⁺CD45RO⁺ cells in PBMCs was examined by using FACS analysis. Immunohistochemical staining of TARC and GM-CSF was performed in the lesional skin of patients with AD.

Results: The serum TARC levels of patients with AD were significantly higher than those of healthy control subjects and patients with psoriasis. The serum TARC levels significantly correlated with eosinophil number ($r = 0.61$), SCORAD score ($r = 0.60$), and serum soluble E-selectin levels ($r = 0.58$) and weakly correlated with serum soluble IL-2 receptor levels ($r = 0.34$) in patients with AD. The TARC levels of patients with AD decreased after the treatment in accordance with the improvement of clinical symptoms. The CCR4 positivity of CD4⁺CD45RO⁺ cells in PBMCs of patients with AD was also higher than that of healthy control subjects. Immunohistochemical staining revealed that TARC was positive in keratinocytes in the epidermis and in vascular endothelial cells, T cells, and dendritic cells in the dermis.

Conclusion: Serum TARC levels are associated with disease

activity of AD, and TARC may play an important role in the pathogenesis of AD. (J Allergy Clin Immunol 2001;107:535-41.)

Key words: Atopic dermatitis, thymus and activation-regulated chemokine, disease activity, CC chemokine receptor 4, GM-CSF

Atopic dermatitis (AD) is a chronic inflammatory skin disease of unknown cause that is associated with elevated serum IgE levels, IgE specific to environmental allergens (eg, mites), and tissue and peripheral blood eosinophilia.¹ AD is characterized by the predominant infiltration of T_H2-type cells, the increased secretion of T_H2-type cytokines in the acute phase of lesional skin,² and the high responsiveness to PBMCs for IL-4.³ In vivo data show that the serum level of soluble E-selectin is increased in AD and that this has a significant correlation with disease activity.⁴⁻⁶

Thymus and activation-regulated chemokine (TARC/CCL17) is a member of the CC chemokine group that is constitutively expressed in the thymus and is produced by monocyte-derived dendritic cells (DCs)⁸⁻¹¹ and endothelial cells.¹² It is a ligand for CC chemokine receptor 4 (CCR4) and CCR8 and serves for the recruitment and migration of these receptor-expressing cells.¹³⁻¹⁵ We have previously shown that in NC/Nga mice, regarded as a mouse model for human AD, TARC is expressed in epidermal keratinocytes (KCs).¹⁶ This indicates that TARC can be secreted by not only DCs and endothelial cells but also by KCs. Indeed, we showed that TARC production by KCs was upregulated by IL-1 β and TNF- α in vitro,¹⁶ suggesting the role of TARC in the pathogenesis of inflammatory skin diseases, such as AD. However, little is known about the relationship between TARC and AD. Very recently, it has been reported that macrophage-derived chemokine (MDC), another ligand for CCR4, is highly detected in the serum of patients with AD.¹⁷

In the present study we quantified the serum levels of TARC and examined the correlation between TARC levels and the disease activity of AD. We also compared the serum TARC levels with other factors, such as serum soluble E-selectin, soluble IL-2 receptor (sIL-2R), and IgE levels and eosinophil numbers in peripheral blood, as well as CCR4 positivity of CD4⁺CD45RO⁺ cells in PBMCs of patients with AD. Moreover, we performed immunohisto-

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Abbreviations used

AD:	Atopic dermatitis
CCR4:	CC chemokine receptor 4
DC:	Dendritic cell
KC:	Keratinocyte
MDC:	Macrophage-derived chemokine
PE:	Phycoerythrin
sIL-2R:	Soluble IL-2 receptor
TARC:	Thymus and activation-regulated chemokine

chemical staining of TARC in the lesional skin of patients with AD. Serum GM-CSF levels and immunohistochemical staining of GM-CSF was also examined.

METHODS

Samples and reagents

Forty patients with AD (mean age $\pm$ SEM, 26.6 ± 8.9 years), 20 healthy volunteers (mean age $\pm$ SEM, 28.4 ± 6.7 years), and 20 patients with psoriasis (mean age $\pm$ SEM, 47.8 ± 5.5 years) were examined. All patients with AD were given diagnoses according to the criteria of Hanifin and Rajka¹⁸ and treated with topical corticosteroids in combination with oral antihistamines. Seventeen of 40 patients had personal histories of respiratory allergy. Twenty healthy control subjects and 20 patients with psoriasis did not have any histories of allergy, and each of their serum IgE levels was within normal range.

Disease activity was determined by the modified SCORAD (SCORing Atopic Dermatitis) system.^{19,20} In our experiment we divided patients with AD into 3 groups (mild, moderate, and severe disease) according to a proposal for severity grading of AD using only objective criteria (mild, < 15 ; moderate, $15-40$; severe, > 40).²⁰

Laboratory data, such as serum soluble E-selectin, sIL-2R, IgE, and GM-CSF levels and eosinophil numbers in peripheral blood were also examined. Moreover, in 10 patients with AD, these laboratory data were examined before and after treatment with topical corticosteroids and oral antihistamines for 2 weeks.

The following antibodies were used: phycoerythrin (PE)-conjugated anti-human CD45RO mAb (PharMingen, San Diego, Calif); Cy-chrome-conjugated anti-human CD4 mAb (PharMingen); and FITC-conjugated anti-human CCR4 mAb (mouse IgG1), the characteristics of which are described elsewhere.²¹ FITC-conjugated mouse IgG2b, PE-conjugated mouse IgG, and Cy-chrome-conjugated mouse IgG were also used as negative controls (PharMingen). For immunohistochemical staining, the following antibodies and isotype controls were used: rabbit anti-human TARC antibody (Peprotec, Rocky Hill, NJ); mouse anti-human GM-CSF mAb (R&D Systems, Minneapolis, Minn); rabbit IgG (Jackson ImmunoResearch, West Grove, Pa); mouse anti-human CD3 mAb (DAKO Co, Glostrup, Denmark); mouse anti-human CD1a mAb (DAKO Co); and mouse IgG (Jackson ImmunoResearch).

ELISA

We used a 96-well polystyrene microplate coated with a murine mAb against human TARC (TECHNE Corp, Minneapolis, Minn). The serum TARC levels were measured in patients with AD, normal healthy control subjects, and patients with psoriasis, according to the standard assay protocols. Serum soluble E-selectin, sIL-2R, and GM-CSF levels were also measured by using an ELISA assay system for soluble E-selectin (MedSystems, Vienna, Austria), sIL-2R (R&D systems), and GM-CSF (R&D Systems).

Immunohistochemical staining of lesional skin of patients with AD and patients with psoriasis and normal skin of healthy control subjects

Biopsy samples were taken from the lesional skin of patients with AD (acute phase of 3 cases and chronic phase of 3 cases), the normal skin of healthy control subjects (2 cases), and the lesional skin of patients with psoriasis (2 cases), with a 5-mm disposable punch. The obtained skin samples were embedded in Tissue-Tek OCT compound (Miles Inc, Elkhart, Idaho) for at least 24 hours at -80°C . These frozen sections were cut with a cryostat set at $6\text{ }\mu\text{m}$ of thickness and fixed in 4% paraformaldehyde. They were incubated overnight with rabbit anti-human TARC antibody or rabbit IgG at 4°C after blocking endogenous peroxidase activity. The samples were incubated with biotin-conjugated anti-rabbit IgG (DAKO Co) for 30 minutes. Then 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. For immunohistochemical staining of GM-CSF in the lesional skin of patients with AD, anti-human GM-CSF mAb or mouse IgG was used. For immunohistochemical staining for CD1a or CD3 in the lesional skin of patients with AD, anti-human CD1a mAb, anti-human CD3 mAb, or mouse IgG was used.

Measurement of CCR4 positivity in CD4⁺ CD45RO⁺ cells in PBMCs of patients with AD, healthy control subjects, and patients with psoriasis

PBMCs were isolated from peripheral blood samples of patients with AD, healthy control subjects, and patients with psoriasis. PBMCs were isolated by means of centrifugation on a Ficoll-Metrizoate density gradient (Pharmacia Biotech, Uppsala, Sweden) at 2000 rpm for 20 minutes at room temperature. PBMCs were washed 3 times with PBS and divided in tubes containing 3×10^5 cells each.

Isolated PBMCs were stained with FITC-conjugated CCR4 mAb, Cy-chrome-conjugated CD4 mAb, and PE-conjugated CD45RO mAb. FITC-conjugated mouse IgG2b, PE-conjugated mouse IgG, and Cy-chrome-conjugated mouse IgG were also used as negative controls. The samples were analyzed on a FACSCalibur (Becton Dickinson, San Diego, Calif) by using propidium iodide to exclude dead cells.

Statistical analysis

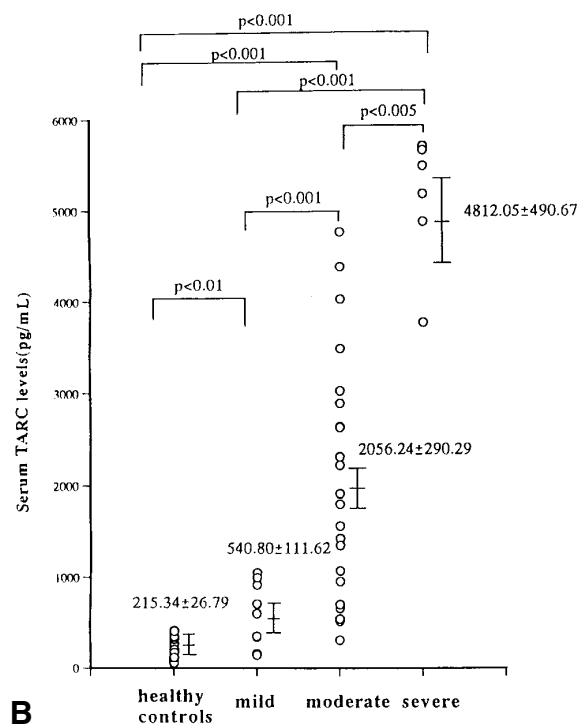
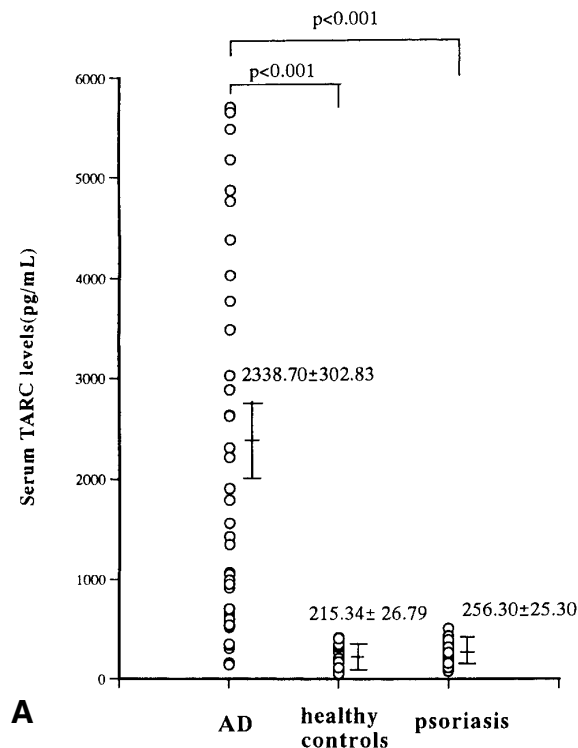
Data were analyzed with the Mann-Whitney *U* test. A *P* value of less than .05 was considered to be statistically significant.

Correlation coefficients were determined by using the Spearman rank correlation test. Because the distributions of measurements of blood samples were skewed, all comparisons were made after logarithmic transformation. Statistical analysis was also performed, and a *P* value of less than .05 was considered to be statistically significant.

RESULTS

Serum TARC levels in patients with AD

The serum TARC levels of patients with AD were more than 10-fold greater than those of healthy control subjects or patients with psoriasis. The average value of serum TARC of patients with AD was 2338.70 ± 302.83 pg/mL, whereas that of healthy control subjects was 215.34 ± 26.79 pg/mL, and that of patients with psoriasis was 256.30 ± 25.30 pg/mL (Fig 1, A). Differences



between patients with AD and healthy control subjects, as well as patients with psoriasis, were statistically significant ($P < 0.001$, respectively).

We next compared serum TARC levels among 3 groups of patients with AD: those with mild, moderate, and severe disease. The serum TARC levels in the group with severe AD were significantly higher than those in the mild and moderate groups ($P < .001$). The average values of TARC in the mild, moderate, and severe groups were 540.80 ± 111.62 pg/mL, 2056.24 ± 290.29 pg/mL, and 4812.05 ± 490.67 pg/mL, respectively (Fig 1, B). No significant difference was observed between the serum TARC levels of patients with respiratory allergy ($n = 17$) and those of patients without respiratory allergy ($n = 23$) (data not shown).

Serum TARC levels of patients with AD and disease severity

The serum TARC levels of patients with AD were compared with serum soluble E-selectin, sIL-2R, IgE, and GM-CSF levels and eosinophil numbers in peripheral blood in addition to comparison of SCORAD scores. The serum levels of soluble E-selectin and sIL-2R were significantly higher in patients with AD than in healthy control subjects, which was consistent with previous reports.⁴⁻⁶ The serum TARC levels significantly correlated with SCORAD scores ($r = 0.60$), serum soluble E-selectin levels ($r = 0.58$), and eosinophil numbers in peripheral blood ($r = 0.61$; Fig 2, A, B, and C), whereas serum sIL-2R levels weakly correlated with TARC levels ($r = 0.34$). Each correlation coefficient between TARC levels and other factors is summarized in Table I. GM-

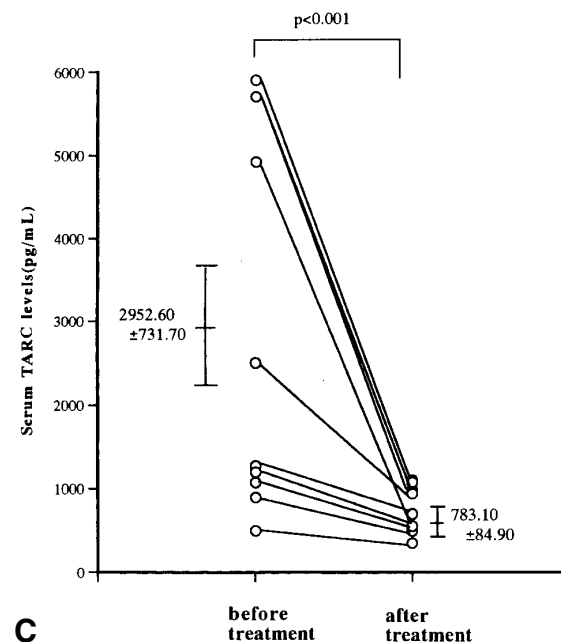


FIG 1. Serum TARC levels were elevated in patients with AD. **A**, Serum TARC levels in patients with AD ($n = 40$), healthy control subjects ($n = 20$), and patients with psoriasis ($n = 20$). **B**, Serum TARC levels among healthy control subjects ($n = 20$) and 3 groups of patients with AD: those with mild ($n = 9$), moderate ($n = 23$), and severe ($n = 8$) disease. Each vertical bar represents mean $\pm$ SE of total subjects in individual categories. **C**, The serum TARC levels of 10 patients with AD were evaluated before and after topical corticosteroid treatment. Serum TARC levels significantly decreased after treatment ($P < .001$).

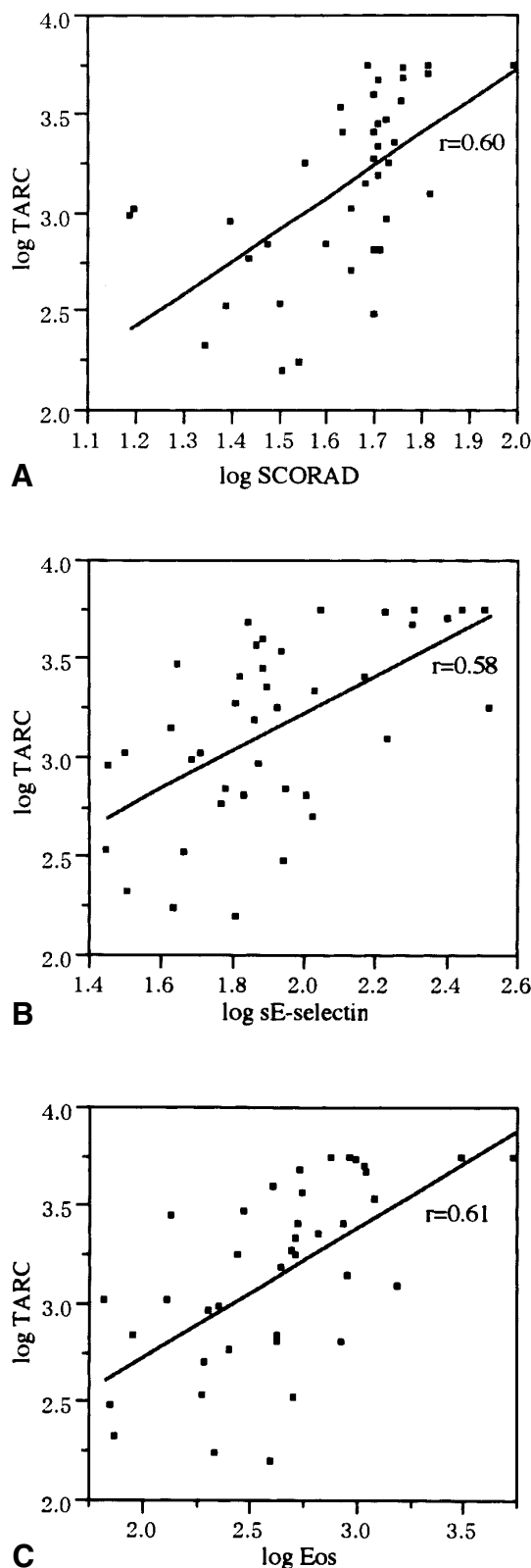


FIG 2. Serum TARC levels in patients with AD correlated with disease activity. Comparison between serum TARC levels and SCORAD scores (A), serum soluble E-selectin levels (B), or eosinophil numbers in peripheral blood (C) in patients with AD.

CSF was not detected in the serum of patients with AD or in healthy control subjects (data not shown).

Serum TARC levels during topical corticosteroid and oral antihistamine drug treatment in patients with AD

We evaluated serum TARC levels in 10 patients with AD during the clinical course. The serum TARC levels were high in patients with AD before treatment (average, 2952.60 ± 731.70 pg/mL) and decreased in all 10 patients during treatment with topical corticosteroids and oral antihistamines in accordance with the improvement of skin conditions (average, 783.10 ± 84.90 pg/mL; Fig 1, C). SCORAD score was 55.6 ± 11.3 before treatment, and it was reduced to 28.4 ± 6.8 after treatment. Serum IgE, soluble E-selectin, and sIL-2R levels and eosinophil numbers in peripheral blood were also measured before and after treatment. The soluble E-selectin levels and eosinophil numbers in peripheral blood, but not serum IgE levels, decreased in parallel with serum TARC levels after treatment (data not shown).

Immunoreactive TARC levels in the lesional skin of patients with AD

Immunoreactive TARC levels were detected in epidermal KCs, dermal infiltrating cells, and endothelial cells in the lesional skin both in the acute and chronic phases of patients with AD (Fig 3, a and b). These dermal infiltrating cells positive for TARC were composed of CD3⁺ T cells and CD1a⁺ dendritic cells (data not shown). When isotype-matched controls were used, no positive staining was observed (data not shown). In contrast, epidermal KCs were virtually negative for TARC in normal skin, and these were weakly positive for TARC in psoriatic skin (Fig 3, c and d). GM-CSF was also detectable in epidermal KCs in the lesional skin of patients with AD, and no reactivity was observed when isotype-matched controls were used (data not shown).

CCR4 positivity of CD4⁺ CD45RO⁺ cells in PBMCs of patients with AD

CCR4 positivity of CD4⁺CD45RO⁺ cells in PBMCs was analyzed in 10 patients with AD, 10 healthy control subjects, and 10 patients with psoriasis. The positivity of CCR4 of CD4⁺CD45RO⁺ cells in PBMCs of patients with AD was significantly higher than that of healthy control subjects ($25.61\% \pm 6.08\%$ vs $5.24\% \pm 1.15\%$, $P < .004$) or patients with psoriasis ($25.61\% \pm 6.08\%$ vs $3.72\% \pm 1.97\%$, $P < .002$; Fig 4, a, b, c, and d). No positivity was detected when isotype controls were used in PBMCs of either healthy control subjects, patients with AD, or patients with psoriasis.

DISCUSSION

TARC/CCL17 is a CC chemokine that attracts CCR4⁺ or CCR8⁺ cells.^{8,13,14} Recent articles revealed the participation of TARC in several diseases, such as Hodgkin's

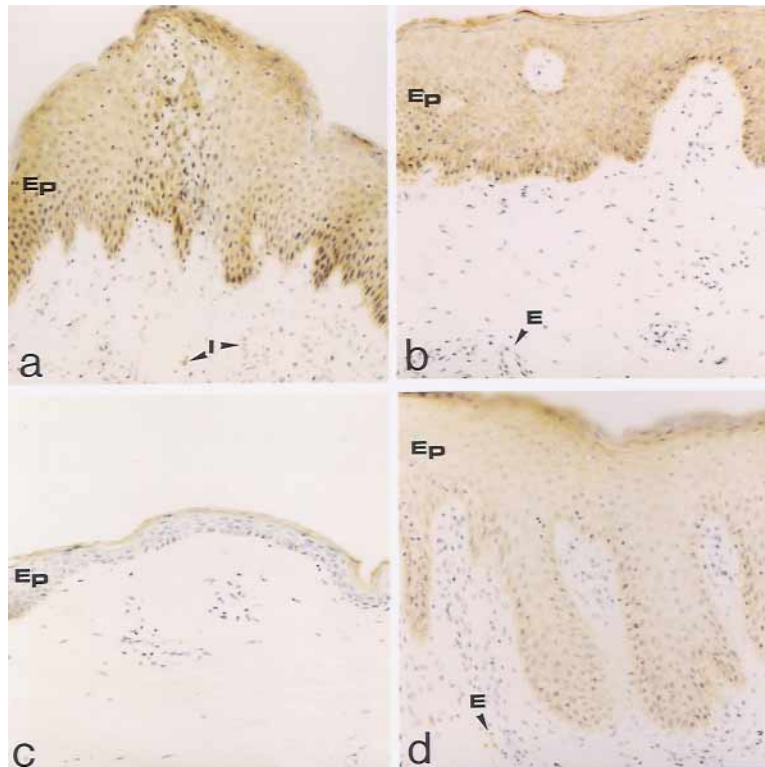


FIG 3. Immunoreactive TARC was detected in the acute (a) and chronic (b) lesional skin of patients with AD and patients with psoriasis (d). It was detectable in epidermal KCs, dermal endothelial cells, and infiltrating cells in lesional skin of patients with AD (b). TARC was virtually negative in normal skin (c) and was weakly positive in epidermal KCs of psoriatic skin (d). Ep, Epidermal KCs; E, endothelial cells; I, infiltrating cells (original magnification, 100 $\times$).

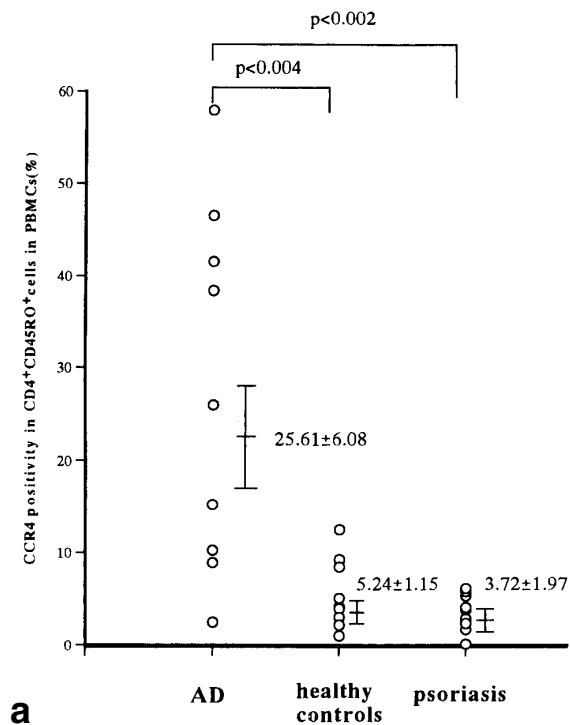
lymphoma²² and fulminant hepatic failure.²³ Very recently, bronchial epithelial cells of asthmatic patients have been reported to produce high levels of TARC protein, indicating the possible role of TARC in the pathogenesis of allergic respiratory diseases.²⁴ However, little is known about the participation of TARC in the pathogenesis of allergic skin diseases, such as AD. We have previously clarified the expression of TARC in the skin of NC/Nga mice, which is a mouse model of human AD.¹⁶ In this report we clearly showed high levels of TARC in the serum of patients with AD and demonstrated that this correlated with the disease activity of AD. The serum TARC levels correlated with soluble E-selectin levels, which is reported to represent disease activity of AD.⁴⁻⁶ Thus our data showed that TARC might also be an important marker for disease activity of AD. As far as we know, this is the first report describing the relationship between serum TARC levels and disease activity of AD.

It is reported that TARC is expressed by endothelial cells,¹² monocyte-derived DCs,⁹⁻¹¹ Reed-Sternberg cells,²² and bronchial epithelial cells.²⁴ In this report we clearly demonstrated the expression of TARC in epidermal KCs, both in the acute and chronic lesional skin of patients with AD by means of immunohistochemical examination. These observations are consistent with those of our previous article, which showed that murine

TABLE I. Correlation coefficient among serum TARC levels and other clinical and laboratory data in patients with AD

	TARC	
	<i>r</i>	<i>P</i> value
Soluble E-selectin	0.58	<.001
sIL-2R	0.34	.003
IgE	0.57	<.001
Eosinophils	0.61	<.001
SCORAD	0.60	<.001

KCs can secrete TARC under stimulation with primary cytokines,¹⁶ although murine KCs are different from human KCs in terms of the responses to cytokines. These observations strongly suggest that KCs can be a source of TARC in the lesional skin of patients with AD and that KCs producing TARC may be involved in the pathogenesis of AD. The regulation of cytokines on TARC production by human KCs is currently under investigation in our laboratory. Recently, MDC, another ligand for CCR4, has been reported to be highly expressed in the serum of patients with AD.¹⁷ We also observed high levels of serum MDC in patients with AD, and this decreased after the treatment in accordance with the improvement of skin severity (data not shown). These



data strongly suggest that TARC and MDC, which are key chemokines for the migration of CCR4⁺ T cells, may play an important role in the allergic skin diseases.

Recent investigation indicates that CCR4 is expressed on T_H2-type cells. However, Sallusto et al¹⁴ reported that CCR4 can be expressed on non-T_H2-type cells by transforming growth factor β in vitro. Campbell et al¹² revealed CCR4 expression in the lesional skin of patients with psoriasis, which is considered to be T_H1-type disease. Thus there is some conjecture as to whether CCR4 expression is especially polarized to T_H2-type cells.

We also examined the positivity of CCR4 in CD4⁺CD45RO⁺ cells in PBMCs of patients with AD and the correlation between CCR4 positivity and serum TARC levels in PBMCs of patients with AD. Our data revealed that CCR4 positivity of CD4⁺CD45RO⁺ cells was high in patients with AD. Furthermore, serum TARC levels significantly correlated with CCR4 positivity of memory helper T cells (data not shown). Although the expression of CCR4⁺ cells on T_H1- or T_H2-type polarization is not fully understood, our data clarified the coordinate relation of TARC and CCR4, which may play some role in the pathogenesis of AD.

We also clarified that serum GM-CSF levels were not detected in patients with AD and that immunoreactive GM-CSF was detected at high levels in the lesional epidermal KCs (data not shown), which was consistent with finding from the previous report.²⁵ Thus these data suggest that GM-CSF and TARC have distinct roles in the pathogenesis of AD.

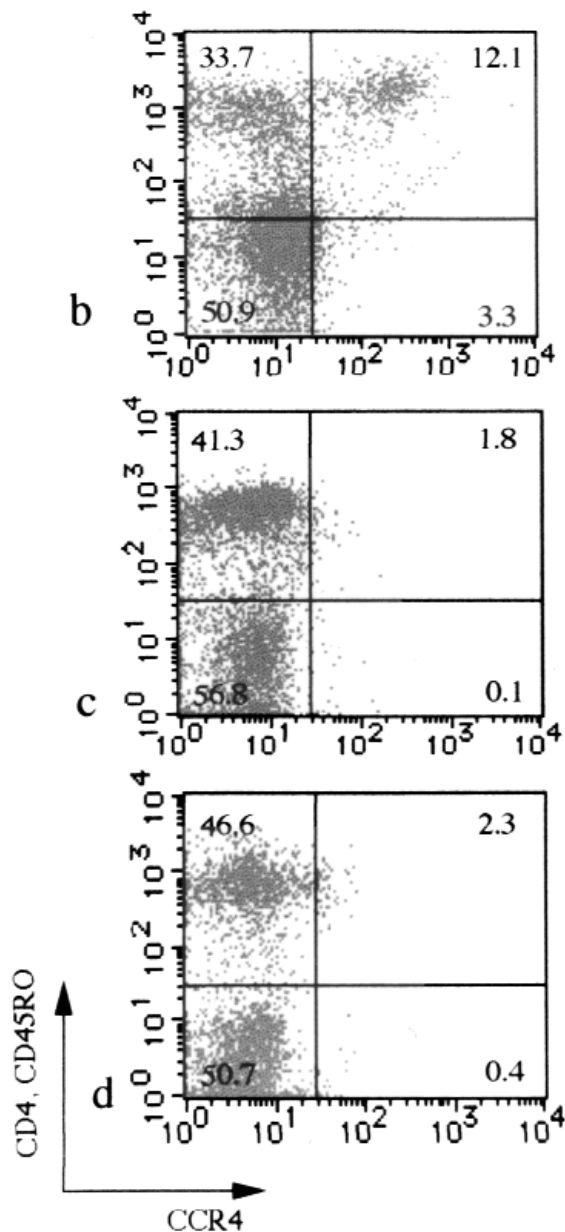


FIG 4. CCR4 positivity of CD4⁺CD45RO⁺ cells in PBMCs of patients with AD ($n = 10$) was higher than that in healthy control subjects ($n = 10$) or patients with psoriasis ($n = 10$, a). Representative data of FACS analysis are shown as follows: b, patients with AD; c, patients with psoriasis; d, healthy control subjects.

In conclusion, we have clarified the fact that serum TARC levels are high in patients with AD and that these reflect the disease activity of AD. Moreover, in the lesional skin of patients with AD, immunoreactive TARC was positive in KCs, dermal endothelial cells, and infiltrating cells. Thus our data strongly suggest that TARC may be one of the important chemokines in the pathogenesis of AD.

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