Expression of fractalkine and its receptor, CX_3CR1, in atopic dermatitis: Possible contribution to skin inflammation

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Background: Fractalkine (FKN) induces activation and adhesion of leukocytes expressing its receptor, CX_3CR1. FKN is released from the cell surface through proteolytic cleavage as soluble FKN (sFKN).
Objective: We sought to assess FKN and CX_3CR1 expression in the skin, serum sFKN levels, and CX_3CR1 expression on blood leukocytes in patients with atopic dermatitis (AD).
Methods: FKN and CX_3CR1 expression in the skin was examined immunohistochemically. mRNA expression of FKN, thymus and activation–regulated chemokine, and macrophage-derived chemokine in the skin was assessed by means of real-time RT-PCR. Serum sFKN levels were assessed by using ELISA. Blood leukocytes were stained for CX_3CR1 by means of flow cytometric analysis.
Results: FKN was strongly expressed on endothelial cells in skin lesions of patients with AD and psoriasis but not in normal skin. FKN mRNA levels in AD lesional skin increased to a similar extent to thymus and activation–regulated chemokine and macrophage-derived chemokine mRNA levels. CX_3CR1-expressing cells in the affected skin of patients with AD or psoriasis increased compared with those in normal skin. Serum sFKN levels were increased in patients with AD but not in patients with psoriasis relative to levels in healthy control subjects. Serum sFKN levels were associated with the disease severity and decreased with the improvement of skin lesions in patients with AD. CX_3CR1^+ cell frequencies and CX_3CR1 expression levels were decreased in $CD8^+$ T cells, monocytes, and natural killer cells from patients with AD, but this was not observed in patients with psoriasis.
Conclusions: These results suggest that through functions in both membrane-bound and soluble forms, FKN plays an important role in the trafficking of CX_3CR1^+ leukocytes during the inflammation caused by AD. (J Allergy Clin Immunol 2004;113:940-8.)

Key words: *Atopic dermatitis, chemokine, fractalkine, CX_3CR1, endothelial cell, leukocyte activation, adhesion molecule, skin inflammation*

Abbreviations used
AD: Atopic dermatitis
FKN: Fractalkine
MDC: Macrophage-derived chemokine
NK: Natural killer
sFKN: Soluble fractalkine
TACE: TNF-α–converting enzyme
TARC: Thymus and activation–regulated chemokine
TIMP: Tissue inhibitors of metalloproteinase

Atopic dermatitis (AD) is a chronic and highly pruritic inflammatory skin disease that manifests as eczematous skin lesions.[1-3] A skin lesion in AD is characterized by preferential infiltration of activated T cells, monocytes-macrophages, and eosinophils. Although it has been proposed that T_H2-type cells play a key role in the pathogenesis of AD,[4-8] recent studies have revealed that T_H1-type cytokines, including IFN-γ and IL-12, are predominantly expressed in chronic lesions of patients with AD.[6,9,10] Furthermore, chronic activation of macrophages-monocytes with increased cytokine secretion is the other immune dysfunction that characterizes AD.[11] The development of cutaneous lesions in patients with AD is closely related to the accumulation of these leukocytes migrating from the blood into the affected skin through the endothelium. This process is highly dependent on expression of chemokines and chemokine receptors, as well as adhesion molecules.[7]

Fractalkine (FKN), or CX_3CL1, is a CX_3C chemokine that is expressed on activated vascular endothelial cells stimulated with IL-1, TNF-α, or IFN-γ.[12,13] FKN is expressed in various organs, including the skin, tonsils, brain, and kidneys,[12,14-16] and interacts with its unique receptor, CX_3CR1, which is expressed on monocytes, natural killer (NK) cells, and some T cells. FKN and CX_3CR1 represent a novel type of leukocyte-trafficking molecule that regulates both adhesive and chemotactic functions.[17] The membrane-bound FKN is able to mediate firm adhesion of CX_3CR1-expressing leukocytes without requiring selectin-mediated rolling or activation of integrins.[18,19] Furthermore, FKN is released from the cell surface by means of proteolytic cleavage as soluble FKN (sFKN), which has potent chemoattractant activity for CX_3CR1^+ leukocytes and activates them.[12,17,20] Because CX_3CR1 is preferentially expressed on T_H1 cells compared with T_H2 cells, cell infiltration through FKN-CX_3CR1 interaction especially promotes T_H1 responses.[13] Recent studies have demonstrated that FKN-CX_3CR1 interaction contributes to the development of various

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inflammatory diseases and vascular injury by recruiting inflammatory cells.[19]

These previous findings suggest the involvement of FKN-CX_3CR1 interaction in the induction and development of inflammatory processes associated with AD. Therefore we evaluated the expression of FKN and CX_3CR1 in the skin, serum sFKN levels, and CX_3CR1 expression on circulating leukocytes in patients with AD. The results of this study suggest that through functions in both its membrane-bound and soluble forms, FKN plays an important role in the trafficking of CX_3CR1^+ leukocytes during the inflammation caused by AD.

METHODS

Patients and control subjects for sFKN measurement

Serum sFKN levels were examined in 32 patients with AD (13 female and 19 male patients; age, 24.7 ± 7.4 years). All patients fulfilled the criteria for AD proposed by Hannifin and Rajka,[1] and they did not have any history of other atopic diseases, such as bronchial asthma and allergic rhinitis. The clinical severity of AD was evaluated by using the scoring system proposed by Rajka and Langeland[21]: 16 patients had moderate AD, and 16 patients had severe AD. The clinical data and serum samples were obtained at the same time. All patients were treated with topical steroids in combination with oral antiallergic drugs. However, none of the patients were treated with systemic steroids or immunosuppressive drugs. In addition, patients with contact dermatitis (n = 15; 8 female and 7 male patients; age, 24.3 ± 2.5 years) or psoriasis vulgaris (n = 23; 10 female and 13 male patients; age, 30.5 ± 5.9 years), both of which are considered T_H1-mediated skin diseases, were examined as disease control subjects in this study. The control subjects were 30 sex- and age-matched healthy Japanese individuals (12 female and 18 male patients; age, 26.7 ± 4.5 years). To assess the effect of treatment on serum sFKN levels, we examined sFKN levels before and after treatment in 23 patients with severe AD (8 female and 15 male patients; age, 22.8 ± 7.2 years). These patients were intensively treated with topical steroids in combination with oral antiallergic drugs during 2 weeks of hospitalization. None of these patients were treated with systemic steroids or immunosuppressive drugs. Serum specimens were placed in aliquots and kept frozen at −70°C before use. The protocol was approved by the Committee at Kanazawa University Graduate School of Medical Science, and informed consent was obtained from all patients and healthy individuals.

ELISA for sFKN

Unless indicated otherwise, reagents were obtained from R&D Systems (Minneapolis, Minn). Human sFKN levels were measured in serum samples by means of specific ELISA. Briefly, 96-well polystyrene plates were coated overnight at 25°C with 2 μg/mL purified goat IgG anti-human FKN antibody. After washing, plates were blocked for 1 hour at 20°C with PBS containing 1% BSA and 5% sucrose. Recombinant human FKN and serum samples were added in triplicate, and the plates were incubated for 2 hours at 20°C. After washing, the plates were incubated with biotinylated goat anti-human FKN antibody (250 ng/mL) for 2 hours at 20°C and then with streptavidin-peroxidase for 1 hour at 20°C. Samples were developed with 0.1 mL per well of tetramethylbenzidine substrate diluted in a citrate-phosphate buffer. Reactions were stopped by adding 1 mol/L H_2SO_4, and the plates were read at 450 nm. Thymus and activation−regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22), which are known as T_H2-type chemokines,[22] were also examined by means of ELISA.

Flow cytometric analysis

CX_3CR1 expression levels by peripheral blood leukocytes were examined in patients with AD (n = 16; 10 with severe and 6 with moderate AD; 7 female and 9 male patients; age, 25.5 ± 5.8 years), patients with psoriasis (n = 8; 4 female and 4 male patients; age, 33.2 ± 7.8 years), and healthy control subjects (n = 10; 4 female and 6 male patients; age, 28.3 ± 6.2 years). Heparinized blood samples were collected and immediately placed on ice. Two-color analysis was performed with a combination of FITC-conjugated anti-CX_3CR1 mAb (Medical & Biological Laboratories Corp, Nagoya, Japan) and phycoerythrin-conjugated anti-CD4 (Coulter Corp, Miami, Fla), anti-CD8 (Coulter Corp), anti-CD14 (Coulter Corp), or anti-CD16 (Coulter Corp) mAbs. Three-color analysis was also conducted with a combination of FITC-conjugated anti-CX_3CR1 (Medical & Biological laboratories Corp), biotinylated anti-cutaneous lymphocyte antigen (BD PharMingen, San Diego, Calif), and peridinin chlorophyll protein−conjugated anti-CD4 (BD PharMingen) mAbs. Phycoerythrin-conjugated streptavidin (Southern Biotechnology Associates Inc, Birmingham, Ala) was used for biotin-coupled antibody staining. The blood samples were stained at 4°C with a predetermined optimal concentration of the test mAb for 20 minutes, as previously described.[23] Blood erythrocytes were lysed with the Coulter Whole Blood Immuno-Lyse kit, as instructed by the manufacturer (Coulter Corp). Cells were washed and analyzed with a FACScan flow cytometer (BD PharMingen). The positive and negative population of cells was determined with the unreactive isotype-matched mAbs (Coulter Corp) as a control for background staining.

Immunohistochemical staining

FKN and CX_3CR1 expression in the skin was determined by using immunohistochemical staining in patients with AD with chronic skin lesions (n = 9; 4 female and 5 male patients; age, 28.0 ± 5.5 years), patients with psoriasis (n = 5; 2 female and 3 male patients; age, 34.1 ± 7.2 years), and healthy control subjects (n = 4; 2 female and 2 male patients; age, 29.6 ± 4.8 years), as previously described.[24] Briefly, formalin-fixed, paraffin-embedded skin tissues were acetone fixed and then incubated with 10% normal rat serum for 10 minutes at 37°C to block nonspecific staining. Sections were stained with goat polyclonal IgG antibody specific for human FKN (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) or rabbit polyclonal IgG antibody specific for human CX_3CR1 (Chemicon Int, Temecula, Calif). Sections were incubated sequentially (20 minutes at 37°C) with biotinylated rabbit anti-goat IgG secondary antibody for FKN staining or with biotinylated goat anti-rabbit IgG secondary antibody for CX_3CR1 staining (Vectastatin avidin-biotin Complex Methods, Vector Laboratories, Burlingame, Calif) and then with horseradish peroxidase−conjugated avidin-biotin complexes (Vector Laboratories). Sections were finally developed with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide and counterstained with methyl green. In a similar way, the serial skin tissues were stained with anti-CD3, anti-CD4, anti-CD8, anti-CD14, anti-CD16, anti-CD20, and anti-CD68 antibodies (DakoCytomation Co Ltd, Glostrup, Denmark) to identify leukocyte subsets of CX_3CR1-expressing cells.

RNA isolation and real-time RT-PCR

Total RNA was isolated from the frozen tissue of 5 chronic skin lesions of patients with AD (3 female and 2 male patients; age, 27.4 ± 4.6 years) and 5 healthy control subjects (3 female and 2 male patients; age, 28.2 ± 4.2 years) with QIAGEN RNeasy spin columns (QIAGEN Ltd, Crawley, United Kingdom). Total RNA from each

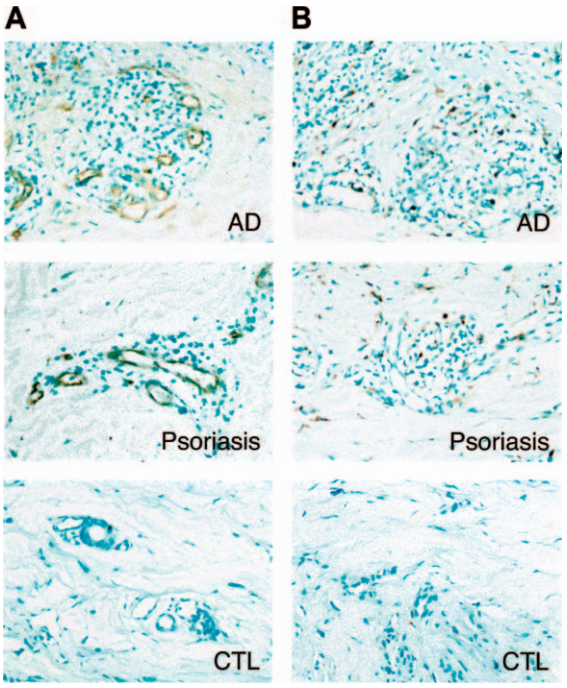

FIG 1. Representative immunohistochemical expression of FKN **(A)** and CX_3CR1 **(B)** in the lesional skin from patients with AD, patients with psoriasis, and healthy control subjects *(CTL)*.

sample was reverse transcribed into cDNA. Expression of FKN, TARC, and MDC was analyzed by using a real-time PCR quantification method, according to the manufacturer's instructions (Applied Biosystems, Foster City, Calif). Sequence-specific primers and probes were designed by Pre-Developed TaqMan Assay Reagents or Assays-On-Demands (Applied Biosystems). Real-time PCR (40 cycles of denaturation at 92°C for 15 seconds and annealing at 60°C for 60 seconds) was performed on an ABI Prism 7000 Sequence Detector (Applied Biosystems). Glyceraldehyde-3-phosphate was used to normalize mRNA. Relative expression of real-time PCR products was determined by using the ΔΔCt method[25] to compare target gene and housekeeping gene *(GAPDH)* mRNA expression. One of the control samples was chosen as a calibrator sample.

Statistical analysis

The Mann-Whitney *U* tests were used to compare variables between 2 groups, and the Bonferroni test was used for multiple comparisons. The Spearman rank correlation coefficient was used to examine the relationship between 2 continuous variables. A *P* value of less than .05 was considered statistically significant. All data are shown as means ± SDs.

RESULTS

Expression of FKN and CX_3CR1 in the skin

We performed immunohistochemical analysis using specific antibodies to assess FKN expression in the affected skin from patients with AD. In the skin from healthy individuals, FKN was not expressed on endothelial cells. In contrast, vascular endothelial cells strongly expressed FKN in the affected skin but not in the unaffected skin of patients with AD or psoriasis (Fig 1, *A*, and data not shown). Then we examined the distribution of CX_3CR1^+ cells in the skin lesions from patients with AD (Fig 1, *B*). CX_3CR1^+ cells were sparsely detected by means of immunohistochemical staining in normal control skin tissues (1% ± 3% of infiltrating mononuclear cells). In contrast, a considerable rate of infiltrating cells expressed CX_3CR1 in the affected skin tissues from patients with AD (27% ± 19%) and patients with psoriasis (23% ± 17%). Most of the CX_3CR1^+ cells were identified as $CD3^+$ T cells in patients with AD (80% ± 32%) and patients with psoriasis (78% ± 23%). The expression ratio of CD4/CD8 in infiltrating $CD3^+$ CX_3CR1^+ cells was similar in patients with AD (1.2 ± 0.6) and patients with psoriasis (1.3 ± 0.6). Thus the endothelial FKN expression was augmented with increased infiltration of CX_3CR1^+ cells in the lesional skin of patients with AD, as well as the lesional skin of patients with psoriasis.

Expression of FKN, TARC, and MDC mRNA in the skin

To determine the relative importance of FKN in comparison with TARC and MDC, T_H2-type chemokines that have been shown to be involved in skin inflammation in patients with AD,[26] we examined mRNA expression of FKN, TARC, and MDC in the lesional skin of patients by means of real-time RT-PCR. FKN mRNA levels in patients with AD were significantly 8.7-fold higher than those in healthy control subjects (14.8 ± 12.2 vs 1.7 ± 1.5, $P < .05$). Similarly, TARC mRNA levels in patients with AD were significantly 7.6-fold higher than those in healthy control subjects (24.7 ± 14.5 vs 3.2 ± 2.0, $P < .05$), whereas MDC mRNA levels in patients with AD were significantly increased by 7.9-fold compared with those of healthy control subjects (13.4 ± 9.0 vs 1.7 ± 1.4, $P < .05$). Thus mRNA expression levels of FKN in the skin of patients with AD were increased to a similar extent to those of TARC and MDC.

Serum sFKN levels

Because FKN is released from the cell surface as a soluble form,[12] sFKN levels were assessed in serum samples from patients with AD (Fig 2). Serum sFKN levels in patients with AD were significantly 5.1-fold higher than those in healthy control subjects (81 ± 153 vs 16 ± 11 pg/mL, $P < .05$). By contrast, patients with psoriasis (34 ± 51 pg/mL) or contact dermatitis (22 ± 20 pg/mL) showed mean sFKN levels similar to those of healthy control subjects. The affected skin area was similar in patients with AD (52% ± 23% of total body) and patients with psoriasis (49% ± 26%), suggesting that sFKN levels were not simply determined by the extent of skin disease. Regarding the severity of AD, patients with severe AD (141 ± 201 pg/mL) exhibited significantly increased sFKN levels compared with patients with moderate AD (21 ± 15 pg/mL, $P < .05$). Furthermore, sFKN levels were significantly increased in patients with severe AD relative to patients with psoriasis ($P < .05$) or contact dermatitis ($P < .05$), as well as healthy control subjects ($P < .005$). However, sFKN levels in patients with

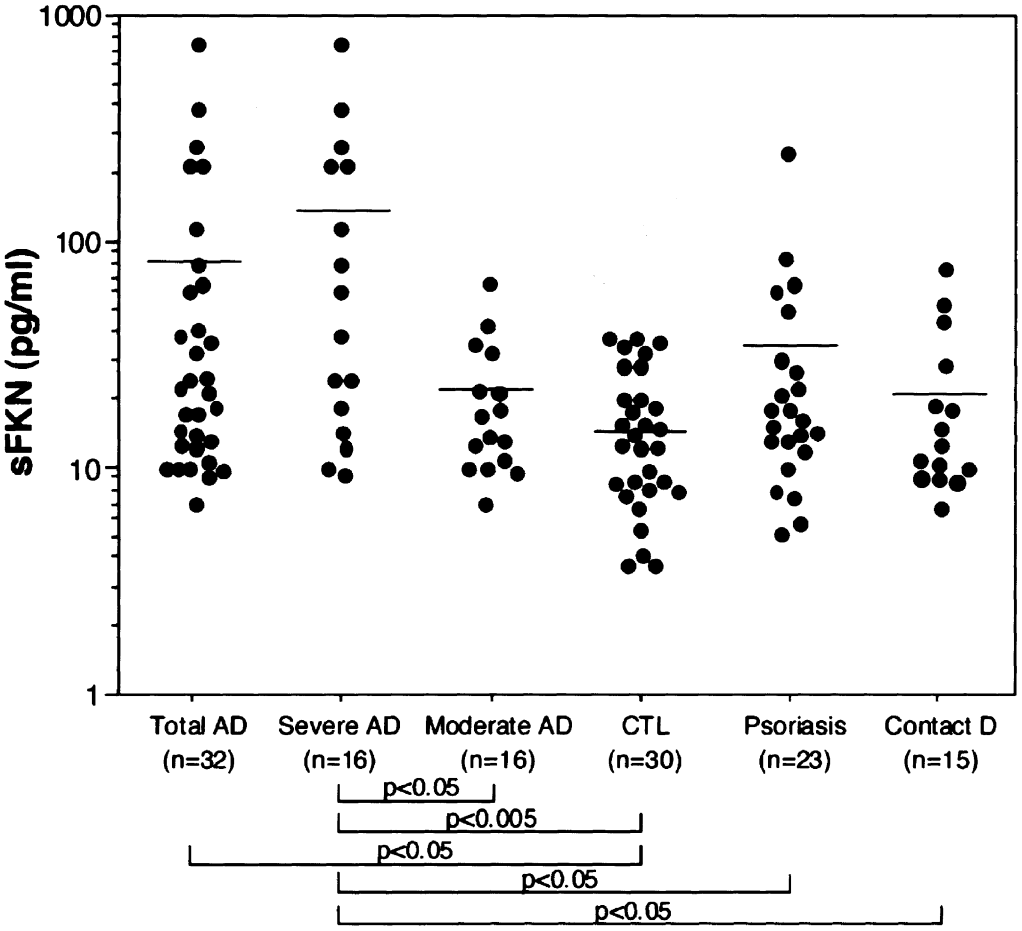

FIG 2. Serum levels of sFKN in patients with AD, patients with psoriasis, patients with contact dermatitis *(Contact D)*, and healthy control subjects *(CTL)*. Patients with AD were grouped into those with severe AD and those with moderate AD. Serum sFKN levels were determined by means of ELISA. The *horizontal bars* represent mean values, with statistically significant differences between groups indicated. Note the logarithmic scale.

moderate AD were comparable with those in healthy control subjects. Blood eosinophil numbers and serum IgE levels did not correlate with the serum sFKN levels in patients with AD (data not shown). We determined correlation of sFKN levels with serum levels of TARC and MDC that are increased in sera from patients with AD.[27,28] Serum sFKN levels correlated positively with serum levels of TARC ($r = 0.757$, $P < .0001$) or MDC ($r = 0.370$, $P < .05$). Thus serum sFKN levels were increased in patients with AD, especially severe AD, but not in other skin disorders, including psoriasis and contact dermatitis.

Twenty-three patients with severe AD were examined for serum sFKN levels before and after treatment to determine the treatment effect on sFKN levels. The skin lesions in all patients significantly improved (the score changed from 7.6 ± 1.2 to 4.7 ± 1.3, $P < .0001$) with intensive therapy of topical steroids and oral antiallergic drugs during 2 weeks of hospitalization. Similarly, serum sFKN levels in patients with AD were significantly decreased by 31% after treatment compared with those before treatment (100 ± 191 vs 69 ± 118 pg/mL, $P < .05$). Thus serum sFKN levels correlated with the disease activity of AD.

Frequency of CX_3CR1-expressing cells in blood leukocytes and CX_3CR1 expression levels on blood leukocytes

CX_3CR1 expression on peripheral blood $CD8^+$ T cells, $CD4^+$ T cells, $CD14^+$ monocytes, and $CD16^+$ NK cells was assessed by means of flow cytometry with 2-color analysis (Figs 3 and 4). The frequency of $CD8^+$ T cells, $CD4^+$ T cells, monocytes, and NK cells was not significantly different among patients with AD, patients with psoriasis, and healthy control subjects (data not shown). The frequency of CX_3CR1-expressing cells in $CD8^+$ T cells was significantly decreased in patients with AD compared with in healthy control subjects ($P < .05$) and patients with psoriasis ($P < .05$). CX_3CR1 expression levels in CX_3CR1-expressing $CD8^+$ T cells were also

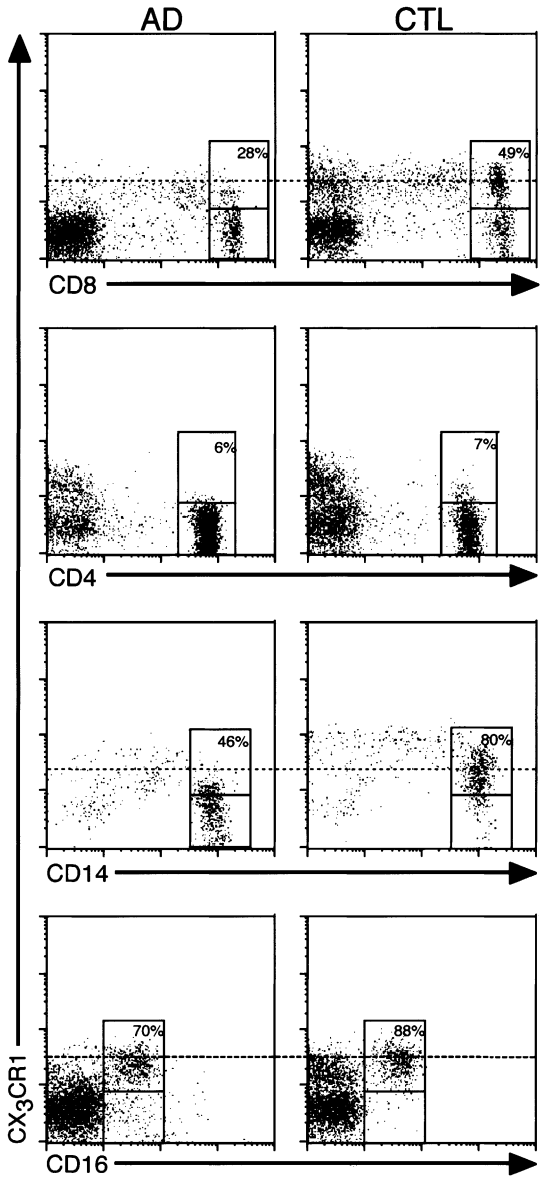

FIG 3. Representative expression of CX_3CR1 on $CD8^+$ T cells, $CD4^+$ T cells, $CD14^+$ monocytes, and $CD16^+$ NK cells in peripheral blood from patients with AD and healthy control subjects *(CTL)*. All samples were stained in parallel by using 2-color immunofluorescent staining of mononuclear cells and analyzed sequentially by means of flow cytometry with identical instrument settings. Quadrants were set according to the staining of control mAbs. The percentage represents the frequency of CX_3CR1^+ cells in each leukocyte subset. *Horizontal dashed lines* in each histogram are provided for reference.

significantly decreased in patients with AD (mean fluorescence intensity, 10 ± 3) compared with in healthy control subjects (mean fluorescence intensity, 16 ± 3; $P < .0005$); however, they were not significantly different from those in patients with psoriasis (mean fluorescence intensity, 13 ± 4). Similarly, patients with AD had a significantly reduced frequency of CX_3CR1^+ cells in monocytes relative to that of healthy control subjects ($P < .05$) and patients with psoriasis ($P < .05$), and CX_3CR1 expression levels in CX_3CR1-expressing monocytes were significantly lower in patients with AD (11 ± 6) than those found in patients with psoriasis (20 ± 5, $P < .005$), as well as healthy control subjects (23 ± 5, $P < .0001$). The percentage of CX_3CR1^+ cells in NK cells was also significantly lower in patients with AD than in healthy control subjects ($P < .05$) and patients with psoriasis ($P < .05$). Patients with AD also exhibited significantly reduced CX_3CR1 expression levels in CX_3CR1^+ NK cells relative to healthy control subjects (25 ± 11 vs 36 ± 6, $P < .05$), whereas there was no significant difference in CX_3CR1 expression levels on CX_3CR1^+ NK cells between patients with AD and patients with psoriasis (31 ± 8). However, the frequency of CX_3CR1-expressing cells in $CD4^+$ T cells was similar for patients with AD, healthy control subjects, and patients with psoriasis, and expression levels of CX_3CR1 in CX_3CR1^+ $CD4^+$ T cells were also similar in patients with AD (4.3 ± 1.4), patients with psoriasis (5.4 ± 1.4), and healthy control subjects (5.6 ± 1.0). Furthermore, the frequency of CX_3CR1^+ cells among cutaneous lymphocyte antigen−positive $CD4^+$ T cells in patients with AD (18% ± 5%) was not significantly different from that in healthy control subjects (16% ± 4%). In contrast, the frequency of CX_3CR1^+ cells in each leukocyte subset from patients with psoriasis was comparable with that from healthy control subjects. Thus the frequency of CX_3CR1-expressing cells and CX_3CR1 expression levels were decreased in some peripheral leukocyte subsets of AD.

DISCUSSION

The current study showed that FKN expression was augmented in the cutaneous vascular endothelial cells of patients with AD. Consistent with the finding that upregulated FKN expression on endothelial cells facilitates the recruitment of CX_3CR1^+ cells to the skin,[17] CX_3CR1-expressing leukocytes were increased in the affected skin of patients with AD. Reflecting the augmented FKN expression in the skin, serum sFKN levels were increased in patients with AD and associated with the disease severity and activity. Released sFKN induces integrin activation and migration of CX_3CR1-expressing cells similar to other soluble chemokines.[17,18,29] Collectively, the results of this study suggest that through functions of both membrane-bound and soluble forms, FKN regulates the CX_3CR1^+ leukocyte trafficking during the development of AD skin lesions and also suggest that the serum sFKN level is a useful clinical marker that reflects both the severity and activity of AD.

Chemokines and their receptors have the capability of regulating the selective migration of T_H1 and T_H2 cells to the target tissues and of controlling the T_H1-type and T_H2-type responses.[30-32] Although the abnormal shift to T_H2 cells in AD has been shown to be clear by means of analysis with peripheral blood,[33-35] direct assessment of

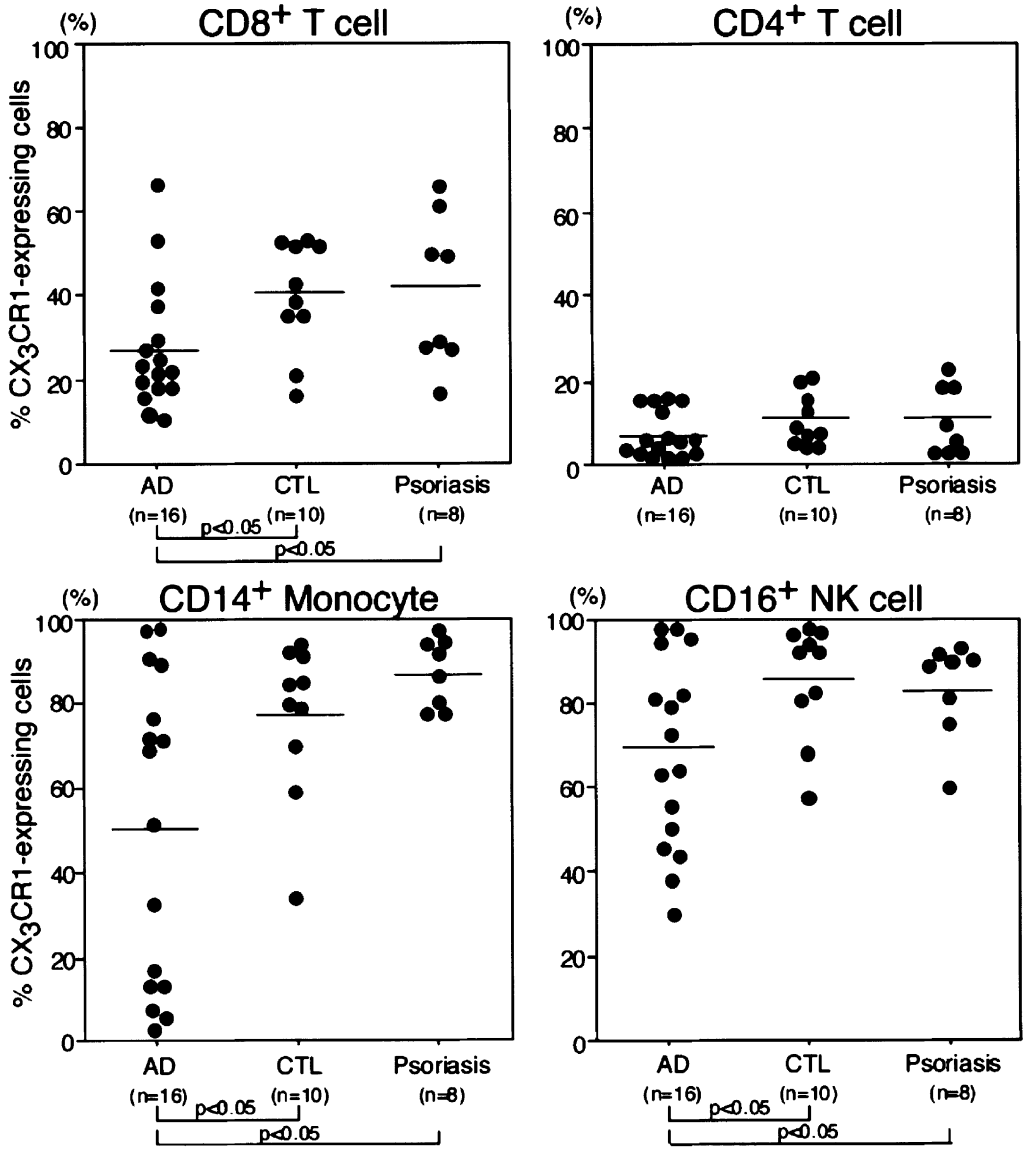

FIG 4. The frequency of CX_3CR1^+ leukocyte subpopulations from patients with AD, patients with psoriasis, and healthy control subjects *(CTL)*. The frequency of CX_3CR1^+ cells was examined on $CD8^+$ T cells, $CD4^+$ T cells, $CD14^+$ monocytes, and $CD16^+$ NK cells by means of 2-color immunofluorescence staining with flow cytometric analysis, using the gates shown in Fig 3. All samples were stained and analyzed sequentially by means of flow cytometry in parallel with identical instrument settings. The *horizontal bars* represent mean values, with statistically significant differences between groups indicated.

infiltrating cells in skin lesions of patients with is less clear cut. Specifically, previous studies with atopy patch tests with house dust mite allergens have demonstrated that a majority of T cells in the lesions express IFN-γ mRNA and protein alone or in combination with IL-4.[10,36,37] Recent studies have revealed that CCR4 plays a critical role in the migration of T_H2 cells from peripheral blood into the skin of patients with AD, whereas T_H1 cells are suggested to migrate from blood into the skin through CXCR3.[38-40] Because FKN is induced by T_H1 cytokines, such as IFN-γ, and CX_3CR1 prefers expression on T_H1 cells compared with T_H2 cells,[13,41] the enhanced FKN expression on endothelial cells might mediate T_H1 cells into skin lesions and thereby participate in the amplification of the polarized T_H1 response in AD. In this study mRNA expression levels of FKN in the lesional skin of patients with AD were increased to an extent similar to that of the T_H2-type chemokines TARC and MDC. Furthermore, serum sFKN levels were significantly associated with serum levels of TARC and MDC. These results suggest that FKN might contribute to the inflammation of AD in concert with TARC and MDC.

Collectively, these findings support the recent hypothesis that T_H1 cytokines, in addition to T_H2 cytokines, play an important role in the development of AD.[7,42]

Patients with AD exhibited increased sFKN levels that significantly decreased with the improvement of skin lesions through treatment. Because sFKN enhances the chemotactic activity of CX_3CR1-expressing cells,[12,43,44] sFKN might promote CX_3CR1^+ cell infiltration into the affected tissue. However, recent studies have revealed that sFKN inhibits the adhesion of CX_3CR1-expressing leukocytes to endothelial cells.[12,29,43,44] A similar function has been reported in other adhesion molecules, including L-selectin.[45,46] Circulating L-selectin likely serves as a biologic buffer system to prevent leukocyte rolling at sites of subacute inflammation.[45,47] In a similar way, the increase in sFKN might reflect the defense system's attempt to avoid excessive inflammatory cell infiltration by inhibiting the adhesion of CX_3CR1^+ leukocytes to endothelial cells in patients with AD. Thus although the biologic significance of sFKN remains unclear, sFKN might be related to the inflammation associated with AD by interfering with cell-to-cell interaction.

The percentage of CX_3CR1-expressing cells and CX_3CR1 expression levels on blood $CD8^+$ T cells, monocytes, and NK cells were decreased in patients with AD. This might be caused by leukocyte activation that sheds or downregulates CX_3CR1, as observed in other adhesion molecules, such as intercellular adhesion molecule 1 and selectins.[46,48-51] Consistent with this possibility, the percentage of $CD8^+$ memory T cells expressing CX_3CR1 is also decreased in patients with rheumatoid arthritis, suggesting that this receptor is shed or downregulated on lymphocyte activation.[52] Alternatively, because expression levels of adhesion molecules generally correlate with the capacity to bind their receptors,[53,54] leukocytes with high levels of CX_3CR1 might selectively infiltrate the affected skin tissues through FKN-CX_3CR1 interaction, which results in decreased blood leukocytes with increased CX_3CR1 levels and increased CX_3CR1^+ cells in the lesional skin of patients with AD. Furthermore, it is possible that circulating sFKN directly binds to blood CX_3CR1^+ leukocytes and thereby downregulates CX_3CR1 expression. Although the mechanisms for decreased CX_3CR1 expression on blood leukocytes of patients with AD remain unknown, it might finally contribute to downregulation of the inflammation by diminishing their migration capacity into the inflamed skin.

The endothelial FKN expression and frequency of infiltrating CX_3CR1^+ cells in the lesional skin were increased in patients with psoriasis, as well as in patients with AD. By contrast, a previous study showed that FKN expression was enhanced in the skin of patients with psoriasis but not in the skin of patients with AD.[13] Although the reason for this discrepancy is unclear, it might be due to the difference in AD populations studied because patients with milder AD generally showed weaker FKN expression (data not shown). Despite the increase in FKN expression and skin CX_3CR1^+ cell infiltration, serum sFKN levels and CX_3CR1 expression on blood leukocytes in patients with psoriasis were similar to those in healthy control subjects, which were distinct from those of patients with AD. Inducible FKN cleavage from the cell surface of endothelial cells depends on TNF-α–converting enzyme (TACE), a member of a family of proteins containing a disintegrin and metalloprotease domain (ADAMS proteins).[55,56] TACE activity is inhibited by tissue inhibitors of metalloproteinase (TIMP)−3, but not by TIMP-1, TIMP-2, and TIMP-4.[57] Because TIMP-3 expression is augmented in psoriasis but not in AD,[58,59] the enhanced TIMP-3 expression in patients with psoriasis might inhibit TACE activity, perhaps resulting in reduced sFKN release and normal serum sFKN levels. These normal sFKN levels might not alter CX_3CR1 expression on circulating leukocytes from patients with psoriasis. Although the mechanisms for the difference in sFKN levels and CX_3CR1 expression on leukocytes between patients with AD and patients with psoriasis remain unknown, the results suggest that the contribution of CX_3CR1-FKN interaction to the inflammation of AD is different from that of psoriasis. However, we cannot exclude the possibility that FKN is related to the development of general skin inflammation because local FKN expression was not significantly different between patients with AD and patients with psoriasis. Further studies will be needed to determine the relative importance of FKN in AD compared with other skin inflammatory diseases.

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