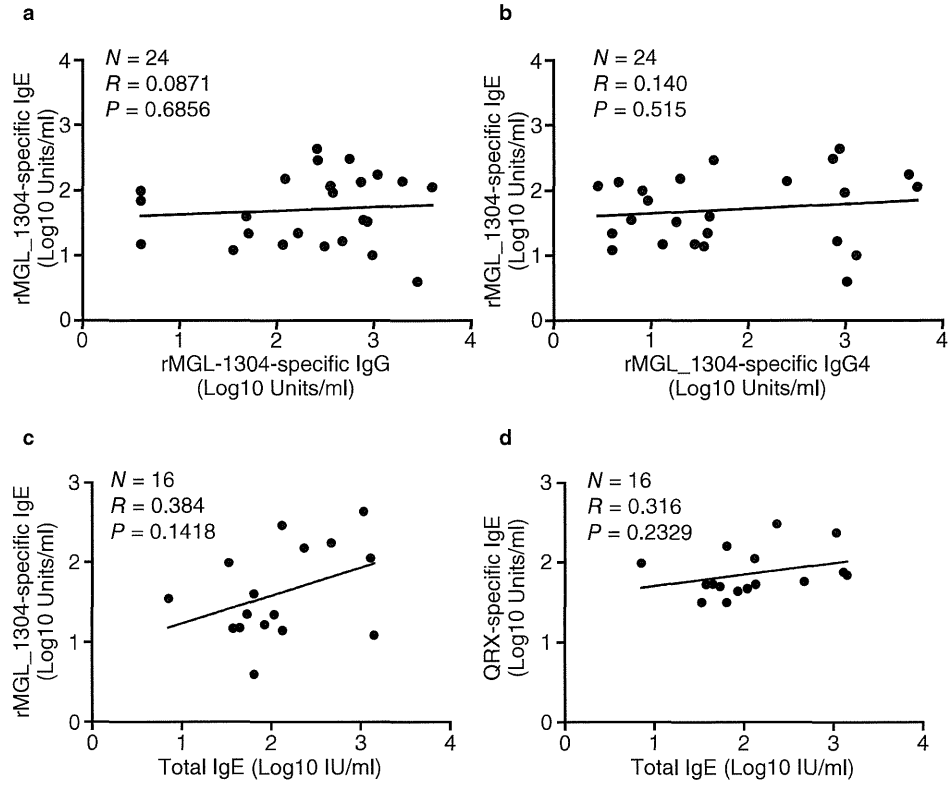

Fig. 6 Correlations among rMGL_1304-specific immunoglobulins in sera of patients with CU. No significant correlations was observed between serum levels of rMGL_1304-specific IgE and IgG (**a**), IgE and IgG4 (**b**) or the specific IgE and total serum IgE (**c**). Likewise, no apparent correlation was observed between serum levels of QRX-specific IgE and total serum IgE in patients with CU (**d**).

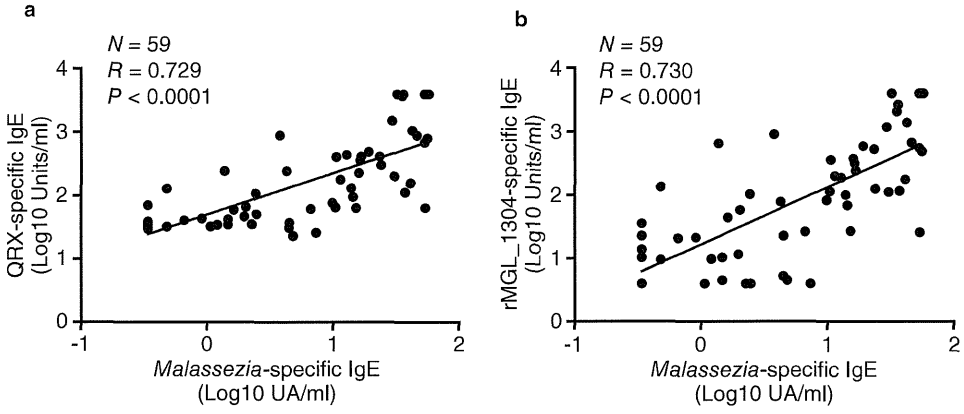

Fig. 7 Correlation between serum levels of MGL_1304-specific IgE and *Malassezia*-specific IgE. Serum levels of *Malassezia*-specific IgE and QRX-specific IgE (**a**) or rMGL_1304-specific IgE (**b**) in patients with AD were significantly correlated.

triguing. In the present study, we revealed that the levels of QRX specific-IgE in patients with CU were also higher than that of NC and BA. Takahagi *et al.*[14] also reported that the levels of basophils histamine release against QR in patients with CU were significantly higher than that of NC. On the other hand, the levels of rMGL_1304 specific-IgG or IgG4 in patients with CU were not correlated with their levels of QRX-

or rMGL_1304-specific IgE. Taking into account that both IgG and IgG4 against MGL_1304 likely neutralize the histamine release activity of MGL_1304, the presence of IgE over IgG and IgG4 in their binding activity to MGL_1304 may be critical in the pathogenesis of CU. Several groups have already performed clinical trials of hyposensitization therapy for CU by the use of autologous sweat.[23-25] Moreover, it is known that antigen-specific immunotherapy for allergic rhinitis induces the increase of antigen-specific IgG4.[26,27] Therefore, the methods to evaluate levels of serum IgG or IgG4 against MGL_1304 might be useful to monitor the effectiveness of immunotherapy on patients with CU.

The levels of QRX specific-IgE in patients with AR tended to be higher than that of NC, although this difference was not statistically significant. It might be due to the colonization of *M. globosa* in nasal cavity,[28] and suggests that MGL_1304 might also be a causative antigen for a certain population of patients with AR.

In conclusion, MGL_1304 is an important antigen in sweat and the methods to quantify the specific IgE against MGL_1304 in sera by ELISA were a useful means to diagnose allergy to MGL_1304 contained in sweat and estimate long term disease severities of AD. The measurement of QRX-specific IgE in patients with CU was also useful for the diagnosis and could be a clue to clarify the mechanism of CU. Further studies on the relationship between AD or CU and MGL_1304 in sweat are needed.

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SUPPLEMENTARY MATERIALS

Supplementary Fig. 1-4 are available online.

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this, we observed that the downstream effectors of the Wnt pathway, i.e., c-myc and Skp2 were not expressed in KSCs by reverse transcription–PCR (Figure 1b). Further, probing cell lysates of WIF1-arrested keratinocytes in western blots revealed that Wnt3A/WIF1 treatment resulted in increased p21 protein levels (Figure 2o) demonstrable quantitatively (Figure 2p). Thus, WIF1 may achieve its cell cycle arrest in keratinocytes at least in part through derepression of p21 transcription.

In conclusion, we report that WIF1 is, to our knowledge, previously unreported as a marker of interfollicular KSCs, and that it inhibits cell cycle progression in human keratinocytes even in the presence of activating Wnt signals (Wnt3A). Although canonical Wnt signaling appears to be dispensable during development in the interfollicular epidermis (Huelsken *et al.*, 2001; Nguyen *et al.*, 2009), our data suggest that inhibition of Wnt signaling may be required for keeping interfollicular stem cells quiescent and differentiating cells from proliferating during homeostasis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Serum Gastrin-Releasing Peptide Levels Correlate with Pruritus in Patients with Atopic Dermatitis

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TO THE EDITOR

Bombesin and two major bombesin-like peptides in mammals, gastrin-releasing peptide (GRP) and neuromedin B, have been shown to elicit various physiological effects. GRP elicits gastrin release and regulates gastric acid secretion and motor function (Merali *et al.*, 1999). This peptide is also involved in the biology of the circadian system. Interestingly, intradermal injections of GRP elicit scratching in mice (Andoh *et al.*, 2011). GRP is expressed in a subset of peptidergic dorsal root ganglion neurons, whereas GRP receptor (GRPR) is expressed in lamina I of the dorsal spinal cord (Sun and Chen, 2007). When lamina I neurons expressing GRPR in the spinal cord were selectively ablated, the mice showed profound scratching deficits in response to all of the itching stimuli tested, irrespective of their histamine dependence (Sun *et al.*, 2009). These data support the labeled line

Abbreviations: AD, atopic dermatitis; GRP, gastrin-releasing peptide; GRPR, GRP receptor; PGP, protein gene product; VAS, visual analog scale

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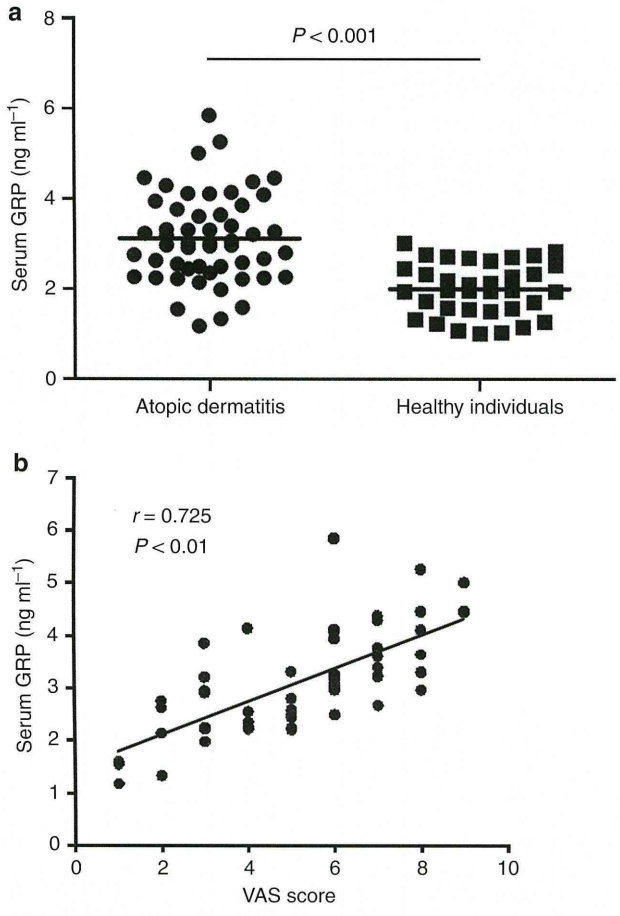

Figure 1. Serum gastrin-releasing peptide (GRP) levels in atopic dermatitis patients. (**a**) Serum GRP levels in 53 atopic dermatitis patients and 35 healthy individuals. Each bar indicates the mean of each group. (**b**) Correlations between serum GRP levels and visual anlog scale (VAS) itch scores.

for itching in the spinal cord and provide an important cellular basis for explaining how a pruritogenic stimulus is perceived by the brain as a major sensation.

Atopic dermatitis (AD) is an inflammatory pruritic skin disorder. Mechanical trauma resulting from extensive scratching that is precipitated by intensive itching results in skin barrier dysfunction and exacerbation of AD (Barnes, 2010). So far, severe pruritus in AD has been attributed to increased release of substance P, nerve growth factor, neurotrophin-3 and 4, brain-derived neurotrophic factor, histamine, and IL-31 (Arck and Paus, 2006; Lee *et al.*, 2006; Scuri *et al.*, 2010; Lee and Yu, 2011). It was recently reported that a proportion of protein gene product (PGP) 9.5^+ nerve fibers expressed GRP in both the epidermis and the dermis of NC/Nga mice, the most popular AD model mice (Tominaga *et al.*, 2009). In this study, we examined serum GRP levels in AD and their correlation with visual analog scale (VAS) itch scores.

A total of 53 AD patients (mean ± SD age: 36.4 ± 12.6 years, 31 men and 22 women) and 35 healthy controls (41.6 ± 16.0 years, 11 men and 24 women) were enrolled in this study. All AD patients were given diagnoses according to the criteria of Hanifin and Rajka (1980). We rated itch by VAS 0–10, asking the patients to mark a point on the

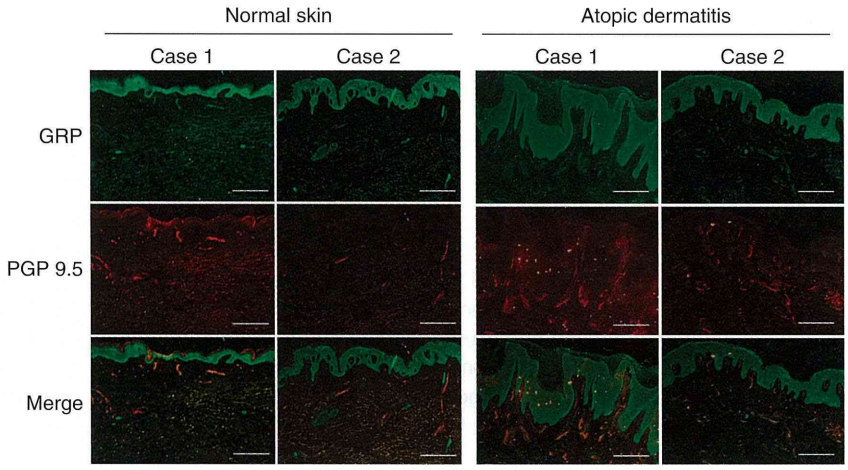

Figure 2. Double immunofluorescence staining for gastrin-releasing peptide (GRP) and protein gene product (PGP) 9.5. Dermal PGP 9.5^+ nerve fibers (red) expressed GRP (green). Increased numbers of double-positive fibers (yellow) in lesional skin of atopic dermatitis compared with normal skin. Representative two cases in each group. Bar = 100 μm.

line corresponding to mean itch during the last 7 days before blood draw. The medical ethical committee of the University of Tokyo approved all described studies, and the study was conducted according to the Declaration of Helsinki Principles. Informed consent was obtained from participants. The 35 healthy controls had no history of allergy or pruritus. Enzyme immunoassay was performed using GRP EIA kit obtained from Phoenix Pharmaceuticals (Burlingame, CA). Biopsy specimens obtained from AD patients ($n=5$) and healthy controls ($n=6$) were snap-frozen, cut into 5-μm-thick cryostat sections, and fixed in acetone. These sections were then stained with rabbit anti-human GRP antibody (number H-027-07, Phoenix Pharmaceuticals) and mouse anti-human PGP 9.5 mAb (13C4/I3C4, Abcam plc, Cambridge, UK), followed by corresponding secondary antibodies labeled with Alexa-Fluor 488 or Alexa-Fluor 594 (Molecular Probes, Eugene, OR). The numbers of double-positive fibers per $\times 100$ field were counted independently by two investigators in a blinded manner. The χ^2 goodness-of-fit test was used to evaluate normality for all parameters. The F-test was used to evaluate the equality of variance between two groups. Welch's t test was used for comparison between two groups. Correlation coefficients were determined by using Spearman's rank correlation test. $P<0.05$ was considered significant.

Serum GRP levels in AD patients were significantly higher than in healthy individuals (3.11 ± 0.98 and $2\pm0.58\,ng\,ml^{-1}$, respectively; $P<0.001$, Figure 1a). When serum GRP levels higher than the mean + 2 SD of the control serum samples were considered to be elevated, the percentage of elevated samples in AD were 45.2%. We compared serum GRP levels with other laboratory data reflecting disease activity of AD, such as serum levels of IgE, sIL–2R, IL-31, CCL17, and numbers of eosinophils in peripheral blood. There was no significant relation between serum GRP levels and these markers (data not shown). By contrast, there was a significant correlation between serum GRP levels and VAS itch scores ($r=0.725$, $P<0.01$, Figure 1b). We next performed double immunofluorescence staining for GRP and PGP 9.5. In both AD lesional skin and normal skin, dermal PGP 9.5^+ nerve fibers expressed GRP (Figure 2), as was previously reported in NC/Nga mice. Absorption experiments suggested that immunoreactivity in the epidermis was false-positive (data not shown). The number of GRP^+ nerve fibers in AD skin (5.0 ± 1.2 per $\times 100$ field) was significantly larger than that of normal skin (2.7 ± 0.8 per $\times 100$ field).

We clearly demonstrated that serum GRP levels in AD patients were significantly higher than in healthy individuals, and that they correlated with VAS itch scores. Transepidermal water loss and serum levels of β-endorphin and IgE are useful biomarkers for itch intensity in AD patients (Lee *et al.*, 2006). IL-31 is also regarded as a key cytokine for the development of AD-induced skin inflammation and pruritus (Bilsborough *et al.*, 2006). However, serum IgE and IL-31 levels did not correlate with VAS itch scores in this study. Although further studies with a large number of cases are required, serum GRP levels could be a better biomarker of itch in AD patients. No significant correlation between serum GRP levels and serum IgE or IL-31 levels in this study has also suggested multiple pathways for itch sensation. GRP-induced scratching was inhibited by the μ-opioid receptor antagonist naltrexone hydrochloride, the GRPR antagonist RC-3095, the H_1 histamine receptor antagonists fexofenadine hydrochloride and chlorpheniramine maleate, and the PAR_2 proteinase-activated receptor antagonist FSLLRY-NH_2 (Andoh *et al.*, 2011). It has recently been reported that central GRPR and neuromedin B receptor act independently to elicit scratching behavior and that there is an additional, unidentified receptor mechanism underlying bombesin-elicited scratching (Su and Ko, 2011). In summary, elevated serum GRP levels and increased numbers of dermal GRP^+ nerve fibers suggested that this peptide could be a therapeutic target for itch in AD patients.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Montrouge, le 12/07/2013

Makoto Sugaya

Vous trouverez ci-après le tiré à part de votre article au format électronique (pdf) :
Serum visfatin levels in patients with atopic dermatitis and cutaneous T-cell lymphoma

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John Libbey Eurotext

Investigative report

Eur J Dermatol 2013; 23(5): 629-35

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Serum visfatin levels in patients with atopic dermatitis and cutaneous T-cell lymphoma

Visfatin, a novel adipocytokine, is related with chronic inflammatory diseases, especially those characterized by T helper (Th)1-type immune responses. In this study, we examined serum visfatin levels in patients with atopic dermatitis (AD) or cutaneous T-cell lymphoma (CTCL), both of which are Th2-dominant diseases. Serum visfatin levels in patients with AD or advanced stage CTCL were significantly elevated compared to healthy controls. In CTCL patients, serum visfatin levels significantly decreased after treatment. Serum visfatin levels correlated with eosinophil counts in AD patients, whereas they correlated with the visual analogue scale itch scores and serum C-C motif ligand (CCL) 11 and CCL26 levels in CTCL patients. Visfatin expression by adipose tissue in lesional skin of AD and advanced stage CTCL was enhanced compared to that of healthy controls. These results suggest that visfatin may also be important in the development of Th2-dominant diseases as well as in Th1-type diseases.

Key words: visfatin, atopic dermatitis, cutaneous T-cell lymphoma, mycosis fungoides, adipose tissue

Adipose tissue has emerged as an important endocrine organ, producing a variety of secreted factors including tumor necrosis factor (TNF)-α [1], interleukin (IL)-6, IL-8 [2], leptin [3], adiponectin [4, 5] and resistin [6]. These adipocytokines are related with obesity, insulin resistance, inflammation and immune systems [7]. Visfatin, a novel adipocytokine, has been initially recognized as a pre-B-cell colony enhancing factor, which enhances differentiation of B-cell precursors in synergy with IL-7 and stem cell factors [8]. Serum visfatin levels were elevated in patients with rheumatoid arthritis (RA), Behcet's disease, inflammatory bowel diseases and psoriasis [9-12], which are characterized by T helper (Th)1-type immune responses.

Mycosis fungoides (MF) and Sezary syndrome (SS) are the most common types of cutaneous T-cell lymphoma (CTCL). Patients with MF typically have a prolonged clinical course and only limited cases progress over years through patch, plaque and tumor stages, followed by lymph node and visceral involvement [13]. Most cases with MF/SS, especially at an advanced stage, show a Th2-dominant phenotype, characterized by increased IL-4, IL-5, IL-10, and IL-13 production [14, 15]. Atopic dermatitis (AD) is also characterized by the presence of a specific immunoglobulin E (IgE) in association with Th2-mediated immune responses [16].

The aim of this study was to examine serum visfatin levels in AD and CTCL patients to discover whether visfatin was also associated with Th2-type diseases. Serum visfatin levels in patients with AD or advanced stage CTCL were elevated compared to healthy controls. Serum visfatin levels correlated with eosinophil counts in AD patients, whereas they correlated with the visual analogue scale (VAS) itch scores and serum C-C motif ligand (CCL) 11 and CCL26 levels in CTCL patients. Visfatin expression by adipose tissue in lesional skin of AD and plaque MF, tumor MF and erythrodermic CTCL was enhanced compared to those in healthy controls and patch MF. These results suggest that visfatin may also be important in the development of Th2-dominant diseases.

Materials and methods

Patients

Forty patients with AD (27 males and 13 females; mean ± standard deviation (SD) age: 37.0 ± 13.3 years; all extrinsic type), 39 patients with MF/SS (22 males and 17 females; 57.6 ± 12.3 years, 16 cases of patch MF, 6 cases of plaque MF, 8 cases of tumor MF, and 9 cases of erythrodermic MF/SS), and 42 healthy control subjects (19 males and 23 females; 42.6 ± 17.3 years) were enrolled in this study. AD was diagnosed according to the criteria of Hanifin and Rajka [17]. The clinical severity of AD was evaluated using the scoring system proposed by Rajka and Langeland [18]. The diagnosis of MF and SS and the stages of CTCL were based on clinical criteria as well as on histologic and immunohistochemical assessment according to World Health Organization classification and the criteria of the International Society for Cutaneous Lymphomas [19, 20]. When classifying the patients into patch MF, plaque MF, tumor MF or erythrodermic MF/SS, the severest skin lesion was taken into consideration. The 42 healthy controls had no history of allergy, AD, CTCL or any skin diseases. We

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excluded patients under treatment for various metabolic and inflammatory disorders other than AD and CTCL. We also analyzed the correlation between serum visfatin levels and body mass index (BMI). We grouped subjects into three sub-groups according to BMI (underweight, BMI<18.5; normal, 18.5-25.0; overweight, > 25.0). All samples were collected after informed consent during daily clinical practice. We rated itch by VAS 0-10, asking the patients to mark a point on the line corresponding to mean itch during the last seven days before blood drawing. The medical ethical committee of the University of Tokyo approved all described studies and the study was conducted according to the Declaration of Helsinki Principles.

Enzyme-Linked ImmunoSorbent Assay

Serum visfatin levels were quantified by Visfatin C-Terminal (Human) Enzyme Immunoassay Kit (Phoenix Pharmaceuticals. Burlingame, CA, USA). Serum CCL11 and CCL26 levels were quantified by human Quantikine ELISA kits (R&D systems. Minneapolis, MN, USA). These assays employ the quantitative sandwich enzyme immunoassay technique. Optical densities were measured at 450 nm using a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The concentrations were calculated from the standard curve generated by a curve-fitting program.

Immunohistochemistry

We performed immunohistochemical staining for visfatin with the lesional skin of AD (n = 5), patch MF (n = 5), plaque MF (n = 5), tumor MF (n = 5), erythrodermic MF/SS (n = 5) and with normal skin (n = 5). The size of the skin biopsies was about two centimeters and the cut ends were the depth of adipose tissue. Briefly, 5 μm-thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were de-waxed and rehydrated. These sections were then stained with rabbit anti-human visfatin polyclonal antibody (Pheonix Pharma), followed by ABC staining (Vector Lab, Burlingame, CA, USA). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer hematoxylin was performed, according to the manufacturers' instructions.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney's U-test and Student's t-test for comparison of two groups. For testing the equality of population means among three or more groups, the Kruskal-Wallis test and Scheffe's F test were used. Correlation coefficients were determined by using the Spearman's rank correlation test. *P*-values of <0.05 were considered statistically significant.

Results

Serum visfatin levels in patients with AD or CTCL

Serum visfatin levels in patients with AD were 3.67 ± 2.79 (mean ± SD) ng/mL, which were significantly higher than those in healthy controls (1.75 ± 0.92 ng/mL) and patients with CTCL (1.89 ± 1.26 ng/mL; *figure 1A*). We next analyzed the serum visfatin levels according to BMI. In AD patients, serum visfatin levels in the underweight group (n = 7), the normal group (n = 23), and the overweight group (n = 10) were 3.08 ± 1.22 ng/mL, 3.38 ± 1.98 ng/mL, and 4.84 ± 3.62 ng/mL, respectively (*figure 1B*). Serum visfatin levels in the overweight group were significantly higher than those in the underweight group (*figure 1B; P*<0.05). In CTCL patients, serum visfatin levels in the underweight group (n = 5), the normal group (n = 25), and the overweight group (n = 8) were 1.10 ± 0.78 ng/mL, 1.73 ± 1.19 ng/mL, and 3.02 ± 1.13 ng/mL (*figure 1C*). Serum visfatin levels in the overweight group were significantly higher than those in the underweight or normal group (*figure 1C; P*<0.05, each). In healthy controls, serum visfatin levels in the underweight group (n = 5), the normal group (n = 26), and the overweight group (n = 11) were 0.57 ± 0.92 ng/mL, 1.85 ± 0.79 ng/mL, and 2.06 ± 0.88 ng/mL (data not shown). Serum visfatin levels in the normal or overweight group were significantly higher than those in the underweight group (*P*<0.05, each, data not shown). There was no significant difference in BMI among AD patients, CTCL patients, and healthy controls. There was no significant correlation between BMI and severity in AD (data not shown). We next analyzed the relationship between serum visfatin levels and the severity of AD. Serum visfatin levels of patients with mild (n = 14), moderate (n = 17), and severe AD (n = 9) were 2.86 ± 0.72 ng/mL, 4.16 ± 3.11 ng/mL, and 4.28 ± 2.61 ng/mL, respectively, all of which were significantly higher than those of healthy controls (*figure 1D*). There was, however, no significant difference in serum visfatin levels among the AD groups. We also analyzed serum visfatin levels in AD patients according to the onset time. Serum visfatin levels in patients with classical AD who had developed the skin lesions in childhood (n = 16) and adult-onset AD (n = 24) were 2.83 ± 0.96 ng/mL and 4.22 ± 2.88 ng/mL, respectively (*figure 1E*). Serum visfatin levels in adult-onset AD patients were significantly higher than in classical AD patients (*P*<0.05)

We next sub-grouped CTCL patients according to the types of skin lesions. Serum visfatin levels in patients with plaque MF (2.82 ± 1.13 ng/mL), tumor MF (2.24 ± 0.84 ng/mL), and erythrodermic MF/SS (2.43 ± 1.20 ng/mL) were significantly higher than patch MF (1.08 ± 1.11 ng/mL; *P*<0.01, *P*<0.05, and *P*<0.05, respectively; *figure 1F*). Interestingly, when patch and plaque MF were regarded as early CTCL, and tumor MF and erythrodermic MF/SS were grouped into advanced stage CTCL, serum visfatin levels in patients with advanced stage CTCL were higher than normal controls (*P*<0.05). Thus, serum visfatin levels were elevated in patients with AD and advanced stage CTCL.

Serum visfatin levels before and after treatment in CTCL patients

We measured serum visfatin levels in five CTCL cases (2 cases of plaque MF, 1 case of tumor MF, and 2 cases of SS) before and after treatment (*table 1*). All patients showed partial remission. Serum visfatin levels after treatment were significantly lower than those before treatment (*P*<0.05). Thus, serum visfatin levels may reflect the disease activity of each patient with CTCL.

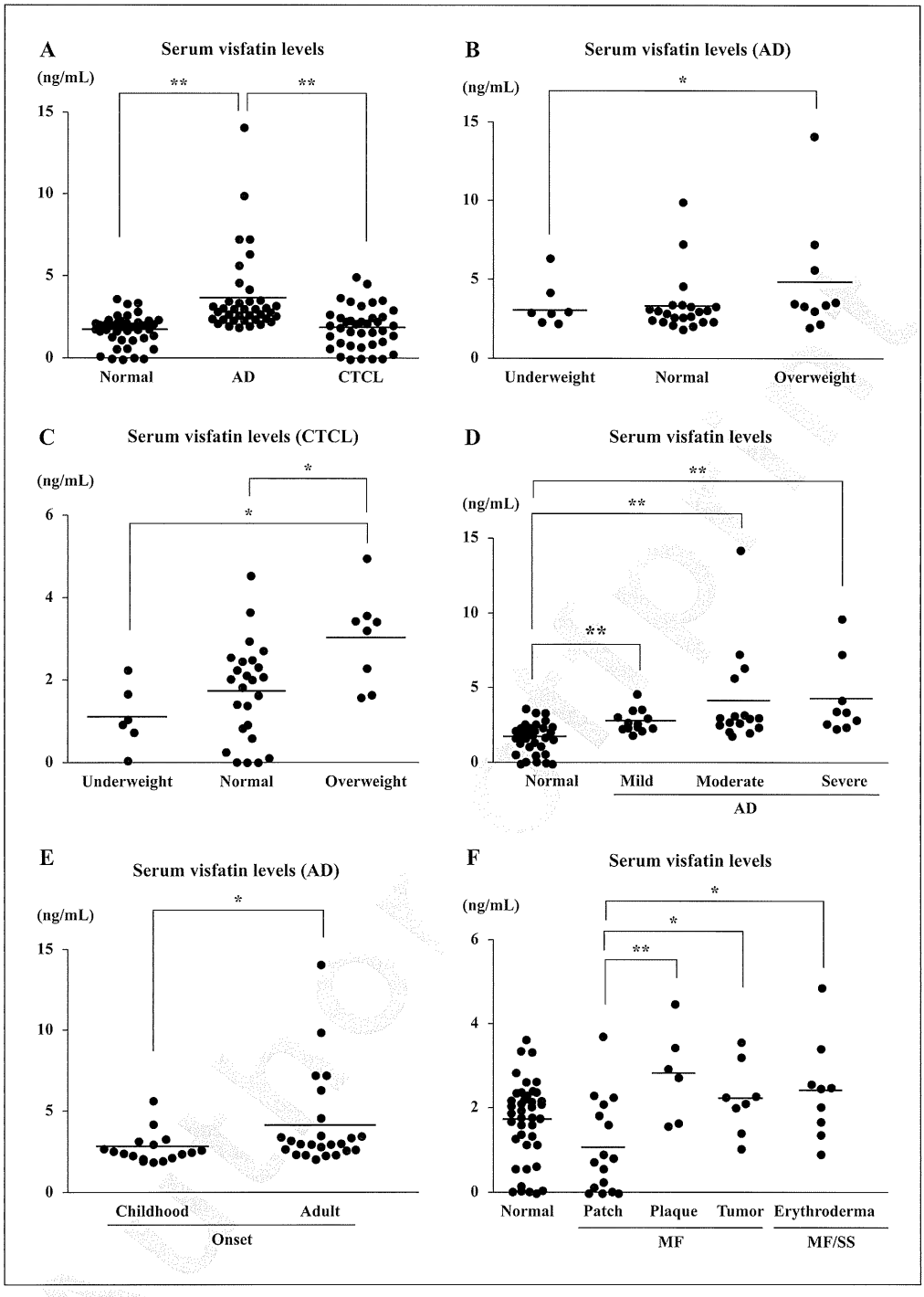

Figure 1. Serum visfatin levels in patients with atopic dermatitis (AD) and cutaneous T-cell lymphoma (CTCL). **A)** Serum visfatin levels in AD patients were higher than normal controls and those in CTCL patients. **B)** Serum visfatin levels in overweight AD patients were higher than underweight AD patients (underweight; body mass index <18.5, normal; 18.5-25.0, overweight; >25.0). **C)** Serum visfatin levels in overweight CTCL patients were higher than underweight or normal weight CTCL patients. **D)** Serum visfatin levels in patients with mild, moderate, and severe AD were higher than normal controls. **E)** Serum visfatin levels in adult-onset AD patients were significantly higher than classical AD patients. *$P<0.05$ and **$P<0.01$. **F)** Serum visfatin levels in patients with plaque mycosis fungoides (MF), tumor MF, and erythrodermic MF/Sezary syndrome (SS) were significantly higher than those in patch MF.

Table 1. Serum visfatin levels before and after treatment in patients with cutaneous T-cell lymphoma.

Age	Sex	Stage	Serum visfatin levels (ng/mL)		Treatment	Observation period
			before	after		
62	M	Plaque MF	1.64	1.04	Topical corticosteroids UV phototherapy, oral etretinate	9 months
59	F	Plaque MF	4.55	3.78	Topical corticosteroids Oral etretinate, VP-16	4 months
43	M	Tumor MF	3.22	1.70	Oral corticosteroids Multiagent chemotherapy	4 months
42	F	SS	4.97	3.63	Oral corticosteroids Multiagent chemotherapy	3 months
66	F	SS	3.33	2.83	UV phototherapy Vorinostat	3 months

Abbreviations: M, male; F, female; MF, mycosis fungoides; SS, Sezary syndrome; UV, ultraviolet

Positive correlations between serum visfatin levels and eosinophil counts, the VAS itch scores, and serum CCL11 and CCL26 levels

We evaluated correlations between serum visfatin levels and eosinophil counts, serum levels of CCL11, CCL17, CCL26, lactate dehydrogenase (LDH), IgE, and soluble interleukin-2 receptor (sIL-2R), and the VAS itch scores. Among above disease markers, only eosinophil counts significantly correlated with serum visfatin levels in AD patients (*figure 2*). In patients with CTCL, serum visfatin levels significantly correlated with the VAS itch scores and serum CCL11 and CCL26 levels (*figure 2*). Thus, serum visfatin levels were related with some markers of Th2-type immune responses.

Enhanced visfatin expression by adipose tissue in lesional skin of AD and advanced stage CTCL

Immunohistochemical staining for visfatin was performed using lesional skin of AD, patch MF, plaque MF, tumor MF, erythrodermic MF/SS and normal skin (*figure 3*). Visfatin expression by adipose tissue in lesional skin of AD, plaque MF, tumor MF and erythrodermic MF/SS was enhanced compared to those in healthy controls and patch MF (*figure 3*). Thus, enhanced visfatin expression by adipose tissue in skin lesion may account for elevated serum visfatin levels in AD and some of advanced stage CTCL patients.

Discussion

In this report, we measured serum visfatin levels in AD and CTCL patients. Serum visfatin levels in patients with AD and advanced CTCL were significantly higher than healthy controls. In CTCL patients, serum visfatin levels significantly decreased after treatment. When we analyzed correlations between serum visfatin levels and other disease markers, serum visfatin levels correlated with eosinophil counts in AD patients, whereas they correlated with the VAS itch scores and serum CCL11 and CCL26 levels in CTCL patients. Enhanced visfatin expression by adipose tissue was detected in lesional skin of AD and plaque MF, tumor MF, and erythrodermic CTCL.

In patients with RA, serum visfatin levels were higher than in healthy controls, reflecting the degree of inflammation and clinical activity [9, 10, 21]. Visfatin messenger RNA (mRNA) expression was significantly elevated in synovial tissue of RA [22]. Similarly, serum visfatin levels in patients with active Behcet's disease were increased compared to healthy controls [9]. These data suggest that visfatin is important for the development of inflammatory diseases which are characterized by Th1-type immune responses. So far, little is known about the possible roles of visfatin in Th2-dominant diseases.

We evaluated correlations between serum visfatin levels and eosinophil counts, serum levels of CCL11, CCL17, CCL26, LDH, IgE, and sIL-2R, and the VAS itch scores, which were all reported to be elevated in AD and CTCL patients [23-30], revealing that serum visfatin levels correlated with the VAS itch scores and serum CCL11 and CCL26 levels in CTCL patients (*figure 2*). CCL11 is a member of the eotaxin family named as eotaxin-1, which is a ligand for CCR3. CCR3 is preferentially expressed on eosinophils and Th2 cells. We recently showed that serum CCL11 levels in patients with CTCL were significantly higher than in healthy controls [24]. Serum CCL11 levels of advanced stage CTCL were higher than early stage CTCL. CCL26 is another member of the eotaxin family, named eotaxin-3. We revealed that cultured fibroblasts isolated from the lesional skin of CTCL expressed higher amounts of CCL26 mRNA compared to those from normal skin [24]. The lesional skin of advanced stage CTCL contained significantly higher levels of CCL26 mRNA compared to early stage CTCL [24]. Serum CCL26 levels of advanced stage CTCL were significantly higher than normal controls [24]. Taken together, CCL11 and CCL26 are associated with the disease activity in CTCL. Moreover, our study showed that serum visfatin levels also correlated with eosinophil counts in AD patients (*figure 2*), leading us to hypothesize that visfatin is important for Th2-type diseases as well as Th1-type diseases. Although we showed that serum visfatin levels were significantly decreased after treatment (*table 1*), we had some limitations. Only five patients were included in this sub-analysis and the patients included were treated with different therapies, including topical corticosteroids, UV phototherapy, oral etretinate and/or systemic chemotherapy. To clarify the correlation between serum visfatin levels and disease activity, further studies would be needed.

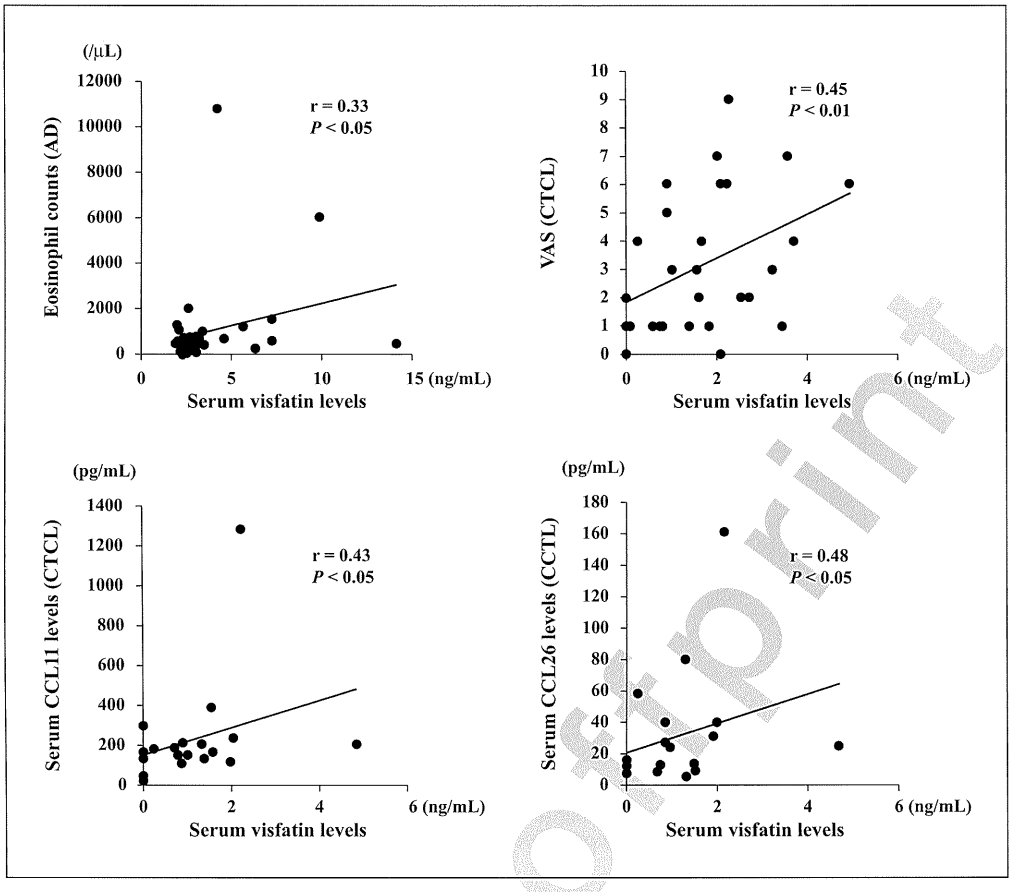

Figure 2. Correlation between serum visfatin levels and eosinophil counts in patients with atopic dermatitis (AD). In patients with cutaneous T-cell lymphoma (CTCL), serum visfatin levels correlated with the visual analogue scale (VAS) itch scores, and serum CCL11 and CCL26 levels.

Visfatin induces cytokine production by human leukocytes [31]. Visfatin exerts pro-inflammatory activities by dose-dependently up-regulating IL-1β, IL-1Ra, IL-6, IL-10, and TNF-μ in human monocytes. These cytokines play a substantial role in a wide range of infectious and inflammatory diseases such as AD [32-34]. Moreover, IL-5, IL-6, IL-10, and IFN-γ are associated with development of MF [35, 36]. Visfatin expression in AD and MF/SS may be related with induction of these inflammatory cytokines, although further study is necessary.

Psoriasis is a chronic inflammatory skin disease characterized by the formation of scaly and erythematous plaques, where Th1-type immune responses are dominant. Serum visfatin levels were elevated in patients with psoriasis [11, 12]. According to the Th1 and Th2 dogma, psoriasis and AD/CTCL are mutually exclusive. As for Th1/Th2 balance in advanced CTCL, we previously revealed that high CCL26 expression and low herpes virus entry mediator expression on dermal fibroblasts contribute to a Th2-dominant microenvironment in advanced CTCL, showing that advanced CTCL is a Th2-dominant disease [24, 37]. However, the dichotomy of Th1 and Th2 in the pathogenesis of psoriasis and AD/CTCL may be overly simplistic. Recent studies suggest that psoriasis and AD shared much genetic similarity [38, 39]. Moreover, lesional skin of AD expresses elevated levels of IL-22 and IL-17, which are key cytokines for the development of psoriasis as well as RA and Behcet's disease [40, 41]. We recently showed that IL-22 was also expressed in CTCL lesional skin, suggesting that IL-22 is a common cytokine for psoriasis, AD and CTCL, responsible for epidermal hyperplasia [42]. The results in this study suggested that visfatin may be a common factor involved in the development of the three skin diseases, as well as IL-22. It was recently reported that serum visfatin levels in children with AD were lower than those in healthy children, which was not compatible with our results [43]. One reasonable explanation is that there would be difference in the underlying mechanism of child AD and adult AD. We analyzed serum visfatin levels according to the onset time, finding that patients with adult-onset AD showed significantly higher serum visfatin levels than those who had developed the skin lesions in childhood (*figure 1E*). Additionally, AD is currently considered a biphasic disease, with Th2-type reaction predominating in acute disease and a switch to Th1-type characterizing chronic disease [44]. Our results suggest that visfatin may be associated with adult-onset AD and classical AD at the chronic phase in adulthood.

In conclusion, visfatin expression is enhanced in the sera and skin lesions of AD and advanced stage CTCL. Serum visfatin levels correlated with eosinophil counts in AD patients, whereas they correlated with the VAS itch scores

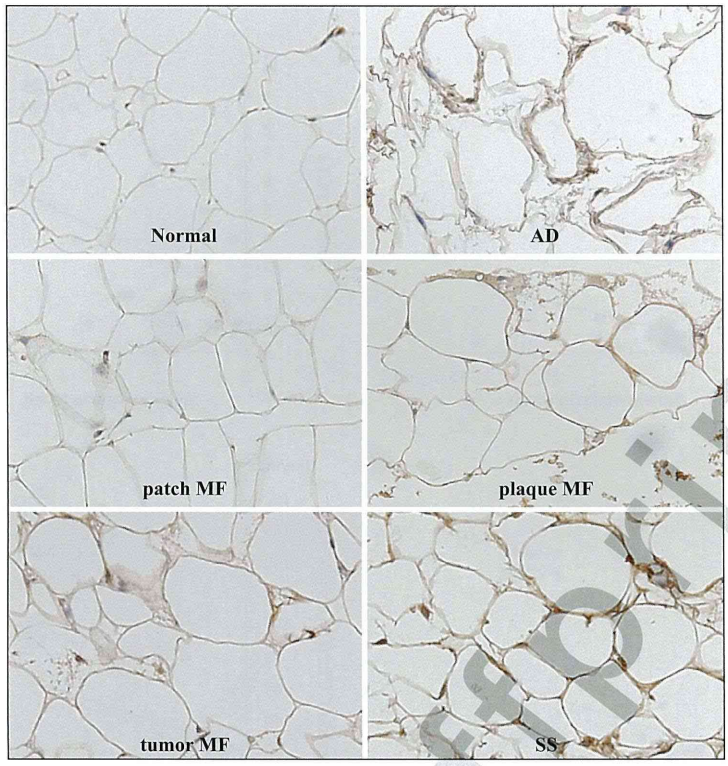

Figure 3. Immunohistochemical staining for visfatin (original magnification ×400). Representative pictures of five cases in each group. Adipose tissue of the lesional skin of atopic dermatitis (AD) and plaque mycosis fungoides (MF), tumor MF, and Sezary syndrome (SS) showed enhanced visfatin expression compared to normal skin and patch MF.

and serum CCL11 and CCL26 levels in CTCL patients, suggesting that visfatin may be involved in Th2-dominant diseases as well as Th1-type diseases such as psoriasis. Further studies for more precise elucidation of the pro-inflammatory activities of visfatin would deepen our understanding of the pathogenesis of these skin diseases. ■

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SHORT COMMUNICATION

Variations in Serum TARC and I-TAC Levels Reflect Minor Changes in Disease Activity and Pruritus in Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic or relapsing inflammatory skin disease. Scratching in AD patients results in proinflammatory cytokine and chemokine production. Thus, serum levels of monocyte chemotactic protein-1 (MCP-1), regulated on activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1β, eotaxin, thymus and activation-regulated chemokine (TARC), and macrophage-derived chemokine (MDC) were increased in AD patients, compared with normal controls (1–4). With regards to Th1 chemokines such as interferon (IFN)-γ-inducible protein 10 (IP-10), IFN-γ-inducible T-cell α-chemoattractant (I-TAC), and monokine induced by IFN-γ (MIG), their expression in lesional AD skin was confirmed by immunohistochemistry (5). They may negatively contribute to the development of AD because macrophages from AD patients produced lower levels of IP-10 compared to cells from healthy controls in response to α-toxin (6) and the expression of MIG and IP-10 was lower in Langerhans cells from patients with AD than from patients with psoriasis, whereas the opposite was observed for TARC and MDC (7).

Visual analogue scale (VAS) is a valuable method to assess pruritus intensity in patients with pruritic dermatoses (8). In this study, we focus on temporal variation of pruritus in each patient and compare serum samples taken at different time points when there were only slight, if any, changes in disease activity. The aim of this study was to highlight the most sensitive chemokine associated with changes in pruritus in AD patients.

MATERIALS AND METHODS

Seventeen Japanese outpatients with moderately-controlled AD, diagnosed according to the criteria of Rajka & Langeland (9), were enrolled in this study (11 men and 6 women, mean age 34.4 ± 10.4 years). Clinical severity: 8 mild cases, 5 moderate cases, and 4 severe cases. Blood samples were collected twice with an 8-week interval after informed consent. We rated itch by VAS 0–10, asking the patients to mark a point on the line corresponding to mean itch during the last 7 days before blood draw. The clinical severity of AD was evaluated using the scoring system proposed by Rajka & Langeland (9). They were treated with topical corticosteroids and moisturizers in combination with oral antihistamine, which were not changed. during the study. None of the patients showed dramatic changes in disease activity during the observation period. All studies were approved by the ethics review board of the Faculty of Medicine, the University of Tokyo and conducted according to the Declaration of Helsinki Principles.

Serum cytokine levels of 12 chemokines, i.e., interleukin-8, MCP-1, RANTES, MIP-1α, MIP-1β, IP-10, I-TAC, MIG, eotaxin, TARC, MDC, and GROα were analysed with Multi-Analyte ELISArray Kits (QIAGEN, Frederick, MD) according to the manufacturer's protocol.

Correlation coefficients between ⊿VAS (VAS at the 2nd visit subtracted by VAS at the 1st visit) and ratio of absorbance (serum chemokine absorbance at the 2nd visit divided by serum chemokine absorbance at the 1st visit) were determined using the Spearman's rank correlation test. *P*-values of < 0.05 were considered statistically significant.

RESULTS

Pruritus VAS score (mean±standard deviation) at the 1st visit in mild, moderate, and severe AD was 4.6 ± 0.74, 6.2 ± 0.8 and 4.8 ± 2.4, respectively (Fig. 1a). Pruritus VAS scores at two different visits are shown in Fig. 1b and example of serum chemokines levels in Fig. 1c and d. After the 8 weeks, pruritus was improved in 10 patients. In 4 patients, itch sensation got worse, while there was no change in pruritus in 3 patients. Only minor changes in chemokine levels occurred (all data not shown). The variations in pruritus correlated positively with the before and after ratio of serum TARC levels and negatively with ratio of I-TAC levels (Fig. 1e, f). On the other hand, absolute VAS scores did not significantly correlate with any serum chemokine levels (data not shown).

DISCUSSION

Variation in pruritus correlated with the ratio of serum TARC levels, and correlated inversely with that of serum I-TAC levels (Fig. 1e, f). Although TARC and I-TAC may not directly regulate pruritus in AD patients, it may be safely said that these chemokines are very sensitive disease markers of AD. Plenty of data have been accumulated to suggest that serum TARC levels reflect disease activity of AD (4, 10). The expression of TARC and MDC was reported to be higher in Langerhans cells from patients with AD than from patients with psoriasis (7). Moreover, TARC was reported to be superior to other markers of AD. When mRNA expression levels of 14 CC chemokines in the skin were examined, TARC and other two chemokines went along with eczema development in AD (11). Simi-

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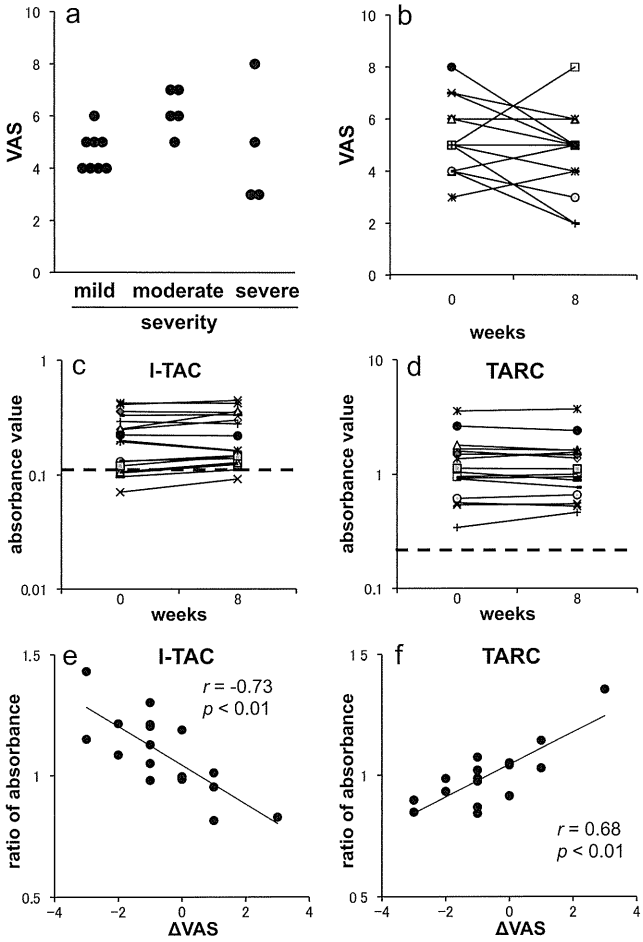

Fig. 1. (a) Pruritus visual analogue scale (VAS) scores at the first visit of AD patients. (b) Pruritus VAS scores at two different visits. (c, d) Absorbance values of IFN-γ-inducible T-cell α-chemoattractant (I-TAC) (c) and thymus and activation-regulated chemokine (TARC) (d) at two different visits. Dotted lines represent the mean level of each chemokine in healthy controls. (e, f) Association between variations in VAS score and ratio of serum absorbance values of I-TAC (e) and TARC (f) at the 2 observation time-points.

lary, out of 10 examined cytokines/chemokines, serum concentrations of TARC were increased in adult AD patients (12). These previous findings, together with our results, suggest that TARC is the most sensitive disease marker of AD. On the other hand, the role of I-TAC in the development of AD is yet to be investigated. It was previously reported that IFN-γ response was attenuated in monocyte-derived dendritic cells in patients with AD (13). Plasma levels of IP-10, another Th1 chemokine, tended to be decreased in severe AD compared to mild AD, although they were higher in AD patients than in normal controls (14). These previous studies suggest that Th1 chemokines might be expressed in response to Th2-mediated inflammation and that decrease in their expression might be associated with deterioration of AD.

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Questionnaire survey of the efficacy of emollients for adult patients with atopic dermatitis

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ABSTRACT

Emollients are useful and important treatment adjuncts for patients with atopic dermatitis (AD). Heparinoid mucopolysaccharide creams or lotions are emulsion ointments for moisturizing the skin. The objective of this study was to investigate the view among adult AD patients regarding the effectiveness of emollients. We developed a questionnaire at our University Hospital to characterize how patients with AD viewed the efficacy of emollients. Patients were asked to participate prior to treatment and the questionnaire was given within 1 month of treatment. The severity of AD was graded as mild, moderate, severe or very severe. The severity scoring was performed only when the participants answered the questionnaire. Of the 110 enrolled AD patients, 103 returned the completed questionnaires. Ninety-eight patients (95.1%) used heparinoid mucopolysaccharide creams or lotions. There was a strong correlation between their view of the efficacy of the emollient and the condition of dry skin, pruritus and eczematous skin. There was a significant correlation between AD severity and the perceived efficacy of the emollient for dry skin, pruritus and eczematous skin. There was a greater sense of efficacy among patients with milder AD than in more severe AD cases. Patients who felt sufficient efficacy of the emollient for pruritus were significantly older than those who felt there was no efficacy. In addition, the age of onset of AD was significantly higher among those who felt sufficient efficacy for pruritus compared to those who felt little efficacy. We speculate that the efficacy of emollients could be demonstrated in the treatment of milder AD, but may only have partial efficacy in more severe cases. Emollient therapy might have lower efficacy for pruritus among younger or earlier onset AD patients.

Key words: adult, atopic dermatitis, emollients, heparinoid mucopolysaccharide, pruritus, questionnaire survey.

INTRODUCTION

Atopic dermatitis (AD) is a frequent, chronic inflammatory disease influenced by local, immunological, genetic and environmental factors. The barrier dysfunction of dry skin is thought to be an important etiological factor in the pathogenesis of AD. Therefore, appropriate use of emollients is an essential part in the management of AD. Emollients are useful and important treatment adjuncts for the daily skin care of patients with dry and inflamed skin associated with AD. After the AD is stabilized, the addition of maintenance treatment with emollients to topical corticosteroid treatment significantly reduces the risk of relapse.[1,2] There have been many clinical studies on the efficacy of emollients by using non-invasive biophysical methods and/or clinician's visual assessment but few clinical studies from the aspect of patients' view. Information about the effectiveness has been lacking and, in this study, we assessed the effectiveness of emollients based on the view of AD patients. To the best of our knowledge, questionnaire survey about AD patients' minds or opinion for the efficacy of emollients has not been reported previously.

Heparinoid mucopolysaccharide creams and lotions are emulsion ointments of the water in oil type and the oil in water type, respectively.[3–5] These topical preparations (Hirudoid; Maruho, Osaka, Japan)

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are used for moisturizing the skin. The active ingredient of Hirudoid is mucopolysaccharide polysulfuric acid ester which is similar to the body's naturally occurring mucopolysaccharides. The drug is a commercial and original product from Japan and can only be obtained by prescription in Japan.

METHODS

Participants

We developed a questionnaire to determine the view of emollients in the treatment of adult AD patients in Japan. One hundred and ten patients with AD were enrolled at the Department of Dermatology, St Marianna University School of Medicine between 2008 and 2009. AD was diagnosed by experienced dermatologists based on the Japanese Dermatological Association criteria for the disease.[6] These criteria are very similar to those of Hanifin and Rajka.[7] The patients used emollients twice a day for 1 month before enrollment in the study. The patients continued using the same antihistamines and the same topical corticosteroid and/or tacrolimus during the period used to evaluate the efficacy of the emollient. The emollients were applied just after the corticosteroid/tacrolimus. We asked the patients to respond to the following questionnaire.

Questionnaire

The questionnaire was given to them by hand and the patients submitted them to the clerks in our clinic on the same day.

Do you feel that the emollient is effective for treating your dry skin due to atopic dermatitis?
Sufficiently/somewhat/no
Do you feel that the emollient is effective for treating your itching due to atopic dermatitis?
Sufficiently/somewhat/no
Do you feel that the emollient is effective for treating your eczematous skin due to atopic dermatitis?
Sufficiently/somewhat/no
In which season are your symptoms the worst?
Spring/summer/autumn/winter/unsure
How old were you at the onset of atopic dermatitis?

Assessments

The severity of AD was graded as mild, moderate, severe or very severe according to the Japanese Dermatological Association criteria for the disease.[6] The patients were assessed for severity on the day they responded to the questionnaire. We also determined their oral and topical treatments for AD.

Statistical analysis

The χ^2-test was used to compare the response rate for each question (Q1–4) and the severity of AD; the level of significance was set at $P < 0.05$ in all cases. The statistics were analyzed by paired Student's *t*-test to compare each question (Q1–4), age and onset age (Q5); the level of significance was set at $P < 0.05$ in all cases. All data are expressed as means ± standard deviation.

This study was based on the ethical principles of Good Clinical Practice and was approved by the St Marianna University School of Medicine Institutional Review Board for Human Subjects Research (no. 1426).

RESULTS

Characterization of patients

Of the 110 enrolled AD patients, 103 returned the completed questionnaires. Ninety-eight of the patients (95.1%) used heparinoid mucopolysaccharide creams or lotions. The response profile for each question in adult AD patients who used the heparinoid mucopolysaccharide cream or lotion is shown in Table 1. Of the 98 patients (61 men, 37 women), 40 (40.1%) had mild symptoms, 42 (42.9%) had moderate symptoms, 11 (11.2%) had severe symptoms and five (5.1%) had very severe symptoms. Almost all of the enrolled AD patients received topical corticosteroid treatment (92 patients, 94.0%).

Views among adult AD patients regarding the effectiveness of emollients

As might be predicted, all respondents felt the emollient was effective or somewhat effective for treating their dry skin (Q1). Patients who felt sufficient efficacy for dry skin (Q1) tended to feel that there was significant improvement in their pruritus (Q2) ($\chi^2 = 8.45$, $P = 0.015$; Table 2). Interestingly, 81 patients (82.7%) felt the emollient was effective or somewhat effective for treating their pruritus. In addition, there was a close relationship between patients who felt sufficient efficacy for their dry skin (Q1) and those who felt

Table 1. Patient characteristics and response rates to the questionnaire

	Patients	Prevalence %
Sex		
Male	61	62.2
Female	37	37.8
Dry skin (Q1)		
Sufficiently	67	68.4
Somewhat	31	31.6
No	0	0.0
Pruritus (Q2)		
Sufficiently	28	28.6
Somewhat	53	54.1
No	17	17.3
Eczematous skin (Q3)		
Sufficiently	33	33.7
Somewhat	34	34.7
No	31	31.6
Season (Q4)		
Spring	6	6.1
Summer	24	24.5
Autumn	3	3.1
Winter	24	24.5
Unsure	27	27.6
Summer + winter	9	9.2
Autumn + winter	2	2.0
Others	3	3.1
Atopic dermatitis severity		
Mild	40	40.8
Moderate	42	42.9
Severe	11	11.2
Very severe	5	5.1
Oral treatment		
Antihistamines	77	78.6
No antihistamines	21	21.4
Topical treatment		
Corticosteroids	63	64.3
Corticosteroids + tacrolimus	29	29.6
No	6	6.1

sufficient efficacy for their eczematous skin (Q3) (χ^2 = 6.98, P = 0.031; Table 2). Sixty-seven patients (68.4%) felt the emollient was effective or somewhat effective for treating their eczematous skin. In other words, patients who felt that the emollients were effective for treating their dry skin also tended to report efficacy for pruritis and eczematous skin. There was a significant correlation between AD severity and perceived efficacy of the emollient for dry skin (Q1), pruritus (Q2) and eczematous skin (Q3) (χ^2 = 19.41, P = 0.00023; χ^2 = 13.61, P = 0.034; χ^2 = 19.13, P = 0.0039, respectively; Table 3). On the other hand, we did not find any significant correlation between AD severity and age of AD. Patients who felt that emollients were effective for treating their pruritus (Q2) were significantly older than those who did not (Q2) (mean age 37.2 ± 7.9 vs 32.1 ± 6.3 years; P = 0.011; Fig. 1). In addition, the mean age of onset of AD (Q5)

Table 2. Correlation of efficacy of emollients for treating dry skin (Q1), pruritus (Q2) and eczematous skin (Q3) in atopic dermatitis patients. Patients who felt that the emollients were effective for treating their dry skin (Q1) tended to report efficacy for pruritus (Q2), and eczematous skin (Q3)

	Dry skin (Q1)			
	Sufficiently	Somewhat	No	Total
P = 0.015				
Pruritus (Q2)				
Sufficiently	25	3	0	28
Somewhat	33	20	0	53
No	9	8	0	17
Total	67	31	0	98
P = 0.031				
Eczematous skin (Q3)				
Sufficiently	28	5	0	33
Somewhat	22	12	0	34
No	17	14	0	31
Total	67	31	0	98

Table 3. Correlation between atopic dermatitis (AD) severity and efficacy of emollients for treating dry skin (Q1), pruritus (Q2) and eczematous skin (Q3). There was a significant correlation between AD severity and perceived efficacy of the emollient for dry skin (Q1), pruritus (Q2) and eczematous skin (Q3)

	AD severity				
	Mild	Moderate	Severe	Very severe	Total
P = 0.00023					
Dry skin (Q1)					
Sufficiently	29	34	4	0	67
Somewhat	11	8	7	5	31
No	0	0	0	0	0
Total	40	42	11	5	98
P = 0.034					
Pruritus (Q2)					
Sufficiently	12	16	0	0	28
Somewhat	22	20	9	2	53
No	6	6	2	3	17
Total	40	42	11	5	98
P = 0.0039					
Eczematous skin (Q3)					
Sufficiently	15	18	0	0	33
Somewhat	13	15	6	0	34
No	12	9	5	5	31
Total	40	42	11	5	98

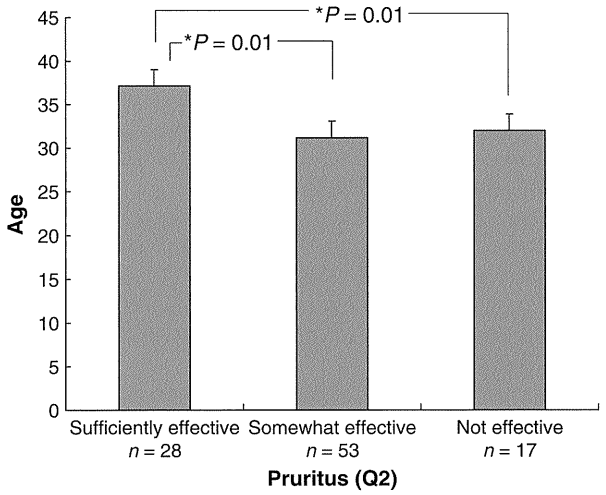

Figure 1. Comparison of efficacy of emollients for treating pruritus (Q2) as a function of age.

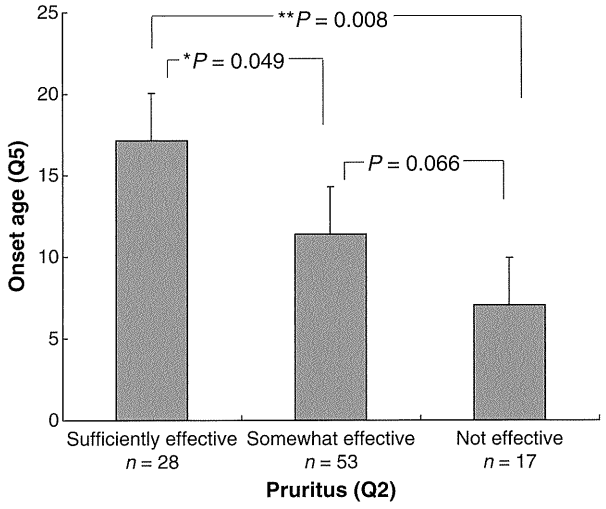

Figure 2. Comparison of efficacy of emollients for treating pruritus (Q2) as a function of mean age of onset of atopic dermatitis (Q5).

was significantly higher among patients who felt sufficient efficacy for pruritus (Q2) (17.1 ± 15.2 years) compared to those who felt there was no efficacy (Q2) (7.0 ± 8.7 years; $P = 0.008$) (Fig. 2).

DISCUSSION

This study was designed to explore the feelings of adult AD patients towards emollients. The AD patients who took heparinoid mucopolysaccharide creams and lotions and felt there was sufficient efficacy for their dry skin tended to also report reasonable efficacy for treating their pruritus and eczematous skin, all of which are important symptoms of AD. Standard treatment of AD is based on topical glucocorticosteroids or calcineurin inhibitors to treat flares combined with moisturizer treatment to alleviate dry skin symptoms. Some studies have suggested that once AD patients are stabilized with topical corticosteroid treatment, the risk of relapse of AD could be significantly reduced by regular emollient therapy in addition to intermittent topical corticosteroids.[1,2] Wirén *et al.*[8] concluded that maintenance treatment with a barrier-improving moisturizer on previous eczematous areas in patients with AD reduced the risk of relapse to approximately one-third of that of no treatment. Based on the view of AD patients in the present survey, emollient therapy in mild to moderate AD patients could prove to be useful in establishing treatment effects. In contrast, none of our AD patients with severe or very severe symptoms felt there was sufficient efficacy of emollients for treating pruritus and eczematous skin. We speculate that the efficacy of emollients could be demonstrated in the treatment of milder AD, but may only have partial efficacy in more severe cases.

According to the questionnaire-based patients' minds or opinion, patients who reported sufficient efficacy of emollients for pruritus tended to be older than those who reported little or no efficacy. In addition, the age of onset of AD was significantly higher among those who felt sufficient efficacy for pruritus compared to those who felt little efficacy. Emollient therapy might have lower efficacy for pruritus among younger or earlier onset AD patients. AD is a multifactorial disease which is increasingly being considered a primary disorder of stratum corneum dysfunction, where major predisposing factors for the eczema are mutations in the filaggrin gene.[9–11] We suggest that dry skin in younger AD patients could be influenced by genetic factors, and therefore emollients would not effectively treat the underlying causes of the dry skin associated with the pruritus. Unfortunately, our data may not be sufficient to discuss the relationship of age with the efficiency of emollients for pruritus in AD patients. The effectiveness of other agents, antihistamines,