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Decrease in circulating Th17 cells correlates with increased levels of CCL17, IgE and eosinophils in atopic dermatitis

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ABSTRACT

Background: Clinical significance of circulating $CD4^+$ T cell subsets, including T-helper (Th)1, Th2, Th17 and regulatory T (Treg) cells, in patients with atopic dermatitis (AD) remains unclear. No previous studies have simultaneously evaluated the four T cell subset profiles in AD.
Objective: The aim of the present study was to explore whether the percentage of these four subsets of $CD4^+$ T cells correlate to the severity parameters of AD patients.
Methods: Intracellular expression of interferon (IFN)-γ, interleukin (IL)-4, IL-17 and forkhead box P3 (Foxp3) in $CD4^+$ T cells was evaluated in peripheral blood mononuclear cells from normal controls and patient with AD as well as with chronic eczema using a flow cytometer. Serum CCL17 levels were measured as an objective severity parameter of AD together with percentage of eosinophils and serum IgE levels.
Results: In AD patients, the number of Th1 (IFN-γ^+) and Th17 (IL-17^+) subsets was significantly decreased, but that of Th2 (IL-4^+) and Treg (Foxp3^+) subsets was similar to that of normal controls. The T cell subset profiles of patients with chronic eczema were not different with those of normal controls. The frequency of Th17cells, particularly that of the IFN-γ^{nega}IL-17^+ subset, showed a significant negative correlation with CCL17, IgE and eosinophil levels in AD patients. This was, however, not the case in Th1, Th2 and Treg cells.
Conclusion: Decreased circulating Th17 cells might contribute to activity of AD.
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1. Introduction

Atopic dermatitis (AD) is a common, chronic or chronically relapsing, severely pruritic eczematous skin disease mostly associated with hyperimmunoglobulinemia E and eosinophilia [1,2]. Specifically, AD is a diathetic and multifactorial disorder which also predisposes to bacterial and viral infections. A complex interaction between susceptibility genes encoding skin barrier molecules and markers of the inflammatory response, environmental factors, host condition, infectious agents, and specific immunologic responses are involved in the pathophysiology of AD [3]. The pivotal role of innate and adaptive immunity in the evolution and persistence of AD is currently fully appreciated. Recently, the subdivision of T cell subsets according to their cytokine-production and/or chemokine receptor expression profiles has revealed a new T-helper (Th) cell classification, namely, Th1, Th2, Th17, and regulatory T (Treg) cell subsets, that plays an important role in autoimmune, infectious and allergic disorders [3,4].

Th1 and Th2 cytokines may differentially contribute to the pathogenesis of acute and/or chronic lesions of AD. The majority of allergen-specific T cells derived from skin lesions that had been provoked by the epicutaneous application of inhalant allergens were found to produce predominantly Th2 cytokines, which was initially considered to be a specific feature reflecting immune dysregulation in AD [5]. However, the cytokine switch from Th2 in the acute phase to Th1 in the chronic phase is now generally accepted for AD and also appears to be relevant in allergic contact dermatitis [6].

Th17 cells have recently been proved to be involved in various autoimmune and inflammatory disorders as well as defense mechanisms against certain extracellular bacteria and fungi [4]. Attention has recently been drawn to a possible role of Th17 cells in allergic contact dermatitis or AD [7]. Interestingly, acute AD lesions showed more Th17 cells than chronic lesions, suggesting that interleukin (IL)-17 functions primarily in the acute Th2 phase rather than in the subsequent Th1-dominated chronic phase of AD [7–9]. Because Th17 cell differentiation is inhibited by the Th2 cytokine IL-4 [10], the question arises as to whether Th17 cells would develop in Th2 conditions. The role of Th17 cells in AD development remains very controversial considering the fact that Th17 cells are highly involved in the development and maintenance of psoriasis, which is classified into a completely different disease spectrum from AD [8].

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$CD4^+CD25^+$ T cells constitute 5–15% of peripheral $CD4^+$ T cells [11], which was referred to as natural Treg cells. However, only 1–3% of $CD4^+$ T cells express CD25 at high levels ($CD25high^+$), and only these cells have been shown to possess suppressor activity [12]. The transcription factor gene, forkhead box P3 (Foxp3), has been considered as one of the most reliable markers of $CD4^+CD25high^+$ Treg cells [13]. The frequency of circulating Treg cells in AD was controversial in previous reports [14–16].

Since the above-mentioned previous studies examined these four T cell subsets in different settings, it is difficult to compare the mutual relationship. In this study, we simultaneously measured Th1, Th2, Th17 and Treg populations in the peripheral blood mononuclear cells (PBMC) of AD patients and normal controls, and analyzed their correlation with the level of serum CCL17, an objective parameter of the disease activity of AD, as well as percentage of eosinophils and serum IgE levels.

2. Materials and methods

2.1. Subjects

Peripheral blood samples were collected from 20 AD patients (5 severe, 4 moderate and 11 mild, evaluated by physicians' global scoring). The mean age ± standard deviation of them was 30.1 ± 12.0 years old. Twenty healthy volunteers (32.3 ± 6.5 years old) were recruited as normal controls. Samples from 7 patients with chronic eczema (5 with wide-spread and 2 with localized eczema; 63.4 ± 20.6 years old) were also examined. AD was diagnosed according to the Japanese Dermatological Association criteria [17]. Routine hematological analyses of peripheral blood and serum IgE levels were also examined. The subjects received no systemic immunosuppressive drugs or corticosteroids. This study was performed after obtaining informed consent from all subjects and was approved by the Ethical Committee of Kyushu University.

2.2. Antibodies and reagents

Anti-CD3-PerCP-Cyanine5.5 (Cy5.5), anti-CD4-[Amcyan, phycoerythrin (PE) and PerCP-Cy5.5], anti-CCR6-PE, anti-CCR4-PE-Cy7, anti-CXCR3-Alexa-488, anti-CD25-Allophycocyanin (APC)-Cy7, anti-CD69-PE, and anti-IL-5-PE monoclonal antibodies were purchased from BD Biosciences (San Jose, CA, USA). Anti-CD45RA-Pacific-Blue, anti-IL-4-APC, anti-IL-17-PE and anti-Foxp3-APC monoclonal antibodies and Foxp3-permeabilization kits were obtained from eBioscience (San Diego, CA, USA). Anti-interferon (IFN)-γ-FITC was procured from Beckman Coulter (Fullerton, CA, USA). Phorbol myristate acetate (PMA), ionomycin and breferdin A were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Cytofluorimetric analysis of cell surface markers and chemokine receptors

PBMC were fleshly isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque™-Plus (GE Healthcare, Björkgatan, Uppsala, Sweden). PBMC were stained with fluorochrome-conjugated anti-CD4, anti-CD25, anti-CD45RA, anti-CXCR3, anti-CCR4, anti-CCR6, and isotype-matched control monoclonal antibodies immediately after isolation. Data were analyzed using a FACSCanto II flow cytometer and FACSDiva (BD Biosciences), and FlowJo (Tree Star, Inc., Ashland, OR, USA) software.

2.4. Analysis of intracellular Foxp3 protein

To identify the Treg population, intracellular staining for Foxp3 was performed following the manufacturer's protocol. Briefly, fleshly isolated PBMC were first incubated with monoclonal antibodies against the surface markers of CD4 and CD25 or isotype-matched controls. After extensive washing, cells were fixed and permeabilized, and then stained with anti-Foxp3 mAb. Data were analyzed using the FACSCant II flow cytometer, FACSDiva and FlowJo software.

2.5. Intracytofluorimetric analysis of cytokine production

For intracellular cytokine staining of IL-4, IFN-γ, IL-17 and IL-5, freshly isolated cells (2×10^6/mL) from PBMC were stimulated with PMA and ionomycin in RPMI-1640 with 5% fetal calf serum in the presence of brefeldin A for 5 h at 37 °C, 5% CO_2. The cells were washed, and fluorochrome-conjugated anti-CD4 mAb was added and incubated. The cells were then washed, fixed and permeabilized using a fixation & permeabilization kit (eBioscience) and stained for intracellular IL-4, IFN-γ, IL-17 and IL-5. Activated lymphocytes were confirmed with the >90% expression of the activation marker CD69 within $CD3^+$ T cells for every examination. Data were analyzed using the FACSCant II flow cytometer, FACSDiva and FlowJo software.

2.6. Quantitative analysis of serum CCL17

Concentrations of CCL17 in sera from subjects were measured using ELISA kits (R&D Systems, Minneapolis, USA) according to the manufactures' instructions. The minimum detectable level of CCL17 was 7 pg/mL.

2.7. Statistical analysis

Data are expressed as mean ± standard deviation, and statistical analyses were performed using the 2-tailed Student's *t* test or Mann–Whitney *U* test for comparison with normal controls. Because the data of total IgE levels in AD patients were not normally distributed, a logarithmic transformation value was used for analysis. Linear regression was used to correlate T-cell subset frequencies with percentage of eosinophils, serum levels of CCL17 and IgE. A P value $< .05$ was considered significant. Calculations were performed with Prism Graph 5.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Decrease in Th17 and Th1 population in AD compared with normal controls

Th1 ($IFN\text{-}\gamma^+$) and Th17 ($IL\text{-}17^+$), but not Th2 ($IL\text{-}4^+$) and Treg ($Foxp3^+$), cell populations significantly decreased in PBMC of AD patients compared to those of normal controls (Table 1). The percentages of these four T cell subsets in chronic eczema were not significantly different compared with those of normal controls

Table 1
Percentages of $CD4^+$ T cell subsets in PBMC from subjects analyzed by flow cytometry.

Population	Normal ($n=16$) (%)	AD ($n=20$) (%)	CE ($n=7$) (%)
Th1; $IFN\text{-}\gamma^+$ cells	20.4 ± 7.2	13.1 ± 6.5**	20.9 ± 4.9
Th2; $IL\text{-}4^+$ cells	3.8 ± 1.4	3.4 ± 1.3	4.7 ± 2.0
Th17; $IL\text{-}17^+$ cells	2.0 ± 0.7	1.4 ± 0.7*	2.1 ± 0.9
Treg; $Foxp3^+$ cells	4.7 ± 1.8[a]	5.4 ± 1.5	5.0 ± 1.1

AD, atopic dermatitis; CE, chronic eczema; Treg, reguratory T; Foxp3, forkhead box P3. Data were presented as mean ± standard deviation.

* $P < .05$ was determined by Mann–Whitney *U* test compared with normal controls.

** $P < .01$ was determined by Mann–Whitney *U* test compared with normal controls.

[a] $n=20$.

Table 2
Percentages of $CD4^+CD45RA^-CD25^-$ memory T cell subsets in PBMC from subjects analyzed by flow cytometry.

Population	Normal ($n=20$) (%)	AD ($n=20$) (%)	CE ($n=7$) (%)
$CXCR3^+$ cells	45.7 ± 7.7	$34.7 \pm 12.2^{**}$	42.1 ± 8.7
$CCR4^+$ cells	23.3 ± 6.5	22.5 ± 10.9	24.2 ± 5.6
$CCR6^+$ cells	40.3 ± 7.1	$32.7 \pm 12.6^{*}$	39.4 ± 5.2

AD, atopic dermatitis; CE, chronic eczema. Data were presented as mean ± standard deviation.
* $P<0.05$ was determined by Student's t test compared with normal controls.
** $P<.01$ was determined by Student's t test compared with normal controls.

(Table 1). We also examined the percentage of IL-5-producing cells within $CD4^+$ T cells but found no significant difference between AD patients and normal controls (data not shown). We could not find out a significant correlation between the percentage of Th1 and Th17 cells in AD patients. In addition, no significant correlation was observed among the proportion of Th17, Th1, Th2 and $IL\text{-}5^+$ cells in AD patients and normal controls.

It was reported that Th1, Th2 and Th17 cells predominantly express CXCR3, CCR4 and CCR6, respectively [18,19]. We then compared the percentage of $CXCR3^+$, $CCR4^+$ and $CCR6^+$ cells in the peripheral $CD4^+CD45RA^-CD25^-$ memory T cells in PBMC from subjects. This phenotypic assay also confirmed that the percentages of $CXCR3^+$ and $CCR6^+$ cells of AD patients were significantly lower than those of normal controls, while there was no significant difference in the percentage of $CCR4^+$ cells between AD patients and normal controls (Table 2). The proportions of these three subsets in chronic eczema were similar to those in normal controls (Table 2).

Among AD subjects, we analyzed the proportion of Th1, Th2, Th17 and Treg cells in different disease severity. Th1 and Th17 cells were tended to decrease according to the severity, and a significant difference was shown in the percentage of Th17 cells between the mild and the severe group. As for the Th2 and Treg cells, there were no differences in their percentages between the groups (Fig. 1).

3.2. Negative correlation between serum CCL17 levels and Th17 population in AD

We next examined the correlation of serum CCL17 levels with the percentage of Th17, Th1, Th2 and Treg cells in AD. The serum CCL17 levels of AD patients were significantly elevated (830.4 ± 595.1 pg/mL) compared to those of normal controls (200.6 ± 99.1 pg/mL, $P<.0001$). As shown in Fig. 2, a significant negative correlation was observed between the percentage of Th17 cells and serum CCL17 levels, but that was not the case between the CCL17 levels and Th1, Th2 or Treg cell number, suggesting the decrease of Th17 cells might preferentially contribute to the disease activity of AD.

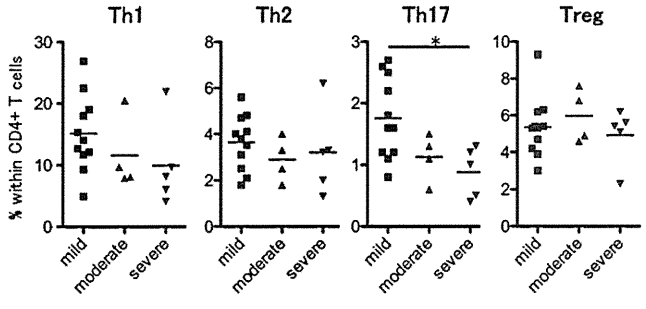

Fig. 1. Percentage of T-cell subsets within $CD4^+$ T cells in different disease severity among AD patients. Severe ($n=5$), moderate ($n=4$) and mild ($n=11$) evaluated by physicians' global scoring. Th subsets (Th1: IFN-γ^+; Th2: IL-4^+: Th17: IL-17^+) were determined by intracellular cytokine production after stimulation by PMA/ionomycin and Treg cells were defined as $Foxp3^+$ cells within $CD4^+$ T cells in PBMC. $^{*}P<.05$ by Student's t test.

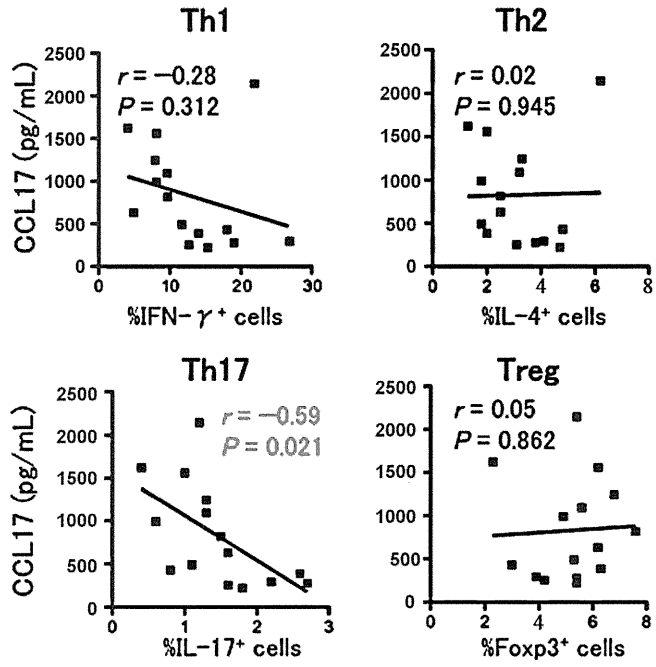

Fig. 2. Correlations of the percentages of T-cell subsets with the serum CCL17 levels in AD patients. We were not able to obtain enough samples for measurement of CCL17 levels in 5 patients ($n=15$). Th subsets were determined by intracellular cytokine production after stimulation by PMA/ionomycin and Treg cells were defined as $Foxp3^+$ cells within $CD4^+$ T cells in PBMC. Linear regression showing correlation between the percentage of Th subsets (Th1: IFN-γ^+; Th2: IL-4^+: Th17: IL-17^+) or Treg ($Foxp3^+$) cells within $CD4^+$ T cells and the levels of serum CCL17.

3.3. Correlations of Th cell subsets with levels of serum total IgE and eosinophilia in AD

Since serum CCL17 levels have been shown to correlate with serum IgE levels and eosinophils number in AD patients [20,21], we also examined their correlation. In our AD patients, the serum CCL17 levels also significantly correlated with the serum IgE levels ($r=0.55$, $P=.034$) and the percentage of eosinophils ($r=0.67$, $P=.007$). As for the correlation of the frequency of T cell subsets to the serum IgE and percentage of eosinophils, we found a significant negative correlation of the percentage of Th17 subset with the percentage of eosinophils and serum IgE levels (Fig. 3). In the case of Th1, Th2 and Treg cells, however, significant correlations were not observed in both percentage of eosinophils and serum IgE levels (Fig. 3).

3.4. IFN-γ^{nega}IL-17^+ and IFN-γ^+IL-17^+ subpopulation in Th17 cells

Recent studies have demonstrated the existence of at least two subsets of Th17 cells; one is IFN-γ^{nega}IL-17^+ mono-producer and the other is IFN-γ^+IL-17^+ co-producer [18,19,22], which were readily detectable in our subjects (Fig. 4A). The former was the major subpopulation in Th17 cells in our subjects as has been previously described (Fig. 4A) [18,19,22]. Though both of the Th17 subpopulations tended to decrease in number in AD patients, the number of the IFN-γ^{nega}IL-17^+ cells was significantly decreased in AD patients compared with normal controls (Fig. 4B). The serum levels of CCL17 and IgE as well as the percentage of eosinophils again demonstrated a negative correlation to the percentage of the IFN-γ^{nega}IL-17^+ subsets in AD patients (Fig. 5). These results suggested that the IFN-γ^{nega}IL-17^+ subset might be significantly involved in the disease activity of AD than the IFN-γ^+IL-17^+ subset.

We also analyzed the percentage of IFN-γ^+IL-17^{nega} Th1 cells in our subjects. This subset was significantly decreased in AD patients

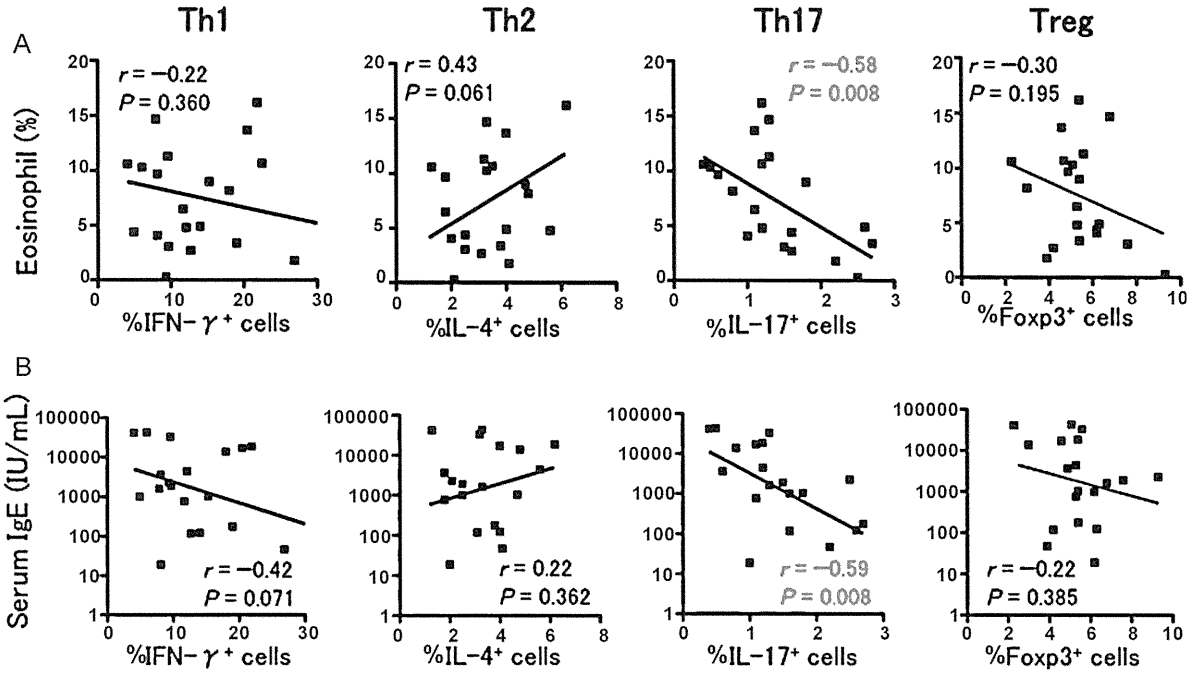

Fig. 3. Correlations of the percentages of T-cell subsets with the laboratory parameters of AD patients. Linear regression showing correlation of the percentages of Th subsets (Th1: IFN-γ+; Th2: IL-4+: Th17: IL-17+) or Treg (Foxp3+) cells within CD4+ T cells in PBMC determined by flow cytometry with the percentage of eosinophils (A) and the levels of serum IgE (B).

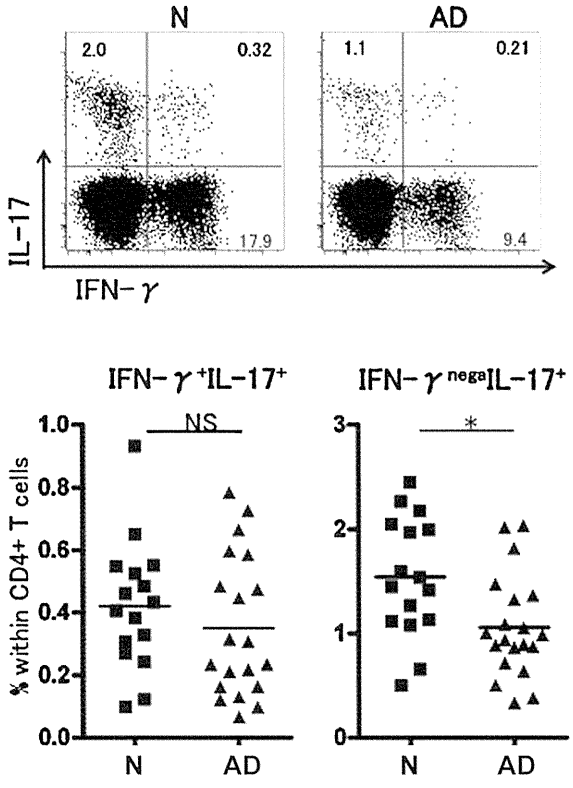

Fig. 4. Percentage of IL-17 subsets within CD4+ T cells in PBMC. (A) Representative intracellular cytokine profiles (IL-17 versus IFN-γ) within CD4+ T cells and determination of the frequency of the IL-17 subsets of IFN-γ+IL-17+ and IFN-γnegaIL-17+ cells in a normal control (N) and an AD patient. Numbers indicate percentage of cells in each quadrant. (B) Percentage of IFN-γ+IL-17+ and IFN-γnegaIL-17+ cells within CD4+ T cells in normal controls (N, $n = 16$) and AD patients ($n = 20$). Black bars show the mean. $^{*}P < .05$. NS indicates "not significant" compared with normal controls as determined by Student's t test.

compared with normal controls (Fig. 6A). However, there found no significant correlation of the percentage of the IFN-γ+IL-17negа subset with the serum levels of CCL17, IgE and eosinophils (Fig. 6B).

4. Discussion

In this study, we examined whether the proportion of circulating Th1, Th2, Th17 and Treg subsets correlate with the disease parameters of AD as assessed by CCL17 levels in AD. We found that a significant decrease in Th17 and Th1 population in AD patients than those of normal controls, as detected by both in the cytokine production assays and the chemokine receptor expression. Moreover, the decrease in Th17 cells significantly correlated with serum CCL17 levels in AD.

CCL17 is a member of CC chemokines that functions as a selective chemoattractant for the recruitment and migration of CCR4+ Th2 cells, and is expressed in the thymus, monocytes, dendritic cells, endothelial cells, bronchial epithelial cells and epidermal keratinocytes [20,21,23]. Many reports have demonstrated that serum CCL17 level is a very useful parameter of disease activity of AD [20,21,23,24]. In addition, the serum CCL17 levels correlate with the levels of serum IgE and eosinophilia in AD [20,21], which have been considered to be mediated by Th2-skewed immunological reaction [3,25]. We demonstrated the number of Th17 cells negatively correlated with CCL17, IgE or eosinophil levels, suggesting a mutual intimate interrelationship among these parameters in AD. Toda et al demonstrated that chronic AD lesions showed a significant increase in the number of eosinophils with a concomitant significant decrease in the number of IL-17 cells compared with acute AD lesions [9], which might also support our findings. Contrary to our data, however, it was reported that serum IL-17 levels were significantly related to clinical symptoms and peripheral eosinophil counts in allergic rhinitis [26]. The cause of this discrepancy is presently unclear, but the different phases of diseases or different methods of evaluating Th17 cells may be responsible.

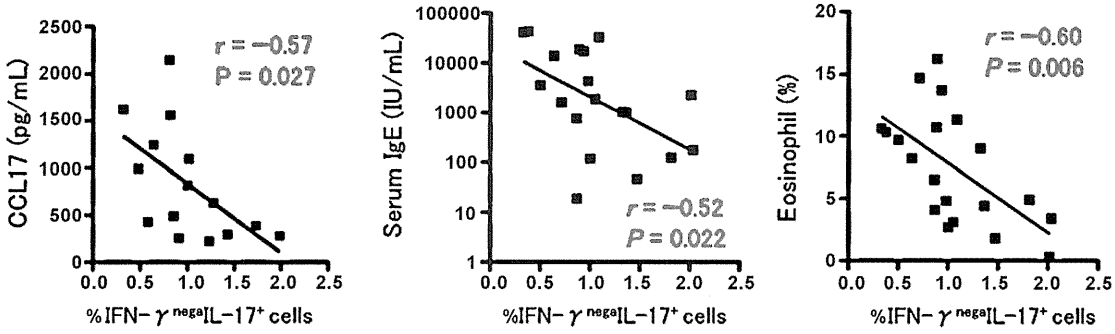

Fig. 5. Correlations of the percentage of IFN-γ^{nega}IL-17^+ subsets with the laboratory parameters of AD patients. Linear regression showing correlation of the percentages of IFN-γ^{nega}IL-17^+ cells within $CD4^+$ T cells in PBMC with the serum levels of CCL17, IgE and the percentage of eosinophils.

Psoriasis and AD have been considered opposite poles of the Th1 vs Th2 paradigm. Psoriasis has been considered a model of Th1 disease, whereas AD has been considered a polar Th2 disease in the acute phase, with a partial shift to Th1 during the chronic phase [27]. However, the classical Th1 and Th2 cell paradigm has recently been challenged with the discovery of Th17 cells. Psoriasis is the first inflammatory skin disease that had been shown to be clearly associated with Th17 cells [28], whereas the participation of Th17 cells in AD remains unclear. Interestingly, acute AD lesions showed more IL-17 cells than chronic lesions, suggesting that IL-17 functions primarily in the acute Th2 phase rather than in the subsequent Th1-dominated chronic phase of AD [7,8]. In contrast, Guttman-Yassky et al. demonstrated that IL-17 expression in AD was much lower than that in psoriasis. From the developmental point of view, Th1 and Th17 cells were closely related under the influence of IL-12 and IL-23. Presently, there is evidence that Th17 cells may be crucial in the pathogenesis of various autoimmune and inflammatory diseases, formerly categorized as Th1-mediated disorders, including rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and airway inflammation [29]. These notions were in accordance with our results, showing the simultaneous down-regulation of Th17 and Th1 cells in AD.

It has been reported that Th1 rather than Th2 predominates in spontaneous or older patch test lesions in AD [30]. However, recent studies have demonstrated the decrease of the levels of IFN-γ mRNA in PBMC, and of the IFN-γ producing skin-homing T cells in chronic AD [31,32]. Teramoto et al. showed a reduced ability of IFN-γ production by PBMCs was associated with an elevated serum IgE levels in AD [33]. Mauchra et al. found that decreased INF-γ production by peripheral blood in AD children was negatively correlated with the number of skin colonization of Staphylococcus aureus and SCORAD index [34]. Meanwhile, Källström et al. showed the decrease in IFN-γ^+ cells did not necessarily correlate with the serum levels of IgE [35]. Our results also revealed a significant decrease in Th1 cells (IFN-γ^+cells as well as IFN-γ^+IL-17^{nega} cells) in AD patients compared with normal individuals. However, the decrease in Th1 population had nothing to do with blood levels of CCL17, IgE and eosinophils in AD patients. The discrepancy between our findings and previous reports may be attributable to the differences in the methods used or investigated patient groups. The alleviative mechanisms of the Th1 axis remain unknown, but recent experiments revealed that activation-induced death of Th1 cells was accelerated in AD patients by enhanced Fas-FasL-mediated apoptosis [36]. Decrease of Th1 cells

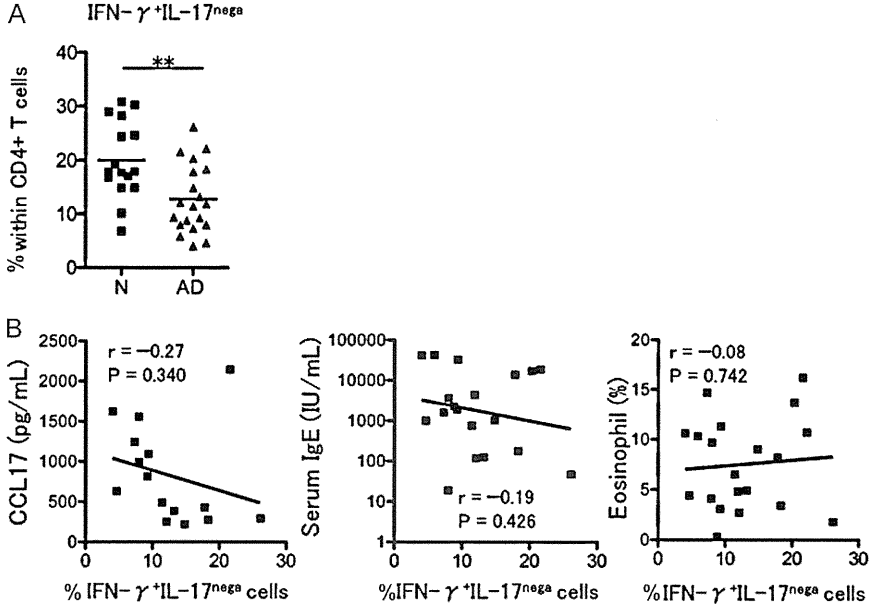

Fig. 6. (A) Percentage of IFN-γ^+IL-17^{nega} cells within $CD4^+$ T cells in normal controls ($n = 16$) and AD patients ($n = 20$). Black bars show the mean. $^{**}P < .01$ by Student's t test. (B) Correlations of the percentage of IFN-γ^+IL-17^{nega} subset with the laboratory parameters of AD patients. The percentage of IFN-γ^+IL-17^{nega} subset did not correlate with either the serum CCL17, IgE levels or the percentage of eosinophils.

may contribute to make cytokine milieu toward Th2-deviated state as has been pointed out by Wong et al. [37].

In the skin, IL-17 is a master regulator of antimicrobial peptides (AMPs) in keratinocytes, playing a central role in host defense against microorganisms at the surface barrier [38]. Decreased IL-17 expression in chronic AD skin has been correlated to reduced expression of key AMPs, potentially accounting for the propensity to skin infections in this disease [28,38]. Decreased circulating Th17 cells in the present study may also contribute to the susceptibility of AD to skin infection. However, we must keep in mind that there remains a possibility that the circulating Th17 cells may decrease as a result of a tissue infiltration of these cells from circulation, because Th17 cells infiltrate to lesional skin during the acute phase of AD [7].

Several studies have demonstrated that IL-17 mono-producers and IL-17/IFN-γ co-producers are consistently detected in Th17 cells in PBMC, synovial and bronchial T cells [18,19,22]. We could also confirm these two subsets, however, the population of IFN-γ^{nega}IL-17^+ was much more abundant than that of IFN-γ^+IL-17^+ in PBMC, as previously reported [22]. Recent reports suggested a common developmental origin between Th1 and Th17 cells, because Th17 clones could be potentiated to produce IFN-γ when cultured in the presence of IL-12 [19,39]. In classical Th1 diseases, IL-17/IFN-γ co-producers were increased, suggesting that both Th1 and Th17 cells and their effector cytokines might substantially contribute to the pathogenesis [39,40]. In our study, IFN-γ^{nega}IL-17^+ subset significantly decreased in AD and it was negatively correlated with the levels of CCL17, IgE and eosinophilia. Although the accurate function of the IFN-γ^{nega}IL-17^+ and IFN-γ^+IL-17^+ subsets is still unclear, the decrease of IFN-γ^{nega}IL-17^+ subset might quantitatively and qualitatively correlate with activity of AD.

With regard to Treg cells, the numbers of Treg cells in AD patients were shown to be similar to or higher than those in healthy controls [14–16], likewise, we could not find a significant difference in Treg population between AD and normal controls. Although our AD patients did not receive systemic steroids or immunosuppressive drugs, we have to exclude a possible influence of standard topical steroid therapy on the interpretation of our results. In order to address this point, we measured Th1, Th2, Th17 and Treg cell subsets in the PBMC from patients with chronic eczema who were treated with long-term topical steroids. However, we found no significant difference in the population of these four subsets in the patients with chronic eczema compared with the normal controls.

In conclusion, the decrease of circulating Th17 cells may contribute to disease activity of AD as assessed by serum CCL17, IgE and eosinophil levels.

Acknowledgments

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ORIGINAL ARTICLE

Clinical comparison of human and canine atopic dermatitis using human diagnostic criteria (Japanese Dermatological Association, 2009): Proposal of provisional diagnostic criteria for canine atopic dermatitis

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ABSTRACT

Atopic dermatitis (AD) is a common skin disease encountered in both humans and dogs. Canine AD can be used in the analysis of naturally occurring AD; however, details of clinical comparison have been lacking. The purpose of this study is to compare those clinical features using the human diagnostic criteria (Japanese Dermatological Association, 2009). Fifty-one dogs with canine AD were evaluated by the human criteria. Prior to this study, canine AD was basically diagnosed by the fulfillment of two authentic canine AD criteria and a positive reaction against *Dermatophagoides farinae* in serum immunoglobulin E levels and/or in intradermal tests. Among the human AD criteria items, behavior corresponding to pruritus was observed in all 51 dogs. Skin lesions corresponding to eczematous dermatitis were seen in 50 dogs, and symmetrical distribution of skin lesions was noted in all 51 dogs. A chronic or chronically relapsing course was observed in 50 dogs. Based on these results, the concordance rate for the criteria was 96% (49/51). Differential diagnoses of AD were also investigated in the same manner. The concordance rate for the criteria was 0% (0/69) in scabies, 2% (1/50) in pyoderma, 0% (0/50) in demodicosis, 0% (0/9) in cutaneous lymphoma, 0% (0/2) in ichthyosis, 25% (2/7) in flea allergy, 48% (24/50) in seborrheic dermatitis and 75% (3/4) in food allergy. Canine AD is thus indicated as a valuable counterpart to human AD in clinical aspects. In addition, the human AD criteria could be applicable, with some modification, as provisional diagnostic criteria for canine AD.

Key words: animal model, atopic dermatitis, comparative dermatology, diagnosis, dog.

INTRODUCTION

An appreciation of the evolutionary history of skin structure and biochemistry, and knowledge of skin diseases in animals, gives perspective to the human condition but may also provide clues to understanding human skin disease.[1] Some diseases appear to be identical or very similar in humans and animals. Although common skin diseases encountered in both humans and dogs are limited, there are several concordant disorders, such as scabies, seborrheic dermatitis and atopic dermatitis (AD).[2] AD is pruritic, eczematous dermatitis in humans, and the symptoms are characterized by chronic fluctuation with remissions and relapses. Most individuals with AD have atopic diathesis, which is related to a personal or family history of atopy (asthma, allergic rhinitis and/or conjunctivitis, or AD) and/or a predisposition to overproduction of immunoglobulin (Ig)E antibodies.[3] Animal models may give some clues about epidemiology,

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pathogenesis or genetic aspects.[1] Mice offer many benefits, including low cost, short time to maturity, availability of reagents and the opportunity to evaluate the effects of specific genetic alterations. While some mice have been used as an animal model of human AD, their contribution is limited in the analysis of naturally occurring AD.[4] For this purpose, canine AD (cAD) can be used as an animal model because dogs live in the human lifestyle environment. It is important to investigate the pathological condition and pathogenesis of cAD in comparison with human AD. In cAD, epidermal barrier functions such as discontinuity of lipid lamellae,[5] decreased transepidermal water loss,[6] filaggrin expression in the epidermis,[7] immunological events such as T-helper cell (Th)1/2 imbalance,[8] expression of chemokines,[9,10] and association with *Staphylococcus*[11,12] and *Malassezia*[13,14] have been investigated, and the majority of findings were similar to those in human AD. In addition, a comparison of the phenotype in human and canine AD is important for investigating the nature of this multifactorial disease, but detailed analyses in terms of clinical comparison have been limited.[15,16] In general, the use of diagnostic criteria for human disease is considered a valuable approach for identifying its counterpart in animals. However, this has not been applied to AD.[17] In human AD, the diagnostic criteria proposed by Hanifin and Rajka[18] in 1980 are used worldwide, but they are difficult to apply to cAD diagnosis because most of the 23 minor features cannot be determined in dogs. These include keratoconus, anterior subcapsular cataract, facial pallor, pityriasis alba, itch when sweating, and intolerance to wool and lipid solvents, which cannot be assessed in animal dermatology clinics. Based on the above, the simple diagnostic criteria established by the Japanese Dermatological Association (JDA) in 2009 appear to be suitable for application to dogs with cAD in Japan.[3] The purpose of this study is to compare the clinical human and canine AD features using the JDA diagnostic criteria to verify cAD as a human counterpart.

Table 1. Diagnostic criteria for atopic dermatitis by the Japanese Dermatological Association, 2009

- Pruritus
- Typical morphology and distribution
  - Eczematous dermatitis
    - Acute lesions: erythema, exudation, papules, vesiculopapules, scales, crusts
    - Chronic lesions: infiltrated erythema, lichenification, prurigo, scales, crusts
  - Distribution
    - Symmetrical
      - Predilection sites: forehead, periorbital area, perioral area, lips, periauricular area, neck, joint areas of limbs, trunk
    - Age-related characteristics
      - Infantile phase: starts on the scalp and face, often spreads to the trunk and extremities
      - Childhood phase: neck, the flexural surfaces of the arms and legs
      - Adolescent and adult phase: tendency to be severe on the upper half of body (face, neck, anterior chest and back)
- Chronic or chronically relapsing course (usually coexistence of old and new lesions)
  - More than 2 months in infancy
  - More than 6 months in childhood, adolescence and adulthood
  - Definitive diagnosis of atopic dermatitis requires the presence of all three features without any consideration of severity. Other cases should be evaluated on the basis of age and clinical course with a tentative diagnosis of acute or chronic, non-specific eczema.
- Differential diagnosis (association may occur)
  - Contact dermatitis, seborrheic dermatitis, prurigo simplex, scabies, miliaria, ichthyosis, xerotic eczema, hand dermatitis (non-atopic), cutaneous lymphoma, psoriasis, immune deficiency diseases, collagen diseases (systemic lupus erythematosus, dermatomyositis), Netherton's syndrome
- Diagnostic aids
  - Family history (bronchial asthma, allergic rhinitis and/or conjunctivitis, atopic dermatitis), personal history (bronchial asthma, allergic rhinitis and/or conjunctivitis), follicular papules (goose-skin), elevated serum immunoglobulin E level.
- Clinical types (not applicable to the infantile phase)
  - Flexural surface type, extensor surface type, dry form in childhood, head/face/neck/upper chest/back type, prurigo type, erythroderma type, combinations of various types are common
- Significant complications
  - Ocular complication (cataract and/or retinal detachment): especially in patients with severe facial lesions, Kaposi's varicelliform eruption, molluscum contagiosum, impetigo contagiosa

METHODS

Fifty-one dogs with cAD were evaluated using the JDA diagnostic criteria (2009) (Table 1).[3,19] All cases were referred to the ASC during the period from February 1997 to August 2009. Prior to this study, cAD was basically diagnosed by the fulfillment of two

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Table 2. Diagnostic criteria for canine atopic dermatitis by Willemse (1986, 1988)

Major features
Patient must have at least three of the following features:
Pruritus
Typical morphology and distribution: facial and/or digital involvement or lichenification of the flexor surface of the tarsal joint and/or the extensor surface of the carpal joint
Chronic or chronic-relapsing dermatitis
Individual or family history of atopy, and/or breed predisposition
Minor features
At least three of the following features should also be present:
Onset of symptoms before 3 years
Facial erythema and cheilitis
Bacterial conjunctivitis
Superficial staphylococcal pyoderma
Hyperhidrosis
Immediate positive intradermal test reaction to inhalants
Elevated serum allergen-specific immunoglobulin E
Elevated serum allergen-specific immunoglobulin G

Table 3. Diagnostic criteria for canine atopic dermatitis by Prélaud *et al.* (1998)

Major criteria
Patient must have at least three of the following five features:
Corticosteroid-sensitive pruritus
Erythema of pinnae
Bilateral cranial erythematous pododermatitis
Cheilitis
Appearance of first signs between the ages of 6 months and 3 years
Differential diagnosis
Flea allergy dermatitis, adverse food reaction, scabies or other pruritic mite infestation, pruritic bacterial folliculitis, *Malassezia* dermatitis, cornification disorders, contact dermatitis

authentic cAD criteria (Tables 2,3)[20,21] and a positive reaction against *Dermatophagoides farinae* in serum IgE levels and/or in intradermal tests (IDT).[22] While the precise pathological role of IgE has not been elucidated in dogs, it is customarily used for diagnosis of cAD.[23] Serum was collected and submitted to a commercial laboratory (Saloon, Kyoto, Japan) to measure IgE levels using an FcεRIα-based enzyme-linked immunosorbent assay (ALLERCEPT Definitive Allergen Panels; Heska, CO, USA). The test result was considered positive when the optical density was over 400 U, according to a previous study.[22,24] In IDT, *D. farinae* at 1/50 000 w/v (Greer Labs, Lenoir, NC, USA) was used as the antigen.[25] The positive control solution was histamine phosphate 1/100 000 v/w, and the negative control solution was physiological saline. Of the control solutions and allergen, 0.04 mL were injected i.d. intradermally at the lateral thorax.[26] IDT reactions were read 10–15 min after injection. Reactions were scored positive with the appearance of wheals equal to or greater than those of the positive control. Prior to IDT, all anti-inflammatory drug therapy, including oral and topical glucocorticoids and antihistamines, was discontinued for at least 3 weeks and 10 days, respectively. In order to clarify the differential diagnosis, all differentials in the criteria and any differentials considered in cAD were evaluated.

RESULTS

Pruritus

Behavior corresponding to pruritus such as licking, scratching, biting, chewing and rubbing was observed in all 51 dogs (100%) with cAD.

Typical morphology and distribution

Eczematous dermatitis

Skin lesions corresponding to eczematous dermatitis, such as erythema with or without lichenification, were seen in 50 dogs (98%) with cAD. In only one dog, the history of skin lesions did not correspond to eczematous dermatitis, but there were hyperpigmentation and alopecia. These lesions could be explained as secondary lesions related to eczematous dermatitis.

Distribution

Symmetric distribution of skin lesions including typical flexure sites was seen in all 51 dogs (100%) with cAD (51/51). Lesions were located at the anterior auricle in 63% (Fig. 1), perioral area in 61% (Fig. 2), periorbital area in 49% (Fig. 3), flexural surface of the limbs in 33%, trunk in 33% and neck in 24%. On the trunk, lesions were observed precisely at the axillae (18%), inguinal area (16%), ventral abdomen (16%) and lumbar area (4%). The forehead is one of the predisposed sites in humans, but it is not clearly defined in dogs. Compared to humans, dogs with AD appear to have several different sites of predilection, including

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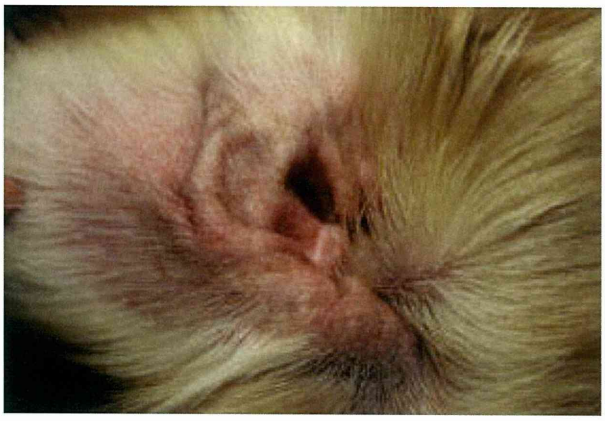

Figure 1. Erythema of the anterior auricle of a dog with canine atopic dermatitis.

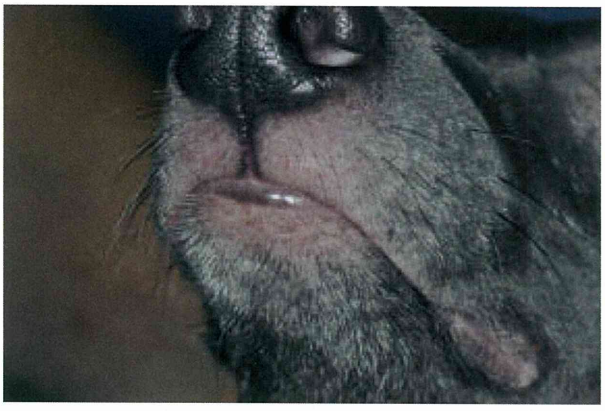

Figure 2. Erythema of the perioral area of a dog with canine atopic dermatitis.

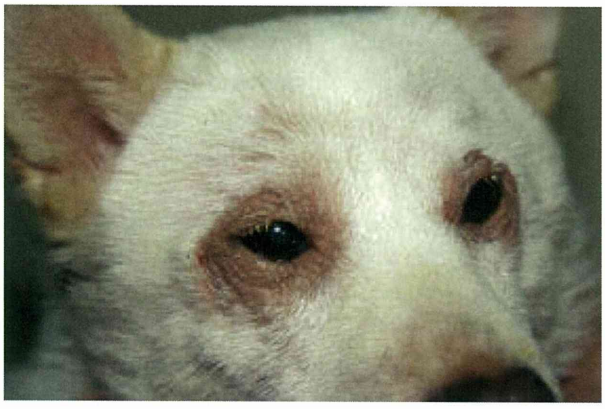

Figure 3. Erythema and lichenification of the periorbital area of a dog with canine atopic dermatitis.

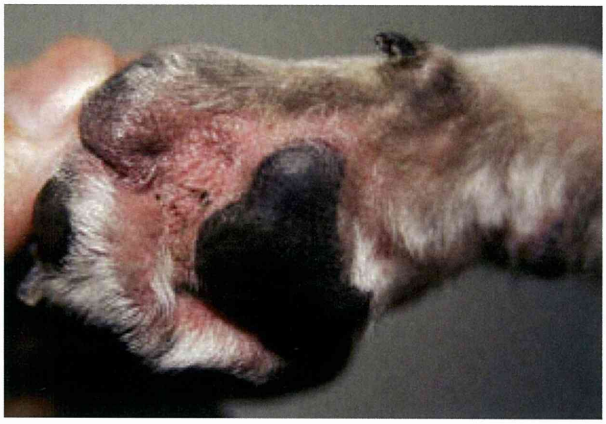

Figure 4. Erythema of the interdigital area of a dog with canine atopic dermatitis.

the interdigital area (61%) (Fig. 4), and perianal, perineum or perigenital region (12%).

Chronic or chronically relapsing course

More than 6 months of a chronic or chronically relapsing course was observed in 50 dogs (98%) with AD. Only one dog failed to show such a course. The case was an 8-month-old dog at the time of examination, with a 5-month history of dermatitis. In this study, the mean age of referrals was 4.7 years. However, the age at onset was less than 2 years old in 31% of the cases, and in no cases in this study did the first onset of skin lesions occur at over 6 years old. In addition, summer seasonality was observed at the first onset year in 27.4% of the cases.

Differential diagnosis

We next investigated the differential diagnosis of cAD compared with other canine skin diseases.[3,21] The concordance rate for the AD criteria was 0% (0/69) in scabies, 2% (1/50) in pyoderma, 0% (0/50) in demodicosis, 0% (0/9) in cutaneous lymphoma, 0% (0/2) in ichthyosis, 25% (2/7) in flea allergy, 48% (24/50) in seborrheic dermatitis and 75% (3/4) in food allergy (Table 4). Contact dermatitis and systemic lupus erythematosus as differentials in human AD are extremely rare in dogs. We did not encounter these disorders in the course of our investigation. Prurigo simplex, miliaria, xerotic eczema, hand dermatitis (non-atopic), psoriasis, immune deficiency diseases, dermatomyositis and Netherton's syndrome are differentials of human AD reported in humans, but not in dogs.

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Table 4. Concordance rate of human criteria for atopic dermatitis in dogs with differentials (%)

	Demodicosis (n = 50)	Scabies (n = 69)	Ichthyosis (n = 2)	Pyoderma (n = 50)	Seborrheic dermatitis (n = 50)	Flea allergy (n = 7)	Food allergy (n = 4)
Pruritic behavior	86	100	50	100	100	100	100
Typical morphology and distribution	22	3	0	8	60	29	75
Eczematous dermatitis	22	4	0	22	60	43	75
Distribution	60	35	100	8	100	29	75
Chronic or chronically relapsing course	46	25	50	52	74	72	75
Correspondence rate	0	0	0	2	48	29	75

Table 5. Provisional diagnostic criteria for canine atopic dermatitis

Pruritic behavior (licking, scratching, biting, chewing or rubbing)
Typical morphology and distribution
  Eczematous dermatitis
    Erythema with or without lichenification that is not characterized by scaling, seborrhea and crusting
  Distribution
    Symmetrical
      Anterior auricle, interdigital area, perioral area, periorbital area, joint area of limbs, ventral abdomen, axilla, inguinal area
Chronic or chronically relapsing course
  More than 6 months
Definitive diagnosis of canine atopic dermatitis requires the presence of all three features without any consideration of severity. Other cases should be evaluated on the basis of clinical course with the tentative diagnosis of acute or chronic, non-specific dermatitis
Differential diagnosis (association may occur)
  Seborrheic dermatitis, flea allergy, food allergy, contact dermatitis
Diagnostic aid
  Elevated immunoglobulin (Ig)E level against *Dematophagoides farinae* (>400 U with the serum allergen-specific IgE test using the high-affinity IgE receptor)

Diagnostic aids

In general, a reliable family history was not available for pet dogs. In personal histories, bronchial asthma was seen in 0% of the cases, allergic rhinitis in 0% and conjunctivitis in 7.8% (4/51). Follicular papules were not observed in dogs with cAD. No significant complications such as cataracts described in human criteria were seen in dogs.

Concordance rate for JDA criteria on cAD

Among the human AD criteria, the concordance rate for the criteria on human AD on these cAD dogs was 96% (49/51). In general, the pruritic behavior seen in cAD was rare in other canine skin diseases except for seborrheic dermatitis, flea allergy, food allergy and contact dermatitis.

DISCUSSION

This study evaluated the clinical features of cAD to verify its applicability as a counterpart to human AD through the use of diagnostic criteria for human AD (JDA, 2009). This was the first approach to AD in terms of comparative dermatology and the human criteria demonstrated an extremely high concordance rate to cAD. It is therefore indicated that cAD is a valuable counterpart to human AD in clinical aspects, while some modification was required for using the criteria. First, we found that pruritus was not an appropriate descriptive term for use with dogs because it is a rather subjective complaint used more for humans. Pruritic behavior indicated by licking, scratching, biting, chewing and rubbing is more appropriate for describing the symptom in dogs. Second, current veterinary dermatology hesitates to use the term eczema, preferring dermatitis as a pathological description rather than a morphological one. In this study, we specified eczema as erythema with or without lichenification related to pruritic behavior because a doctor can easily recognize these skin symptoms. Another concern was evaluating the distribution of skin lesions because dogs have a different anatomical structure compared to humans. Interestingly, the common sites of skin lesions in cAD were similar to those in human AD, although some lesions seemed to be dog specific. In this regard, sites on the trunk in cAD were precisely the ventral abdomen,

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axillae and inguinal, even though the incidence rate was not high. In addition, the interdigital area was predominant in dogs, but not in humans. However, the ventral abdomen and interdigital area were all considered as flexure sites. Distribution of skin lesions in human AD is affected by several factors including barrier dysfunction, local flexion and/or self-rubbing behavior.[27,28] It is suggested that this scenario is operative in dogs as well as in humans. The last consideration was the difference in lifespan of dogs relative to humans, which might affect the evaluation of their clinical course. In this study, most of the dogs had more than a 6-month history because all cases were referrals with a chronic course. However, approximately one-fourth of the cAD dogs had summer seasonality at least in the first episode. In such cases, follow-up observation is required to finalize the diagnosis of cAD.

The criteria did rule out various non-atopic dermatoses with pruritic behavior, such as scabies, pyoderma, demodicosis and ichthyosis, but not seborrheic dermatitis, flea allergy, food allergy and contact dermatitis. Seborrheic dermatitis is a common dermatitis in humans.[27] Its distinctive morphology is characterized by red, sharply-demarcated lesions covered with oily-looking scales, and a distinctive distribution on well-supplied areas of sebaceous glands.[29] In dogs, seborrheic dermatitis is characterized by scaling and greasiness, with gross evidence of local or diffuse inflammation.[23] The skin lesions of seborrheic dermatitis in dogs are similar to those in humans, even though their distribution is more generalized, particularly at the flexural and intertriginous area because in dogs the entire body is completely covered with pilosebaceous units.[23] In dogs, the distribution of seborrheic dermatitis is quite similar to that in cAD, but it is crucial to note that seborrheic dermatitis shows predominant scaling and seborrhea compared to cAD. After careful evaluation, we could differentiate seborrheic dermatitis from cAD. Flea allergy is a pruritic skin disease caused by hypersensitivity reactions against flea salivary antigens. The most common onset age is 3–5 years,[23] and skin lesions are typically confined to the dorsal lumbosacral area, caudomedial thighs, ventral abdomen and flanks. Clinical features are basically different from those in cAD, although some cases of cAD may show concurrent features. Food allergy is an adverse reaction to foods or food additives, and is an important exacerbation factor in cAD.[30] Although classic cases were scarce, we must always consider the association with cAD. Contact dermatitis is another important differential diagnosis of cAD. It may share its preponderant sites with cAD such as perioral area, anterior auricle, axillae, inguinal area, ventral abdomen and interdigital area, and there is a difficulty in taking a detailed history for contactants.[23] However, symmetrical distribution and chronic or chronically relapsing course in multifocal preponderant regions are the unique diagnostic prerequisites for cAD. Eventually, patch test is a valuable diagnostic tool to conclude contact dermatitis in dogs as well as in humans.

There are several diagnostic criteria for identifying cAD, and two authentic criteria were used in this study. Favrot *et al.*[31] recently reported a low concordance rate of these two cAD criteria. No reports have compared human AD and cAD. In the present study, we demonstrated that human AD criteria by JDA are readily acceptable for use in dogs, and may be even more useful with some modification and an appendix of diagnostic aids, particularly in dogs with first occurrence. As for diagnostic aids, elevated serum *D. farina*-specific IgE level might be useful for cAD. The clinical value of the serum allergen-specific IgE test using the high-affinity IgE receptor (ALLERCEPT) in cAD was investigated in our previous study.[24] ALLERCEPT against *D. farinae* was measured in 50 dogs with classic cAD and 151 non-cAD dogs with pruritic behavior. Sensitivity was 98% and specificity was 80.8% on cAD with 400 U as the cut-off. Because elevated IgE against *D. farinae* is useful for identifying atopic diathesis, it may be considered a diagnostic aid. We would like to propose provisional diagnostic criteria for cAD based on JDA criteria (Table 5).

In conclusion, cAD is indicated as a valuable counterpart to human AD in its clinical phenotype. Furthermore, the proposed provisional diagnostic criteria could be applicable for identifying cAD, but their validity must be confirmed in additional cases, including mild forms, in veterinary primary care.

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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

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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) ($\chi^2 = 6.98$, $P = 0.031$; 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) ($\chi^2 = 19.41$, $P = 0.00023$; $\chi^2 = 13.61$, $P = 0.034$; $\chi^2 = 19.13$, $P = 0.0039$, 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)

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

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)

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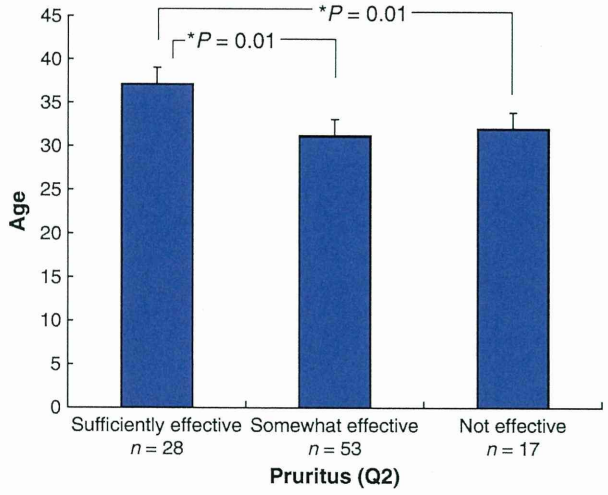

Figure 1. Comparison of efficacy of emollients for treating pruritus (Q2) as a function of age.

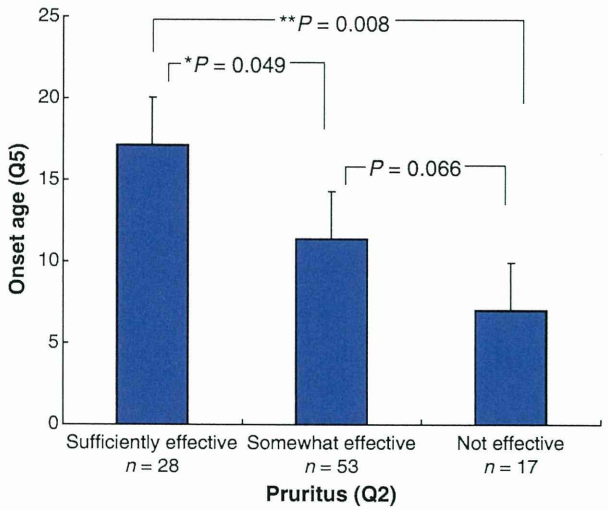

Figure 2. Comparison of efficacy of emollients for treating pruritus (Q2) as a function of mean age of onset of atopic dermatitis (Q5).

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,

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topical corticosteroids and tacrolimus, may have influenced these results. Further studies are required to confirm the emollient therapy for the pruritus. We expect that the results of our questionnaire analysis will be useful to some extent for improving the ability of dermatologists to determine the appropriate role of emollients in the treatment regimen for AD.

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Cysteinyl leukotriene receptor 2 gene polymorphism -1220 A/C is not associated with atopic dermatitis or psoriasis vulgaris in Japanese patients

Dear Editor,

The cysteinyl leukotrienes (CYSLT), such as leukotriene C4 (LTC4), leukotriene D4 (LTD4) and leukotriene E4 (LTE4), are bronchoconstrictors and pro-inflammatory mediators of the asthmatic response.[1] CYSLT act through two G protein-coupled receptors, CYSLT receptor 1 (CYSLTR1) and CYSLTR2.[2] It was reported that CYSLT released from leukocytes isolated from atopic dermatitis (AD) patients are increased compared to those from healthy controls.[3] LTC4 has been found in the skin of AD patients using the suction blister technique.[4]

Pillai *et al.*[5] revealed a significant association of 601 A/G CYSLTR2 single nucleotide polymorphism (SNP) with asthma, a T-helper cell (Th)2 cytokine-mediated lung disease. Thompson *et al.*[6] reported that the 601 A/G SNP of CYSLTR2 was associated with atopic disease in patients of Tristan da Cunha. However, Fukai *et al.*[7] did not detect the 601 A/G SNP in Japanese asthmatics, and identified eight SNP in CYSLTR2, and one polymorphism in intron 2 (-1220 A/C SNP) was associated with the development of asthma in a Japanese population. To the best of our knowledge, no data on other ethnicities are available regarding -1220 A/C SNP. In addition, enhanced synthesis of CYSLT was also reported in psoriasis vulgaris (PsV), a Th1/Th2 cytokine-mediated skin disease.[8]

The purpose of this study was to evaluate whether the -1220 A/C CYSLTR2 SNP is a predisposing genetic factor for AD or PsV in a Japanese population.

We evaluated 158 unrelated Japanese patients with AD (mean age 28.3 years) who were diagnosed according to the generally accepted criteria of Hanifin and Rajka, and 153 unrelated Japanese patients with PsV (mean age 51.8 years), diagnosed by clinical and histopathological findings. In the AD patients, 18 patients had asthma and 25 patients had history of asthma. None of the PsV patients had asthma or history of asthma. One hundred and four Japanese individuals served as control subjects (mean age 32.2 years). There was no history of atopic diseases such as AD, asthma or seasonal allergies nor any history of PsV in the control group.

Venous blood was drawn from each individual, and genomic DNA was extracted from peripheral blood leukocytes using a QIAamp blood kit (Qiagen, Hilden, Germany). The -1220 A/C SNP was genotyped by

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