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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 Th17 cells, particularly that of the IFN- γ ^{neg}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 (CD25^{high}), 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⁺CD25^{high} 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 $\pm$ 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 brefeldin A were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Cytofluorimetric analysis of cell surface markers and chemokine receptors

PBMC were freshly isolated from heparinized venous blood by density gradient centrifugation on Ficoll-PaqueTM-Plus (GE Healthcare, Björksgatan, 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, freshly 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 FACSCanto 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% CO₂. 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 FACSCanto 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 $\pm$ 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- γ ⁺) and Th17 (IL-17⁺), but not Th2 (IL-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- γ ⁺ cells	20.4 $\pm$ 7.2	13.1 $\pm$ 6.5**	20.9 $\pm$ 4.9
Th2; IL-4 ⁺ cells	3.8 $\pm$ 1.4	3.4 $\pm$ 1.3	4.7 $\pm$ 2.0
Th17; IL-17 ⁺ cells	2.0 $\pm$ 0.7	1.4 $\pm$ 0.7*	2.1 $\pm$ 0.9
Treg; Foxp3 ⁺ cells	4.7 $\pm$ 1.8 ^a	5.4 $\pm$ 1.5	5.0 $\pm$ 1.1

AD, atopic dermatitis; CE, chronic eczema; Treg, regulatory T; Foxp3, forkhead box P3. Data were presented as mean $\pm$ 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 ± 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 ± 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-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.

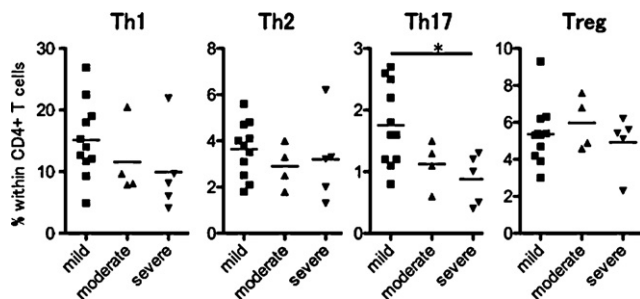


Fig. 1. Percentage of T-cell subsets within CD4⁺ T cells in different disease severity among AD patients. Severe ($n = 5$), moderate ($n = 4$) and mild ($n = 11$) evaluated by physicians' global scoring. Th subsets (Th1: IFN- γ ⁺; Th2: IL-4⁺; Th17: IL-17⁺) were determined by intracellular cytokine production 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.

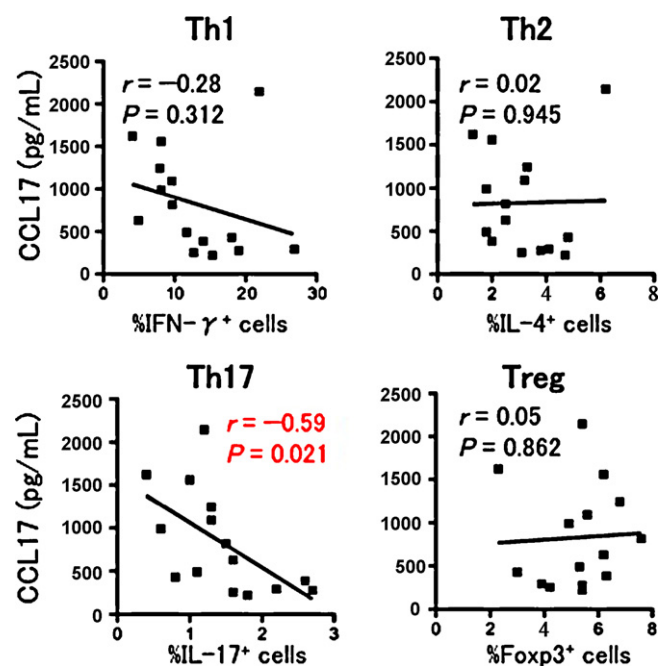


Fig. 2. Correlations of the percentages of T-cell subsets with the serum CCL17 levels in AD patients. We were not able to obtain enough samples for measurement of CCL17 levels in 5 patients ($n = 15$). Th subsets were determined by intracellular cytokine production after stimulation by PMA/ionomycin 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 ^{neg}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 ^{neg}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 ^{neg}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 ^{neg}IL-17⁺ subsets in AD patients (Fig. 5). These results suggested that the IFN- γ ^{neg}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^{neg} Th1 cells in our subjects. This subset was significantly decreased in AD patients

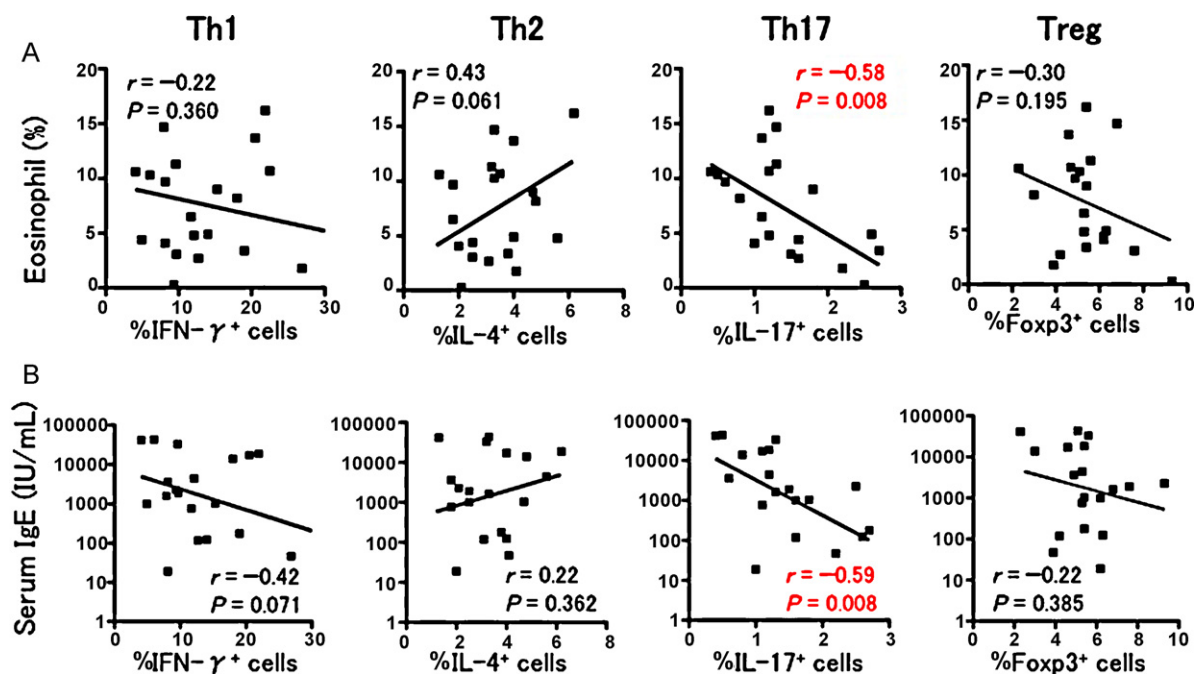


Fig. 3. Correlations of the percentages of T-cell subsets with the laboratory parameters of AD patients. Linear regression showing correlation of the percentages of Th subsets (Th1: IFN- γ ⁺; Th2: IL-4⁺; Th17: IL-17⁺) or Treg (Foxp3⁺) cells within CD4⁺ T cells in PBMC determined by flow cytometry with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

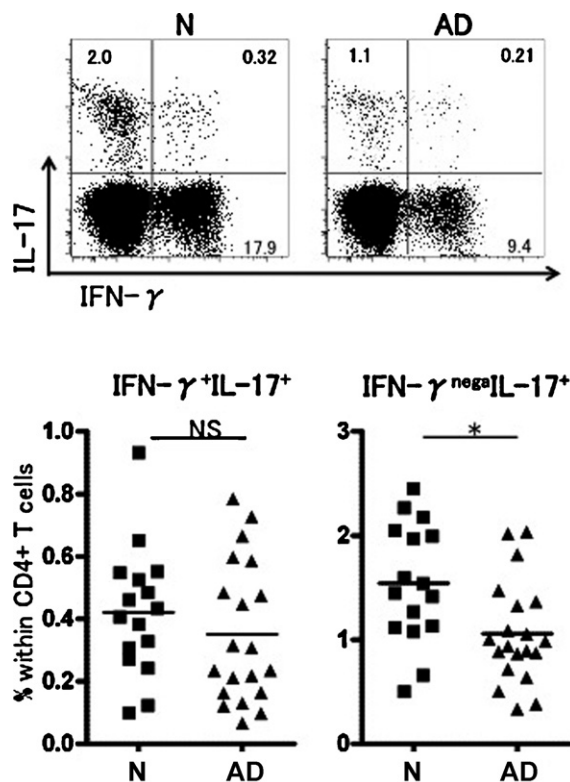


Fig. 4. Percentage of IL-17 subsets within CD4⁺ T cells in PBMC. (A) Representative intracellular cytokine profiles (IL-17 versus IFN- γ) within CD4⁺ T cells and determination of the frequency of the IL-17 subsets of IFN- γ ⁺IL-17⁺ and IFN- γ ^{neg}IL-17⁺ cells in a normal control (N) and an AD patient. Numbers indicate percentage of cells in each quadrant. (B) Percentage of IFN- γ ⁺IL-17⁺ and IFN- γ ^{neg}IL-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-17^{neg} 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.

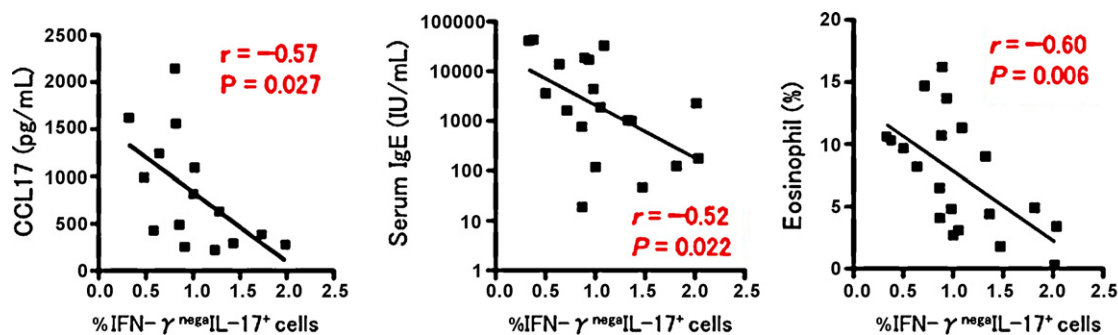


Fig. 5. Correlations of the percentage of IFN- γ^{neg} IL-17 $^{+}$ subsets with the laboratory parameters of AD patients. Linear regression showing correlation of the percentages of IFN- γ^{neg} IL-17 $^{+}$ cells within CD4 $^{+}$ T cells in PBMC with the serum levels of CCL17, IgE and the percentage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

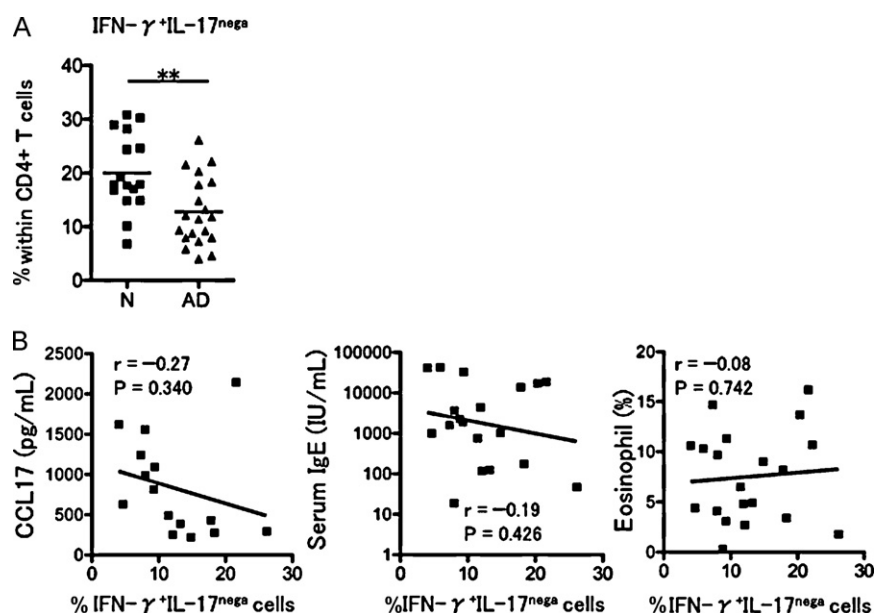


Fig. 6. (A) Percentage of IFN- γ^{+} IL-17 $^{\text{neg}}$ cells within CD4 $^{+}$ T cells in normal controls ($n = 16$) and AD patients ($n = 20$). Black bars show the mean. $^{**}P < .01$ by Student's t test. (B) Correlations of the percentage of IFN- γ^{+} IL-17 $^{\text{neg}}$ subset with the laboratory parameters of AD patients. The percentage of IFN- γ^{+} IL-17 $^{\text{neg}}$ 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 ^{neg}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 ^{neg}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 ^{neg}IL-17⁺ and IFN- γ ⁺IL-17⁺ subsets is still unclear, the decrease of IFN- γ ^{neg}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.

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