

ORIGINAL ARTICLE

The Imbalance of Th17 cells and CD4⁺CD25^{high}Foxp3⁺ Treg cells in patients with atopic dermatitis

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Abstract

Background Th17/Treg imbalance is involved in several autoimmune, inflammatory and allergic reactions. Nevertheless, the possible contribution of Th17/Treg imbalance in atopic dermatitis (AD) remains unknown.

Objective To explore the possible role of Th17/Treg imbalance in AD.

Methods Th17 and Treg cells percentage in peripheral blood mononuclear cells (PBMCs) and skin specimens, specific transcription factor retinoic acid-related orphan receptor (ROR) γ t and Foxp3 mRNA levels in PBMCs, as well as Th17- and Treg-related cytokines mRNA levels in PBMCs, serum concentrations, and expression levels in PBMCs culture supernatant after recombinant Dermatophagoides pteronyssinus antigen stimulation were detected in AD patients. Controls included patients with psoriasis, allergic contact dermatitis (ACD) and healthy donors.

Results Th17 cells percentage, ROR γ t, IL-17 and IL-23 levels in peripheral circulation of AD patients were significantly higher than those in ACD patients and healthy controls, but lower than those of psoriasis patients. Treg cells percentage, Foxp3 and TGF- β mRNA levels were reduced in AD patients compared with healthy controls, while there were no significant differences among AD, ACD and psoriasis patients. Th17 cells percentage, IL-17 and IL-23 levels were increased, while Treg cells percentage and TGF- β level were decreased in AD lesion and PBMCs culture supernatant respectively. There was a negative association between Th17 and Treg cells percentage in AD patients. AD severity score positively correlated with Th17 cells percentage and Th17/Treg ratio, while negatively correlated with Treg cells percentage. Serum IgE levels positively correlated with Th17/Treg ratio.

Conclusion In AD, there exists an immune imbalance in Th17 and Treg cells, which may contribute to its pathogenesis and development.

Received: 12 March 2013; Accepted: 16 September 2013

Conflicts of interest

None declared.

Funding sources

None declared.

Introduction

Atopic dermatitis (AD) is a common, chronic relapsing, inflammatory skin disease characterized by pruritus and eczematous lesions, which usually occurs in early infancy but can also start or persist in adulthood. It is well known that AD is attributed to the interaction of multiple genetic and immunological factors.^{1,2} Experimental and clinical data have indicated that CD4⁺ T helper cells play an important role in the pathogenesis of AD. Originally, CD4⁺ T helper cells are thought to be differentiated into Th1 and Th2 subsets based on their cytokine expression

profiles and immune regulatory functions. Recently, there has been new recognition in the basic understanding of immunology following the discovery of two new subsets of T helper cells, IL-17-expressing T cells (termed Th17) and CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells (termed Treg), which have opposite effects on autoimmunity and inflammation.

Th17 cells play a critical role in the development of autoimmunity, as well as inflammatory and allergic reactions by producing IL-17. The retinoic acid-related orphan receptor (ROR) family transcription factor ROR γ t is the subset-determining transcription factor of Th17 cells, which is essential for Th17 development and function.^{3–5} IL-23, an important proinflam-

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matory cytokine, has been shown to play a significant role in the differentiation and maintenance of Th17 cells and is absolutely required for disease pathogenesis in many models of Th17-cell-mediated autoimmune and inflammatory diseases, thus it is also called IL-23-Th17 cell pathway.^{6–8} Treg cells, characterized by constitutive expression of transcription factor forkhead/winged helix transcription factor (Foxp3), play a central role in immune homeostasis by regulating the maintenance of self-tolerance, releasing anti-inflammatory cytokines, such as transforming growth factor (TGF)- β , and exhibiting their inhibitory actions on effector T cells.^{9–12} Therefore, the Th17/Treg balance may control the development of autoimmunity and inflammation. Accordingly, Th17/Treg imbalance has been proved to widely exist in autoimmune, inflammatory and allergic diseases.^{13–15}

Recently, the possible roles of Th17 and Treg cells in the pathogenesis of AD have been reported, respectively, however, the results were not completely consistent.^{16–19} And, to the best of our knowledge, a possible role of Th17/Treg imbalance in AD has not been explored. Thus, we detected Th17 and Treg cells frequencies in peripheral circulation and skin specimens, evaluated mRNA levels of their specific transcription factors (ROR γ t and Foxp3) in peripheral blood mononuclear cells (PBMCs), measured Th17- and Treg-related cytokines (IL-17, IL-23 and TGF- β) mRNA levels and serum concentrations, as well as investigated the expression change in IL-17, IL-23 and TGF- β by PBMCs after specific antigen stimulating, to explore the possible role of Th17/Treg imbalance in the pathogenesis of AD.

Materials and methods

Subjects and skin samples

Thirty-five AD patients were included in the study; they were diagnosed according to the criteria defined by Rajka and Langeland,²⁰ and the clinical severity was evaluated by SCORing Atopic Dermatitis (SCORAD) index.²¹ Exclusion criteria included the following points: patients were treated with systemic or local glucocorticoids within 1 month or 1 week, respectively, prior to entering the study; patients were treated with other immunosuppressive agents or desensitization therapy within 6 months prior to entering the study; patients had bacterial or virus infections within 2 weeks prior to entering the study; patients got vaccination within 1 month prior to entering the study; patients complicated with significant abnormalities in hematopoietic system, cardiovascular system, liver, kidney, brain; patients complicated with malignant neoplasm, psychiatric disorders, autoimmune diseases or other skin diseases. All the 35 AD peripheral blood samples and 5 AD skin tissues (including lesional and perilesional samples) were acquired. Peripheral blood samples from patients with allergic contact dermatitis (ACD, $n = 8$) and psoriasis vulgaris ($n = 20$), as well as normal healthy subjects ($n = 31$), who did not have the above abnormalities or atopic disorders so far were used as control groups. Clinical

characteristics of AD, ACD and psoriasis patients as well as healthy controls are listed in Table 1. The study was guided by the Declaration of Helsinki and approved by the Research Ethics Committee of Binzhou Medical University Hospital. Informed consent was obtained from all individuals and/or their parents.

Flow cytometric analysis of Th17 and Treg cells percentage

Sample preparation Skin samples were incubated at 4 °C overnight in 0.3% dispase solution (Invitrogen, Carlsbad, CA, USA). After peeling off the epidermis, the dermis was cultured for 96 h at 37 °C in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% foetal calf serum (FCS), 100 U/mL penicillin, 100 μ g/mL streptomycin and 1% 1 M Hepes buffer (Sigma, St Louis, MO, USA). Single cell suspensions were obtained from the culture medium for flow cytometry analysis.

PBMCs were collected by Ficoll-Hypaque density gradient centrifugation, suspended in the medium as stated above and analysed using flow cytometry.

Intracellular cytokine staining and Multicolor flow cytometric analysis

For analysis of Treg cells, about 10^6 prepared cells were added to each tube and stained with fluorescein isothiocyanate (FITC)-labelled CD4 and allophycocyanin-labelled CD25 antibodies, incubated at 4 °C for 30 min in dark, and then washed in cold flow cytometry staining buffer. For further Foxp3 staining, cells were firstly fixed and permeabilized by a commercial cell fixation/permeabilization kit, then incubated with phycoerythrin (PE)-labelled Foxp3 antibody.

For analysis of Th17 cells, prepared cells were stimulated with 25 ng/mL phorbol myristate acetate and 1 μ g/mL ionomycin in the presence of 2 mmol/mL monensin at 37 °C under a 5% CO₂ environment. After incubation for 5 h, cells were transferred to each tube, washed once with phosphate-buffered saline (PBS), and incubated with FITC-labelled CD4 antibody at 4 °C for 30 min in dark. After surface staining, cells were fixed and permeabilized according to the manufacturer's instruction, then stained with PE-labelled IL-17 antibody.

All flow cytometry antibodies and reagents were from eBioscience company (San Diego, CA, USA) and isotype controls were used to correct non-specific binding. Flow cytometric analysis was performed on a FACScanto flow cytometer (BD Biosciences, San Jose, CA, USA) using winMDI 2.9 software.

Real-time quantitative RT-PCR analysis of ROR γ t, Foxp3, IL-17, IL-23 and TGF- β mRNA levels

Total RNA was isolated from PBMCs with Trizol reagent (Invitrogen) following the manufacturer's instructions. The quality of RNA samples was assessed by inspecting the 28S and 18S bands after 1.5% agarose gel electrophoresis, as well as the 260/

Table 1 Clinical characteristics of AD, ACD and psoriasis patients as well as healthy controls

	AD	ACD	Pso	HC
Gender (F/M)	15/20	3/5	11/9	14/17
Age (years)				
Range	6–18	16–41	14–45	6–17
Mean $\pm$ SD	11.49 $\pm$ 3.00	11.58 $\pm$ 2.32	25.63 $\pm$ 8.33	28.15 $\pm$ 9.89
Disease duration				
Range	1–13 (year)	6–181 (day)	0.5–15 (year)*	–
Mean $\pm$ SD or median	6.71 $\pm$ 3.02	54.38 $\pm$ 58.94	3.50	–
25 th –75 th percentile	–	–	2.00–6.00	–
IgE levels (U/mL)				
Range	544–3249	396–1552	–	–
Mean $\pm$ SD	2027.37 $\pm$ 809.59	838.25 $\pm$ 376.77	–	–
EOS ($\times 10^9$ L ⁻¹)				
Range	0.41–0.96	0.23–0.81	–	–
Mean $\pm$ SD	0.62 $\pm$ 0.15	0.50 $\pm$ 0.21	–	–
SCORAD index				
Range	16–46*	–	–	–
Median	29	–	–	–
25th–75th percentile	21–42	–	–	–
Atopic background	12 cases	–	–	–

Serum IgE was measured by the method of BA-ELISA (the upper limit of normal: 521 U/mL).

*According to the results of normal distribution test (Shapiro-Wilk test), data were expressed as mean $\pm$ SD or median (25th–75th percentile).

AD, atopic dermatitis; ACD, allergic contact dermatitis; Pso, psoriasis; HC, healthy controls; EOS, eosinophil cell count.

Table 2 List of ROR γ t, Foxp3, IL-17, IL-23, TGF- β and β -actin primers for real-time quantitative RT-PCR

Primers	Sequence (5' to 3')	Product length
ROR γ t	Forward: 5'-CCTGGGCTCCTCGCCTGACC-3' Reverse: 5'-TCTCTCTGCCCTCAGCCTTGCC-3'	170 bp
Foxp3	Forward: 5'-CTGACCAAGGCTTCATCTGTG-3' Reverse: 5'-ACTCTGGGAATGTGCTGTTTC-3'	175 bp
IL-17	Forward: 5'-TGTCACCATGTGGCCTAAGAG-3' Reverse: 5'-GTCCGAAATGAGGCTGTCTTTGA-3'	119 bp
IL-23	Forward: 5'-ACAACCTGAGGGAACCAACCA-3' Reverse: 5'-CAGCAGCAACAGCAGCATTAC-3'	172 bp
TGF- β	Forward: 5'-AGCGACTCGCCAGAGTGGTTA-3' Reverse: 5'-GCAGTGTGTTATCCCTGCTGTCA-3'	130 bp
β -actin	Forward: 5'-AGTTGCGTTAC ACCCTTTCTTG-3' Reverse: 5'-TCACCTTCACCGTTCCAGTTT-3'	150 bp

280 absorbance ratio was between 1.9 and 2.0. The Prime-ScriptTM RT reagent kit (TaKaRa, Shiga, Japan) was used for complementary DNA synthesis. The expression levels of ROR γ t, Foxp3, IL-17, IL-23 and TGF- β were quantified by SYBR Premix Ex TaqTM II (TaKaRa) with the given primers (Table 2), and β -actin was used as an endogenous reference. The amplification was performed on a Rotor-Gene 3000 (Corbett

Research, Sydney, Australia) and analysed using the Rotor-Gene Real-Time Analysis Software 6.0. The reaction condition was 95 °C for 2 min, followed by 45 cycles of 95 °C for 20 s, 60 °C for 30 s and 68 °C for 30 s, and each reaction was performed in triplicate.

ELISA for IL-17, IL-23 and TGF- β concentrations Peripheral venous blood was collected after an overnight fast in the morning between 8:00 and 9:00 am. Serum was isolated within 30 min of blood collection and stored at –80 °C for further measurement of cytokines levels. The concentrations of IL-17, IL-23 and TGF- β in serum were measured using enzyme-linked immunosorbent assay (ELISA kit; R&D, Minneapolis, MN, USA). All samples were measured in triplicate.

PBMCs culture PBMCs from 12 AD patients who had a positive result of Dermatophagoides pteronyssinus-specific IgE (UniCap, Pharmacia, Stockholm, Sweden) and 8 healthy controls were cultured in 24-well plates at 2×10^6 cells/mL per well in RPMI 1640 with 5 μ g/mL recombinant Dermatophagoides pteronyssinus antigen (rDerP, Prospec Tany, Rehovot, Israel) at 37 °C, 5%CO₂ for 48 h. The cell culture supernatant was collected and the concentrations of IL-17, IL-23 and TGF- β were measured by the method of ELISA.

Statistical analysis

According to the results of normal distribution test (Shapiro–Wilk test), data are expressed as mean $\pm$ SD or median[25th–75th percentile], such as SCORAD index, peripheral and perilesional Th17/Treg ratio of AD patients, and serum IL-17 concentration of healthy controls, as well as the disease duration, Th17 cells percentage, TGF- β mRNA level and serum concentration of psoriasis patients. The one-way ANOVA (Holm–Sidak test), Independent-Samples *t*-test and Pearson Correlation were used to analyse the normally distributed data, and Kruskal–Wallis test followed by the Mann–Whitney *U*-test and Spearman's test were used to analyse the abnormally distributed data, with the level of significance set at $P < 0.05$.

Results

Th17 and Treg quantification in peripheral blood and lesional skin

The presence of IL-17⁺ and CD25^{high}Foxp3⁺ within the CD4⁺ circulating compartment was analysed in AD, respectively, and compared with healthy controls, ACD and psoriasis patients. Representative pictures for Th17 cells percentage (CD4⁺IL17⁺/CD4⁺T cells%) and Treg cells percentage (CD4⁺CD25^{high}Foxp3⁺/CD4⁺T cells%) are shown in Fig. 1a and 1b. Circulating Th17 cells percentage was significantly higher in AD patients than that in ACD patients ($P = 0.013$) and healthy controls ($P = 0.004$), and lower than that in psoriasis patients ($P < 0.001$, Fig. 1c). In contrast, circulating Treg cells percentage was similar in patients with AD, ACD and psoriasis ($P > 0.05$). Both AD and control diseases showed significantly reduced Treg cells percentage compared with that of healthy controls (all $P < 0.01$, Fig. 1d). The above results are listed in Table 3.

The analysis of Th17 and Treg cells percentage in skin specimens was conducted in AD patients. There was a parallel increase in Th17 cells percentage (4.89 ± 1.50 vs. 1.84 ± 0.65 , $P < 0.01$) and decrease in Treg cells percentage (1.82 ± 0.53 vs. 3.07 ± 0.74 , $P < 0.01$) in AD lesional skin compared with perilesional skin respectively. The ratio of Th17/Treg was also compared between AD lesions and perilesional skin samples, and there was a significant difference (2.99 ± 1.34 vs. 0.53

$[0.42\text{--}1.04]$, $P < 0.01$), which is in agreement with the results of peripheral blood in AD patients and healthy controls ($0.68 [0.54\text{--}0.99]$ vs. 0.12 ± 0.03 , $P < 0.01$).

Correlation analysis in AD patients

Figure 1e showed that AD circulating Th17 cells frequency negatively correlated with the corresponding percentage of Treg cells ($r = -0.425$, $P = 0.011$). SCORAD index in AD patients positively correlated with circulating Th17 cells percentage ($r = 0.400$, $P = 0.017$) and Th17/Treg ratio ($r = 0.462$, $P = 0.005$), but negatively correlated with Treg cells percentage ($r = -0.428$, $P = 0.018$). Serum IgE levels positively correlated with Th17/Treg ratio ($r = 0.520$, $P = 0.001$).

ROR γ t, Foxp3, IL-17, IL-23 and TGF- β mRNA levels in PBMCs

There was a distinct increase in ROR γ t, IL-17 and IL-23 mRNA levels in AD patients compared with ACD patients and healthy controls (all $P < 0.05$), and decreased levels compared with psoriasis patients (all $P < 0.05$, Fig. 1c). Foxp3 and TGF- β mRNA levels were reduced in AD patients compared with healthy controls (both $P < 0.01$), while there were no significant differences among AD, ACD and psoriasis patients (all $P > 0.05$, Fig. 1d). The above results are listed in Table 3.

Serum concentrations of IL-17, IL-23 and TGF- β

IL-17 and IL-23 serum concentrations in AD patients were significantly elevated compared with those in ACD patients and healthy controls (both $P < 0.05$), and reduced compared with psoriasis patients ($P < 0.05$). The TGF- β serum level in AD patients was slightly lower than that in ACD patients ($P = 0.066$) and healthy controls ($P = 0.056$), even if this difference was not significant, it was higher than that in psoriasis patients ($P = 0.041$). The above results are presented in Fig. 2a and list in Table 3.

IL-17, IL-23 and TGF- β production in PBMCs cultured with rDerp

The concentrations of IL-17 and IL-23 in PBMCs culture supernatant were markedly higher in AD patients than those in healthy

Figure 1 Th17 and Treg cells percentage, their specific transcription factors and effective cytokines mRNA levels in AD, control diseases and healthy controls as well as correlation analysis in AD patients. Representative pictures for Th17 cells percentage (CD4⁺IL17⁺/CD4⁺T cells%) and Treg cells percentage (CD4⁺CD25^{high}Foxp3⁺/CD4⁺T cells%) are presented in a and b (a1 and b1: Th17 and Treg cells percentage of a 17-year-old male healthy person respectively; a2 and b2: Th17 and Treg cells percentage of a 16-year-old male AD patient respectively). Results of Th17 cells percentage, ROR γ t, IL-17 and IL-23 mRNA levels in AD, control diseases and healthy controls are presented in Fig. 1c (* $P < 0.01$, # $P < 0.05$). Results of Treg cells percentage, Foxp3 and TGF- β mRNA levels in AD, control diseases and healthy controls are presented in Fig. 1d (* $P < 0.01$). Association analysis of AD circulating Th17 cells percentage with Treg cells percentage, SCORAD index with Th17 cells percentage, Treg cells percentage and Th17/Treg ratio as well as serum IgE levels with Th17/Treg ratio are presented in Fig. 1e ($r = -0.425, 0.400, -0.428, 0.462$ and 0.520 , respectively, all $P < 0.05$).

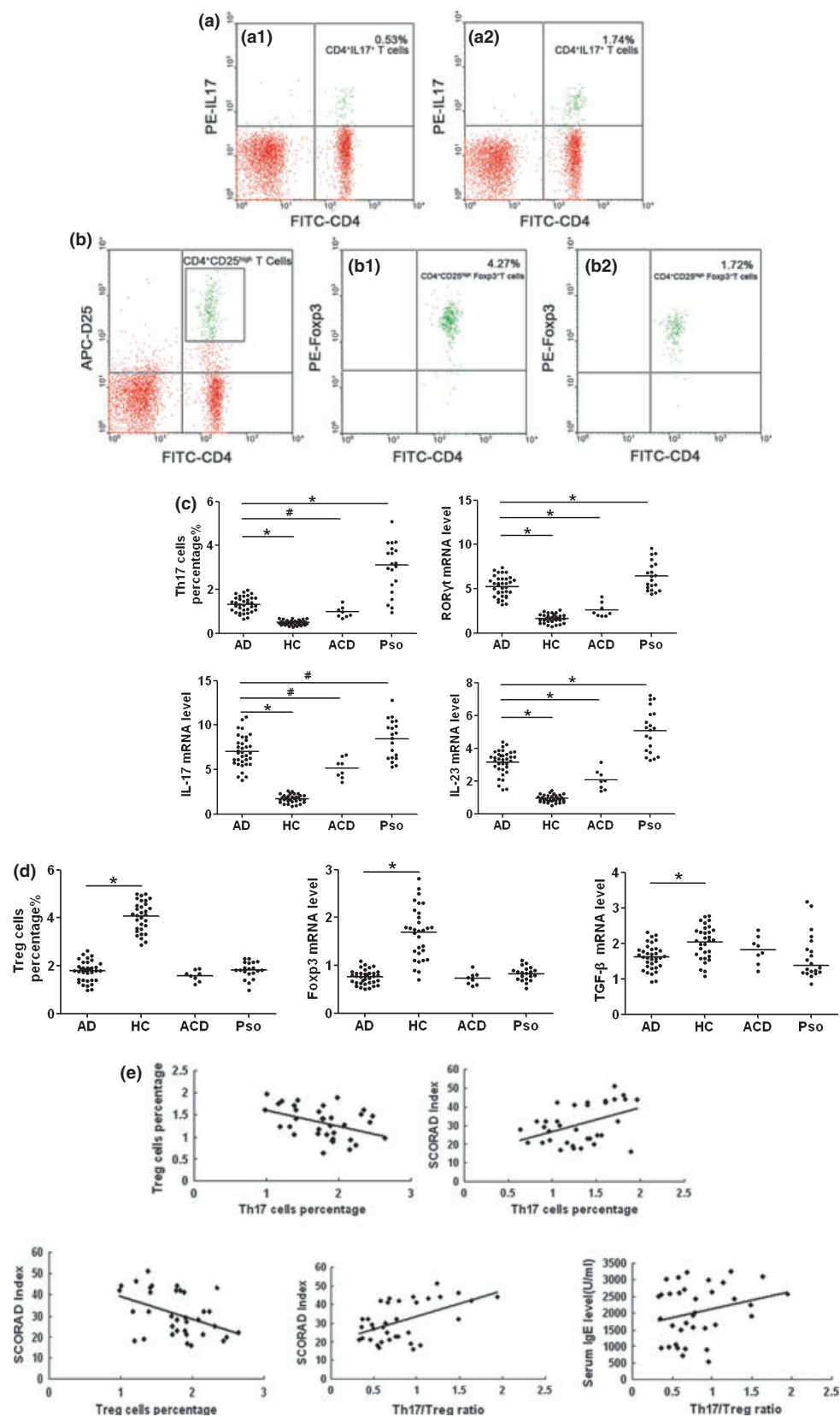


Table 3 Peripheral blood Th17 and Treg cells percentage, specific transcription factor and cytokines expression levels

	AD	ACD	Pso	HC
Th17 cells (%)	1.32 ± 0.32	0.94 ± 0.17	3.12* [1.96–3.84]	0.49 ± 0.11
Treg cells (%)	1.78 ± 0.43	1.59 ± 0.24	1.82 ± 0.35	4.06 ± 0.63
ROR mRNA level	5.26 ± 1.16	2.60 ± 0.82	6.43 ± 1.62	1.64 ± 0.50
IL-17 mRNA level	7.05 ± 1.77	5.15 ± 1.12	8.42 ± 2.17	1.74 ± 0.46
IL-23 mRNA level	3.16 ± 0.73	2.07 ± 0.60	5.06 ± 1.32	0.93 ± 0.22
Foxp3 mRNA level	0.75 ± 0.15	0.72 ± 0.13	0.83 ± 0.15	1.68 ± 0.54
TGF-β mRNA level	1.70 ± 0.45	1.82 ± 0.39	1.37* [1.17–2.01]	2.03 ± 0.54
IL-17 serum concentration (pg/mL)	33.39 ± 5.51	25.84 ± 6.02	38.52 ± 5.88	12.81* [11.96–14.50]
IL-23 serum concentration (pg/mL)	53.42 ± 9.55	45.09 ± 6.62	59.30 ± 8.08	19.48 ± 4.11
TGF-β serum concentration (ng/mL)	15.47 ± 2.72	17.74 ± 2.80	13.66* [12.23–15.02]	17.05 ± 3.17

*According to the results of normal distribution test (Shapiro–Wilk test), data were expressed as mean ± SD or median [25th–75th percentile].

AD, atopic dermatitis; ACD, allergic contact dermatitis; Pso, psoriasis; HC, healthy controls.

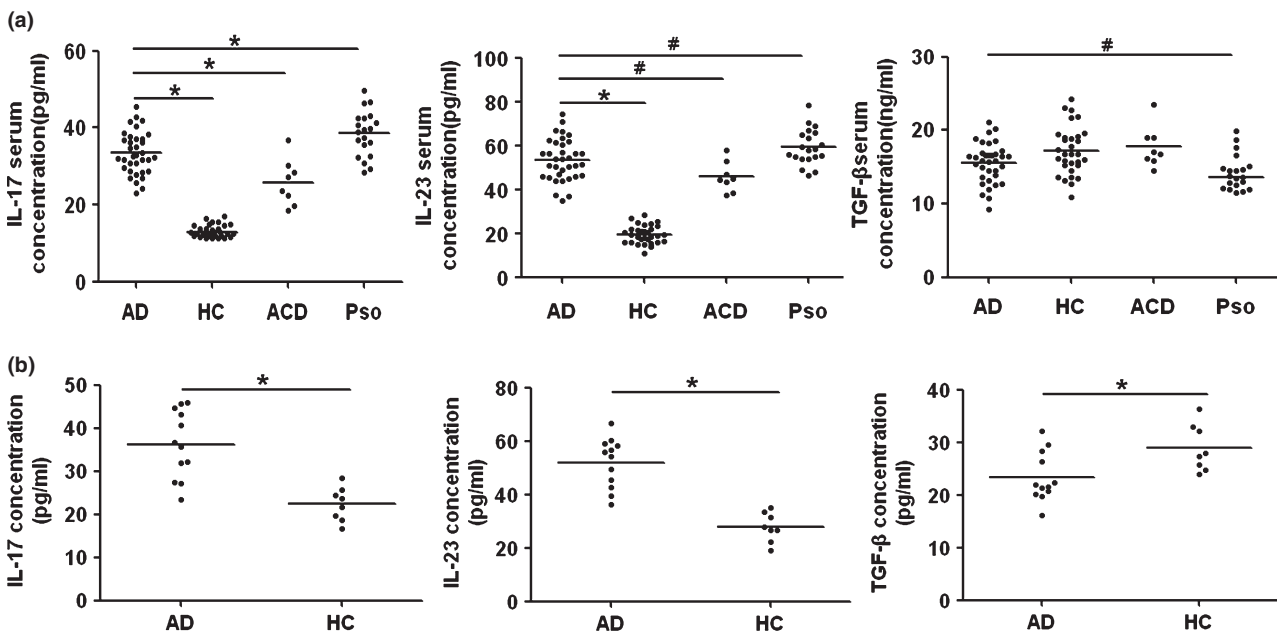


Figure 2 Th17- and Treg-related cytokines serum concentrations in AD, control diseases and healthy controls as well as expression levels in PBMCs culture supernatant by rDerP stimulation. Results of IL-17, IL-23 and TGF-β serum concentrations in AD, control diseases and healthy controls are presented in Fig. 2a (* $P < 0.01$, # $P < 0.05$). Results of IL-17, IL-23 and TGF-β concentrations in PBMCs culture supernatant by rDerP stimulation are presented in Fig. 2b (* $P < 0.01$).

controls (IL-17: 36.19 ± 7.84 pg/mL vs. 22.38 ± 3.91 pg/mL; IL-23: 52.06 ± 9.34 pg/mL vs. 27.81 ± 5.55 pg/mL, both $P < 0.01$), while the concentration of TGF-β was lower in AD patients than that in healthy controls (23.39 ± 4.69 pg/mL vs. 28.86 ± 4.44 pg/mL, $P = 0.018$, Fig. 2b).

Discussion

In this study, our results provide direct evidence that AD patients exhibit a significant increase in circulating Th17 cells percentage and Th17-related cytokines (IL-23 and IL-17) mRNA and serum levels, and a dramatic decrease in frequency of Treg

cells and Treg-related cytokine (TGF-β) mRNA level when compared to healthy controls. We also evaluated the proportion of Th17 and Treg cells in skin specimens, which showed the first clue that there were statistical differences between AD lesions and perilesional skin tissues in the two newly identified CD4⁺T helper cells. Moreover, the elevated Th17/Treg ratio and the imbalance of Th17/Treg cells were firstly found both in peripheral blood and skin lesion of AD patients. RORγt and Foxp3 were the subset-determining transcription factors of Th17 and Treg cells, respectively, in this study, the increase in RORγt expression and decrease in Foxp3 level were also detected, which

confirmed that a Th17/Treg imbalance occurred in AD. On the other hand, though we measured increased expression levels of Th17 cells percentage, ROR γ t, and Th17-related cytokines as well as similar frequency of Treg cells, Foxp3 and TGF- β mRNA levels in psoriasis patients compared with AD patients, it is still an argument to say whether the Th17 signature is less strong in AD than in psoriasis, which should be clarified in further study.

Th17 cells and Treg cells present mutually antagonistic function. Th17 cells may be the driving force in the pathogenesis of autoimmune and inflammatory disorders, while Tregs cells play a critical role in maintaining self-tolerance and preventing autoimmunity, allergy and inflammatory reactions. TGF- β is a major pluripotential cytokine with a pronounced immunosuppressive effect.²² Murine experiments have indicated that both the differentiation of Treg cells and Th17 cells from naïve T-cell precursors need the presence of TGF- β , while the factors that determine the expression of IL-17 in human CD4⁺T cells are completely different from those in mice.²³ TGF- β is not necessary for the development of human Th17 cells^{24,25} and TGF- β -induced Foxp3 can also suppress Th17 cells differentiation by antagonizing ROR γ t and inhibiting ROR γ t-mediated IL-17 mRNA transcription and IL-17 production.^{26,27} In addition, IL-23, the promoting cytokine of Th17 cells responses, can restrain the induction and development of Foxp3⁺ CD4⁺ T cells.²⁸ In agreement, we found in this study that AD circulating Th17 cells frequency negatively correlated with the corresponding percentage of Treg cells, as well as significantly lower TGF- β mRNA level, slightly lower serum TGF- β concentration and especially higher IL-23 and IL-17 mRNA levels and serum concentrations have also been detected in peripheral circulation. Thus, we consider that the relatively lower level of TGF- β failure to effectively promote Treg cells development, lack of sensitivity to TGF- β make it easy for Th17 cells to escape the immune inhibition of Treg cells, as well as the elevated expression of IL-23 antagonizing the suppressive effect of Foxp3 on ROR γ t may lead to the decrease in Treg cells and increase in Th17 cells in AD together, and as a result, the imbalance of Th17 and Treg cells occur. More importantly, for the first time, we have reported that the disease severity of AD patients (determined by SCORAD index) was associated with increased Th17 cells percentage and decreased Treg cells levels, and that it positively correlated with Th17/Treg ratio, further indicating a potential role of the shift of Th17/Treg cells in the pathogenesis of AD.

It is thought that AD may be caused by the induction of Th2 phenotype and IgE responses specific for common environmental allergens in susceptible individuals. In accordance with the mRNA levels and serum concentrations of Th17- and Treg-related cytokines in peripheral circulation, we found significant increase in IL-17 and IL-23 expression as well as obvious decrease in TGF- β production in PBMCs culture supernatant of AD patients by stimulating with the given allergen of rDerp. It has been suggested that Treg cells may actively prevent Th2

responses to allergens occurring in healthy non-atopic individuals,²⁹ while in allergic patients, the inhibiting function of Treg cells on Th2 cytokine production may be impaired.^{29,30} In X-linked autoimmunity-allergic dysregulation syndrome, patients are deficient in Treg cells and suffer from allergic manifestations with severe eczema, elevated IgE levels, eosinophilia and food allergy.³¹ On the other hand, in asthma, another Th2-mediated allergic disease, evidence has showed that IL-23 and Th17 cells can not only induce Th17-cell-mediated neutrophilic airway inflammation but also up-regulate Th2-cell-mediated eosinophilic airway inflammation.³² In this study, we found a positive association between Th17/Treg ratio and serum IgE levels. So, combined with the above study results, we may believe that in AD, the imbalance of Th17/Treg cells may trigger and/or aggravate its disease process by interacting with Th2 cells.

In conclusion, we found a marked immune imbalance of Th17 and Treg cells in AD patients, as well as the positive association of elevated Th17/Treg ratio with AD severity and serum IgE levels, which may contribute to the pathogenesis and development of AD.

Acknowledgements

This study was funded by Shandong Province Higher Educational Science and Technology Program of China (no. J10LF90), Shandong Province Natural Science Foundation of China (no. ZR2010HQ013), Shandong Province Medical Science and Technology Development Project of China (no. 2011QZ003), Shandong Province Science and Technology Development Project of China (no. 2011YD18069) and Binzhou City Science and Technology Development Project of Shandong Province, China (no. 2011ZC0914).

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