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Original Article

The profiles of T lymphocytes and subsets in peripheral blood of patients with chronic idiopathic urticaria

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Abstract: Chronic idiopathic urticaria (CIU) is defined as persistent symptoms of urticaria for 6 weeks or more. It is associated with autoimmunity in approximately 35-40% of the patients. Immunological dysfunction has been described to occur in the patients with CIU, most notably in association with an inflammatory process. Apart from the factors of humoral immunity such as autoantibody, there may be some cellular immunity factors usually mediated by T lymphocytes involved in the pathogenesis of CIU. However, the actions of T lymphocytes and lymphocyte subsets in the pathogenesis of CIU are not fully delineated so far. This present study was aimed to investigate the roles of T lymphocytes and lymphocyte subsets in the pathogenesis of CIU. Thirty patients with CIU and 30 healthy controls were randomly enrolled. Flow cytometry was used to analyze the constituent ratios of $CD3^+$, $CD4^+$, $CD8^+$, Th1, Th2, Th17 and Treg lymphocyte subsets in peripheral blood. The results showed that, as compared with the control group, the ratios of $CD8^+$ and Th2 lymphocyte subsets in patients with CIU significantly increased ($P<0.05$), while the ratio of Th17 cells decreased significantly ($P<0.05$). No obvious changes were observed in the constituent ratios of $CD3^+$, $CD4^+$, Th1 and Treg lymphocyte subsets ($P>0.05$). There were imbalance of $CD4^+/CD8^+$, Th1/Th2 and Th17/Treg cell populations in patients with CIU ($P<0.05$). The results suggest that the imbalance of lymphocyte subset plays a role in the pathogenesis of CIU.

Keywords: Chronic idiopathic urticaria, chronic urticaria, T lymphocyte, lymphocyte subset, helper T cells, regulatory T cells, pathogenesis

Introduction

Chronic urticaria (CU) is a chronic inflammatory skin disease characterized by short-lived, pruritus swellings of the skin, mouth, and genitalia due to transient leakage of plasma from small blood vessels into the surrounding connective tissue [1]. As patients often experience itching, the physical and mental health of the patients with CU is greatly influenced [2]. 35-40% of the patients with CU have circulating autoantibodies against the α-chain of the high affinity receptor for immunoglobulin (Ig)E (FcεRI) or against the IgE molecule [3, 4]. However, these patients have no obvious causes and are considered to have chronic idiopathic urticaria (CIU) [5]. There is a clear association of a subpopulation of such patients with autoimmunity and the symptoms are generally more severe, and therefore more difficult to treat [3, 5]. Functional properties of antibodies can be examined *in vivo* by intradermal injection of autologous serum (autologous serum skin test, ASST), which can induce a wheal-and-flare response in patients with CIU. Further evidence could be seen *in vitro* with the release of histamine from basophils and mast cells elicited by the addition of CIU patient serum [6, 7].

The etiology and pathogenesis of CIU are complicated and still remain to be elucidated. Apart from the factors of humoral immunity such as autoantibody, there may be some cellular immunity factors involved in the pathogenesis of CIU, although there are few literatures about it [8, 9]. The histopathological lesions of the skin in CIU are usually marked by dermal edema and perivascular mononuclear cell infiltration predomi-

nantly with lymphocytes, eosinophils and mast cells. The actions of these infiltrating lymphocytes in the pathogenesis of CIU as well as the linkage between the infiltrating lymphocytes and other inflammatory cells in CIU are not fully delineated so far [9, 10]. The cellular immunity response presenting with spontaneous wheals exhibits pro-inflammatory characteristics, involving a prominent role of lymphocytes with a mixed Th1/Th2 response. Consistent with this finding, similar results suggest specific and non-redundant roles of T lymphocyte and subsets in the initiation and maintenance of chronic inflammatory responses in CIU [11, 12]. To understand the proinflammatory process in CIU or the possible autoimmune basis in some patients, it is essential to search for mechanisms that could be involved in immunoregulation. In the present study, we examined the expression profiles of T lymphocyte and subsets in peripheral blood of CIU patients to investigate the possible actions of the lymphocytes and subsets in the pathogenesis of CIU.

Material and methods

Study subjects

This study included 30 CIU patients (13 males and 19 females; median age 49 ± 12.5 years; aged 20-67 years), randomly enrolled from the out-patient-department of dermatology of the 306 Hospital of PLA (Beijing, China). The average disease course of these patients was 40.6 months (2 months to 14 years). All the patients met the following inclusion and exclusion criteria of CIU [13]. Additionally, 30 healthy volunteers (14 males and 16 females; median age 48 ± 11.3 years; aged 22-64 years) were enrolled in this study as healthy controls, who themselves and whose immediate family members had no history of allergy. All patients and control subjects signed informed consent form, and the study protocol was approved by the institutional ethics committee of the 306 Hospital of PLA.

Inclusion and exclusion criteria of CIU

The inclusion criteria of CIU were: 1) The patient was over 18 years old. 2) The wheals were seen when enrolled in the study, and the patient had a history of recurrent wheals over 6 weeks with frequencies of 4 times a week or more. 3) The allergy to food or drug was ruled out and there was no definite cause clinically. 4) The patient had no history of allergic diseases such as rhinitis, asthma or atopic dermatitis, and no history of autoimmune diseases or parasite infection. 5) Serum ASST was positive as detected. 6) Serological examination for hepatitis B and C showed negative results. 7) Serum test for anti-*Helicobacter pylori* antibodies, antithyroid autoantibodies and antinuclear autoantibodies showed negative results.

The exclusion criteria of CIU were: 1) The wheals lasted over 24 h. 2) The patient had other types of urticaria such as physical urticaria, hereditary angioedma, drug-induced urticaria or urticarial vasculitis. 3) Pregnant or lactating woman. 4) The patient had taken corticosteroids or immunomodulants during the past 4 weeks or taken antihistamines during the past 3 days. 5) The patient had concomitant allergic contact dermatitis, atopic dermatitis, eczema or other pruritic skin diseases. 6) The patient's test result of blood hematology tests, blood chemistry tests, urine analysis or stool analysis was abnormal.

ASST

The ASST was performed according to standard methods [6, 7], using fresh autologous serum injected intradermally into the volar forearm skin. A positive test was defined as a serum-induced weal with a diameter of ≥1.5 mm, or more than that elicited by the saline-induced response after 30 min.

Flow cytometry

Venous blood of 6 mL from CIU patients and control subjects was collected, anti-agglutinated with ethylenediaminetetraacetic acid (EDTA) and diluted with an equal volume of cold Hanks solution.

All fluorescence measurements were performed by multicolour flow cytometry (FACScan; Becton-Dickinson, San Jose, CA, USA). Sample preparation and detection methods were conducted in strict accordance with reagent instructions. Standard emission filters were used and the CellQuest software was used to process the data, analyze the constituent ratios of $CD3^+$, $CD4^+$, $CD8^+$, Th1, Th2, Th17 cells and regulatory T cells (Treg) lymphocyte subsets in peripheral blood and calculate the ratios of $CD4^+/CD8^+$ and Th1/Th2 cells.

Statistical analysis

Statistical analysis was carried out in SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). All data were expressed as mean ± SE. The differ-

Table 1. The percentages of T lymphocyte subsets in peripheral bloods from CIU patients and healthy controls ($\bar{x} \pm s$)

Croups	T cells (%)	$CD3^+CD4^+$ T cells (%)	$CD3^+CD8^+$ T cells (%)	$CD4^+/CD8^+$
Control group	61.15 ± 9.05	31.95 ± 5.57	23.70 ± 6.51	1.45 ± 0.50
CIU group	65.90 ± 9.25	31.25 ± 7.41	30.00 ± 9.17*	1.15 ± 0.49

*P<0.05 versus control.

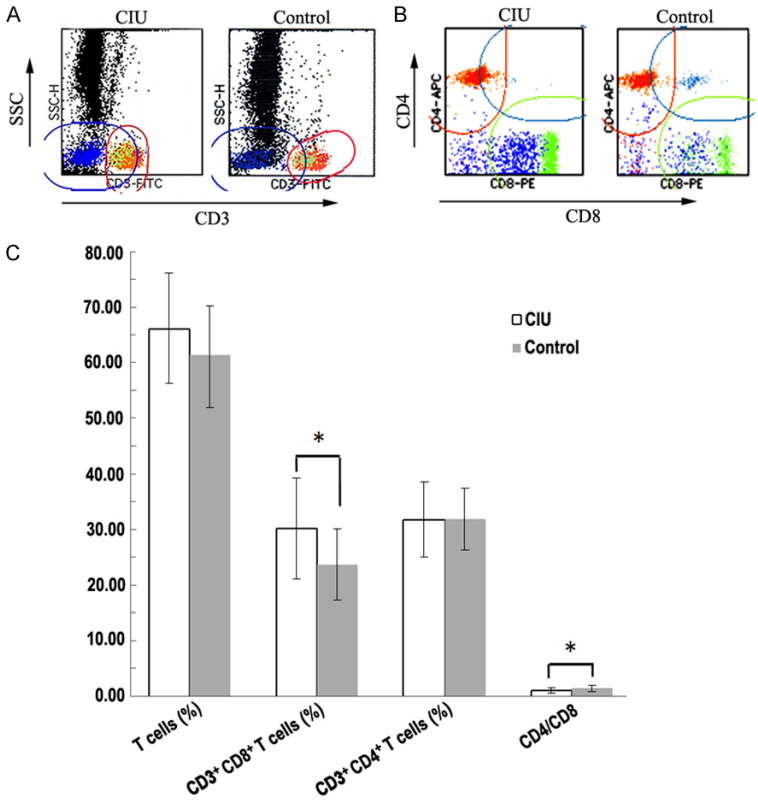

Figure 1. Increased circulating $CD3^+CD8^+$ T cells and decreased ratio of CD4/CD8 in CIU patients. A and B: Representative scatter grams of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in peripheral bloods from CIU patients and healthy controls. C: The comparison of total T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells and CD4/CD8 ratio between CIU patients and healthy controls. Data are shown as median (range). *P<0.05 versus control.

ences between CIU patients and healthy controls were analyzed by the *t*-test. *P* values less than 0.05 were considered significant.

Results

The constituent ratios of $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes in peripheral blood

The constituent ratios of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocyte subsets were detected by flow cytometric analysis. All the figures are in **Table 1**. While the results showed no obvious changes in the constituent ratios of $CD3^+$ T lymphocyte and $CD4^+$ T lymphocyte in patients with CIU (P>0.05), they indicated that the constituent ratio of $CD8^+$ T lymphocyte significantly increased as compared with that of the control group (P<0.05), and the ratio of $CD4^+/CD8^+$ T lymphocyte decreased significantly in patients with CIU (P<0.05) (**Figure 1**).

The constituent ratios of Th1 and Th2 cells in peripheral blood

$CD4^+$ helper T cells, which differentiate into Th1, Th2 and Th17 lymphocyte subsets, were to play a different immune response. The constituent ratios of Th1 and Th2 cells were detected by flow cytometric analysis. All the figures are in **Table 2**. The results showed that, as compared to the control group, no obvious changes were observed in the constituent ratio of Th1 cells (**Figure 2A**, **2E**) in patients with AIU (CIU?) (P>0.05), but the ratio of Th2 cells (**Figure 2B**, **2E**) significantly increased (P<0.05) and the ratio of Th1/Th2 significantly decreased (P<0.05) (**Figure 2E**).

The constituent ratios of Th17 cells and Treg in peripheral blood

Treg are a subpopulation of effector T cells devoted to the maintenance of immune response. Th17 immune cells and Treg have the opposing immunomodulatory functions in the inflammatory process of several skin diseases. Our results showed that the constituent ratio of Th17 cells significantly decreased as compared with the control group (P<0.05) (**Figure 2C**, **2E**), while no obvious changes were observed in the constituent ratio of Treg (P>0.05) (**Figure 2D**, **2E**). This indicated that there were unbalanced Th17/Treg responses in the inflammatory process of CIU (P<0.05) (**Figure 2E**).

Table 2. The percentages of Th1, Th2, Th17 and Treg cells, and the ratios Th1/Th2 and Th17/Treg in peripheral bloods from CIU patients and healthy controls ($\bar{x} \pm s$)

Groups	Th1 (%)	Th2 (%)	Th1/Th2	Th17 (%)	Treg (%)	Th17/Treg
Control group	21.14 ± 4.68	2.28 ± 0.96	10.40 ± 3.57	3.33 ± 0.97	5.49 ± 2.14	0.87 ± 0.36
CIU group	20.31 ± 4.78	3.32 ± 0.97*	6.43 ± 1.93*	2.33 ± 0.96*	4.96 ± 1.45	0.58 ± 0.34*

*P<0.05 versus control.

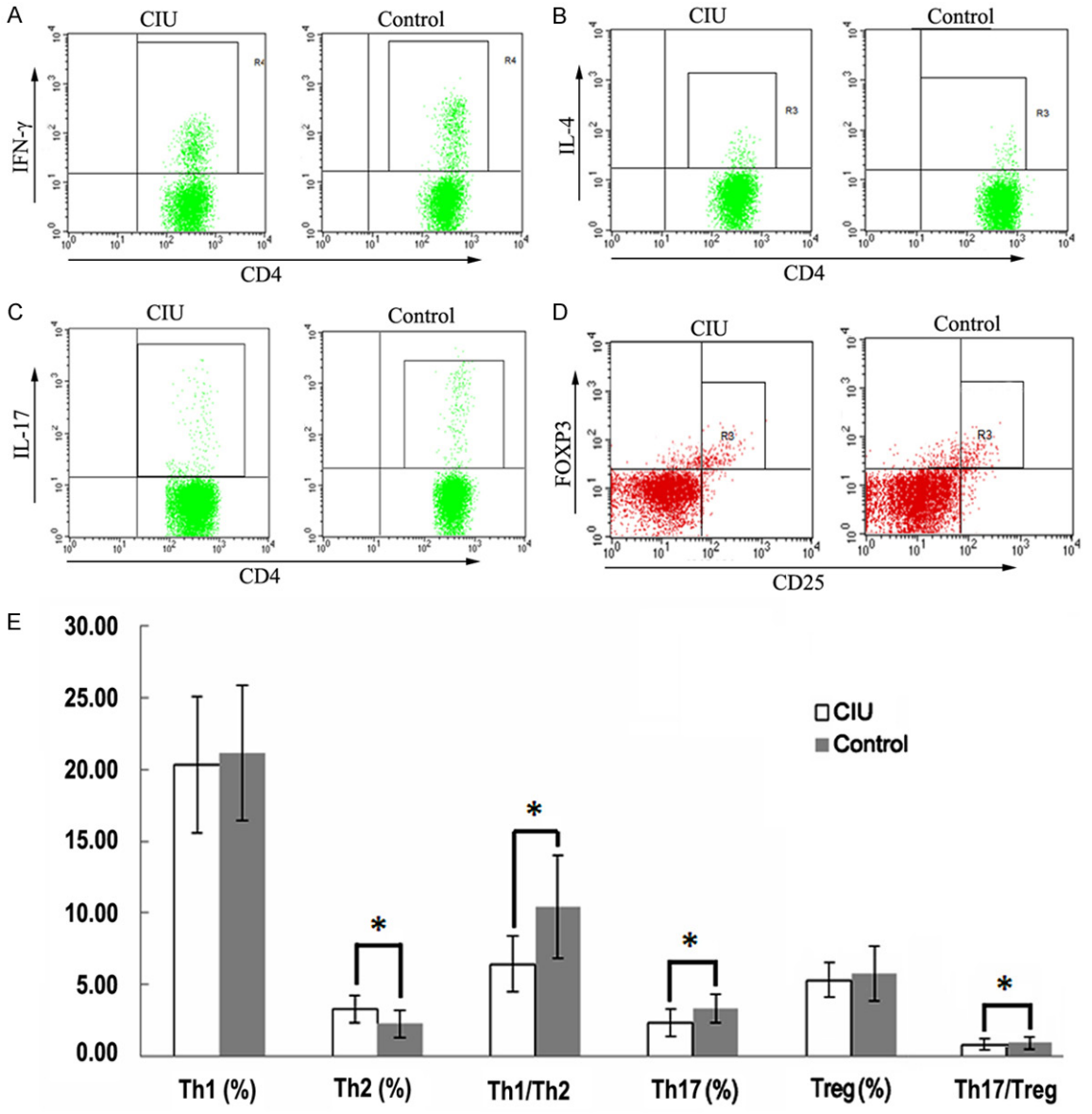

Figure 2. Increased circulating Th2 cells and decreased Th17 cells, ratios of Th1/Th2 and Th17/Treg in CIU patients. Peripheral blood cells from CIU patients and healthy controls were stimulated with PMA, ionomycin and BFA for 5 hrs, and then stained with fluorescently labeled anti-human antibodies for analyzing Th1, Th2 and Th17. Peripheral blood cells from CIU patients and healthy controls were directly stained with labeled anti-human antibodies for analyzing Treg cells. A-D: Representative scatter grams of Th1 ($CD4^+$ INF-γ^+), Th2 ($CD4^+$ IL-4^+), Th17 ($CD4^+$ IL-17^+) and Treg ($CD4^+$ $CD25^+$ $Foxp3^+$) cells in peripheral bloods from CIU patients and healthy controls. E: The comparison of Th1, Th2, Th17 and Treg cells, ratios Th1/Th2 and Th17/Treg between CIU patients and healthy controls. Data are shown as median (range). *P<0.05 versus control.

Discussion

The pathogenesis of CIU is not well delineated and the treatment is palliative as it is not tied to the pathomechanism. It is believed that the cellular immune response is an important factor in the pathogenesis of CIU. The possible role of T lymphocytes in the autoimmune pathogenesis of CIU has been investigated by various researchers. Studies focused on the profiles of circulating cytokines in CIU serum note increased IL-4, IL-6, IL-1β, and IL-12 as compared to healthy controls [14, 15]. Isolated PBMC PHA-stimulated cytokine profiles have also been examined and results have been variable. As compared to control subjects, some studies revealed no difference in IFN-γ, IL-10, TNF-α and reduced IL-4 expression predominantly in ASST positive hosts [14, 16], whereas high levels of IL-10 and TNF-α were seen with mitogen in a second study [12]. Given the prominence of T lymphocytes in CIU lesions, further studies are necessary to clarify their role in the disease.

In this study, T lymphocyte subgroups in the peripheral blood from CIU patients were analyzed by flow cytometry and compared with those from the control subjects. We found that the number of $CD8^+$ T cells from the patients with CIU was significantly increased as compared with the controls ($P<0.05$), while the ratio of $CD4^+/CD8^+$ T lymphocyte subsets in patients with CIU was significantly lower than that in the controls ($P<0.05$). The wheal in CIU was caused by increased capillary permeability that allows proteins and fluids to extravasate. Capillary permeability results in the increased release of histamine from the mast cells around the capillarits. There were no differences in the numbers of $CD3^+$ cells and $CD4^+$ cells between CIU and the controls ($P>0.05$). The results were consistent with those reported by Dos Santos JC who documented that there were cellular immunity disorders in CIU [14]. $CD3^+$ cells represent mature T lymphocyte while $CD4^+$ cells represent T helper lymphocytes and $CD8^+$ cells represent T suppressor and cytotoxic lymphocytes. There was T lymphocyte dysfunction in these patients with CIU. It is generally believed that most of the CIU which resulted in dysfunction of immunity are caused by Type I hypersensitivity, and fewer by Type II or III. Although mast cells are thought of as main effector cells in the pathogenesis of CIU, lymphocytes may play a role in the formation of wheal by secreting histamin-releasing factors. Histamin receptors are found in the T lymphocytes and it is speculated that histamin may have some effect on cellular immunity through its interaction with the histamin receptor [17, 18]. Our results indicated that the cell-mediated immune response in patients with CIU was significantly suppressed.

$CD4^+$ T helper cells were originally classified into two subgroups: Th1 and Th2 cells. The Th1 cells secrete IL-2 and IFN-γ which induce cellular immune responses and inhibit Th2 cells. On the other hand, Th2 cells produce IL-4, IL-5, IL-13, TGF-β and IL-10 which inhibit Th1 cells. Th1 cytokines are proinflammatory and Th2 cytokines are anti-inflammatory. Each type of cytokines downregulates the others' responses [19, 20]. For that reason, Ferrer and co-workers questioned whether the immunologic profile reflects the predominance of a Th1 or Th2 phenotype [15, 21]. They measured INFγ as a representative of a Th1 profile, and then measured IL-4 and IL-5 as representatives of a Th2 subtype. They found that IL-4 was higher in the sera of patients with CIU compared to controls while IL-5 and IFN-γ levels were normal. Significant differences were found in the ability of $CD4^+$ lymphocytes to produce IL-4 and INF-γ upon PMA-Ionomycin stimulus when healthy controls were compared to CIU patients. These authors analyzed skin biopsies from CIU patients by in situ hybridization, and revealed a higher mRNA expression of IL-4, IL-5 and INF-γ in CIU patients than in healthy controls [22, 23]. Likewise, some experimental studies have shown that T cells in skin lesions typically express Th2 cytokines like IL-4, IL-5, IL-6, and IL-10 but fail to produce Th1 cytokines including IFN-γ [24]. In our study, we found that the ratio of Th1/Th2 lymphocyte subsets decreased significantly in patients with CIU as compared with that of the control group ($P<0.05$). This profile indicates a direct contribution of the lymphocytes in the Th1/Th2 imbalance observed frequently in CIU patients and suggests their potential role in cell-mediated immunity.

Another $CD4^+$ T helper cell subset that has emerged is the Th17 subset which produces high levels of IL-17 along with IL-17 B through F. TH17 cells promote inflammation and play an important role in autoimmunity. Activated T

cells send signals to B lymphocytes through cytokines such as IL-2, IL-4, IL-5, and IL-6, resulting in their activation, proliferation, and differentiation [25, 26]. TH17 cells are probably involved in protection against bacterial pathogens and in pathogenesis of Th1-mediated chronic inflammatory diseases. As CIU is believed to be a Th2-mediated disease, the role of TH17 cells in its pathogenesis is still under investigation [27]. In a recent study, the percentage of Th17 cells was shown to be lower in the peripheral blood of CIU patients and was associated with the severity of CIU [28]. However, the data are still discrepant, because recently it was shown by Dos Santos JC et al [14] that the dysfunctional innate immune response in CIU consequent to functional impairment of pDC to TLR9 activation disturbs the cytokine production by T cells, mainly of IL-17A and IL-10 but that no obvious changes were observed in the number of Th17 cells in CIU patients. Our study showed the number of Th17 cells significantly decreased as compared with the control group ($P<0.05$). It is speculated that the increase of the ratio of Th2 lymphocyte subsets results in suppression of TH1 cells response, induction of immune tolerance, and a reduced Th17 effector response.

Treg are a subpopulation of effector T cells devoted to the maintenance of immune tolerance, and hence, their expansion and maintenance are critical in the resolution of inflammation and in preventing sustained tissue damage or autoimmunity [29]. These cells are characterized by the expression of the transcription factor foxp3, which is the master regulator of the immune suppressive activity of Treg. Therefore, the mechanisms that induce foxp3 expression and sustain Treg activity have gained a great interest in recent biomedical research [27, 30]. Functional assays and phenotype analysis have revealed that Treg isolated from patients of autoimmune disorder exhibit reduced regulatory function as opposed to those from healthy controls [31, 32]. It may be concluded that reduced percentage of CD4(+) CD25(+) FOX P3(+) regulator T-cells contributes to the autoimmune pathogenic process of CIU [33]. However, in our study, there were no differences in the numbers of Treg between CIU patients and the controls ($P>0.05$). The results were consistent with those reported by Piconi S et al [12] who exhibited a normal number of Tregs and foxp3 expression but reduced suppressive activities of these cells in PBMCs from CIU patients when compared to healthy controls. Taken together, these findings show that the role of Treg in CIU remains to be elucidated.

Th17 immune cells and Treg have opposing immunomodulatory functions in the inflammatory process of several skin diseases [34]. The opposing functions of Th17 immune cells and Treg have led many researchers to propose that unbalanced Th17/Treg responses may be involved in the pathogenesis of chronic inflammatory diseases [30, 31]. These findings were confirmed by our experiments in which the constituent ratio of Th17 cells significantly decreased as compared with the control group ($P<0.05$), but no obvious changes were observed in the constituent ratio of Treg ($P>0.05$). The imbalance of Th17/Treg cell populations has been suggested to exist in CIU patients and be involved in CIU pathogenesis. We speculate that inflammation can be inhibited by the balance of Th17/Treg cell populations under normal settings. However, the mechanism behind this phenomenon remains unclear. Further study is needed to determine exactly how the regulation of the balance of Th17/Treg cells in CIU patients is globally controlled, and how each factor interplays to exhibit the disease profile in these immune cells.

In conclusion, the present study revealed an increased ratio of $CD8^+$ and Th2 lymphocyte subsets and a decreased ratio of Th17 cells in the peripheral blood of CIU patients. These imbalances could play a role in the pathogenesis of CIU and need further study. T lymphocyte and subsets play important roles in cutaneous tissues. Newer insights into their roles in CIU have provided researchers with important clues to the etiological factors implicated not only in the pathomechanism but also in the responses to therapies in CIU and has led to the development of a whole new genre of drugs known as biological, which has revolutionized the therapy of CIU.

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Disclosure of conflict of interest

None.

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