

ORIGINAL ARTICLE

Role of the subgroups of T, B, natural killer lymphocyte and serum levels of interleukin-15, interleukin-21 and immunoglobulin E in the pathogenesis of urticaria

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ABSTRACT

The immunological characterization in the pathogenesis of urticaria, mainly regarding cytokine profile, needs more investigation. In this study, subgroups of the T, B and natural killer (NK) lymphocyte from peripheral blood and serum levels of interleukin (IL)-15, IL-21 and immunoglobulin (Ig)E were examined in patients with acute urticaria (AU) and chronic urticaria (CU). Moreover, symptom scores and course of the patients were assessed. The percentage of NK cells and the ratio of CD4⁺/CD8⁺ increased, however, CD8⁺ decreased in CU compared to controls ($P < 0.01$). But no significant changes of T, B and NK lymphocyte were found in AU. IL-15 and IL-21 significantly decreased in AU and CU, but IgE increased. CU with a positive autologous serum skin test were more likely to be associated with longer course and higher CD3⁺, B cells and IL-21, and lower IgE ($P < 0.01$). Weak negative correlations were demonstrated between CD3⁺, CD8⁺ and scores in CU ($r = -0.23, -0.25, P < 0.05$). Significant correlations were found between B cells and scores and course in CU ($r = 0.49, 0.65, P < 0.01$). Moreover, a significant correlation was found between IL-21 and IgE ($r = 0.42, P < 0.01$) in CU. But no significant correlations were found in AU. Our findings supported the concept that both humoral immunity and cellular immunity dysregulation in the pathogenesis of urticaria – mainly related to the decrease of the serum levels of IL-15 and IL-21 – may induce the increasing expression of IgE produced by B cells.

Key words: interleukin-15, interleukin-21, lymphocyte subgroups, pathogenesis, urticaria.

INTRODUCTION

Urticaria is the most common cutaneous disorder seen in outpatient allergy clinics,¹ characterized by short-lived swellings of the skin, mouth and genitalia related to a transient leakage of plasma from small blood vessels into the surrounding connective tissues. Approximately 15–20% of people develop at least one episode of urticaria in their lives and in less than 20% of them an etiological factor has been identified.² It can occur acutely or evolve in a chronic

mode.³ Urticaria results from the activation of cutaneous mast cells.⁴ The release of mast cell-derived mediators may be caused by both immune and non-immune mechanisms.⁵ In recent years, anti-high affinity immunoglobulin (Ig)E receptor's autoantibody and anti-IgE autoantibody were found to play an influential role in the pathogenic role of chronic urticaria (CU).⁶ However, Oswalt pointed that anti-FcεRI antibody titers were not associated with clinical control or autoantibodies.⁷ Recent work has demonstrated that T-cell activation is proportional to mast

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cell deregulation in these patients with CU⁸ and T cells infiltrating the skin can participate in the chronicity of the lesions.³

For over 35 years, immunologists have divided T-helper (Th) cells into functional subsets.⁹ The Th1/Th2 hypothesis was that so-called type 1 CD4 T-helper cells (Th1) drive cell-mediated immune responses leading to tissue damage, and drive antibody-mediated responses in certain subclasses of the G isotype of Ig antibody, specifically termed IgG_{2a}. In contrast, type 2 CD4 T-helper cells (Th2) drive certain antibody-mediated responses, particularly those that are involved in allergy dominated by the IgE isotype.^{10,11} This simplistic dichotomous view of T-cell differentiation has recently been challenged by the discovery of a new lineage of T cells characterized by the ability to preferentially secrete a pro-inflammatory cytokine, interleukin (IL)-17 and thus designated Th17 cells.¹² The cytokine profile found in CU did not reflect either Th1 or Th2 predominance.^{13,14} So, we were interested in investigating whether Th17 was associated with urticaria.

The roles of IL-15 in allergic responses remain elusive.¹⁵ On one hand, it is generally thought to be a Th1 cytokine,¹⁶ for promoting γ -interferon (IFN- γ) production from murine Th1 clones through upregulation of IL-12 receptor β 1,¹⁷ regulating the activation, proliferation and cytokine release of T, natural killer (NK), mast and B cells,^{18–21} and its reduction related to allergic diseases such as asthma, allergic dermatitis and allergic rhinitis.^{15,22,23} On the other hand, IL-15 has been reported to induce IL-5 production by allergen-specific Th2 clones²⁴ and promote the survival of B cells,²⁵ mast cells²⁶ and eosinophils.²⁷

Interleukin-21 is a newly described cytokine, produced by activated CD4⁺ T cells. IL-21 augments the proliferation of CD4⁺ and CD8⁺ T lymphocytes and regulates the profile of cytokines secreted by these cells, drives the differentiation of B cells into memory cells and terminally differentiated plasma cells, and moreover, enhances the activity of NK cells.²⁸ Recent researches discovered that IL-21 is a product of Th17 cells, and serves as an autocrine factor that promotes or sustains Th17 lineage commitment.²⁹ Moreover, IL-21 appeared to be a critical cytokine to keep low IgE levels under physiological and pathological conditions.³⁰ Therefore, we were interested in determining

whether IL-15 or IL-21 production is decreased in urticaria.

METHODS

Subjects

One hundred and sixty patients (acute urticaria [AU] $n = 80$, male/female 22/58, mean age 35 years, age range 17–59 years; CU $n = 80$, male/female 19/61, mean age 37 years, age range 17–62 years) were studied. As opposed to AU, CU is defined by spontaneous wheals for more than 6 weeks.⁶ A complete retrospective history of disease and drug therapy was taken from each patient. We excluded patients with lesions suspicious for urticarial vasculitis, patients with focal infection, physical urticaria or food allergy, or those who were taking drugs that could exacerbate CU. None of our patients took doxepin. Antihistamine treatment was stopped at least 4 days before, and corticosteroid or immunosuppressive therapy at least 2 months before serum samples were collected.³¹ The scoring system was based on the assessment of key urticaria symptoms: (i) wheals and pruritus (0 = none, 1 = mild [<20 wheals/24 h], 2 = moderate [21–50 wheals/24 h], 3 = intense [>50 wheals/24 h or large confluent areas of wheals]); and (ii) pruritus assessment (0 = none, 1 = mild, 2 = moderate, 3 = intense). Patients documented 24-h self-evaluation scores.⁶ The control group consisted of 80 healthy blood donors (male/female 20/60; mean age 36 years, age range 18–58 years). There was no statistical significant difference in sex and age in each group enrolled.

Instruments and reagents

Phenotypic data was acquired by FACSCalibur (Becton-Dickinson, Franklin Lakes, NJ, USA). CD45/CD14, IgG1/IgG2a, CD3/CD19, CD3/CD4, CD3/CD8, CD3/CD16⁺CD56, bi-color straight bidding sheet clonal antibody and hemolytic agents were purchased from Becton Dickinson. CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD16⁺56⁺ represented total T cells, Th cells, Ts cells or destructive T, B and NK cells. Supplies of pH 7.2 (0.01 mol/L) contained 1 g/L nitrite sodium phosphate buffered saline. The enzyme that exempted the meter was the Alisei Quality, a completely automatic enzyme which exempts the analyzer, was bought from Italian SEAC (Calenzano,

Florence, Italy), while IL-15, IL-21 and the IgE examination reagent boxes were bought from R&D Systems (Minneapolis, MN, USA), eBioscience (San Diego, CA, USA) and the BioCheck Biology (Foster City, CA, USA), respectively.

Experimental methods

The specimen process was executed according to the reagent instruction booklet. Flow cytometry was used to determine and analyze. The FACScan flow cytometer was first prepared for sample analysis using CaliBRITE beads and AutoCOMP software. Whole blood samples were first stained with LeucoGATE (CD45/CD14) through lysing solution, and then run on the flow cytometer and analyzed with Simul-test IMK-Lymphocyte software. The LeucoGATE tube was used to gate lymphocytes. The software automatically collected a sufficient number of events to obtain 5000 lymphocytes within the lymphocyte gate. Enzyme-linked immunosorbent assay (ELISA) to examine was used strictly according to the reagent instruction booklet. The autologous serum skin test (ASST) was performed in CU patients, using i.d. skin test with 0.05 mL of sterile, fresh autologous serum and with histamine 10 µg/mL and 0.9% sterile saline as positive and negative controls, as described by Sabroe.³²

Statistical analysis

The results of normally distributed data were expressed as mean $\pm$ standard deviation. ANOVA followed by post-hoc Dunnett's T3 was applied to compare the data among the three study groups. An independent sample Student's *t*-test was applied to compare the data between positive and negative groups of ASST in CU patients. The correlations were assessed with Spearman correlation coefficients. $P < 0.05$ was taken as significant.

RESULTS

Patient characteristics

The general characteristics of the patients were as follows: symptom scores were AU 3.56 ± 0.91 and CU, 3.36 ± 0.75 ; and course was AU 10.58 ± 10.19 days and CU 36.23 ± 68.11 months. ASST positivity was found in 31 (nine men, 22 women) of 80 CU patients (38.75%).

Results of flow cytometry

No differences in the percentage of CD3⁺, CD4⁺, CD8⁺ T cells, B cells, or NK cells were detected between AU patients and normal controls ($P > 0.05$), as well as the ratio of CD4⁺ to CD8⁺ (data not shown). The percentage of NK cells was significantly higher in CU patients compared to controls ($P < 0.01$), as well as the ratio of CD4⁺ to CD8⁺ (data not shown). But CD8⁺ T cells significantly lower in CU patients compared to controls ($P < 0.01$). No significant differences in the percentage of CD3⁺, CD4⁺ T cells or B cells were observed between CU patients and normal controls ($P > 0.05$). The percentage of CD8⁺ T cells was significantly higher in AU patients compared to CU patients ($P < 0.01$), yet the ratio of CD4⁺/CD8⁺ was significantly lower (data not shown) ($P < 0.01$). The percentage of CD3⁺, CD4⁺, B cells and NK cells were not significantly different when AU and CU patients were compared ($P > 0.05$) (Fig. 1).

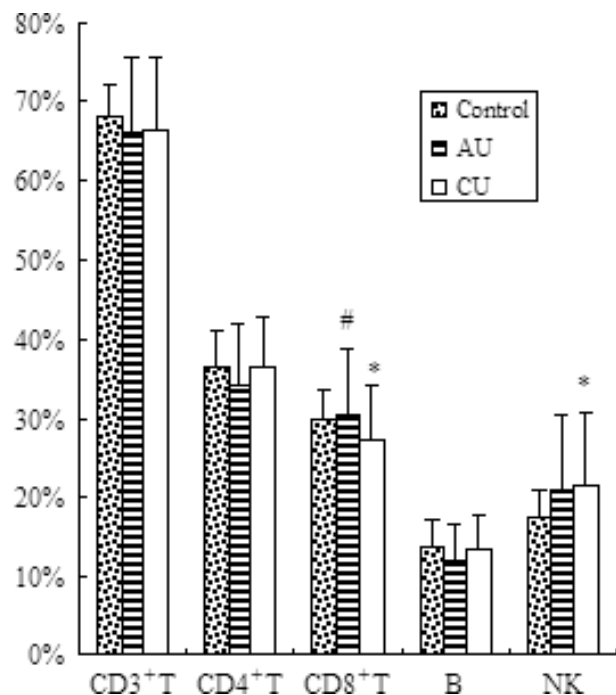


Figure 1. Result of subgroups of the T, B and natural killer (NK) lymphocyte in acute urticaria (AU), chronic urticaria (CU) and control groups. * $P < 0.05$ for control group vs AU or CU group; # $P < 0.01$ for AU vs CU group.

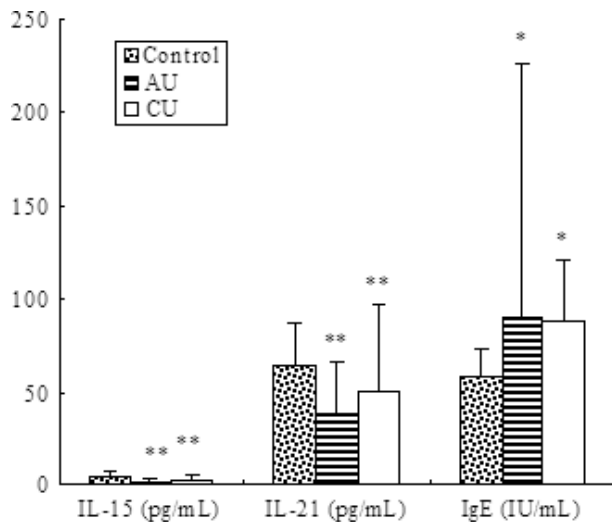


Figure 2. Serum levels of interleukin (IL)-15, IL-21 and immunoglobulin (IgE) in acute urticaria (AU), chronic urticaria (CU) and control groups. * $P < 0.05$ for control group vs AU or CU group; ** $P < 0.01$ for control group vs AU or CU group.

Results of ELISA

Notably, serum levels of IL-15 and IL-21 significantly decreased in AU and CU patients compared to controls ($P < 0.01$). However, the level of IgE was in contrast ($P < 0.05$). The serum levels of IL-15 and IgE were not significantly different when AU and CU patients were compared ($P > 0.05$) (Fig. 2).

Correlation analysis

No significant correlations were found between the percentage of CD3⁺, CD4⁺, CD8⁺ T cells, B cells, NK cells, CD4⁺/CD8⁺, serum levels of IgE, IL-15, IL-21 and clinical scores and course when individual AU patients were assessed ($P > 0.05$) (Table 1). Nor were any significant correlations found between the serum levels of IL-15, IL-21 and IgE in AU patients. Weak negative correlations were demonstrated between the percentage of CD3⁺, CD8⁺ T cells and scores of symptoms in CU patients ($r = -0.23$, -0.25 , $P < 0.05$). Significant positive correlations were found between the percentage of B cells and scores of symptoms and course in CU patients ($r = 0.49$, 0.65 , $P < 0.01$) (Table 2). A significant negative correlation was found between serum level of IL-21 and IgE ($r = -0.42$, $P < 0.01$), but not between IL-15 and IgE ($r = 0.14$, $P > 0.05$) in CU patients.

Differences between CU patients with a positive ASST and a negative ASST

A significant increase in the course (63.93 ± 82.12 vs 18.71 ± 51.96 months), percentage of CD3⁺ T cells ($69.55 \pm 8.05\%$ vs $64.05 \pm 9.27\%$), B cells ($15.85 \pm 5.09\%$ vs $12.02 \pm 3.29\%$) and serum levels of IL-21 (56.29 ± 26.85 vs 40.16 ± 20.63 pg/mL) were observed in CU patients with a positive ASST (ASST⁺)

Table 1. Correlation with subgroups of the T, B and natural killer (NK) lymphocyte and serum levels of interleukin (IL)-15, IL-21 and immunoglobulin (IgE) in acute urticaria patients

Subject	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺	B cells	NK cells	IL-15	IL-21	IgE
Score									
<i>r</i>	-0.04	-0.05	-0.06	-0.02	0.18	-0.11	-0.22	0.12	-0.09
<i>P</i>	0.69	0.67	0.57	0.85	0.11	0.34	0.05	0.25	0.45
Course									
<i>r</i>	0.07	-0.05	0.13	-0.18	-0.09	0.02	0.08	0.13	-0.07
<i>P</i>	0.56	0.66	0.24	0.30	0.45	0.86	0.47	0.25	0.54

Table 2. Correlation with subgroups of the T, B and natural killer (NK) lymphocyte and serum levels of interleukin (IL)-15, IL-21 and immunoglobulin (IgE) in chronic urticaria patients

Subject	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺	B cells	NK cells	IL-15	IL-21	IgE
Score									
<i>r</i>	-0.23	-0.09	-0.25	0.10	0.49	-0.00	-0.13	-0.08	0.10
<i>P</i>	0.04	0.43	0.03	0.36	0.00	0.99	0.27	0.50	0.38
Course									
<i>r</i>	-0.17	-0.06	-0.12	-0.03	0.65	-0.12	-0.09	-0.12	0.16
<i>P</i>	0.13	0.61	0.30	0.82	0.00	0.25	0.42	0.27	0.14

as compared to those with a negative ASST (ASST⁻), yet the serum levels of IgE significantly decreased (52.95 ± 25.24 vs 83.09 ± 62.46 pg/mL) ($P < 0.01$). No significant differences in the clinical scores (3.55 ± 0.68 vs 3.24 ± 0.78), percentage of CD4⁺ ($37.13 \pm 6.82\%$ vs $35.08 \pm 6.61\%$), CD8⁺ T cells ($26.45 \pm 7.59\%$ vs $27.69 \pm 6.83\%$), NK cells ($20.29 \pm 10.24\%$ vs $21.28 \pm 8.61\%$), ratio of CD4⁺/CD8⁺ (1.47 ± 0.59 vs 1.43 ± 0.60) or serum levels of IL-15 (2.12 ± 2.05 vs 2.61 ± 3.15) were observed between ASST⁺ and ASST⁻ patients ($P > 0.05$).

DISCUSSION

In this study, we examined the subgroups of the T, B and NK lymphocyte from peripheral blood. We demonstrated that the percentage of NK cells and the ratio of CD4⁺/CD8⁺ increased in CU patients. Furthermore, interestingly, we found that the increasing ratio of CD4⁺/CD8⁺ in CU patients was because the percentage of CD8⁺ decreased in CU patients. An impaired CD8⁺ T-cell development in allergic asthma had been found by Bratke.³³ In the present study, we found CD8⁺ T-cell subpopulations decreased in CU patients. It seems that CU is like a viral infection associated disease, but further investigations are needed. We also found weak correlations between CD3⁺, CD8⁺ T cells and the scores of symptoms in CU patients. Furthermore, we also found significant positive correlations between the percentage of B cells and scores of symptoms and course in CU patients. On the other hand, no significant change and correlation were found in AU patients. Our results exhibit the imbalances of the immune system at CD3⁺, CD8⁺ T cells, B cell and NK cell composition. These results prompt the difference of cellular immunity between AU and CU patients. On the basis of our results we suggest that the cellular immunity would be much more tightly connected with CU patients than AU patients.

B cells are traditionally considered to have a close relationship with urticaria as cytoplasmic cells' first somatic cells. Toubi discovered that proliferations of B cells in CU patients elevate and apoptosis rates drop.³⁴ Apoptosis plays a critical role in B-cell development and homeostasis and is tightly regulated by many cytokines, such as IL-15 and IL-21. We found

no significant differences in B cells of AU and CU patients and the normal control group, which was similar to Garmendia *et al.*'s experimental result.³⁵ The relevance between the constitution ratio of B cells and scores of symptoms and the course was positive in CU patients, but such a result could not be seen in AU patients. The reason might be that B cells gradually differentiate and multiply in CU along with the course, and affect patients' symptoms. Moreover, an inflammatory reaction and the increase of the present antigen in the early stages may make B cells apoptotic.

We also examined the serum levels of IL-15, IL-21 and IgE and found that IL-15 and IL-21 significantly decreased in AU and CU patients, but IgE increased. Moreover, we found a negative correlation between serum levels of IL-21 and IgE in CU patients. Our data indicate for the first time that patients with urticaria are characterized by reduced serum levels of IL-15 and IL-21. Moreover, the pattern and/or secretion of their Th17 cytokines were also altered. Our finding support the concept of an immune dysregulation resulting in cytokine imbalance underlying or associated with the development of urticaria. It was found that the serum IgE level in patients with chronic urticaria was higher than that of the normal healthy subjects.³⁶ Our results also indicated that serum levels of IL-15, IL-21 and IgE were of no significant difference between AU and CU patients, which implies that the serum levels of IgE, IL-15 and IL-21 are closely related to the pathogenesis of AU and CU. Moreover, the reduction of the levels of IL-15 and IL-21 relieved the suppression of the multiplication of B cells, triggering the increasing production of IgE, thus resulting in the disease occurring. IL-21 may induce the apoptosis of antigen special B lymphocyte.³⁷ So, the decrease of IL-21 might result in the B lymphocytes increasing along with the course of CU. Moreover, lower serum level of IL-15 and IL-21 of AU and CU patients, and the decreased expression of Th1 and Th17 cells, may play an important role in the pathogenesis of urticaria, particularly in CU patients. The reason may be that, as the expression of Th1 and Th17 cells decrease, the ability to suppress Th2 cells drop out, then the relative hyper-function of Th2 cells and promotion of the secretion of IgE may occur. Moreover, we found that percentage of NK cells

was significantly higher in CU patients as the IL-21 decrease. This supported Liu's research that IL-21 might suppress NK-cell activation and survival.³⁸

Furthermore, we found that ASST⁺ CU patients were more likely to be associated with longer course and higher percentage of CD3⁺ T cells, B cells and serum levels of IL-21, and lower serum levels of IgE. Chomiciene had showed that increased total IgE was seen in two-fifths of CU patients and was more often noted in patients without functional autoantibodies.³⁹ The serum levels of IgE of our patients was similar to Chomiciene's experimental results. The reason may be that the lack of IL-21 dramatically enhanced IgE isotype switch and antigen driven IgE⁺ cell clonal expansion.³⁰ IL-21 decrease, triggering the increased production of IgE, is more closely related to the pathogenesis of CU patients with ASST⁺.

Our report supports the concept of immunological dysregulation in urticaria, reflected by the systemic inflammatory profile in association with a disturbance of T-cell-induced cytokine production and the humoral immunity profile in association with a disturbance of B-cell-induced IgE production. At present, B-cell-targeted therapies in chronic autoimmune urticaria are caused increasingly by autoantibodies, which may be a more direct and effective therapy than neutralizing IgE with the hope of downregulating FcεRI expression.⁴⁰ Moreover, IL-15 and IL-21 are used in treatment of allergic diseases with certain effect.^{15,41} On the basis of these results, we propose that the design of successful therapy for urticaria may target a regulatory pathway linking B cells, T cells, IgE, IL-15 and IL-21.

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