

Correlation between T-Cell and Mast Cell Activity in Patients with Chronic Urticaria

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Key Words

Chronic urticaria · sIL2R · Tryptase · FcεRIα-specific autoantibody

Abstract

Background: The presence of autoantibodies reacting with the high affinity IgE receptor (FcεRIα) usually indicates a more severe form of chronic urticaria (CU). Previously, we showed an increased lymphocyte reactivity in CU patients; however, the relation between enhanced cellular immunity and the presence of anti-FcεRIα-specific autoantibodies has not been investigated. **Methods:** Cellular and humoral immune reactivity of 50 CU patients and 28 healthy controls was studied. Serum sIL-2R, neopterin, and tryptase levels were measured to assess T-cell, monocyte/macrophage and mast cell activity, respectively. *Helicobacter pylori* (HP)-specific IgG antibody, and IgE levels were also tested. Anti-FcεRIα-specific autoantibody was determined by Western blotting. In vivo histamine-releasing activity of patients' sera was assessed by the autologous serum skin test (AST). **Results:** 17/50 CU patients, who both had IgG-type anti-FcεRIα-antibodies by Western blotting and a positive AST response, were classified as autoimmune CU. All patients with CU had significantly higher serum sIL-2R and tryptase levels than healthy controls ($p = 0.000257$,

$p = 0.000166$, respectively), indicating T-cell and mast cell activation. Patients with higher sIL-2R levels also had higher tryptase levels; the strongest correlation was shown in the autoimmune subgroup of patients ($p = 0.688$, $p = 0.002$). There was a tendency towards higher tryptase levels in the autoimmune subgroup, as compared to the nonautoimmune CU patients. While the serum IgE was significantly lower in autoimmune than in nonautoimmune CU ($p = 0.000836$), there was no significant difference in their sIL-2R, neopterin and HP-specific IgG antibody levels. CU patients with a positive AST response (38/50) had significantly higher tryptase levels ($p = 0.0107$) when compared to the negative skin test group. **Conclusions:** The significant correlation between sIL-2R and tryptase levels in patients with CU indicates that T cell activation is proportional to mast cell degranulation in these patients. The increased level of tryptase in autoimmune CU may suggest more severe disease.

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Introduction

The pathogenesis of chronic urticaria (CU) is still unknown, although a number of theories exist. In recent years, histamine-releasing high-affinity IgE receptor alpha-chain (FcεRIα)-specific autoantibodies have been

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found in about one third to one half of patients. Based on the presence or absence of autoantibodies, CU patients can be subdivided into an autoimmune and a nonautoimmune group [1–7]. The presence of autoantibodies against the FcεRIα usually indicates more severe disease [8, 9]. Previously, we demonstrated an increased lymphocyte activation in CU patients by phytohemagglutinin (PHA) and *Helicobacter pylori* (HP) stimulation. We hypothesized that the increased T-cell activity is a result of the presence of autoreactive T-cells in CU [10]. To support this hypothesis, we set out to simultaneously detect markers of T-cell and mast cell activation in CU patients as a whole, as well as in different subgroups. As measures of cellular and humoral immune reactivity, serum sIL-2R, neopterin and tryptase (to assess T-cell, monocyte/macrophage and mast cell activity, respectively) as well as HP-specific IgG antibody and IgE levels (markers of humoral immunity) were determined in CU patients and healthy controls. The presence of anti-FcεRIα-specific autoantibody was studied by Western blotting as well. Histamine-releasing activity of the patients' sera was tested *in vivo* by skin inoculation of autologous serum.

Material and Methods

Patients

All patients with severe continuous CU were seen at a tertiary care referral center at the Department of Dermato-Venereology, Semmelweis University, Budapest. They have had wheals daily, or almost daily, for 6 weeks or longer. Urticaria vasculitis, or physical urticaria were excluded. A history was taken; duration and diurnal variation of urticaria, occurrence of angioedema (at least once a week), history of atopy and autoimmune diseases, presence of gastrointestinal symptoms were recorded. Routine laboratory parameters (total blood count, erythrocyte sedimentation rate, liver and kidney function test, and urinalysis), antinuclear antibody, thyroid antimicrobial antibody were determined, ¹³C urea breath test and AST were performed. Antihistamines were withdrawn 72 h before the study. No patients had been taking corticosteroids for at least 4 weeks. The patients were on a pseudoallergen-free diet (free of artificial additives, and of known naturally occurring pseudoallergens).

Serum samples from 50 patients with CU (11 males and 39 females, aged: 20–71 years; mean: 54.3) and 28 healthy controls (11 males and 17 females aged 16–62 years; mean 47.2) were collected and stored in aliquots at –20°C.

Methods

AST were performed by one of the authors, according to Sabroe [11]. The test qualified as positive, if the serum-induced red wheal was 1.5 mm greater than the saline-induced control at 30 min. Antihistamines were withdrawn 72 h before testing.

Serum sIL2R, HP-specific IgG antibody, IgE and tryptase levels were evaluated in 50 patients and 28 healthy controls, serum neopterin in 18 patients and 26 healthy controls. Serum neopterin, sIL-

2R, HP-specific serum IgG antibody, and IgE levels were determined by ELISA according to the manufacturers' instructions (ELitest Neopterin, Henning Berlin GmbH, Berlin; RIL-2s/ sIL-2R, Immunotech, Marseille, France; Varelisa *Helicobacter pylori* Abs, Pharmacia & Upjohn, Freiburg, Germany; and Sevatest ELISA IgE Micro II, Sevapharma, Praha, Czech Republic). The lower detection limits of the assays were as follows (in the order of the above list): 2 nmol/l, 5 pmol/l, 0.1 U/ml, 1 IU/ml. Serum total tryptase levels were detected with UniCap Tryptase Fluoro-enzyme immunoassay (Pharmacia & Upjohn AB, Uppsala, Sweden) with a lower limit of detection of 1 ng/ml. Since the literature reflects significant population differences in normal tryptase levels, we determined the normal range in the Hungarian healthy population in 28 controls, and found the mean ± SD to be 2.4 ± 2.8 ng/ml. Anti-FcεRIα-specific autoantibodies were determined by Western blotting in 50 patients and 9 healthy controls. This method was published by Fiebiger et al. [2]. Recombinant soluble (s) FcεRIα chain was genetically fused to human serum albumin. After 5 min of heat denaturation at 95°C, the purified protein migrated as a single 32-kD band. Recombinant FcεRIα or BSA control (10 µg/lane) was run on 10–13% gradient polyacrylamide gels (BioWhittaker Molecular Applications, Rockland, Me., USA). Gels were blotted onto PVDF membranes (Bio-Rad Laboratories) in a SEMI-PHOR dry blotting chamber (Hoefer Scientific Instruments), and were blocked with 5% dry milk/0.05% Tween 20 (Sigma Chemical Co.)/PBS for at least 6 h. To selectively denature the IgE molecule, the patients' serum samples were incubated for 3 h at 56°C. The PVDF membranes were incubated with the patients' sera (diluted 1:50), or with chimeric IgE (Serotec, Oxford, UK, diluted 1:1,000) positive control, as well as a positive patient control. An IgG2a isotype monoclonal antibody served as a negative control. The membrane-bound first-step human antibodies were detected with either horseradish peroxidase-labeled rabbit anti-human IgG (diluted 1:100,000) or rabbit horseradish peroxidase-labeled anti-human IgE (diluted 1:2,000, Jackson ImmunoResearch Laboratoris, Richmond, Calif., USA). The blot was then developed in an ECL system (Amersham International, UK), and exposed to Kodak X-Omat S films.

Statistical Analysis

Statistical analysis was performed using the multivariate ANOVA and Student-Newman Keuls post hoc test (Statistica for Windows 1999 Edition 5.5 StatSoft Inc.). Correlations between parameters were assessed by the Spearman Rank test (table 2, fig. 1, 2).

Results

Clinical Characteristics

The mean duration of continuous CU was 24.8 months (range 2–240). Angioedema occurred in 28/50 (56%) of the patients; 17/50 (34%) patients reported night/early morning diurnal variation of urticaria. Autoimmune diseases were reported by only 4/50 (8%) of the patients, including autoimmune thyroid disease, vitiligo, rheumatoid arthritis and Sjögren syndrome. A personal history of atopy was obtained in 7/50 (14%) of all patients. 31/50

Table 1. Mean ($\pm$ SD) serum levels of sIL-2R, neopterin, total IgE, HP-specific IgG antibodies, and tryptase of patients with chronic urticaria (subgroups: anti-Fc ϵ RI α -autoantibody-negative/positive and AST-negative/positive) and healthy controls

Groups	sIL-2R pmol/l	Neopterin nmol/l	HP antibody titer, U/ml	Total IgE IU/ml	Tryptase ng/ml
CU patients (50)	46.6 $\pm$ 26.5 (50)	6.6 $\pm$ 1.8 (18)	43.42 $\pm$ 59.9 (50)	116.4 $\pm$ 266.3 (50)	7.1 $\pm$ 5.5 (50)
Fc ϵ RI α -Ab-positive CU (17)	51.4 $\pm$ 22.3 (17)	6.0 $\pm$ 1.41 (6)	41.9 $\pm$ 58.13 (17)	21.9 $\pm$ 24.3 (17)	8.2 $\pm$ 5.7 (17)
Fc ϵ RI α -Ab-negative CU (33)	44.1 $\pm$ 28.5 (33)	6.9 $\pm$ 2.0 (12)	44.26 $\pm$ 61.78 (33)	159.4 $\pm$ 312.8 (33)	6.6 $\pm$ 5.4 (33)
AST-negative CU patients (12)	46.12 $\pm$ 34.81 (12)	6.66 $\pm$ 0.57 (3)	53.96 $\pm$ 85.80 (12)	237 $\pm$ 461.4 (12)	4.97 $\pm$ 4.41 (12)
AST-positive CU patients (38)	46.7 $\pm$ 23.96 (38)	6.6 $\pm$ 2.02 (15)	44.21 $\pm$ 52.62 (38)	76.28 $\pm$ 147.33 (38)	7.82 $\pm$ 5.68 (38)
Healthy controls (28)	26.2 $\pm$ 12.4 (28)	5.8 $\pm$ 1.7 (26)	44.34 $\pm$ 62.84 (28)	63.5 $\pm$ 91.3 (28)	2.4 $\pm$ 2.8 (28)

For pairwise comparison, multivariate ANOVA and the Student-Newman Keuls post hoc tests were used. Significance (p) between groups: CU vs. healthy controls, p = 0.000257, significant for sIL-2R, and p = 0.000166 for tryptase; Fc ϵ RI α Ab+ CU vs. Fc ϵ RI α - CU, p = 0.000836, significant for total IgE; AST- CU vs. AST+ CU, p = 0.0107, significant for tryptase.

Table 2. Correlations between serum sIL-2R and tryptase levels in patients with CU (Fc ϵ RI α -autoantibody-negative/positive and AST-positive subgroups)

Groups	Correlation coefficient
CU patients (50)	Spearman ρ = 0.53 p = 0.00007
Fc ϵ RI α -Ab-positive CU (17)	Spearman ρ = 0.69 p = 0.0022
Fc ϵ RI α -Ab-negative CU (33)	Spearman ρ = 0.44 p = 0.01
AST-positive CU patients (38)	Spearman ρ = 0.56 p = 0.000233

Numbers of patients in the different groups are shown in parentheses.

(62%) of the patients complained of epigastric pain, while 21/50 (42%) of them had IgG anti-HP-specific antibody. In 17/21 (81%) of the HP seropositive patients with CU, the infection was confirmed by a ^{13} C-urea breath test. Routine laboratory parameters were within the normal range. Antinuclear antibody and thyroid microsomal antibody were found in 9/50 (18%), and 7/50 (14%) of patients, respectively. There were no differences between patients with autoimmune and non-autoimmune CU in the mean duration of urticaria, the occurrence of angioedema or autoimmune diseases, incidence of atopy, gastrointestinal complaints, or antinuclear antibody positivity. A trend towards increased incidence of thyroid antimicrosomal antibody 5/17 (29%) was found in CU patients with Fc ϵ RI α -specific autoantibodies, as compared to the

nonautoimmune group (2/33; 6%). None of the patients had clinical evidence of thyroid disease.

Laboratory Data

CU patients had significantly higher serum sIL-2R (46.6 $\pm$ 26.5 vs. 26.2 $\pm$ 12.4 pmol/l) and tryptase levels (7.1 $\pm$ 5.5 ng/ml vs. 2.4 $\pm$ 2.81 ng/ml) than did healthy controls (p = 0.000257 and p = 0.000166), respectively. There were no significant differences in serum IgE (116 $\pm$ 266 vs. 63.5 $\pm$ 91.3 IU/ml), and neopterin levels (6.6 $\pm$ 1.8 vs. 5.8 $\pm$ 1.7 nmol/l), or in the concentration of HP-specific circulating antibodies (43.42 $\pm$ 59.9 vs. 44.34 $\pm$ 62.84 U/ml) between the CU and healthy control groups (table 1). Patients with higher serum sIL-2R levels also had higher serum tryptase levels, and the correlation was statistically significant (Spearman ρ = 0.53, p = 0.00007) (table 2, fig. 1).

In our study, (38/50) 76% of patients with CU showed in vivo histamine-releasing activity by AST. One third (17/50) of the patients and 0/9 normal controls had IgG-type anti-Fc ϵ RI α reactivity by Western blot. All Fc ϵ RI α -antibody-positive patients had a positive AST response and, thus, were determined to have autoimmune CU (17/50).

Serum IgE level was significantly lower in patients with autoimmune CU (21.9 $\pm$ 24.3 IU/ml) compared to the nonautoimmune patients (159.4 $\pm$ 312.8 IU/ml, p = 0.000836). There were no significant differences between the autoimmune and non-autoimmune subgroups with regard to the sIL-2R (51.4 $\pm$ 22.3 vs. 44.1 $\pm$ 28.5 pmol/l) and neopterin (6.0 $\pm$ 1.41 vs. 6.9 $\pm$ 2.0 nmol/l) or to the concentration of circulating HP-specific IgG antibody levels (41.9 $\pm$ 58.13 vs. 44.26 $\pm$ 61.78 U/ml) (table 1).

1

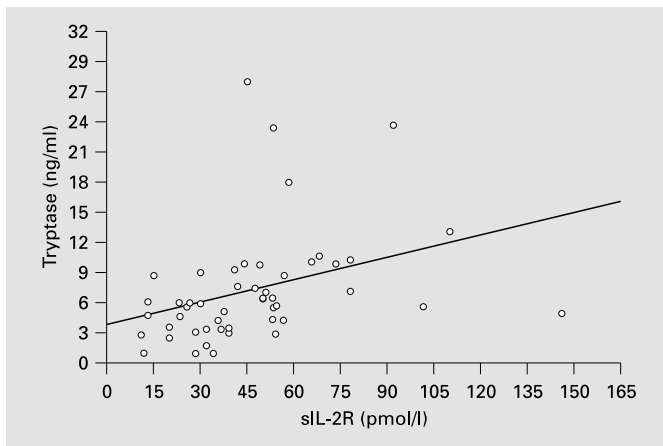
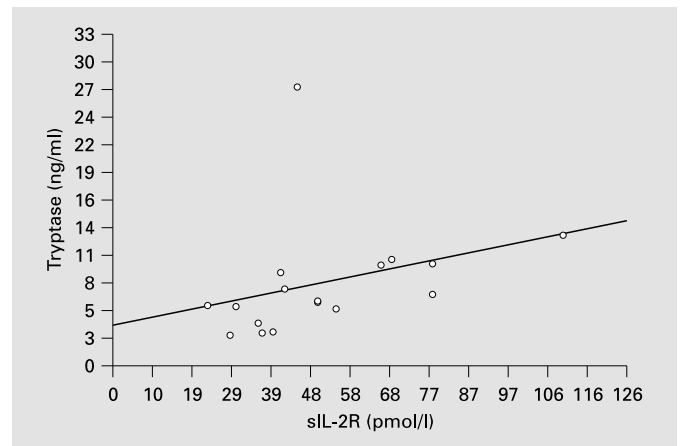


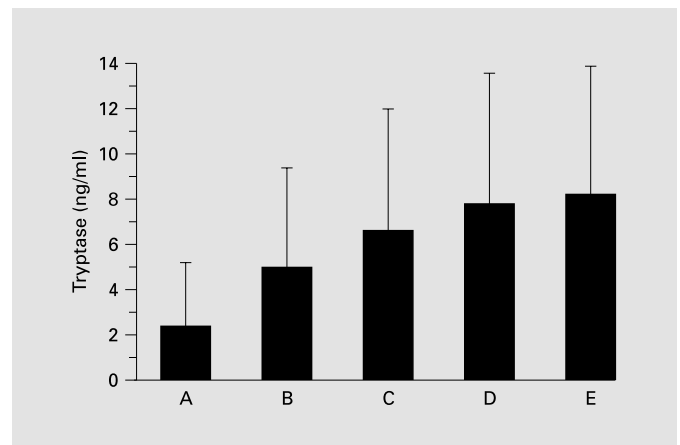
Fig. 1. Correlation between serum sIL-2R and tryptase levels in patients with CU (Spearman $\rho = 0.53$ $p = 0.00007$).

Fig. 2. Correlation between serum sIL-2R and tryptase levels in patients with Fc ϵ RI α -autoantibody-positive CU (Spearman $\rho = 0.69$, $p = 0.0022$).

Fig. 3. Mean ($\pm$ SD) serum tryptase levels (ng/ml) of healthy subjects and patients with CU (Fc ϵ RI α autoantibody-negative/positive and AST-negative/positive subgroups). A = Healthy subjects; B = AST-negative patients; C = Fc ϵ RI α -specific-autoantibody-negative patients; D = AST-positive patients; E = Fc ϵ RI α -specific-autoantibody-positive patients. Tryptase levels tended to increase with the highest mean value in the Fc ϵ RI α -specific-autoantibody-positive subgroup.



2



3

There was a tendency to higher tryptase levels in the autoimmune subgroup (8.2 ± 5.7 vs. 6.6 ± 5.4 , fig. 3). Also in patients with autoimmune CU, there was a strong positive correlation between serum tryptase and sIL-2R levels (Spearman $\rho = 0.69$, $p = 0.0022$, table 2, fig. 2).

In the patient population, the 38/50 AST-positive patients had a lower serum IgE level compared to the skin-test-negative group (76.28 ± 147 vs. 237 ± 461.4 IU/ml), although the difference did not quite reach statistical significance. Patients with in vivo histamine-releasing activity had significantly higher tryptase levels (7.82 ± 5.68 vs. 4.97 ± 4.41) ($p = 0.0107$) when compared to the negative skin test group (table 1, fig. 3).

Discussion

The main goal of our cross-sectional study was to simultaneously investigate cellular and humoral immunity of patients with CU, and determine possible differences

between the autoimmune and nonautoimmune subgroups.

Immunoblot analysis of high affinity IgE receptor alpha chain (Fc ϵ RI α)-specific autoantibodies is a widely accepted method; however, nonfunctional autoantibodies can be detected in other skin-related immune diseases by this assay [1]. Fiebiger et al. [2] found autoantibody positivity in 37% of CU patients, most of them with in vitro functional activity. Opinions about functional versus binding assays, as well as about the relationship between positive patient groups widely differ, and the choice of test depends on local expertise. Kikuschi et al. [3] prefer a functional assay, the basophil histamine-release test, as a screening method. In a recent publication, Sabroe et al. [12] concluded that patients with histamine-releasing autoantibodies against either the Fc ϵ RI α , or IgE, as well as patients with a mast-cell-specific histamine-releasing factor had more severe CU than patients without such serum activity. Furthermore, the authors suggest that degranulation of mast cells rather than basophils is important in

determining CU severity. A good correlation of AST with disease severity was published previously [8]. In our experiments, all patients with positive immunoblot results had *in vivo* histamine-releasing activity by AST. Functional activity of sera from 11 patients with negative immunoblot but positive AST results may be due to other histamine-releasing factors, anti-IgE autoantibodies, cytokines, enzymes or complement fragments [3]. To further assess the activity of mast cells, the major primary effector cell in CU, a specific protein, tryptase, was measured. Tryptase, a neutral protease, is selectively concentrated in mast cells, is released by degranulation along with histamine, and induces leukocyte infiltration, possibly via complement activation [13, 14]. In a recently published study, higher tryptase levels were found in anaphylactic patients with urticaria than in those without, and the extent of urticaria was a predictor of elevated tryptase [15]. We have shown higher levels of tryptase in sera of patients with CU, predominantly in the autoimmune and the AST-positive subgroups (fig. 3), indicating that patients with autoimmune CU have a more extensive mast cell activation. However, histological studies did not find significant differences in the intensity of cutaneous inflammatory cell infiltrate and lesional cytokine pattern (IL-4, IL-5 and IFN- γ) between patients with autoimmune and nonautoimmune CU [16, 17]. Previously, we demonstrated an increased peripheral lymphocyte reactivity in patients with CU [10]. In the present study, all CU patients, irrespective of the presence or absence of Fc ϵ RI α -specific autoantibodies, had significantly higher serum sIL-2R concentration, indicating T-cell activation in this disorder. Thus, T-cell activation in CU may not be related to the presence of autoantibodies.

Serum neopterin, a good marker of cellular immune activation, was also evaluated in our patients. Serum neopterin is produced by activated monocytes and macrophages in response to IFN- γ released from antigen-stimulated CD4-positive T-cells. Psoriasis, dermatomyositis, and systemic lupus erythematosus are examples of autoimmune diseases with usually elevated neopterin levels [18, 19]. Neopterin levels were within normal range in our CU patients, suggesting that monocytes and macrophages are not the primary effectors in the pathogenesis of CU. sIL-2R is a proteolytic fragment of the membrane IL-2R molecule. sIL-2R levels are elevated in various inflammatory and autoimmune conditions [20]. sIL2R levels are elevated as well in many inflammatory skin diseases, such as psoriasis, eczema, and erythroderma [21, 22].

Similar to Sabroe et al. [8], we measured significantly lower serum IgE levels in the Fc ϵ RI α -antibody-positive

urticaria subgroup compared to the seronegative group. This may reflect a shift towards Th1 predominance in autoimmune CU, as suggested by Fiebiger et al. [1], although more recent data suggest a Th0 profile [17].

In summary, not much is known about the connection between T-cell activation and mast cell degranulation in CU. In the current paper, we demonstrate a close correlation between serum sIL-2R and tryptase levels in CU patients, not only indicating that both T cell activation and mast cell degranulation are characteristic of the disease, but also that they might be related events. Also, in patients with autoimmune CU, there was a strong positive correlation between serum tryptase and sIL-2R levels (fig. 2). Although the rate of cellular immune activation is elevated in both autoimmune and non-autoimmune CU, as reflected by the high serum sIL-2R, the mast cell activation is more pronounced in patients with autoimmune-CU, which might be taken as evidence that patients with autoimmune CU have more severe disease.

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