

Association of TG2 from mast cells and chronic spontaneous urticaria pathogenesis



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ABSTRACT

Background: Mast cells and their mediators play important roles in chronic spontaneous urticaria (CSU) pathogenesis. Transglutaminase 2 (TG2) is expressed in activated mast cells and contributes to airway inflammation in allergic asthma.

Objective: To investigate the role of TG2 in CSU.

Methods: Patients with CSU (n = 72) and healthy controls (n = 51) were evaluated. Skin biopsy specimens were obtained from 5 patients with CSU and 2 healthy controls. Cord blood–derived human mast cells and peripheral blood–derived human mast cells were activated with IgE. TG2 activity and inflammatory mediators, such as histamine, leukotriene C4, and cytokines, were measured in serum or supernatant from cultured mast cells by enzyme-linked immunosorbent assay. Colocalization of mast cells and TG2 was determined in skin tissues by immunofluorescence.

Results: TG2 activity was significantly higher in serum samples from patients with CSU than in serum samples from healthy controls ($P < .001$). Colocalization of mast cell surface marker c-kit and TG2 was significantly increased in the lesional skin of patients with CSU compared with that in healthy controls. The levels of histamine, leukotriene C4, tumor necrosis factor α , transforming growth factor β , and interleukins 4, 5, and 6 were significantly higher in patients with CSU than in healthy controls ($P < .001$). Serum TG2 levels had positive correlations with each inflammatory mediator ($P < .001$). TG2 activity was increased in cord blood–derived human mast cells (CBMCs) and peripheral blood–derived human mast cells activated with IgE compared with those without activation ($P < .05$).

Conclusion: Our findings suggest that TG2 expressed in and released from mast cells plays an important role in CSU pathogenesis.

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Introduction

Chronic spontaneous urticaria (CSU) is defined by recurrent hives lasting more than 6 weeks and affects 0.5% to 5% of the general population.^{1,2} Although the exact underlying cause of CSU has not been identified, an autoimmune mechanism, termed *chronic autoimmune urticaria*, is suggested in half of patients with CSU.^{1–4} Approximately 35% to 45% of patients with CSU have

circulating IgG autoantibodies against IgE and/or against the high-affinity IgE receptor α , which activate cutaneous mast cells and basophils.^{1–4} Chronic autoimmune urticaria can be diagnosed using a combination of autologous serum skin test (ASST), basophil mediator release assay, and immunoassay for anti-IgE and/or anti-high-affinity IgE receptor α antibody in clinical practice.^{3,4} Mast cells are major effector cells in urticaria. However, besides the involvement of autoantibodies, the cause of the spontaneous activation of mast cells in CSU remains unknown.

Transglutaminases are a family of structurally and functionally related proteins that catalyze the Ca^{2+} -dependent posttranslational modification of proteins by deamidation and amine incorporation.^{5,6} The biological functions of TG2, which is the most widely distributed transglutaminase among tissue and cell types in humans, include scaffolding of the cytoskeleton to maintain membrane integrity, regulation of cell adhesion, and modulation of

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signal transduction.⁶ TG2 has been implicated in various inflammatory conditions, such as rheumatoid arthritis, allergic asthma, exercise-induced bronchoconstriction, pulmonary fibrosis, and celiac disease.^{7–11} TG2 also serves as a key initiator of airway inflammation in allergic asthma by activating the T_H2 immune response via the induction of interleukin (IL) 33 expression in airway epithelial cells¹² and induces eicosanoids or inflammatory cytokines by activating phospholipase A₂ (PLA₂) and nuclear factor (NF)- κ B, respectively.^{8,9} Recently, we found that TG2 expressed in mast cells increased IgE production and mediator release by interacting with mast cells and B cells via CD40/CD40L in a murine asthma model¹³ and that TG2 expressed or activated in mast cells from skin and bone marrow contributed to the development of systemic mastocytosis.¹⁴ Thus, it can be inferred that TG2 may have a role in CSU in which mast cells are key effector cells.

Cytokines and chemokines from other inflammatory cells promote immune cell recruitment and activation and play an integral role in the coordination and persistence of chronic inflammation. The roles of circulating cytokines and chemokines, such as IL-4, IL-6, IL-13, IL-18, interferon γ , thymic stromal lymphopoietin (TSLP), and eotaxin, have been investigated in the pathogenic mechanism of CSU and for the development of useful biomarkers for its diagnosis, treatment, and prognosis.^{15–21} However, no cytokines or chemokines have a consistent association with CSU. In one study, IL-17, IL-23, and tumor necrosis factor α (TNF- α) were elevated in patients with CSU compared with healthy controls, and they were significantly associated with disease activity and a positive response to the ASST.²² However, these results have not been replicated in other studies. It has been reported that cytotoxic T cells, mainly the T_C1 and T_C17 subsets, were involved in chronic urticaria, especially in the ASST-positive group.²³ Peripheral blood mononuclear cells from patients with CSU had increased production of cytokines and chemokines and expression of adhesion molecules.^{15,17,24,25}

In this study, we examined the role of TG2 in CSU by measuring TG2 activity and various inflammatory mediators in serum samples from patients with CSU and healthy controls. We also investigated whether TG2 is expressed in mast cells from the lesional skin of patients with CSU and whether it is released from cultured human mast cells.

Methods

Study Participants

Seventy-two patients with CSU (40.3% male; mean [SD] age, 37.3 [12.0] years) and 51 healthy controls (17.6% male; mean [SD] age, 29.5 [8.9] years) were enrolled. CSU was diagnosed by the daily appearance of wheals and an associated itching sensation that lasted at least 6 weeks, after all known causes of chronic urticaria were ruled out by medical history and physical and laboratory evaluations. We excluded patients with clinical evidence of urticaria vasculitis and physical urticaria only, such as cholinergic urticaria or cold urticaria. In most cases, serum total IgE and antinuclear and antithyroid antibodies, including anti-thyroid peroxidase (reference range, <75 IU/mL), antithyroglobulin (reference range, <150 IU/mL) and anti-thyrotropin receptor antibodies (reference range, <150 IU/mL), were evaluated.

Atopy was defined as 1 or more positive reactions to 12 common inhalant allergens by allergy skin prick tests or simultaneous multiple-allergen tests (AdvanSure AlloScreen inhalant panel; LG, Seoul, South Korea): *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, birch, oak, grass mix, ragweed, mugwort, Japanese hop, *Alternaria*, *Aspergillus*, and dog and cat epithelium.

This study was approved by the institutional review board of Hallym University Dongtan Sacred Heart Hospital, and all participants provided written informed consent.

Assessment of Disease Severity

Patients were divided into 4 subgroups by disease severity, which was determined according to the level of treatment, modified from the guidelines for the treatment of chronic urticaria²⁶: level 1, well controlled by 1 or 2 second-generation antihistamines; level 2, well controlled by 3 or more second-generation antihistamines; level 3, well controlled by the addition of leukotriene receptor antagonist and/or histamine₂-antagonist; and level 4, patients whose urticaria was not adequately controlled with level 3 treatment and who were additionally treated with cyclosporine or omalizumab.

Autologous Serum Skin Test

The ASST was performed following the method recommended by European Academy of Allergology and Clinical Immunology/Global Allergy and Asthma European Network at least 3 days after discontinuing use of all medications for treating urticaria, including antihistamines and corticosteroids,²⁷ and the remaining serum was preserved at -80°C until the measurements were performed. Briefly, venous blood was collected in sterile glass tubes and allowed to clot at room temperature for 30 minutes. Serum was separated by centrifugation at 500g for 10 minutes. Fifty microliters of autologous serum was injected intradermally into the patients' forearm to perform the ASST. Skin prick tests with 10 mg/mL of histamine and intradermal tests with 0.9% sterile saline were used as positive and negative controls, respectively. The diameter of a wheal was calculated as the mean of the 2 longest perpendicular wheal diameters. The ASST result was considered positive when a serum-induced wheal had a diameter 1.5 mm greater than that of the negative control surrounded by erythema at 30 minutes.

TG2 Activity Assay

The TG2 activity in serum or the supernatant of culture media (for cultured human mast cells) was determined using the TG-Covtest kit (Covalab, Villeurbanne, France).¹³ Briefly, microplates were coated with 200 μL of purified anti-TG2 antibodies overnight, supplemented with human serum (100 μL) or culture medium supernatant, and then incubated for 2 hours. After incubation, each well was treated with 100 μL of streptavidin–horseradish peroxidase and 50 μL of horseradish peroxidase substrate, and then the absorbance was read at 450 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader. TG2 activity was calculated via standard curves generated using a specific TG2 standard and is expressed in microunits per milligram of protein. All assays were performed in triplicate. The lowest detection limit for TG2 activity was 0.59 $\mu\text{U}/\text{mg}$.

Determination of Mast Cells or Eosinophils in Skin Tissues

Skin tissues were obtained from urticarial lesions on the trunk or a proximal extremity in 5 of the patients with CSU by punch biopsy. Normal skin tissues were donated from patients who underwent benign abdominal surgery ($n = 2$). Skin tissues fixed immediately in 4% paraformaldehyde solution were prepared by freezing and slicing and were sectioned at 3 μm . The skin tissue sections were stained with May–Grünwald Giemsa (Merck, Darmstadt, Germany) and toluidine blue (Sigma-Aldrich, St Louis, Missouri). The numbers of mast cells and eosinophils located within the dermis were determined at 10 sites that measured 200 $\times$ 200 μm in each slide under a microscope (Axiophot; Carl Zeiss, Oberkochen, Germany).⁹

Immunofluorescence

Skin tissue sections (3 μm) were washed in ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in

methanol for 5 minutes. Slides were blocked with goat serum for 1 hour and then incubated with antibodies against c-kit (green) and TG2 (red) in blocking solution overnight (1:25 dilution for antibodies). The slides were treated with the corresponding fluorescein isothiocyanate- or Texas Red-conjugated anti-IgG (1:100; Bethyl Laboratories, Montgomery, Texas) for 1 hour at room temperature. Washing steps between each stage were performed for 1 to 2 min. After washing, the slides were mounted using aqueous mounting medium (Thermo Shandon, Pittsburgh, Pennsylvania) and examined using a confocal microscope (Carl Zeiss).¹⁴

Measurement of Histamine, Cytokines, or Leukotriene C₄ by ELISA

Histamine or individual cytokines (TNF- α , transforming growth factor β [TGF- β], IL-4, IL-5, and IL-6) were identified in human serum samples using human ELISA kits (Cayman Chemical, Ann Arbor, Michigan, and Life Technologies, Gaithersburg, Maryland, respectively), in accordance with the manufacturers' instructions. The resultant color intensity was read at 405 nm for histamine or 450 nm for the individual cytokines using an ELISA plate reader. The lowest detection limit was 0.39 ng/mL for histamine, 15.6 pg/mL for TNF- α , 31.2 pg/mL for TGF- β , and 7.8 pg/mL for IL-4, IL-5, and IL-6.^{11,12} The leukotriene C₄ (LTC₄) contents in human serum samples were determined using the human LTC₄ ELISA kit (Antibodies Online, Aachen, Germany), in accordance with the manufacturer's instructions. The lowest detection limit for LTC₄ was 50 pg/mL.¹³

Preparation and Activation of Cultured Human Mast Cells

For developing primary cultured human mast cells, cord blood was taken from 3 healthy donors, and peripheral blood was taken from 3 CSU patients. Cord blood-derived human mast cells (CBMCs) and peripheral blood-derived human mast cells (PDBMCs) were developed from CD34⁺ progenitors isolated from cord blood and peripheral blood, respectively, using the CD34 MicroBead Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), as described previously,^{28,29} with a few modifications in the case of PDBMC culture.³⁰ Briefly, isolated CD34⁺ progenitor cells were grown in complete StemPro medium (Invitrogen, Carlsbad, California) supplemented with 10 ng/mL of IL-3 (only for the first week of culture; Peprotech, London, England), 100 ng/mL of stem cell factor, 10 ng/mL of IL-6 (50 ng/mL for PDBMCs; Peprotech), 2 mM L-glutamine (Sigma-Aldrich), 100 IU/mL of penicillin, and 100 μ g/mL of streptomycin (Sigma-Aldrich) for 6 to 8 weeks. Mast cell maturity and purity were evaluated by staining for a mast cell-specific protease, tryptase, for which a level greater than 95% was used to define mature cells ready for the experiments.

For mast cell activation, 1-mL cell suspension at a concentration of 1×10^6 /mL in complete culture medium was placed in each well of a 6-well culture plate. Cells were sensitized with biotin-conjugated IgE at a concentration of 2 μ g/mL (Enzo Life Sciences Inc, Farmingdale, New York) for 24 hours. Then, the cells were stimulated with 500 ng/mL of streptavidin-peroxidase (Sigma-Aldrich) for another 24 hours in an incubator (37°C, 5% carbon dioxide, 21% oxygen). After centrifugation, cell supernatants were collected and stored at -80°C until the measurement of TG2.

Statistical Analysis

Data are presented as means (SD) or median (interquartile range). Comparisons between the 2 groups were performed using χ^2 and Fisher exact tests for categorical variables and the *t* test for continuous variables. Penalized logistic regression was used for the control of age and sex as covariates. Correlations were calculated using the Spearman correlation coefficient. *P* < .05 was considered statistically significant. Statistical analyses were performed using SPSS statistical software, version 21 (SPSS Inc, Chicago, Illinois).

Table 1

Clinical Characteristics of the Patients With Chronic Spontaneous Urticaria

Characteristic	Finding (n = 72) ^a
Age, mean (SD), y	38.4 (13.00)
Male	29 (40.3)
Disease duration, median (IQR), mo	4.0 (2.0–12.0)
Presence of dermatographism	15/66 (22.7)
Presence of angioedema	25/44 (36.2)
Presence of atopy	31/49 (63.3)
Total IgE, median (IQR), kU/L (n = 67)	190.0 (88.0–329.0)
Blood eosinophils, median (IQR), /mm ³ (n = 67)	120.0 (80.0–210.0)
Antithyroid antibody positive, any	13/72 (18.1)
Antithyroglobulin antibody	6/61 (9.8)
Antithyroid peroxidase antibody	8/61 (13.1)
Antithyrotropin receptor antibody	5/58 (8.6)
ANA positive	1/65 (1.5)
ASST positive	35/63 (55.6)
Treatment level (n = 70)	
1	47 (67.1)
2	13 (18.6)
3	6 (8.6)
4	4 (5.7)

Abbreviations: ANA, antinuclear antibody; ASST, autologous serum skin test; IQR, interquartile range.

^aData are expressed as number (percentage) of patients unless otherwise indicated. In the case of missing data, the number of patients for whom data are available is indicated next to the value.

Results

Characteristics of the Study Participants

The characteristics of the patients are summarized in Table 1. Twenty-five patients with CSU (36.2%) had recurrent angioedema, and 63.3% of the patients were atopic. Thirty-five patients (55.6%) had a positive reaction on ASST. Thirteen patients (18.1%) had a positive response to at least 1 of the antithyroid antibodies, such as antitransglutaminase, anti-thyroid peroxidase, and anti-thyrotropin receptor antibodies. Six of 10 patients with CSU (60%) with a positive response to antithyroid antibodies had positive ASST results, and 6 of 29 CSU patients (20.7%) with ASST positivity also tested positive for antithyroid antibodies. Forty-seven patients (67.1%) had conditions that were well controlled with 1 or 2 antihistamines, and 5.7% of the patients had refractory chronic urticaria.

Serum TG2 Activity in Patients With CSU

TG2 activity was significantly increased in the serum samples of patients with CSU compared with those in the healthy controls (255.5 [74.48] vs 26.7 [8.10] μ U/mg, *P* < .001) (Fig 1). The *P* value remained significant after controlling for age and sex using penalized logistic regression (*P* < .001). However, serum TG2 activity did not differ between the ASST-positive and -negative groups (263.3 [70.10] vs 232.2 [77.93] μ U/mg, *P* > .05). Serum TG2 activity did not differ according to the level of treatment.

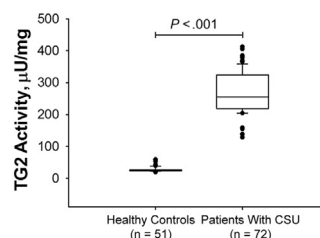


Figure 1. Serum TG2 activity in the study participants. CSU indicates chronic spontaneous urticaria.

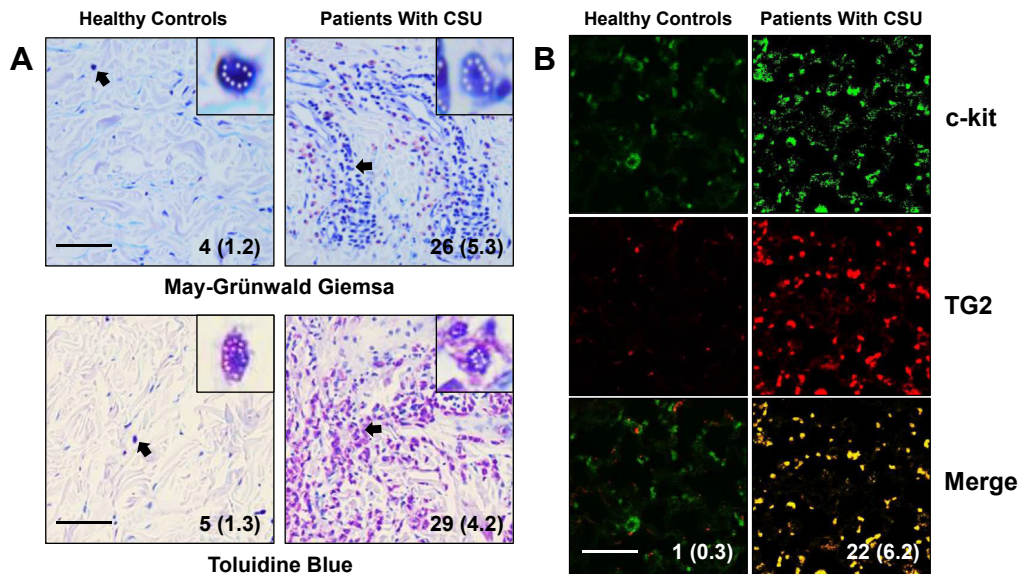


Figure 2. A, May-Grünwald Giemsa and toluidine blue staining for mast cells in the dermis of patients with chronic spontaneous urticaria (CSU) and healthy controls (original magnification $\times 400$). Small boxes in the upper right corner indicate enlarged mast cells (original magnification $\times 1,000$). An arrow indicates mast cells. Scale bar in healthy controls represents $50 \mu\text{m}$. Values in the lower right corner indicate the numbers of mast cells. B, Colocalization of c-kit (surface marker of mast cells) and TG2 in the skin tissues of patients with CSU and healthy controls. Colocalization (yellow) of c-kit (green) and TG2 (red) was determined by confocal microscopy. One representative experiment is shown from 2 healthy controls and 5 patients with CSU. Values in the lower right corner indicate the number of colocalized cells.

TG2 Expression of Mast Cells in the Skin of Patients With CSU

Skin biopsy specimens were obtained from the lesional skin of 5 patients with CSU (4 male; mean [SD] age, 46.2 [13.2] years; range, 31–64 years) who had various disease duration and severity. Mean (SD) duration of CSU was 30 (13.2) months (range, 2–120 months). Two of them were treatment level 4 (both treated with cyclosporine), 1 was level 2, and the other 2 were level 1.

There was marked infiltration of mast cells (26 [5.3] vs 4 [1.2] cells by May-Grünwald Giemsa staining; 29 [4.2] vs 5 [1.3] cells by toluidine blue staining) or eosinophils (11 [6.1] vs 0 [0.8] cells by May-Grünwald Giemsa staining) into the dermis of the lesional skin in patients with CSU compared with that of normal skin in the healthy controls (Fig 2A). However, mast cells infiltrated into the lesional skin more than eosinophils did. The mast cell surface marker c-kit (green) and TG2 (red) were colocalized in the dermis of lesional skin. Moreover, the colocalization (22 [6.2] vs 1 [0.3] cells) of c-kit and TG2 was increased in the lesional skin in patients with CSU compared with that in the healthy controls (Fig 2B).

In Vitro Study of TG2 Release in Human Mast Cells by IgE Activation

We performed an in vitro study using primary human mast cells to determine whether activated mast cells released TG2. TG2 activity (1.0 [0.17] vs 0.5 [0.23] $\mu\text{U}/\text{mg}$, $P < .01$) was significantly higher in the supernatant of CBMCs after IgE activation than in controls. Furthermore, TG2 activity (0.5 [0.06] vs 0.2 [0.06] $\mu\text{U}/\text{mg}$, $P < .05$) was significantly increased in the supernatant of activated PBDMCs compared with that of controls (Fig 3). These findings imply that TG2 can be released by activated mast cells.

Inflammatory Mediators in Patients With CSU

We measured various inflammatory mediators, such as a preformed granular mediator (histamine), a newly synthesized lipid mediator (LTC_4), and cytokines ($\text{TNF-}\alpha$, $\text{TGF-}\beta$, IL-4, IL-5, and IL-6), which are released from activated mast cells. The serum concentrations of all mediators were significantly higher in patients with CSU than in the healthy controls (all $P < .001$) (Fig 4). Each P value remained significant after controlling for age and sex

using penalized logistic regression (all $P < .001$). None of the inflammatory mediators, except $\text{TNF-}\alpha$, differed significantly between the serum levels of the ASST-positive and -negative groups (all $P > .05$). The serum level of $\text{TNF-}\alpha$ was significantly higher in the ASST-positive group than in the ASST-negative group (412.6 [22.57] vs 389.3 [43.87] pg/mL , $P < .05$). None of the inflammatory mediator serum levels differed according to the level of treatment.

Correlations Between TG2 and Other Inflammatory Mediators in CSU Patient Serum Samples

Serum TG2 levels were significantly positively correlated with the serum levels of histamine, LTC_4 , $\text{TNF-}\alpha$, $\text{TGF-}\beta$, IL-4, IL-5, and IL-6 (all $P < .001$) (Fig 5). TG2 and other inflammatory mediators were not correlated with the disease duration, peripheral blood eosinophil count, or serum total IgE level in patients with CSU.

Discussion

In the present study, we found that TG2 and various inflammatory mediators, including histamine, LTC_4 , $\text{TNF-}\alpha$, $\text{TGF-}\beta$, IL-4, IL-5, and IL-6, were increased significantly in the serum samples of patients with CSU. We also found that TG2 expression was increased in mast cells in the lesional skin of patients with CSU and

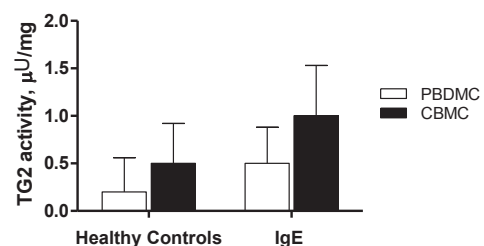


Figure 3. TG2 activity from cultured human mast cells after IgE activation. Cord blood–derived human mast cells (CBMCs) or peripheral blood–derived human mast cells (PBDMCs) developed from CD34^+ progenitors were activated by IgE. Values are presented as the mean from 3 individual experiments. Error bars indicate SDs.

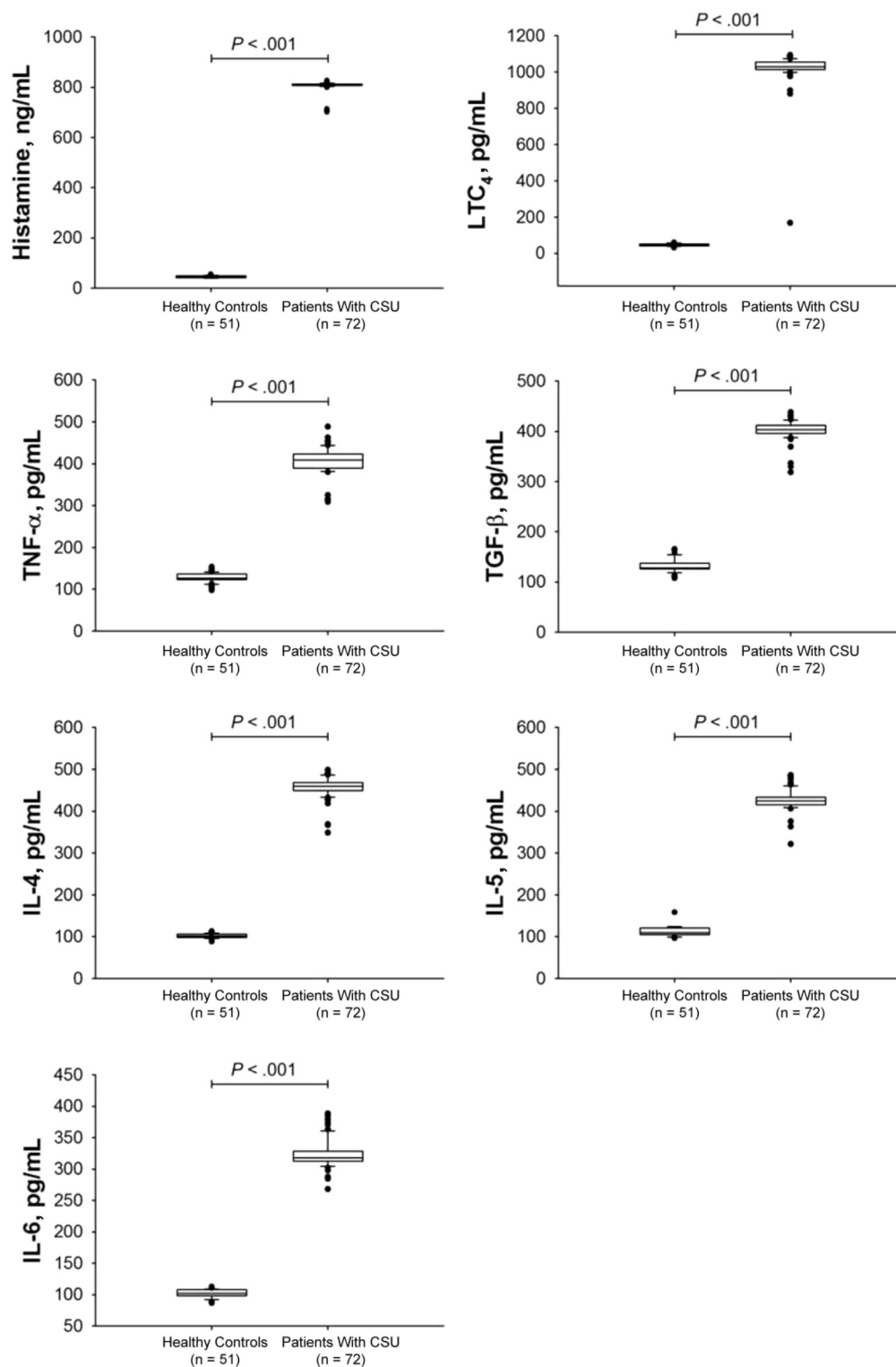


Figure 4. Measurement of histamine, LTC₄, and other inflammatory cytokines in serum samples from the study participants. CSU indicates chronic spontaneous urticaria; LTC₄, leukotriene C₄; IL, interleukin; TGF- β , transforming growth factor β ; TNF- α , tumor necrosis factor α .

that cultured human mast cells derived from peripheral blood or cord blood released TG2 during activation. Our results suggest that TG2 expressed in and released from mast cells may have an important role in CSU pathogenesis.

TG2 is a posttranslational, protein-modifying enzyme that is ubiquitously expressed in various cells, including mast cells. It has pleiotropic functions in blood coagulation, skin-barrier formation,

hardening of the fertilization envelope, extracellular matrix assembly, and other important biological process, which may contribute to the development of various autoimmune, inflammatory, and degenerative diseases.^{5,6} The *TG2* gene is overexpressed in the epithelial cells of the asthmatic airway, and the level of TG2 is increased in the airway lining fluid of patients with asthma, in which the TG2 increase was more prominent in asthma

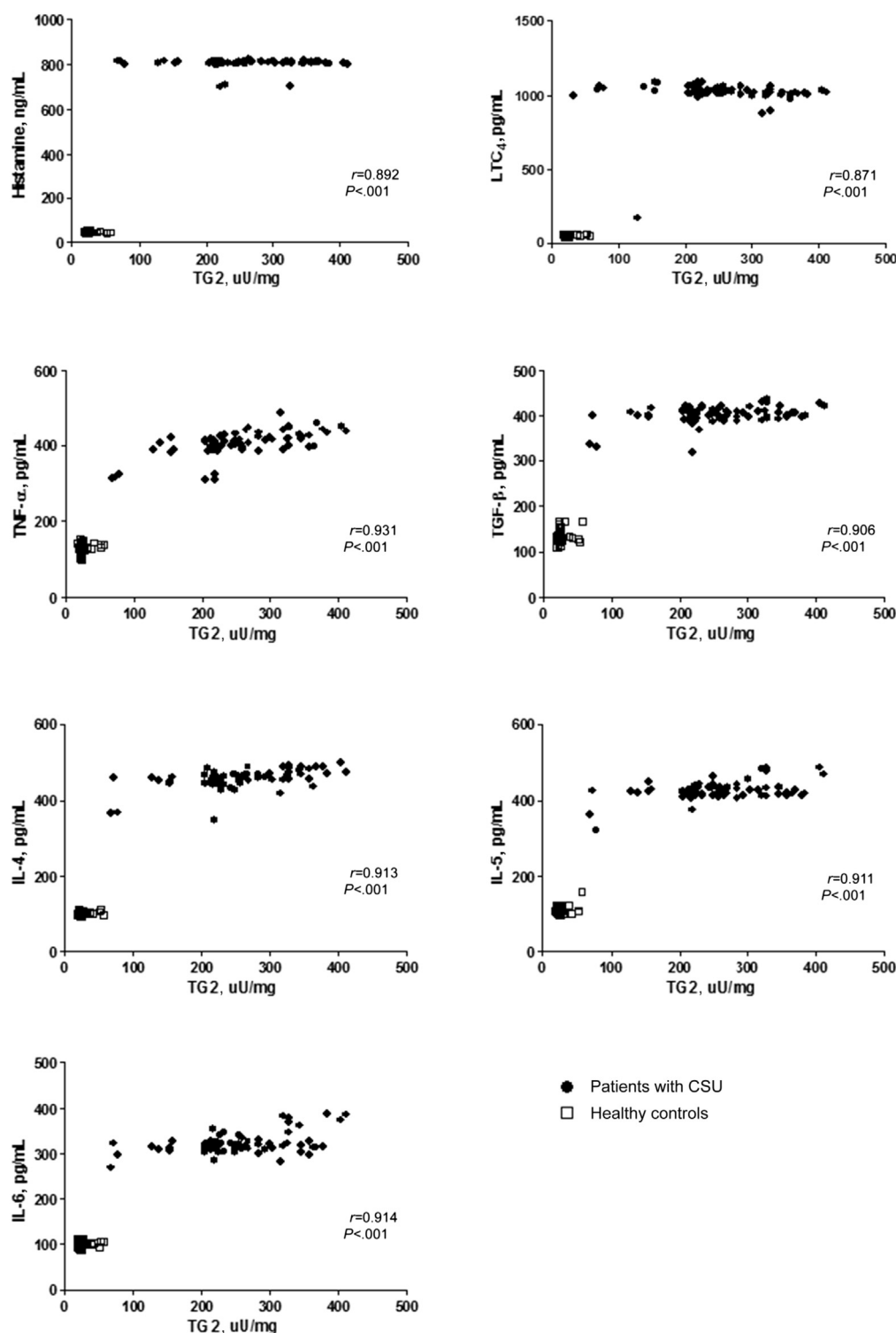


Figure 5. Correlations of TG2 activity with histamine, LTC₄, and other inflammatory cytokines in serum samples from study participants. CSU indicates chronic spontaneous urticaria; LTC₄, leukotriene C₄; IL, interleukin; TGF- β , transforming growth factor β ; TNF- α , tumor necrosis factor α .

with exercise-induced bronchospasm.⁸ TG2 increases sPLA₂-X enzymatic activity, leading to an increase in eicosanoids, which are important mediators of allergic inflammation.⁸ It also played an important role in initiating the allergic T_H2 response by inducing IL-33 and downstream molecules, leading to T_H2 differentiation in an ovalbumin-induced murine asthma model.¹² TG2 inhibitor has also been reported to reduce allergic inflammation in

an ovalbumin-induced murine asthma model by regulating NF- κ B and PLA₂ activities.⁹

We previously reported that TG2 was expressed in activated mast cells from a murine asthma model, which enhanced IgE production by interacting with B cells and the release of inflammatory mediators, such as TNF- α , leukotrienes, and various cytokines.¹³ Recently, our group also found TG2 expression in human

mast cells from pediatric mastocytosis.¹⁴ Our data suggest that serum TG2 activity has a critical role in CSU pathogenesis, in which TG2 from mast cells may initiate allergic inflammation by the release of various mediators via NF- κ B and PLA₂ activities⁹; this was shown by increased TG2 activity in the serum samples of patients with CSU, greater colocalization of TG2 and mast cells in the lesional skin of patients with CSU, and the release of TG2 from human mast cells activated in vitro. Thus, TG2 could be a new therapeutic target for treating CSU.

Mast cells are key effector cells in CSU, which is intensified by an increase in the number of such cells and supported by the histamine levels in the skin of patients with CSU.^{31,32} We found high numbers of mast cells and moderate numbers of eosinophils using May-Grünwald Giemsa staining and c-kit staining in the lesional skin of CSU. Increased serum levels of mast cell mediators, such as histamine and LTC₄, further support the increased number of mast cells and mast cell activation in patients with CSU. On the other hand, other studies using tryptase as a marker did not find an increased number of mast cells.^{33,34} Ying et al.³⁴ found significant infiltration of other inflammatory cells, such as T lymphocytes, eosinophils, neutrophils, basophils, and macrophages, in skin biopsy specimens from patients with CSU compared with healthy controls, whereas the number of mast cells was decreased.³⁴

Serum levels of T_H2-related cytokines, such as IL-4, IL-13, TSLP, IL-33, and IL-25, have been reported to be increased in patients with CSU.^{15,16,35} TNF- α in the serum samples of patients with CSU, which was positively correlated with disease activity, was significantly higher in patients with positive ASST results than in patients with negative ASST results.²² The plasma IL-6 concentration was significantly elevated in CSU, which was correlated with disease activity and serum C-reactive protein level, suggesting that CSU is associated with systemic inflammatory responses.¹⁸ TNF- α , IL-1 β , IL-12p70, and IL-6 are elevated in the serum samples of patients with CSU.¹⁷ These various cytokines were also elevated in human mast cells (CBMCs)^{36,37} and in murine mast cells.^{13,38} In addition, the TG2 gene promoter contains an IL-6-specific cis-regulatory element and a TGF- β 1 response element. TGF- β 1, IL-6, and TNF induce TG2 expression.⁵ In fibroblasts, TG2 increases TGF- β messenger RNA and protein expression via an NF- κ B signaling mechanism.³⁹ This results in a positive feedback loop in which the expressions of TGF- β and TG2 are reciprocally activated.⁴⁰

In this study, although all the cytokines, including TGF- β , TNF- α , IL-4, IL-5, and IL-6, were significantly increased in patients with CSU compared with healthy controls, the cytokines were not correlated with disease severity or ASST positivity, except for TNF- α . The serum level of TNF- α was significantly higher in patients with positive ASST results than in patients with negative ASST results, which is consistent with the results of another study.²² However, Metz et al.²⁰ recently reported that no levels of inflammatory mediators, including histamine, TNF, C5a, IL-6, IL-9, IL-18, IL-31, IL-33, TSLP, neopterin, and vascular endothelial growth factor, were elevated in CSU.²⁰ This inconsistency among studies may be attributed to ethnic differences, small numbers of patients, the timing of serum sampling, or the particular medications used before sampling.

Taking all the findings together, TG2-expressing mast cells may induce the production of cytokines in an autocrine and paracrine manner, resulting in the activation of other inflammatory cells, such as T cells and eosinophils, which contributes to the pathogenesis of CSU. Further study will be needed to elucidate the complex network of inflammatory mediators and their associations with disease activity, which may be helpful for developing useful biomarkers for the diagnosis, prognosis, and treatment of CSU.

Unfortunately, we could not find significant correlations between disease severity and the serum levels of TG2 or other inflammatory mediators. The failure to measure the Urticaria Activity

Score at the time of serum sampling may explain this. Moreover, statistical analysis according to disease severity seems to be inappropriate in this study because most of the patients were at treatment level 1 (67.1%). These might be other limitations of our study. Although we did not determine the Urticaria Activity Score, we measured the disease severity according to the level of treatment, which was stratified based on the medication requirements. If the serum levels of TG2 or inflammatory mediators were correlated with the medication requirements, TG2 might have a potential role of prognostic marker of CSU. For verification of this role, we need to perform further study to investigate the correlation between the serum level of TG2 and Urticaria Activity Score and to determine serial serum TG2 levels in accordance with treatment of urticaria.

In conclusion, we found that the serum level of TG2 is increased in patients with CSU and that TG2 is expressed in mast cells in the lesional skin of these patients. The increased level of TG2 was well correlated with other inflammatory mediators, including histamine, LTC₄, TGF- β , TNF- α , IL-4, IL-5, and IL-6, suggesting that both mast cells and various inflammatory cells are involved in CSU pathogenesis. Although we could not find any significant association between TG2 and clinical parameters, such as ASST positivity or disease severity, TG2 might be a good candidate as a biomarker and new therapeutic target of CSU, which should be further investigated in the near future.

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