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In chronic spontaneous urticaria, IgE against staphylococcal enterotoxins is common and functional

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Abbreviations:

ASST.....	autologous serum skin test
CSU.....	chronic spontaneous urticaria
HC.....	healthy control
UAS.....	urticaria activity score
sIgE.....	specific IgE
SEs.....	<i>Staphylococcus aureus</i> enterotoxins
SEA.....	staphylococcal enterotoxin A
SEB.....	staphylococcal enterotoxin B
TSST-1.....	Toxic shock syndrome toxin -1

Abstract

Background: Chronic spontaneous urticaria (CSU) is a frequent disorder with recurrent itchy wheals and/or angioedema. Despite the known effectiveness of omalizumab therapy, the relevant IgE antigens are largely unknown. Recently, increased rates of elevated levels of IgE towards *Staphylococcus aureus* enterotoxins (SEs) were described in CSU.

Aim: To assess the prevalence and functional relevance of IgE to SEs in CSU.

Method: We investigated serum levels of IgE against SEs in 49 CSU patients and in 15 CSU patients additional specific IgE to SE components and basophil histamine release (BHR). Sera of 15 healthy controls (HCs) served as control group.

Results: 25 (51%) of the CSU patients had detectable levels of SE-IgE as compared to 5 (33%) of HCs. Specific IgE to one of the SEs, Staphylococcus enterotoxin B (SEB), was present in 5 (33%) of 15 randomly selected CSU patients vs 3 (20%) of HC. Total IgE serum

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levels in CSU patients were significantly correlated with SE-IgE ($r = 0.52$, $p < 0.001$) and SEB-IgE ($r = 0.54$, $p = 0.04$) serum concentrations. Interestingly, SEB-IgE levels were strongly correlated with disease activity (UASday) in CSU patients ($r = 0.657$, $p = 0.01$). Furthermore, BHR in response to SEB was significantly higher in basophils loaded with the serum of CSU patients compared to HC ($p < 0.05$) and was clinically correlated with duration of disease ($r = 0.51$, $p < 0.05$).

Discussion: IgE against SEs may contribute to the pathogenesis of CSU in a subpopulation of patients. Its role and relevance in the pathophysiology of CSU needs to be further analysed.

Introduction

Chronic spontaneous urticaria (CSU) is a frequent and disabling disease characterized by recurrent itchy wheals and/or angioedema that result from the activation of cutaneous mast cells (MCs) [1]. The results of recent studies of the underlying etiopathogenesis of CSU suggest that two types of signals, MC secretagogues and MC modulators, are important for the activation of skin MCs in CSU [2]. MC secretagogues induce MC degranulation, the process that leads to the development of CSU signs and symptoms. MC modulators change the susceptibility of MCs to the effects of MC secretagogues, by lowering or increasing the MC threshold to respond with degranulation [3]. The effects of MC priming by these threshold modifiers, together with those of MC secretagogues, result in the release of histamine and other proinflammatory mediators by skin MCs, which induce subsequent vasodilatation and extravasation as well as nerve activation, resulting in the typical itchy wheal and flare type reactions.

Several independent lines of evidence suggest that IgE, via its high affinity IgE receptor, FcεRI, is the major MC secretagogue in CSU: i) Total IgE is elevated in CSU, and CSU patients have a higher prevalence of elevated IgE levels as compared to controls [4, 5].

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ii) The activation of skin MCs via IgE and its receptor leads to urticaria signs and symptoms, e.g. in skin prick testing, as a side effect of specific immunotherapy, or in acute allergic urticaria [6, 7]. iii) Treatment with Omalizumab, a monoclonal humanized anti-IgE antibody, is very effective in CSU [8, 9].

MCs degranulate when FcεR1s on their cell surface aggregate and crosslink. This happens when two or more FcεR1-bound IgE molecules that are all specific for the same antigen bind this allergen, a pollen allergen for example [10]. Allergen-induced IgE/FcεR1-mediated MC degranulation is the hallmark pathogenic feature of type I allergies, such as allergic rhinoconjunctivitis [11, 12]. CSU, in contrast is not a classical allergy and typical exoallergens are not involved in the degranulation of MCs in CSU. As of yet, the antigens to which CSU-relevant IgEs are specific, are largely unknown. Although specific IgE against classical common allergens, i.e. aeroallergens and food allergens [13-16], or to less common allergens such as fungi [5, 17] can be detected in some patients with CSU, they are not regarded as pathophysiological relevant for the development of the signs and symptoms of CSU [14, 15, 18-20]. Autoallergens might be the targets for the IgE molecules in CSU, but there is very little evidence as of yet to support this [21-23].

What could be the specific targets of the IgE that induces skin MC degranulation in CSU patients? Some older studies [24, 25] have shown that bacterial antigens may be the target of IgE, and that MCs with antibacterial IgE on their surface IgE receptors degranulate when exposed to IgE-specific bacterial antigens such as staphylococcal enterotoxins (SEs).

Indeed, IgE to staphylococcus toxins has been demonstrated to be elevated and linked to disease activity and exacerbation in several MC-driven diseases including atopic dermatitis [26], nasal polyposis and allergic rhinitis [27, 28]. Leung et al. [29] have demonstrated that 57% of patients with atopic dermatitis have serum IgE against staphylococcal toxins and that their basophils release histamine upon exposure to the relevant SEs. Sharma *et al* reported that antibacterial treatment reduced urticaria symptoms

in colonized patients with chronic urticaria [30].

Most interestingly, Ye and co-workers detected an increased prevalence of specific IgE against staphylococcal enterotoxin A and B and toxic shock syndrome toxin TSST-1 (13.7%, 12.0% and 18.0%, respectively) in Korean patients with chronic urticaria, and described associations with aspirin sensitivity, elevated total IgE serum levels, higher rates of atopy and specific HLA haplotypes (HLA DQB1*0609 and DRB1*1302) [31]. Recently a second study of the same group described, in a larger cohort of Korean patients, increased rates of IgE against SE. These were significantly elevated for SEB and TSST-1 compared to healthy controls (SEA 10.0% vs. 6.1%, n.s.; SEB 21.3% vs. 6.3%, $p<0.001$; and TSST-1 26.75% vs. 12.3%, $p<0.001$) [32].

In this study, we wanted to confirm these findings in Caucasian CSU patients and address the question if the IgE against SE is functional and relevant in CSU.

Materials and Methods

Study subjects

In this retrospective study, 49 sera of CSU patients from the Department of Dermatology and Allergy, Charité - Universitätsmedizin, Berlin were analyzed and correlated with clinical parameter of the patients. 15 healthy control sera were analyzed in the study. Study subjects' details are given in Table 1. Patient sera and data used for this study were exclusively derived from the blood obtained for the routine clinical management of patient and their medical chart documentation, respectively. None of the enrolled participants had taken systemic corticosteroids or applied topical antihistamines, corticosteroids or mast-cell stabilizers for 2 weeks and not taken oral antihistamines at least 72 hours prior to routine assessment.

Questionnaire

Patients were asked to keep a symptom diary and to document, every day, their itch score (0= none, 1= mild, 2=moderate, 3=severe) and their numbers of wheals (0= none, 1= little intensity, 2= moderate intensity, 3= severe intensity), in order to assess their disease severity. By adding these scores the weekly urticaria activity score (UAS7, range 0-42) of the last seven days before blood drawing were calculated. As 32% of the CSU had taken second generation antihistamines (median 2 Tabl.) in the week before blood drawing, we also calculated the daily urticaria activity score (UASday, range 0-6) on the day of blood drawing, where the patients had stopped AH treatment at least 72h before.

Autologous serum skin test (ASST)

The ASST was routinely performed in CSU patients as described before [33]. The ASST was regarded positive if a serum-induced weal occurred with a diameter of ≥ 1.5 mm bigger than the saline-induced response at 30 min.

Serum analysis

By using the Immuno CAP System Phadia Laboratory Systems, Thermo Fisher Scientific Inc, Uppsala, Sweden, the following parameters were determined:

General:

- total-IgE
- sIgE Grass Mix (gx3)
- sIgE Dermatophagoides pteron. (d1)

Staphylococcus specific:

- sIgE Staphylococcus enterotoxin B (m81)
- SE-IgEs (URL, Ghent University, not commercially available)

Total IgE levels >100 kU/l were considered as elevated (as given in the manual of Immuno CAP). Detection level of SE-IgE was 0.1kU/l.

Basophil histamine release (BHR)

15 randomly selected CSU sera (8 SE-IgE detectable, 7 SE-IgE below detection limit; 5 SEB-IgE detectable, 10 SEB-IgE below detection limit) were used for BHR. Clinical characteristics of the randomly selected CSU patients did not differ from the whole 49 CSU patients (see Supplemental Table 1)

Passive sensitization was performed using heparinized blood from the same single healthy non-allergic donor, with the following characteristics: 1. confirmed strong reaction to anti-IgE (histamine released: 52 ± 5 ng/mL) and 2. No response to SEA or SEB. To remove the IgE bound from the basophils surface, 3 mL of blood samples were treated with 10 mL of stripping buffer (0.14 M NaCl, 0.005 M KCl and 0.0134 M lactic acid) for 10 min at 4°C. Thereafter the cells were washed with PIPES buffer (10 mM piperazine-N,N(bis-ethane sulfonic acid), 140 mM sodium acetate, 5 mM potassium acetate, 0.6 mM CaCl_2 , 1.1 mM MgCl_2 , 1 mg/mL glucose, 0.3 mg/mL HSA, 15 IE/mL heparin) and resuspended to a total volume of 3 mL in the PIPES buffer. Then, 130 μL of sera from patients or controls were incubated with 3 mL of the “IgE-stripped” donor blood for 60 min at 37°C. Aliquots of 25 μL of passively sensitized blood in the presence of IL-3 (2 ng/mL blood) were incubated in the glass fibre-prepared microtiter plates (RefLab ApS, Copenhagen, Denmark), with 25 μL of SEA or SEB at a concentration of 100 and 30 $\mu\text{g/mL}$ for 60 min at 37°C. The SEA and SEB concentration chosen for this test were the highest values that did not lead to unspecific histamine release in a dose-response curve. This level corresponded to the mean + 3 x SD of the histamine release values measured in 5 non-allergic subjects without a specific

stimulus. As a positive control of the passive sensitization was used 25 μ l of anti-IgE (KPL, USA, MD) in a concentration of 5 μ g/mL.

After incubation, the microtiter plate was washed with H₂O for 1 min to remove cells and interfering substances. Thereafter, 75 μ l of NaOH/ortho-phthalaldehyde (OPA) mixture was added to each well allowing glass fiber bound histamine to be released and coupled to OPA. After 10 min the coupling reaction was stopped and histamine-OPA complexes stabilised by adding 75 μ l of 0.59% HClO₄. Histamine was determined fluorometrically in the Histareader™ 501 manufacturing company (RefLab). Results were expressed as ng/mL of histamine released.

Statistical Analyses

Statistical analysis was performed using Graph Pad Prism Version 6 for Windows, 2012. D'Agostino & Pearson omnibus normality test was used to test for normal distribution. Parametric data are depicted using mean and standard error of mean values, non-parametric data using median and (interquartile) range.

Binominal variables were analyzed using Chi-square test. Parametric variables were compared using unpaired t-test. Non-parametric continuous variables were compared using Mann-Whitney test. Correlations were calculated using the Spearman's rank test. $P < 0.05$ was considered to indicate statistical significance.

Results

In CSU patients, total IgE levels are elevated, but specific IgE levels against staphylococcal enterotoxin are comparable to those of healthy controls.

In our study, CSU patients had significantly elevated levels of total IgE (median 95 kU/l) as compared to healthy control subjects (median 29.5 kU/l, $p=0.026$, Figure 1a). We also found specific IgE against a mix of staphylococcal enterotoxins (SEs) in the blood of 51% of CSU patients versus 33% of healthy controls, but IgE-anti-SE-levels were not significantly higher in the CSU group as compared to the healthy control group (Figure 1b).

Specific IgE to one of the SEs, Staphylococcus enterotoxin B (SEB), was present in 33% of CSU patients vs 20% of healthy controls. Again, the median serum levels in CSU patients and healthy controls were comparable (Figure 1b).

Detectable specific IgE against staphylococcal enterotoxins are significantly correlated with total IgE serum levels.

SE-IgE serum concentrations were significantly correlated with total IgE serum levels in both, CSU and healthy controls (CSU: $r = 0.52$, $p<0.001$, healthy: $r = 0.74$, $p = 0.002$). In contrast, SEB-IgE and total IgE were significantly correlated only in CSU patients ($r = 0.54$, $p = 0.04$), but not in healthy controls ($r = 0.39$, $p = 0.155$). In both populations, SEB-IgE was correlated very well with SE-IgE ($r = 0.87$, $p < 0.001$).

In CSU patients, levels of SEB-IgE, but not SE-IgE, correlate with disease activity.

In CSU, SE-IgE did not correlate with any assessed clinical features such as duration of disease, disease activity (determined by UASday or UAS7) or ASST/APST-wheal diameter (Table 2a). In contrast, SEB-IgE levels were strongly correlated with disease activity

(UASday) in CSU patients ($R_s=0.657$, $p=0.01$, Table 2b). Interestingly, most CSU patients with elevated SEB-IgE levels were ASST negative (80%). The significant correlation of SEB-IgE with disease activity (UASday) was pronounced in ASST negative CSU patients ($R_s=0.702$, $p=0.043$, $n=9$) but absent in ASST positive patients ($R_s=0.791$, $p=0.400$, $n=5$). There was no significant correlation with the UAS7 score.

SEB-IgE from CSU patients induces basophil histamine release

When we exposed donor basophils first to the serum of 15 randomly selected CSU patients or healthy control sera and then to SEB, histamine release was significantly more pronounced in the basophils sensitized with CSU sera (*Figure 2a-c*). SEB-induced histamine release was significantly correlated with levels of total IgE and with the disease duration in CSU patients (*see Table 3*).

Basophil exposure to SEA or TSST-1 did not result in histamine release neither in basophils sensitized with the serum of CSU patients nor healthy controls (data not shown).

Discussion

This study shows that many CSU patients have IgE to staphylococcal toxins. The levels of IgE to one of these toxins, SEB, are correlated with total IgE levels and CSU activity. Also, the SEB-specific IgE of CSU patients has histamine-releasing effects, which are linked to disease activity and duration.

CSU patients frequently have IgE against staphylococcal enterotoxins

In comparison to the study of Ye et al. in Korean CSU patients (31.8%) [31] we detected a higher prevalence of about 50% sIgE against staphylococcal enterotoxins in our Caucasian

CSU patients. In this study also, the healthy controls had lower prevalences (13%) compared to our study (33%). In comparison to this publication we also detected more frequently IgE against Staphylococcus enterotoxin B (SEB). Compared to the study of Ye et al. it was present in 33% of our CSU patients (compared to 12%) vs 20% of healthy controls (compared to 4.3%). To our knowledge it is not known if Asians overall have lower sIgE against staphylococcal enterotoxins rates compared to Caucasians. As in both studies the same test system had been used, these differences are not the result of different test sensitivities.

The mean levels of specific IgE against staphylococcal enterotoxins including SEB was not significantly higher compared to the healthy control groups (Figure 1b). If looking at the specific IgE levels in the positive CSU patients and healthy controls, we did see higher sIgE levels in CSU patients (SE-IgE mean 0.84 kU/l $\pm$ 0.14 compared to 0.49 kU/l $\pm$ 0.08 in healthy controls; SEB-IgE mean 0.65 kU/l $\pm$ 0.05 compared to 0.32 kU/l $\pm$ 0.06 in healthy), but this differences missed statistical significance, due to the low number of patients. Ye et al. in comparison did not report the levels of IgE against staphylococcal enterotoxins.

Our results confirm earlier studies showing that CSU patients have elevated serum levels of total IgE [4, 5, 21, 34]. Comparative to other studies [35-37], we also saw in our study a trend to more frequent detectable levels against perennial or seasonal inhalant antigens as compared to healthy controls (see Supplemental Table 1). The highest detection rates had been described for house dust mite allergens [13, 14, 16], for which we could also detect specific IgE in almost half of our patients. In line with a previous study [14], overall the prevalence was not significantly different to those of healthy controls (see Supplemental Figure 1). The clinical significance of these specific IgE antibodies is still not clear. In the literature these sensitisations are mostly regarded as irrelevant to CSU symptoms [15, 18-20].

Aspirin intolerance has not been assessed in our patient population. Previously, chronic urticaria patients with aspirin intolerance [31], aspirin intolerant patients with nasal polyposis [38] and aspirin intolerant asthma patients [39], have been reported to show higher rates of specific IgE to superantigens. Thereasons for this are not completely understood yet. None of the studies was able to provide evidence for a direct link between IgE to *Staphylococcus aureus* and aspirin intolerance, and further studies are needed.

IN CSU, IgE-anti-SEB is linked to total IgE and disease activity

In line with the paper Ye et al. [31] we also detected a correlation of SE-IgE and SEB-IgE with the total IgE levels. This fact, that serum IgE-anti-SEB levels in CSU patients were strongly correlated to total IgE levels, suggests that the elevation of total IgE in CSU may be explained, at least in part, by the production of IgE to SEB and other bacterial antigens.

As of now it is largely unclear why CSU patients have elevated total IgE levels. In some publications IgE against classical common allergens, i.e. aeroallergens and food allergens [13-16], or to less common allergens such as fungi [5, 17] had been detected in some patients with CSU. Nevertheless, they are not regarded as pathophysiological relevant for the development of the signs and symptoms of CSU [14, 15, 18-20], as the symptoms occur independently of exoallergen contact. This is in contrast to contact urticaria, where symptoms occur (immediately) upon contact and can be detected via BHR [40]. We have shown in a former publication that some CSU patients do have IgE against autoantigens such as TPO [21]. How these autoreactive IgE molecules evolve in CSU is unknown. It can be speculated that they develop as a result of cross-reactivity e.g. after infectious diseases, as infections are a common cause of acute and chronic urticaria [41]. *Staphylococcus aureus* is a common skin colonizing bacteria known for causing various infectious diseases. Accordingly, *Staphylococcus aureus* specific antibodies including IgE might occur as a result

of such infections, which then might lead to the development of allergic symptoms, as seen in CSU.

That IgE-anti-SEB levels are correlated with disease activity in CSU patients indicates this specific IgE may contribute to the mast cell degranulation occurring in CSU skin. Neither SE-IgE nor SEB-IgE were correlated with ASST positive reactivity, suggesting that sensitisations to staphylococcal antigens are not associated with autoreactivity in CSU. In contrast, elevated SEB-IgE levels were more often seen in ASST negative patients and in this subpopulation the correlation with the UASday was significant, in contrast to the ASST positive population, probably indicating that SEB-IgE can be a driver of disease in the non-autoreactive patient population. SEB-IgE did not correlate with the UAS7 in our CSU patients, but in this tested selected population, 27.7% of the patients had taken antihistamines in the week before blood drawing, probably affecting the UAS7 score, thus leading to missed correlations due to a low total patient number.

Interestingly, the sensitisation against staphylococcal antigens seemed to have developed irrespective of the sensitisations against environmental antigens in CSU patients. This was in contrast to the healthy controls, where the occurrence of staphylococcus specific IgE, if present, was highly correlated with specific environmental IgE levels (Supplemental Table 2). Thus, sensitisations against staphylococcal antigens seem to evolve by a different route in CSU compared to healthy controls, e.g. via (recurrent) bacterial infections or (constant) colonisation. In CSU patients, rates of skin colonisation with *Staphylococcus aureus* are reportedly very low, 3% in one study [42]. In contrast, nasal colonization rates were found to be increased in two independent studies, 53% in CSU vs. 13% of healthy controls in one study [43]; and 56% in CSU vs. 28% of healthy controls in the other [30]). Interestingly, antibiotic treatment of CSU patients with nasal colonization with *S. aureus* reportedly reduced urticaria symptoms, in one study [30].

SEB induced histamine release is significantly higher in CSU patients, but limited.

In CSU patients the SEB induced histamine release on sensitized basophils was significantly higher compared to healthy controls, but the total histamine release was relatively low compared to the histamine released evoked via anti-IgE induced histamine release (e.g. mean 12.1% SEB 100µg/ml compared to the anti-IgE induced histamine release, mean 5.4% SEB 30µg/ml; Figure 2c). Interestingly, the SEB-induced histamine release was significantly correlated with the disease duration in CSU patients. This can either indicate that SEB-IgE (and possible recurrent SE contact) can prolong CSU disease duration, or that long lasting CSU and a pro-IgE switched immune system preferably produces (also) IgE to SE.

Accordingly, high SEB-level could also induce histamine release via unspecific basophil activation. Overall, the contribution to the total histamine release in CSU patients is limited, indicating that SEB-IgE induced mast cell activation might not be sufficient to solely evoke the clinical symptoms of CSU. Probably several specific IgE stimulations in parallel have to occur in CSU (sum effect), or the specific IgE primes the effector cells to histamine release to other (unspecific) trigger factors (priming effect). The significant correlation of the SEB induced histamine release with the total IgE levels could point towards the first assumption, that several specific IgEs (and their respective antigens) are needed in CSU to provoke a clinical significant skin reaction. Currently we have no clear explanation, why SEA and TSST-1 did not result in basophil activation. This is in contrast to reports on atopic dermatitis where basophil histamine release to SEA or TSST-1 was seen [29]. We speculate that levels and rates of specific IgE are lower in CSU patients as compared to AD, and that this may affect the sensitivity of ex vivo basophil activation assays.

Beside the seen direct effect on basophils and mast cells in CSU, staphylococcal superantigens could also contribute to the eosinophilic and mixed Th1/Th2 cell-mediated inflammatory response seen in histologic examinations of CSU patients [44] [45]. In mice exposed to staphylococcal superantigens, mixed Th1/Th2 cellular infiltration was reported [46], and intracutaneous injection of SEB in wild type mice, but not in T cell deficient mice, elicited mast cell degranulation and inflammatory responses, indicating that both, superantigens and T cells are required for mast cell degranulation [47]. Taken together, these findings suggest that IgE directed against staphylococcal superantigens may trigger and perpetuate inflammation in at least some CU patients by effects on T cells and other non mast cell populations.

Limitations of the study are the limited number and patient material of CSU patients and healthy control subjects, the retrospective analysis of the patients' data and the missing information of aspirin intolerance and the patients' colonisation status. Nevertheless, we were able to detect specific IgE against SE's and give first hints of functional relevance of IgE-antibodies directed against staphylococcal enterotoxins, and especially the antigen SEB, in CSU. IgE against staphylococcal enterotoxin B is functional in CSU patient sera and linked to a prolonged course of CSU as well as daily disease activity.

The overall frequent detection of SE-IgE also in healthy controls, the relatively low serum levels and mild histamine release of basophils do need further prospective investigations to clarify the relevance of this finding. Our results should encourage the further assessment of these types of IgE antibodies and their role and relevance in the pathophysiology of CSU.

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Figures and Tables

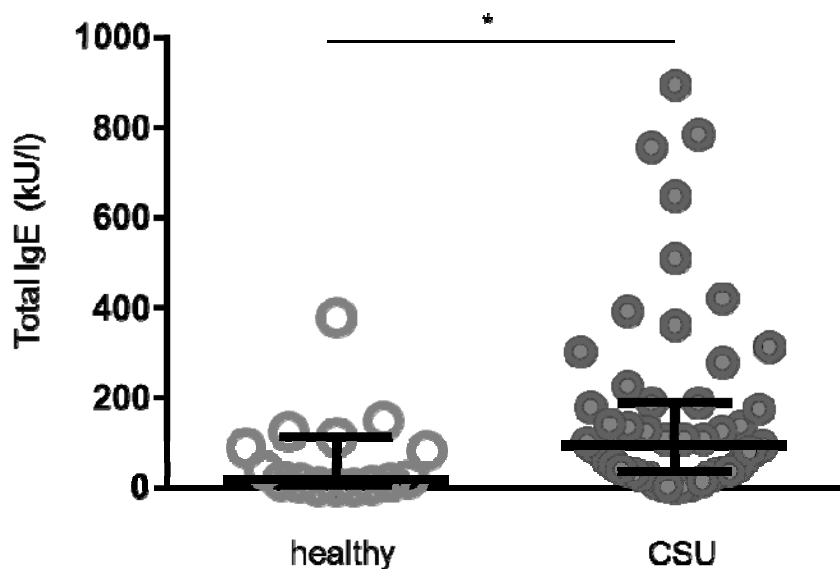
Table1: Clinical characteristics of study participants

	CSU patients n=49	healthy controls n=15
Age median (range)	43 (15 - 82)	28 (22 - 59)
Sex M:F	15:34	5:10
Duration of disease (years) median (range)	1.6 (0.1 -31)	-
UASday (0-6 points) median (range)	(n = 48) 2.5 (0 - 6)	-
UAS7 (0-42 points) median (range)	(n = 48) 17 (0 - 42)	-
ASST Negative : Positive*	(n = 44) 34:10	-

* wheal to autologous serum $\geq 1,5$ mm compared to saline

Figure 1: Individual subjects are depicted as open (healthy; = controls) or filled (CSU; = patients with chronic spontaneous urticaria) circles. Median values (line) and Interquartile range (whiskers) are depicted. * $p < 0.05$, n.s.=not significant. (a) CSU patients ($n = 49$) have significantly elevated total IgE serum levels compared to healthy controls ($n = 15$). (b) CSU patients (SE: $n = 49$; SEB: $n = 15$) have comparable specific IgE serum levels in SE/SEB-IgE positive subjects against staphylococcal enterotoxins (SE) including SEB as healthy controls ($n = 15$). SEB-IgE measurements were performed in 15 randomly selected CSU patients.

a)



b)

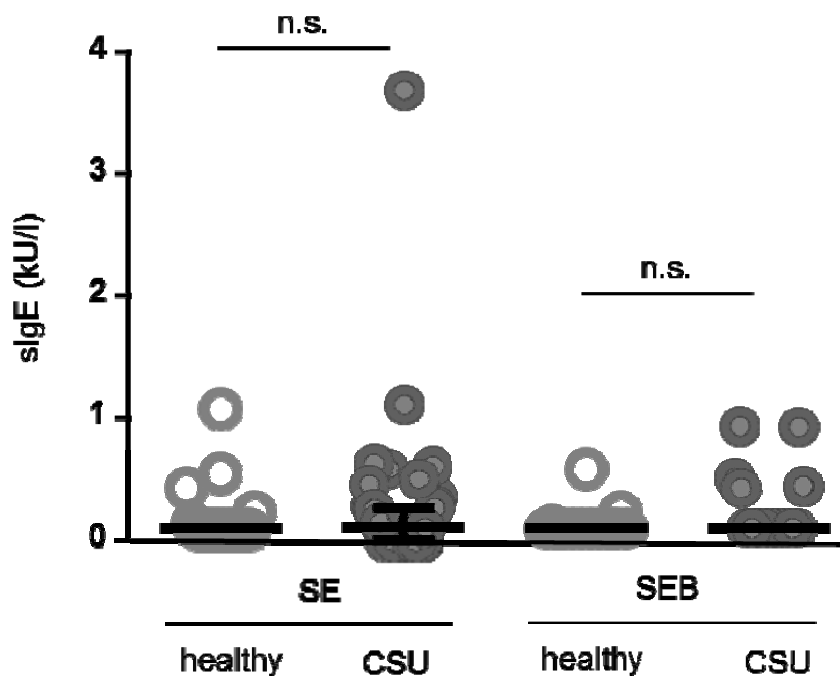


Table 2a: Correlation of **SE-IgE** with selected clinical parameter

	N=	CSU patients r (correlation) p value	N=	healthy controls r (correlation) P=
Age	49	-0.0005 0.99	15	-0.163 0.561
Sex	49	-0.04 0.785	15	-0.429 0.11
Duration of disease	49	0.021 0.88	-	-
UASday	48	0.074 0.61	-	-
UAS7	48	-0.072 0.63	-	-
ASST	44	-0.2	-	-

		0.132		
Total IgE	49	0.516 < 0.001	15	0.74 0.002
SEB-IgE	15	0.87 <0.001	15	0.71 <0.001

Table 2b: Correlation of **SEB-IgE** levels of randomly selected CSU patients* and healthy controls n=15 each

	N=	CSU patients Rs p	N=	Healthy controls Rs p
Age	15	0.17 0.54	15	-0,25 0,01
Sex	15	0.20 0.44	15	-0,7 <0.001
Duration of disease	15	0.22 0.43	-	
UAS day	14	0.657 0.01	-	-
UAS7	15	0.305 0.269	-	-
ASST	15	-0.11 0.02	-	-
Total IgE	15	0.54 0.04	15	0.391 0.155
SE-IgE	15	0.87 <0.001	15	0.71 <0.001

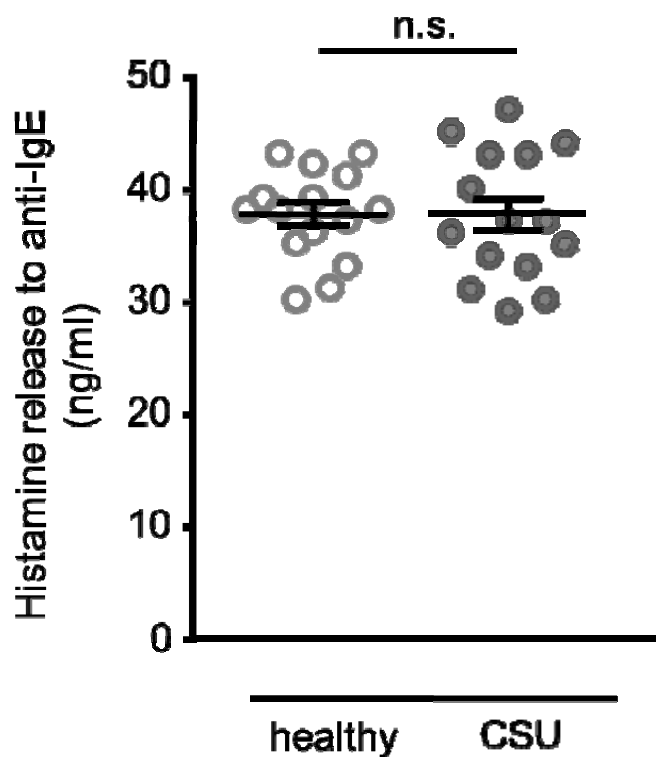
Figure 2: Individual patients are depicted as open (healthy controls) or filled (CSU) circles. Mean values and standard error of mean are depicted. * $p < 0.05$, n.s.=not significant. Basophil histamine-release to SEB antigen (100 μ g or 30 μ g) is more pronounced in CSU patients compared to healthy controls.

(a) absolute histamine release to anti IgE (positive control); healthy controls mean 37.5 ng/ml $\pm$ 1.04, CSU mean 37.6 ng/ml $\pm$ 1.49 .

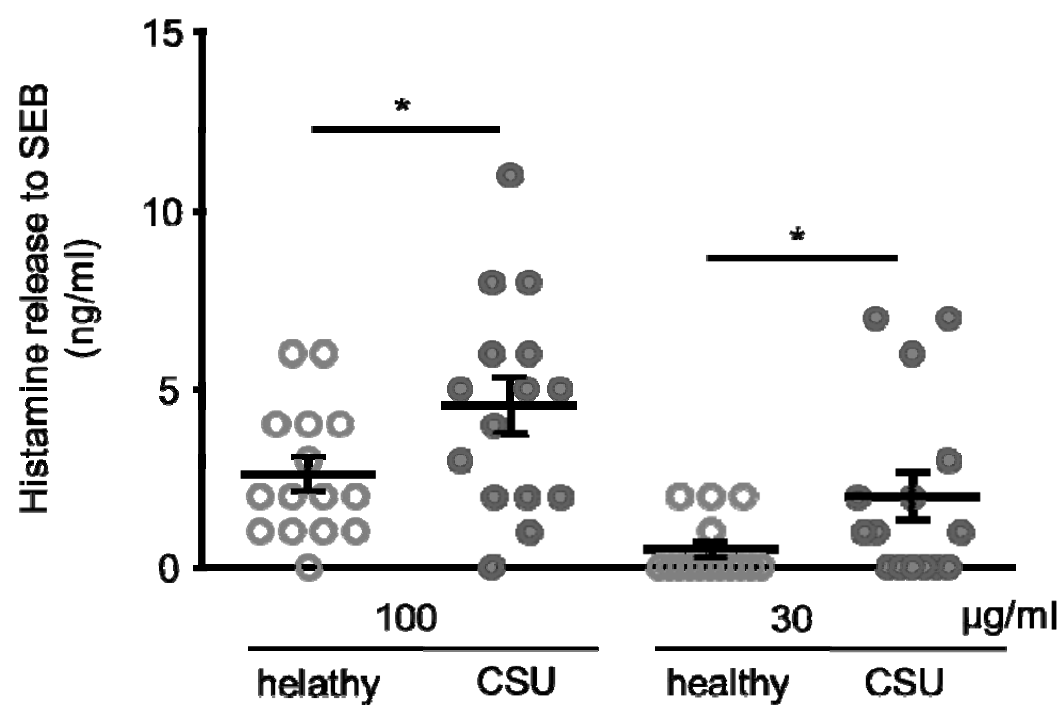
(b) absolute histamine release to 2 concentrations of SEB antigen (100 μ g/ml and 30 μ g/ml); 100 μ g SEB: healthy controls mean 2.6 ng/ml $\pm$ 0.05, CSU mean 4.53 ng/ml $\pm$ 0.77, $p=0.042$); 30 μ g SEB: healthy controls mean 0.4 ng/ml $\pm$ 0.65, CSU mean 2.0 ng/ml $\pm$ 0.67, $p=0.038$).

(c) relative histamine release to 2 concentrations of SEB antigen (100 μ g/ml: healthy controls mean 7.2 % $\pm$ 0.33, CSU mean 12.1 % $\pm$ 0.56, $p=0.079$; 30 μ g/ml: healthy controls mean 1.3 % $\pm$ 0.16, CSU mean 5.4 % $\pm$ 0.45, $p=0.048$) and anti-IgE histamine release (%).

a)



b)



c)

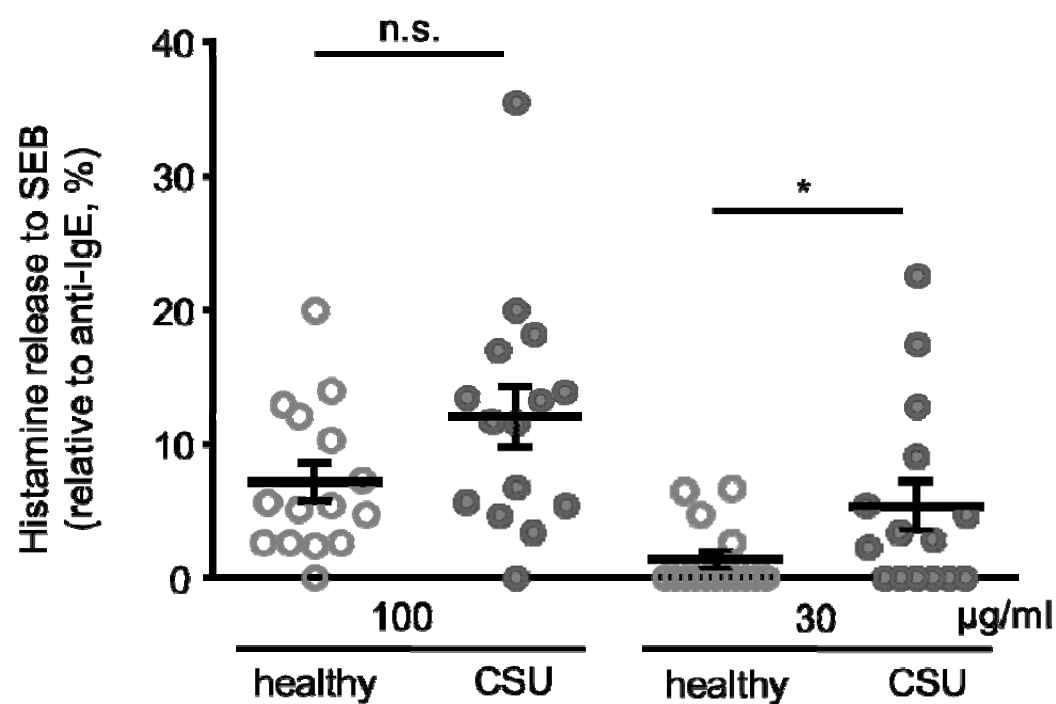


Table 3: Correlation of SEB specific histamine release with other clinical data

Correlation	absolute histamine release to SEB 100µg in selected CSU patients n=15 (if not given else) Rs= p=	absolute histamine release to SEB 30µg in selected CSU patients n=15 (if not given else) Rs= p=	% histamine release to SEB 100µg in selected CSU patients n=15 (if not given else) Rs= p=	% histamine release to SEB 30µg in selected CSU patients n=15 (if not given else) Rs= p=
Duration of disease	0.61 0.016	0.54 0.038	<i>0.51</i> <i>0.054</i>	<i>0.51</i> <i>0.053</i>
UAS day	n= 14 0.20 0.503	n= 14 0.34 0.240	n= 14 0.17 0.565	n= 14 0.27 0.342
UAS 7	n= 14 -0.13 0.637	n= 14 -0.11 0.693	n= 14 -0.10 0.711	n= 14 -0.11 0.693
ASST	-0.51 0.054	0.35 0.195	-0.48 0.074	-0.32 0.239
Total IgE	0.66 0.007	0.55 0.034	0.64 0.010	0.54 0.039
SE-IgE	0.22 0.438	0.38 0.167	0.23 0.418	0.35 0.198

Significant correlations are depicted in bold. Correlations that marginally missed significance are depicted in cursive.

- 1 **Supplemental Table 1:** Data of randomly selected CSU patients in comparison with
- 2 all CSU patients and healthy controls

	CSU patients	Randomly selected CSU patients*	healthy controls n=15	P1= P2=
Age median (range)	43 (15 - 82) n=49	44 (24 - 54) n=15	28 (22 - 59)	0.02 0.06
Sex M:F	15:34	7:8	5:10	1 0.288
Duration of disease (years) median (range)	1.6 (0.1 - 31) n=49	2 (0.1 - 19) n=15	- -	- -
UASday (0-6 points) median (range)	2.5 (0 - 6) n = 48	2 (0 - 5) n=14	- -	- -
UAS7 (0-42 points) median (range)	17 (0 - 42) n = 48	15 (0 - 38) n=15	- -	- -
ASST Negative:Positive	34:10 n = 44	10:5 n=15	- -	- -
Total IgE median (range)	95 (1 - 896) n = 49	191 (2 - 896) n=15	29.5 (2 - 378)	0.026 0.015
SE-IgE (kU/l) number of persons with detectable levels (%)	51 n=49	53.3 n=15	33.3	0.36 0.46
median (range)	0.11 (0-3.69) n=49	0.11 (0-3.69) n=15	0 (0-1.08)	0.39 0.24
SEB-IgE (kU/l) number of persons with detectable levels (%)	-	33.3 n=15	20.0	- 0.68
median (range)	-	0 (0-0.04) n=15*	0 (0-0.59)	- 0.273
Der.p.-IgE (kU/l) number of persons with elevated levels (%)	-	46.6 n=15	20.0	- 0.24
median (range)	-	0 (0-11.3) n=15	0 (0-2.4)	- 0.15
Grassmix – IgE number of persons	-	53.3	26.6	-

with elevated levels (%)		n=15		0.26
median (range)	-	0.15 (0-13.7) n=15	0 (0-36.6)	- 0.38

P1= significance niveau between all CSU patients and healthy controls

P2= significance niveau between selected CSU patients and healthy controls

*15 CSU patients were randomly selected with enough patient material left for SEB-IgE determination and the histamine release assay.

Supplemental Table 2: Correlation of **SE-IgE** and **SEB-IgE** with environmental allergens

	N=	CSU patients Rs P=	N=	healthy controls Rs P=
SE-IgE				
Der.p. IgE	15	0.03 0.9	15	0.83 <0.001
Grassmix-IgE	15	0.27 0.316	15	0.91 <0.001
SEB-IgE				
Der.p. IgE	15	0.22 0.423	15	0.56 0.03
grassmix IgE	15	0.18 0.507	15	0.38 0.158

Supplemental Figure 1: Specific IgE levels to environmental allergens were comparable in CSU patients (n=15) and healthy controls (n=15). Individual patients are depicted as open (healthy controls) or filled (CSU) circles. Median values (lines) and interquartile range (whiskers) are depicted. * p<0.05, n.s.=not significant.

