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IgM and IgA in addition to IgG autoantibodies against FcεRIα are frequent and associated with disease markers of chronic spontaneous urticaria

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ABSTRACT

Background: IgG autoantibodies against the high-affinity IgE receptor, FcεRIα, contribute the pathogenesis of autoimmune chronic spontaneous urticaria (CSU). However, it is not known whether such patients also exhibit IgM or IgA autoantibodies against FcεRIα. To address this question we developed an ELISA to assess serum levels of IgG, IgM, and IgA autoantibodies against FcεRIα and investigated whether their presence is linked to clinical features of CSU including the response to autologous serum skin testing (ASST).

Methods: Serum samples of 35 CSU patients (25 ASST-positive) and 52 healthy control individuals were analyzed using a newly developed competitive ELISA for IgG, IgM, and IgA autoantibodies to FcεRIα.

Results: One in four CSU patients (8/35, 24%) had elevated serum levels of IgG-anti-FcεRIα compared with (3/52, 6%) healthy controls. More than half of patients had IgM (21/35, 60%) and IgA (20/35, 57%) vs (3/52, 5%) each in healthy controls. Elevated IgM, but not IgG or IgA, autoantibodies were significantly more frequent in ASST-positive CSU patients (18/25, 72%) compared with ASSTnegative patients (3/10, 33%, $P = .022$). Also, elevated levels of IgM-anti-FcεRIα, but not of IgG or IgA against FcεRIα, were linked to low blood basophil ($r = .414$, $P = .021$) and eosinophil ($r = .623$, $P < .001$) counts.

Conclusions: Increased serum levels of IgM-anti-FcεRIα are common in patients with CSU and linked to features of autoimmune CSU. The role and relevance of autoantibodies to FcεRIα in CSU can and should be further characterized in future studies, and our novel assay can help with this.

KEYWORDS

autoantibodies, autoimmunity, chronic spontaneous urticaria, FcεRIα, IgM

Abbreviations: Ab, antibody; ASST, autologous serum skin test; BAT, basophile activation test; BHRA, basophil histamine release assay; CRP, C-reactive protein; CSU, chronic spontaneous urticaria; DLQI, dermatology life quality index; ELISA, enzyme-linked immunosorbent assay; FcεRIα, high-affinity IgE receptor α; MC, mast cell; SD, standard deviation; TSH, thyroid-stimulating hormone; UAS, urticaria activity score.

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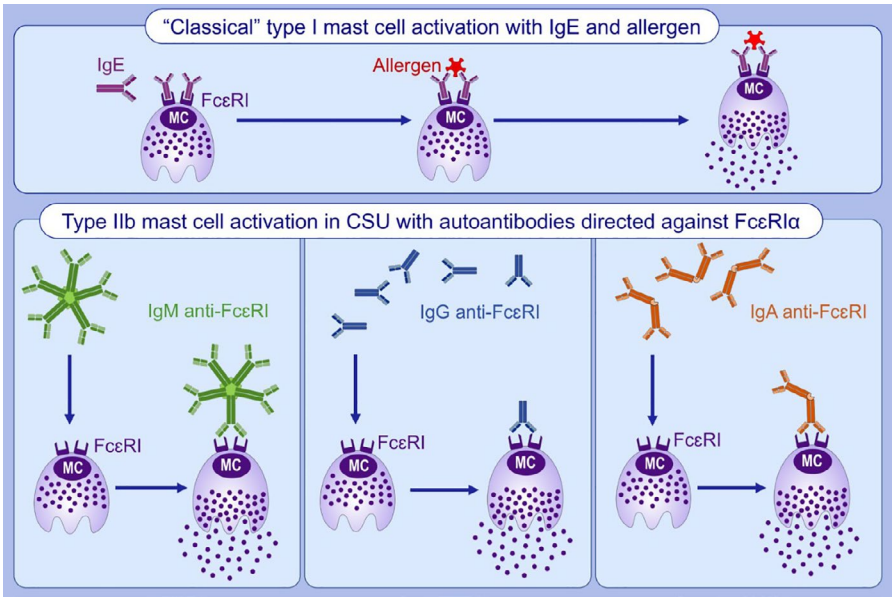

GRAPHICAL ABSTRACT

Beside IgG against the high-affinity IgE receptor, patients with CSU can also have IgM and IgA directed against the receptor. IgM antibodies are associated with positive autologous serum skin tests, a feature of type IIb autoimmunity. FcεRI cross-linking by these autoantibodies, in skin mast cells, could lead to their degranulation, which drives the development of the clinical symptoms of CSU.

Abbreviations: CSU, chronic spontaneous urticaria; FcεRI, Fcε receptor I

1 | INTRODUCTION

Chronic spontaneous urticaria (CSU) is a mast cell–driven skin disease characterized by the recurrence of transient wheals, angioedema, or both for more than 6 weeks.[1] Mast cell degranulation induced via the high-affinity IgE receptor (FcεRI) is considered to be one of the most frequent initiating factors in CSU.[2] This is evidenced by the effectiveness of omalizumab (anti-IgE) in reducing the symptoms of the disease.[3] In CSU, FcεRI-dependent mast cell degranulation may occur in either of two ways. First is type I autoimmunity (aka autoallergy) in which a patient's IgE is directed to autoallergens, such as thyroid peroxidase[4] and interleukin-24.[5,6] Second is type II autoimmunity in which autoantibodies to IgE or FcεRI initiate mast cell activation.[7,8] Beside these well-characterized mechanisms further, yet unidentified, serum-derived mast cell activation factors could be involved in the pathomechanism of CSU.[9-11]

The initial suggestion of autoantibodies to IgE or FcεRI arose more than 30 years ago from the autologous serum skin test (ASST) in which a positive response was the development of a wheal at the site of injection of a patient's own serum.[12] Studies by Grattan and colleagues identified these autoantibodies of the IgG class.[8,13,14] More recent studies have indicated them to be IgG_1 and IgG_3, which are able to cause degranulation of skin mast cells and activate complement.[15-18]

Whether or not CSU patients exhibit IgM or IgA autoantibodies to FcεRI is presently unknown as immunoblotting assays with purified IgG[18-20] and ELISAs[15,21] for the detection of IgG-anti-FcεRI or IgG-anti-IgE are not yet well-established for other antibodies. Although a rapid and specific dot-blot immunoassay to detect autoantibodies against FcεRIα was introduced by Lee and colleagues in 2014,[22]it is not available for routine clinical use.

In this study, we aimed to develop a simple and practical, yet highly specific, ELISA test to detect autoantibodies against FcεRIα in the serum of patients with CSU. We did this for two reasons. First, we wished to facilitate the identification of CSU patients with type IIb autoimmune urticaria by providing a test that is suitable for use in routine clinical practice. Second, we wished to analyze the serum of CSU patients for IgM and IgA autoantibodies against FcεRIα and assess if their presence is linked to positive ASST responses, disease activity, and other clinical parameters.

2 | METHODS

2.1 | Patients

Serum samples and clinical information were obtained from 35 patients with CSU seen at the Urticaria Center of Reference and Excellence (UCARE) at the Department of Dermatology and Allergy, Charité–Universitätsmedizin Berlin.[23] CSU was diagnosed, and its severity assessed according to the recent EAACI/GA^{2}LEN/EDF/WAO guideline for urticaria.[24] Patient blood samples and data were obtained as part of their clinical diagnostic workup, and surplus serum from clinical analyses was stored at −80°C until used for this study. All patients provided prior informed consent on the use of left-over serum as well as their clinical data for research purposes. As all analyses were performed

retrospectively and anonymously, additional ethics approval was not needed or obtained. Patients had not taken systemic steroids and H_1 antihistamines in the last 2 weeks and 3 days before sampling, respectively.

The control group consisted of 52 serum samples from healthy controls with a negative history of chronic urticaria, autoimmune disease, or clinically relevant allergic disease. Within this group, a sex- and age-matched healthy subgroup (n = 16) was analyzed in detail, to rule out differences due to demographics. Clinical characteristics and demographics of all patients with CSU and healthy controls are presented in Table S1.

2.2 | Development of an ELISA-based assay for the detection of anti-FcεRIα autoantibodies

An ELISA for IgM, IgG, and IgA autoantibodies against FcεRIα was developed and used to test sera of patients with CSU and healthy controls. Recombinant soluble FcεRIα expressed from yeast (MyBiosource) was coated at 50 ng/mL onto 96-well microtiter ELISA plates with 0.1 M bicarbonate buffer pH 9.6 overnight at 4°C. The plates were blocked with 10% fetal bovine serum (FBS) in phosphate buffered saline with 0.1% Tween-20 (PBST) for 2 hours. CSU and healthy control sera (1:100 with 10% FBS-PBST) were preincubated with FcεRIα at 100 ng/mL or with respective volume PBST of for 1 hour at room temperature. Preincubated and mock-incubated sera were loaded into the plates in parallel. After 2 hours of incubation at room temperature, the plates were washed with PBST (0.05% Tween-20) three times, followed by incubation with appropriately diluted AP-labeled goat-anti-human IgG, IgM, or IgA (KPL) for 1 hour at room temperature. The plates were washed five times prior to adding the blue phosphatase substrates for colorimetric development and read with a wavelength of 650 nm. The reduction of signal by preincubation with FcεRIα was considered to indicate the presence of antibodies to FcεRIα of the respective immunoglobulin group. Since those assays detect the presence of autoantibodies (IgG, IgM, IgA) against FcεRIα via signal differences with and without the preincubation with soluble FcεRIα (ratio of signal without and with FcεRIα), its results are largely independent of background signals and lower ratios reflect higher levels of autoantibodies (Figure S1).

Selected sera were measured repetitively (10 times) to assess the intra-assay variation coefficient (Figure S2). The normal range of assay results was determined by the use of healthy control sera. No significant differences between the autoantibodies values and ranges were seen when unselected healthy controls or selected age- and sex-matched cohorts were analyzed (Table S1). The 95% percentile was identified to give the best discriminative values (Table S2) and set as the cutoff for elevated levels of anti-FcεRIα autoantibodies. The 95% percentile cutoff values set with all healthy controls for IgM were determined at a ratio of 0.50, for IgA at 0.49, and for IgG at 0.46.

2.3 | Routine laboratory assessments

Routine laboratory assessments were performed in the central laboratory of the Charité-Universitätsmedizin Berlin. Routine assessments included C-reactive protein (CRP), complete blood count, total serum IgG, IgA, IgM, and IgE, electrolyte levels (sodium, potassium), laboratory values of the kidney (creatinine, urea) and liver (aspartate transaminase, alanine transaminase, gamma-GT), thyroid-stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), thyroid autoantibodies (anti-TPO, anti-TG), and IgG to hepatitis A, B, and C. Presence of Helicobacter pylori antigen was analyzed via PCR in the feces.

2.4 | Autologous serum skin test (ASST)

The ASST was performed as described previously.[25] Reactions were evaluated after 15 and 30 minutes and wheal diameter of at least 1.5 mm greater than that of the control wheal induced by the saline solution was recorded as positive. Because a higher prevalence of IgG-anti-FcεRIα was expected in ASST-positive patients, CSU patients were preselected into 25 ASST-positive and 10 ASST-negative patients.

2.5 | Assessment of urticaria activity and quality of life

Urticaria activity was assessed using the 7-day urticaria activity score (UAS7). [1]Assessment of disease control over the previous 4 weeks on signs and symptoms, quality of life impairment, efficacy of treatment, and overall disease control was performed using the urticaria control test (UCT).[26,27]Quality of life was assessed by the dermatology life quality index (DLQI) using the validated "band descriptors"[28]as follows: DLQI: 0-1 = no effect on patient's life, 2-5 = small effect, 6-10 = moderate effect, 11-20 = very large effect, and 21-30 = extremely large effect.

2.6 | Statistics

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 and nonparametric data using median and interquartile (IQR) range. Variation coefficient was calculated for repetitive measurements. For the cutoff determination of IgA, IgM, and IgG, the 0.05 percentile of the healthy population was calculated. The cutoffs assessed this way resulted in excellent specificity and were close to expert suggestions of a cutoff of 0.5, and therefore considered best practice for the analysis. Binominal variables were analyzed using chi-square test. Parametric variables were compared using the unpaired *t* test. Nonparametric continuous

TABLE 1 Patients with CSU more frequently display elevated levels of autoantibodies of different subclasses against FcεRIα

Aabs		CSU patients n = 35	Healthy controls n = 52	*P* value
IgG	IgG-anti-FcεRIα positive Number (%) of patients	8 (24%) n = 34[a]	3 (6%)	.037
	IgG-anti-FcεRIα (ratio) median (q1; q3) [range]	0.82 (0.48; 1.02) [0.19 - 1.61] n = 34	0.79 (0.67; 1.01) [0.4; 1.37]	.661
IgM	IgM-anti-FcεRIα positive Number (%) of patients	21 (60%)	3 (6%)	<.001
	IgM-anti-FcεRIα (ratio) median (q1; q3) [range]	0.47 (0.37; 0.98) [0.01 - 1.39]	0.88 (0.74; 0.99) [0.19 - 1.79]	.001
IgA	IgA-anti-FcεRIα positive Number (%) of patients	20 (57%)	3 (6%) n = 51[a]	<.001
	IgA-anti-FcεRIα (ratio) median (q1; q3) [range]	0.47 (0.24; 0.76) [0 - 1.77]	0.87 (0.74; 1.03) [0.39 - 1.66] n = 51[a]	<.001

Note: For IgG-anti-FcεRI, IgM-anti-FcεRI, and IgA anti-FcεRI (all assessed by the use of a competitive ELISA), subjects with values below the cutoff of 0.46, 0.59, and 0.49 were considered positive, respectively. Rates of patients or healthy controls with elevated autoantibody levels, that is, values below the cutoff, are given in the upper panel, and median and interquartile ranges of ratios are shown in the lower panel of each Ig subclass.

n is given if not all patients of the group were included.

q1 25% percentile.

q3 75% percentile.

[a]Single sample missing due to a technical problem.

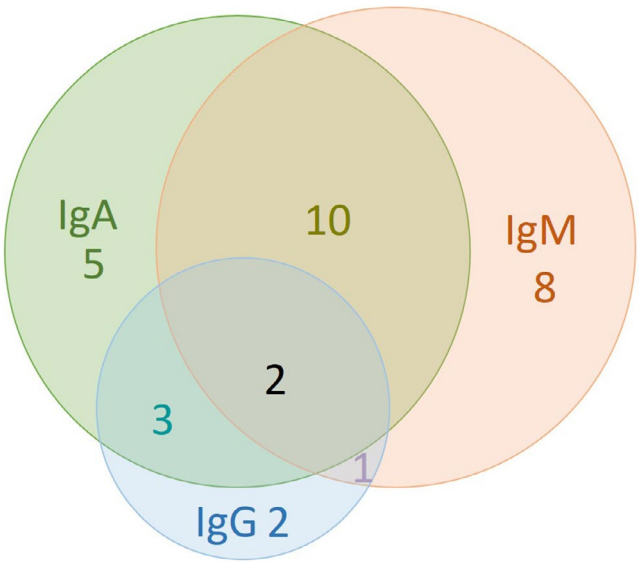

FIGURE 1 Venn diagram of co-occurrence of anti-FcεRIα antibodies of the three different subclasses. Numbers in the graph give the number of single-, double-, or triple-positive CS

variables were compared using the Mann-Whitney test. Correlations between two continuous variables were calculated using Spearman's rank correlation coefficient. $P < .05$ was considered to indicate statistical significance.

3 | RESULTS

3.1 | CSU patients have elevated levels of IgG, IgM, and IgA autoantibodies against FcεRIα

Of 35 patients with CSU, 8 (24%) had elevated serum levels of IgG autoantibodies against FcεRIα (IgG-anti-FcεRIα), compared with 6% of the healthy control group ($P = .037$, Table 1). Among all tested sera, IgG-anti-FcεRIα levels, on average, were comparable in CSU patients and healthy subjects (CSU median 0.82 [IQR 0.48-1.02] vs healthy median 0.79 [IQR 0.67-1.01], $P = .661$).

More than half of the CSU patients analyzed, 60% and 57%, respectively, had elevated IgM antibodies and IgA antibodies against FcεRIα as compared to 5% each of healthy controls. The CSU patient cohort also had significantly higher average levels of IgM-anti-FcεRI and IgA-anti-FcεRI (as reflected by lower ratios) compared with healthy controls ($P \leq .001$, Table 1).

Only four CSU patients (11%) did not have elevated levels of anti-FcεRIα autoantibodies of any subclass, and two patients (6%) were positive for all three subclasses. Six of the eight IgG-anti-FcεRIα-positive patients also had elevated levels of antibodies of other subclasses (IgM: n = 3; IgA: n = 5). A large proportion of CSU patients (n = 12; 35%) had both elevated IgM and IgA antibodies against FcεRIα (Figure 1).

Across all sera tested (CSU patients and healthy control subjects), IgG-anti-FcεRIα levels correlated weakly but significantly with

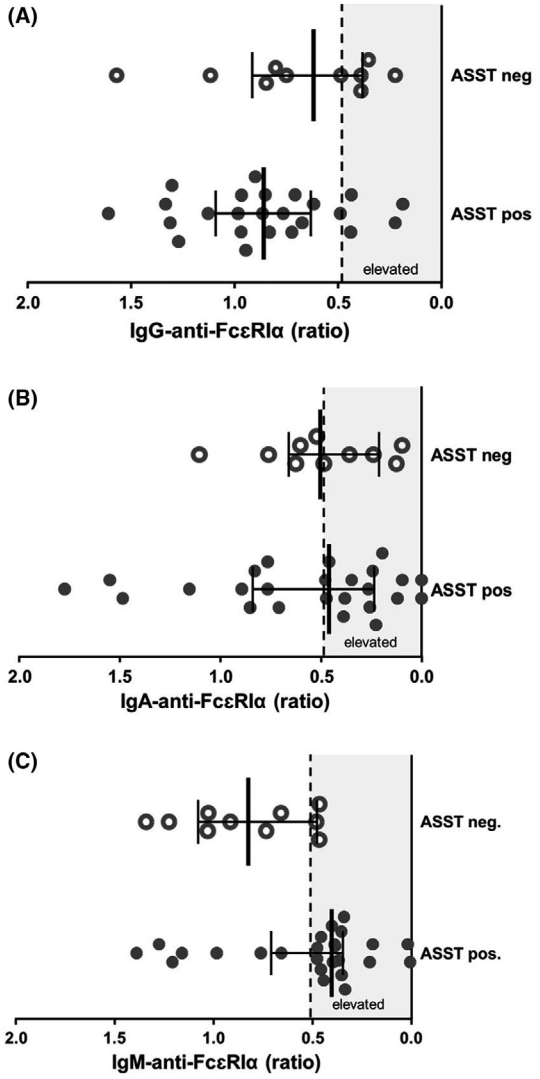

FIGURE 2 Autoantibodies to FcεRIα in the ASST-positive (n = 24) and ASST-negative (n = 10) CSU patient subgroups. All three panels of this figure show ratios of individual patients (every dot represents one patient). Low ratios reflect high autoantibody titers. In addition, median and interquartile ranges are shown for the ASST subgroups for (A) IgG-anti-FcεRIα, (B) IgA-anti-FcεRIα, and (C) IgM-anti-FcεRIα. Cutoffs of the individual assays are depicted as dotted line and areas of elevated levels are depicted in light gray

IgA-anti-FcεRIα (r = .349, P = .001), but not with IgM-anti-FcεRIα (r = .066, P = .547). IgM-anti-FcεRIα and IgA-anti-FcεRIα were also significantly correlated, albeit weakly (r = .375, P < .001).

3.2 | In patients with CSU, a positive ASST is linked to elevated levels of IgM against FcεRIα, but not IgG or IgA against FcεRIα

The analyzed CSU patient cohort contained 25 ASST-positive (71%) and 10 ASST-negative (29%) patients. IgG-anti-FcεRIα levels were elevated in four of the ASST-positive (17%) and four of the

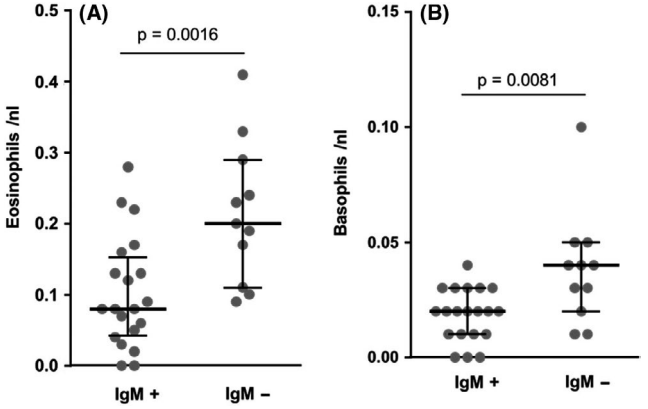

FIGURE 3 The presence of elevated levels of IgM antibodies against FcεRIα is linked to low basophil (3a) and eosinophil numbers (3b). Ratios of individual patients are displayed as dots, and median and interquartile ranges are shown for the anti-FcεRIα IgM-positive (n = 20) and anti-FcεRIα IgM-negative subgroups (n = 11)

ASST-negative (40%) patients (P = .154, Figure 2A). IgA-anti-FcεRIα antibodies were elevated in about half the patients who were ASST-positive (n = 13, 52%) and ASST-negative (n = 6, 60%, P = .668) (Figure 2B). In contrast, IgM-anti-FcεRIα autoantibodies were elevated significantly more often in ASST-positive patients (n = 18, 72%) compared with ASST-negative patients with CSU (n = 3, 30%; P = .022) (Figure 2C).

3.3 | In patients with CSU, IgM against FcεRIα, but not IgG or IgA against FcεRIα, is linked to basopenia and eosinopenia

The presence of elevated levels of IgM-anti-FcεRIα, but not IgG-anti-FcεRIα or IgA-anti-FcεRIα, correlated with low basophil numbers (r = .414, P = .021). IgM-anti-FcεRIα-positive CSU patients had significantly lower blood basophil numbers (median 0.02/nL [IQR 0.01-0.03]) as compared to IgM-anti-FcεRIα-negative patients (median 0.22/nL [IQR 0.13-0.32] P = .0081, see Figure 3A).

The presence of elevated levels of IgM-anti-FcεRIα also showed a clear correlation with the patients' eosinophil numbers (r = .623, P < .001). Again, IgM-anti-FcεRIα-positive CSU patients had significantly lower absolute eosinophil numbers (median 0.11/nL [IQR 0.04-0.15]) compared with IgM-anti-FcεRIα-negative patients (median 0.2/nL [IQR 0.12-0.32] P = .0016; see Figure 3B). The presence of elevated levels of IgG-anti-FcεRIα or IgA-anti-FcεRIα was not linked to eosinopenia, and patients who were positive for these autoantibodies did not have reduced blood eosinophil numbers as compared to patients who were not (data not shown).

A weak, but significant correlation of IgM-anti-FcεRIα positivity, but not IgG-anti-FcεRIα positivity or IgA-anti-FcεRIα positivity, was also seen with lymphocyte numbers (r = .38, P = .028).

Indicators of disease activity (UAS7), disease control (UCT), or quality of life (DLQI) in our CSU patient cohort were not significantly different or correlated with any of the anti-FcεRIα subclasses

tested. There was also no difference or link to other cells assessed in the blood count, electrolyte levels, CRP levels, kidney or liver markers, thyroid hormones or thyroid autoantibodies or total IgE. Furthermore, IgG, IgM and IgA autoantibodies against FcεRIα were not correlated with total serum IgG, IgM and IgA levels, respectively.

4 | DISCUSSION

In this study, we present a new method for the detection of autoantibodies against FcεRIα. To our knowledge, this is the first report to show that CSU patients, in addition to having IgG autoantibodies, have IgM and IgA autoantibodies against FcεRIα. This new ELISA-based test is inexpensive and easy to perform, suitable for high-throughput routine analysis. The assay has proved to be robust in repetitive measurements and circumvents the common problem of serum background signals, by using specific competitive signal inhibition by soluble FcεRIα preincubation.

IgG autoantibodies against FcεRIα were first described in patients with CSU more than 20 years ago.[18] Despite this, no routine testing of these autoantibodies is currently available. Some of the methods used require IgG purification of the serum before detecting via Western blot techniques,[18,19,29-33] which is time-consuming. Using our newly developed ELISA, the prevalence of elevated levels of IgG autoantibodies against FcεRIα was 24% in the analyzed CSU patient sera. These results are in line with many other reports in the literature where rates of 25% up to 52% had been reported in CSU patients.[18,19,29-33]

More interestingly, the majority of our analyzed sera also contained elevated levels of IgM (60%) and IgA autoantibodies (57%) to FcεRIα. Little is known about the prevalence and function of IgM and IgA autoantibodies in patients with CSU. Gruber et al[34] analyzed the prevalence of IgG and IgM autoantibodies directed against IgE in distinct subsets of urticaria, namely CSU and cold urticaria as well as urticaria vasculitis. IgG-anti-IgE antibodies were found in 50% of patients with CSU, in 55% of patients with cold urticaria, and in 50% of patients with urticarial vasculitis. IgM-anti-IgE antibodies with histamine-releasing activity were found exclusively in cold urticaria (22%), but not in patients with CSU.

The ASST is a well-established test to investigate serum autoreactivity in patients with CSU[35] and has been associated with IgG antibodies against the FcεRIα or against IgE (Type IIb autoimmunity).[7,33] Also, basophil activation tests and basophil histamine release tests have been shown to be a good surrogate marker for autoimmunity in CSU,[7] but are not commonly available as routine analysis and had not been assessed in this study cohort. Surprisingly, in our cohort, ASST-positive patients did not show a higher rate of elevated levels of IgG to FcεRIα compared with ASST-negative patients. This finding is somewhat contradictory those of other groups, who reported up to sixfold higher rates of IgG autoantibodies to FcεRIαin ASST-positive patients compared to ASST-negative CSU patients.[29,33] However, our cohort had overall small numbers, especially in the ASST-negative CSU patient group, which limits the interpretation of our results. Furthermore, other studies used different methods for IgG antibody detection, which might explain, in part, the large differences reported among different studies. In previous reports, it had been shown that the IgG autoantibodies in urticaria patients belong predominately to the IgG1 and IgG3 subtype and, to a lesser extent, IgG4,[15,36] who have complement fixing and basophil activation abilities. Asero and colleagues have shown that >100kDa serum fractions (that include IgG) are able to degranulate basophils and that at least in some cases complement seems essential for histamine-releasing activity of serum from patients with CIU.[37] In our study, we have not further discriminated the IgG subtypes or complement fixation ability.

One of the most interesting findings of our study is that elevated levels of IgM-anti-FcεRIα are markedly more common in ASST-positive patients (72%) compared with ASST-negative patients (30%) (Figure 2B).

Antibodies involved in autoimmunity usually belong to the IgG and IgM subclasses. In CSU, Grattan et al[8] showed that the IgM fraction of three CSU sera led to some histamine release from healthy donor basophils, but the specificity of these IgM antibodies remains elusive. To our knowledge, there are no identified IgM autoantibodies with known specificity in patients with CSU in the literature. As IgG autoantibodies to IgE or to FcεRIα exist in CSU, the presence of IgM autoantibodies to IgE or to FcεRIα is feasible and could play a significant role in the pathogenesis of CSU. Their presence might explain ASST reactivity in IgG-anti-FcεRIα and IgG-anti-IgE negative CSU patients, especially as there were only a few patients (n = 3) who were double positive for IgG and IgM autoantibodies. Specific IgM is thought to precede IgG formation,[38] and thus, IgM-positive CSU patients might become IgG positive in the course of their disease. Longitudinal studies following CSU patients over longer time periods assessing IgG or other autoantibodies are currently missing.

Additionally, IgA autoantibodies to FcεRIα were also frequently elevated (57%) in our CSU cohort. Of note, all of the four patients in our cohort who tested positive for Helicobacter antigen displayed IgA autoantibodies (data not shown), and five out of eight IgA-positive patients had IgG antibodies against hepatitis A, but none of the seven IgA negative patients, whose serological hepatitis were available (Fisher's exact; $P = .007$), indicating that present or past gastrointestinal or mucosal associated infections could be a trigger for the development of IgA autoantibodies. These autoantibodies showed a correlation with IgM autoantibodies, and more than one third (35%) of the CSU patients were double positive for both autoantibodies. In contrast to the IgM autoantibody titers, there was no significant difference between the ASST-positive and negative CSU patient cohorts (Figure 2C).

FcεRIα is not only expressed on mast cells, but also on basophils[39] and in some inflammatory diseases, also on eosinophils.[40,41] In CSU, basopenia has been shown to be correlated with disease activity and is restored upon successful treatment with omalizumab.[42,43] In a subgroup of CSU patients, eosinopenia can also be observed.[44] Serum-induced basophil activation, which can be observed with many CSU sera samples, is thought to be due to autoantibodies

against IgE or FcεRIα, leading to basopenia.[45] In our study, IgM but not IgG autoantibodies against FcεRIα were significantly correlated with basopenia and eosinopenia. IgM, like some IgG subclasses, is a complement fixing antibody that can directly activate the complement system with subsequent cell destruction,[46] possibly leading to the observed low cell count.

In our CSU patient cohort, the presence of autoantibodies of any class was not associated with higher disease activity, disease burden, or less disease control. It has been reported that the presence of IgG autoantibodies against FcεRIα is linked to more severe symptoms and a poor response to conventional antihistamine therapy, as well as a slower response to anti-IgE treatment.[19,30,47] In a large multicenter trial using this new assay for IgG-anti-FcεRIα determination, along with many other autoimmune markers, higher UAS7 scores in autoimmune type II CSU patients were seen, but not in other scorings.[7] We cannot explain why we did not see this correlation in our study but the small sample size or the preselection of patients may have been relevant. Of note, there was also no correlation with the total serum IgG, IgM, or IgA values, which strengthens the idea that the detected autoantibodies are rather specific.

The overall limitations of this study are the retrospective analysis and the limited number of preselected patients from a specialized tertiary care center. Nevertheless, we were able to demonstrate, for the first time, the presence of new immunoglobulin class antibodies against FcεRIα and the association with autoimmune disease features. Further limitations are given by the fact that this test assesses the occurrence of the autoantibodies, but does not directly answer the question of functional relevance of these autoantibodies. Here, further studies are needed to prove this.

In conclusion, this study reports a new and easy method for the detection of IgG, IgM, and IgA autoantibodies against FcεRIα. Elevated levels of IgM and IgA class autoantibodies against FcεRIα were more frequent than those of IgG. The detection of these new autoantibody classes in CSU and, in the case of IgM, the association with clinical features such as the ASST and basopenia add to our understanding of the role of autoantibodies in CSU.

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CONFLICT OF INTEREST

All authors have no conflict of interests in relation to this work.

AUTHORS' CONTRIBUTION

Sabine Altrichter has coordinated the study, collected patient data, was involved in statistical analysis, and drafted the manuscript. Vasiliki Zampeli was involved in manuscript preparation and proofreading of the manuscript. André Ellrich was involved in the statistical analysis and proofreading of the manuscript. Ke Zhang performed laboratory tests and was involved in the proofreading of the manuscript. Martin Church was involved in manuscript preparation and proofreading of the manuscript. Marcus Maurer was the overall study coordinator and was involved in manuscript preparation and proofreading of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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