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IL-24 is a common and specific autoantigen of IgE in chronic spontaneous urticaria

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1 **IL-24 is a common and specific autoantigen of IgE in chronic**

2 **spontaneous urticaria**

3

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15

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17

18 **Running title:** IL-24 is a specific IgE autoantigen in CSU

19

21 **Background:** The efficacy of omalizumab (anti-IgE) and elevated IgE levels
22 in chronic spontaneous urticaria (CSU) suggest autoallergic mechanisms.

23 **Objective:** To identify autoallergic targets of IgE in CSU patients.

24 **Methods:** Sera of CSU patients together with idiopathic anaphylaxis patients
25 and healthy controls (seven of each) were screened for IgE-autoantibodies
26 using an array of >9,000 proteins. Sera of 1062 CSU patients and 482 healthy
27 controls were used in an IgE-anti-Interleukin-24 (IL-24) specific ELISA to
28 investigate the association of IgE-anti-IL-24 and CSU.

29 **Results:** By array analyses, over 200 IgE-autoantigens were found in CSU
30 patients that were not in controls. Of the 31 IgE-autoantigens detected in
31 >70% of patients, eight were soluble or membrane bound and expressed in
32 skin. Of these, only IgE-autoantibodies to IL-24 were found in all CSU
33 patients. *In vitro* studies showed IL-24 to release histamine from human mast
34 cells sensitized with purified IgE of CSU patients but not controls. By ELISA
35 analyses, the mean $\pm$ SD levels of IgE-anti-IL-24 were 0.52 ± 0.24 IU/ml in
36 CSU and 0.27 ± 0.08 IU/ml in controls with 80% of CSU patients but only 20%
37 of controls having levels >0.33 IU/ml ($P < 0.0001$). IgE-anti-IL-24 showed
38 acceptable predictive properties for CSU, with a likelihood ratio of 3.9.
39 Clinically, IgE-anti-IL-24 levels showed an association with disease activity as
40 assessed by the urticaria activity score and with reduced basophil counts.

41 **Conclusion:** Our findings show that CSU patients frequently exhibit IgE-
42 autoantibodies against many autoantigens and that IL-24 is a common,
43 specific, and functional autoantigen of IgE-antibodies in CSU.

44

- 46 • CSU patients have IgE to many different autoantigens, including IL-24.
- 47 • 80% of CSU patients have elevated IgE-anti-IL-24 levels.
- 48 • IgE-anti-IL-24 is functional and levels are linked to disease activity.

49 **Capsule Summary**

50 From an array of >9,000 proteins, IL-24 was identified as a common, specific,
51 and functional autoantigen of IgE-antibodies in chronic spontaneous urticaria.
52 Higher IgE-anti-IL-24 levels were associated with higher disease activity and
53 lower basophil counts.

54

55 **Key words:** IL-24; IgE; autoantibody; autoallergen; autoallergy; type I
56 hypersensitivity; chronic spontaneous urticaria; wheal and flare; protein
57 microarray; urticaria activity score

58

- 60 AA, autoantigen
61 ASST, autologous serum skin test
62 CSU, chronic spontaneous urticaria
63 dsDNA, double stranded DNA
64 ELISA, enzyme linked immunoassay
65 GFP, green fluorescent protein
66 HSA, humane serum albumin
67 IdA, idiopathic anaphylaxis
68 IgE, immunoglobulin E
69 IgE-AA, autoantigen recognized by IgE = autoallergen
70 IgE-AAb, IgE autoantibody
71 IU, international units
72 PSCMC, Peripheral CD34+ stem cell-derived mast cell
73 SLE, systemic lupus erythematosus
74 TPO, thyroid peroxidase
75 UAS7, 7-day urticaria activity score
76

78 It is now 75 years since it was first suggested that the production of wheals
79 urticaria may have an underlying allergic mechanism¹. Certainly, the
80 effectiveness of H₁-antihistamines in urticaria² would support this hypothesis
81 even though no obvious extrinsic allergens have been identified. This
82 prompted Rorsman³ to suggest urticaria to be an autoallergy, i.e. type I
83 hypersensitivity to self-allergens. The involvement of IgE in urticaria was
84 suggested from two sources: First is the association of chronic spontaneous
85 urticaria (CSU) with high levels of total IgE and with elevated levels of IgE
86 directed, for example, thyroid antigens and double stranded DNA (dsDNA)⁴⁻⁶.
87 Second is the effectiveness of omalizumab (anti-IgE) in relieving the
88 symptoms of CSU⁷.

89 In other autoimmune conditions, such as systemic lupus
90 erythematosus (SLE), the disease is complex and characterized by the
91 production of autoantibodies to a broad range of self-antigens⁸. For example,
92 in SLE, IgE antibodies specific for dsDNA have been suggested to stimulate
93 plasmacytoid dendritic cells, an immune cell linked to viral defence, to secrete
94 substantial amounts of interferon-alpha⁹.

95 We postulate that CSU is an autoimmune disease in which IgE
96 recognizes several autoantigens, which are probably primarily dermal in
97 origin. Low-level diffusion of these into the bloodstream would be consistent
98 with the basopenia seen in CSU^{3, 10}. Furthermore, periods of increased local
99 generation of such allergens would explain the localized exacerbations of
100 CSU.

101 To test this hypothesis, a large-scale screening of autoreactive IgE in
102 the serum of CSU patients was used to explore potential targets of IgE

103 autoantibodies in this disease. IgE present in the serum from idiopathic

104 anaphylaxis (IdA) patients and healthy individuals was used as control. The
105 association of the primary IgE-autoantibody was further investigated both *in*
106 *vitro* and clinically.

107

108 **Material and Methods**

109 *Serum from CSU patients and control subjects*

110 We analysed 1062 CSU patients (73% female), 7 IdA patients (57%
111 female) and 482 healthy controls (61% female). There was no significant
112 difference between the ages of CSU patients (mean $\pm$ SD, 44.0 $\pm$ 14.7 years),
113 IdA patients (44.4 $\pm$ 13 years) and healthy controls (46.9 $\pm$ 16.7 years).

114 All patients were treated at our specialized outpatient clinic for
115 dermatological allergology. They were diagnosed with CSU and idiopathic
116 anaphylaxis in line with the current World Allergy Organization Guidelines for
117 the Assessment and Management of Anaphylaxis and the
118 EAACI/GA²LEN/EDF/WAO urticaria guideline, respectively. None of the CSU
119 patients had concomitant IdA, and none of the IdA patients had CSU, thus
120 excluding overlap in the patients analysed by microarray analyses. We did not
121 systematically assess autoimmune comorbidities in the study subjects.

122 In our CSU patients, the mean duration of disease was 5.0 years, and
123 the mean seven-day urticaria activity score (UAS7²) was 16.7. Fifty percent of
124 CSU patients had angioedema, and 26% had positive ASST reactions,
125 defined as being greater than 2 mm compared to the negative control.

126 Blood was taken from all study subjects, and serum was separated
127 after clot formation without the use of a clotting enhancer. After separation,

serum was frozen immediately and stored at -80°C until used for microarray,

ELISA or IgE purification for functional studies.

Ethics Statement

Blood samples taken from patients and all material investigated in this study were used after written informed consent as approved by the ethics committee of the Charité - Universitätsmedizin Berlin (EA1-329-14 and EA1-292-14) according to the Declaration of Helsinki (59th WMA General Assembly, Seoul, October 2008).

Auto-IgEome analyses by microarray

One ml serum from seven CSU patients, seven IdA patients and seven healthy controls was incubated on a ProtoArray® Human Protein Microarray version 5.1 (ThermoFisher, Waltham, USA) at room temperature (RT) for 1h. This microarray contains 9,374 unique, full-length human proteins. The slide was then washed three times with TPBS (PBS with 0.05% Tween-20, Sigma, Munich, Germany) and incubated with 1 ml staining solution for 30 minutes in the dark (1 µg/ml each of mouse anti-human IgG-PE (clone G18-145) and mouse anti-human IgE-APC (clone MHE18) (both Biolegend, San Diego, USA) in 2% human albumin (clinical grade, Baxter, Unterschleißheim, Germany) in TPBS). Thereafter, the slide was washed extensively with TPBS followed by three washes with water and measurement at 2 µm resolution in a Powerscanner (Tecan, Männedorf, Switzerland). Specificity of the monoclonal antibodies was tested on non-serum treated chips to remove off-target bindings. The signal was calculated as mean or Z score and signals

corresponding to immunoglobulin-binding proteins (TRIM-domain containing

or FcR proteins) were removed.

Quantification of IgE-anti-Interleukin 24 function

The functionality of IgE-anti-Interleukin 24 (IL-24) was tested using histamine release from peripheral stem cell derived mast cells (PSCMCs), which were prepared as previously described¹¹. Briefly, CD34 positive cells (Stem Cell Technologies, Cologne, Germany) were cultured with recombinant IL-3 and SCF (both from Miltenyi, Bergisch Gladbach, Germany) for two weeks in SFEM medium containing human LDL (Stem Cell Technologies). Beads for IgE purification were prepared with 1 mg anti-IgE mab (clone: MB10-5C4, Miltenyi) conjugated to NHS-mag sepharose (GE, Frankfurt am Main, Germany) and blocked with 100 mM ethanolamine in 1 M Tris, pH 8.0 (Sigma). Total IgE was purified from 1 l pooled CSU or healthy serum by using these beads after 24 h incubation at 4°C. Beads were washed with TPBS and IgE was eluted with 0.1 M citrate buffer (Sigma) at pH 3 and stabilized with 1 M Tris pH 8.0. PSCMCs were diluted to 2×10^5 /ml in 10 ml SFEM medium (Stem Cell Technologies) containing 250 μ l (=10 μ g) total IgE preparation and incubated over night at 37°C. Ninety-six well plates were coated overnight at 37°C with 0.5 μ g IL-24 (Miltenyi) per well in carbonate buffer, pH 9. Plates were blocked for 2 h at room temperature with 2% HSA in PBS. IgE loaded PSCMCs were washed with RPMI (Thermofisher) and added to the plates to a final concentration of 80k cells per well in 0.2 ml SFEM. In addition to the coated wells, further wells were submitted to anti-IgE (1 μ g/well), 1% triton-x or nothing for 30 min at 37°C. Thereafter, plates were centrifuged at 400 x g for 8 minutes to deplete cells. Supernatants were

179 stored in -80 °C until histamine measurement in a Histareader (Reflab,

180 Copenhagen, Denmark).

181
182 *IgE-anti-IL-24 measurements by ELISA*

183 Sixteen µg of anti-IgE (MHE18, Biolegend) were dispensed in 10 ml
184 Coating Buffer (0.1 M carbonate at pH 9, Sigma) and 25 µl of this solution
185 was used per well in a black NUNC Maxisorb 384-well plate (Thermofisher) to
186 coat at 4°C overnight. After blocking with 2% HSA in TPBS again overnight at
187 4°C, human sera were added from a 96-well plate as four 25 µl/well replicates
188 and incubated at 4°C overnight followed by three washes with TPBS and
189 three washes with water. Thereafter, 25 µl/well antigen-solution containing 4
190 µg BHHCT (Sigma) conjugated (molar ratio of 1:30) and europium (Sigma)
191 saturated IL-24 (Miltenyi) per 10 ml 50 µM DTPA-purified BSA (Perkin Elmer,
192 Waltham, USA) in 50 mM Tris buffer pH 8 was added and incubated at 4°C
193 for 1 h. After washing ten times with water, 25 µl DELFIA enhancer solution
194 (Perkin Elmer) was added to each well. The plates were measured in a Victor
195 V reader (Perkin Elmer) with standard settings.

196 We report mean values of a total of 1062 CSU patients and 482
197 healthy controls, with each individual tested up to four times in four
198 independent experiments with similar results, one of which is shown in figure
199 4.

200
201 *Calculation of IgE-anti-IL-24 units, fractions, and cut-off*

202 Recombinant IgE-anti-GFP (Bio-Rad, Munich, Germany) was used to
203 generate a standard curve, to calculate IgE-anti-IL-24 in IU/ml serum
204 measured by ELISA and IgE-anti-IL-24 IU/protein spot by microarray. The

sum of autoreactive IgE as detected by the microarray was calculated based

on the IgE-anti-IL-24 protein spots, which allowed to determine the percentage of IgE-anti-IL-24 of all detectable autoreactive IgE. Due to several calculation steps the values may be viewed as an approximation. Total IgE levels measured by the Central Laboratory of Charité – Universitätsmedizin Berlin were used to calculate the percentage of ELISA-based IgE-anti-IL-24. The cut-off for elevated IgE-anti-IL-24 ELISA values was calculated according to the ROC curve using the Youden index.

Assessment of basophil numbers

Blood basophil numbers were determined by the Central Laboratory of the Charité - Universitätsmedizin Berlin by use of an automated haematocytometer.

Statistical analyses

Normal distribution was tested with D'Agostino-Pearson omnibus test, and an approximately Gaussian distribution served as basis to perform Student's t and ANOVA tests corrected for multiple testing by the Holm-Sidak method. The null hypothesis of random results was rejected if the *P* value was very small <0.05.

Results

CSU patients frequently exhibit IgE against many autoantigens

All seven CSU patients, IdA patients and healthy controls exhibited IgE autoantibodies (IgE-AAbs) against multiple autoallergens (AAs) as assessed by microarray analyses of serum reactivity to 9,374 human proteins (Fig. 1).

231 IgE-AAbs from CSU patients recognized a mean $\pm$ SD of 95 ± 66 AAs, IdA

232 patients 105 ± 63 and healthy controls 28 ± 11 . The distribution of AAs
233 recognized by each group is shown in the Venn diagram (Fig. 2; Tables 1 and
234 2). Of the total 226 IgE-AAbs recognized by at least one CSU patient, only four
235 were also recognized by an IdA patient's IgE and none by IgE from any
236 healthy control. IgE from CSU patients was more heterogenous and
237 recognized more different AAs than IgE from IdA patients (226 vs. 161).

238 Considering the IgE-AAbs recognized only by CSU serum, Tables 1 and
239 2 show that 222 AAs were recognized by at least one patient. As the number
240 of patients increased, the number of common AAs decreased, so that the IgE
241 of half of the patients reacted to 77 AAs, while 31 AAs were recognized by
242 71% of patients, and only three AAs are fully shared AAs, recognized by all
243 CSU patients.

244

245 *Accessibility of CSU-specific IgE-AAbs to AAs that are expressed in the skin*

246 Out of the 31 AAs to which most (71%) of the CSU patients had IgE-
247 AAbs, eight were accessible, four as soluble and four as membrane-bound
248 AAs. In contrast, the other 23 IgE-AAbs were intracellular and, therefore, are
249 unlikely to be available. Of the eight accessible IgE-AAbs, all can be expressed
250 in the skin, but only one, interleukin-24 (IL-24) was detected by IgE-AAbs of
251 all seven CSU patients (Fig. 1).

252

253 *IgE-anti-IL-24 is the most abundant IgE-AAb in CSU patients*

254 In all CSU patients analysed by protein array, IgE-anti-IL-24 showed
255 the highest serum levels of all IgE-AAbs detected. Up to 1.1% (mean $\pm$ SD:
256 $0.6\% \pm 0.24\%$) of the total detectable IgE-AAbs in CSU patients was IgE-anti-

257 IL-24. IgE-anti-IL-24 serum levels did not correlate with total IgE ($r = 0.002$, P
258 $= 0.840$) or total IgE-AAb levels. None of the seven CSU patients showed
259 detectable IgG-anti-IL-24 serum levels.

260

261 *IgE-anti-IL-24 and IL-24 induce mast cell degranulation*

262 Peripheral stem cell derived mast cells (PSCMCs) were sensitized with
263 purified total IgE of CSU patients or normal control subjects, or with purified
264 IgE-anti-IL-24. IL-24 induced degranulation in cells that had been
265 preincubated with the purified IgE of CSU patients but not normal control
266 subjects (Fig. 3A). Mast cells also showed degranulation after sensitization
267 with purified IgE-anti-IL-24 and challenge with IL-24 (Fig. 3B).

268

269 *IgE-anti-IL-24 is a sensitive and specific marker of CSU*

270 To further investigate the association of IgE-anti-IL-24 and CSU, we
271 developed and used an IgE-anti-IL-24-specific ELISA to measure serum
272 levels in CSU patients and healthy controls. In combined data from all tested
273 CSU patients ($n = 1062$) and healthy controls ($n = 482$), the mean $\pm$ SD IgE-
274 anti-IL-24 serum levels were 0.52 ± 0.24 IU/ml (median 0.49, range 0.00 –
275 2.33) and 0.27 ± 0.08 IU/ml, respectively (median 0.26, range 0.10 - 0.61; $P =$
276 0.001; Fig. 4). 847 of 1062 CSU patients (80%) exhibited higher than normal
277 IgE-anti-IL-24 serum levels as compared to 99 of 482 healthy controls (20%,
278 $P < 0.0001$), based on the calculated cut-off, 0.33 IU/ml. IgE-anti-IL-24
279 showed acceptable predictive properties for CSU, with a sensitivity of 80%
280 (76-83 CI 95%), a specificity of 80% (75-84% CI 95%) and a likelihood ratio of
281 3.89 (Fig. 4).

282

284 Microarray-based IgE-anti-IL-24 levels correlated with CSU activity as
285 assessed using the seven-day urticaria activity score, UAS7 ($P = 0.03$; Fig.
286 5A). ELISA-based measurements also showed that relative IgE-anti-IL-24
287 levels were linked to disease activity, albeit weakly (Fig. 5B and C). Relative
288 IgE-anti-IL-24 levels showed a negative association with blood basophil
289 counts (Fig. 5D).

291 Discussion

292 This study shows that CSU patients produce IgE against a wide array
293 of diverse autoantigens that are distinct from those in patients with idiopathic
294 anaphylaxis and healthy controls. Further analyses showed IL-24 to be a
295 common, functional and specific target of IgE in CSU patients. These findings
296 support a role for IgE directed to dermal autoantigens, of which IL-24 is the
297 first to be identified, in the pathogenesis of CSU.

298 This study has performed full-scale screening of IgE autoreactivity in
299 CSU using >9,000 AAs. Previous studies had identified only single IgE
300 reactivities to self (e.g. IgE against thyroperoxidase or DNA) and did not use
301 screening assays for IgE^{5, 12-19}. Our present study shows that the IgE of CSU
302 patients is directed to many more AAs than that of healthy control subjects,
303 226 vs. 34 AAs.

304 CSU patients also exhibit IgE reactivity to a larger set of different AAs
305 than patients with IdA, who show similar levels of total IgE as CSU patients.
306 This supports the conclusion that the IgE reactivities detected are specific,
307 rather than artefacts due to elevated total IgE levels. An important question
308 that needs to be addressed by future studies is whether each AA in CSU is

309 detected by one specific IgE or whether there are some IgEs that recognize
310 several AAs. Because we cannot answer this question at present, we have
311 assumed in our calculation of IgE-anti-IL-24 levels that all recognized AAs are
312 bound by a mono-specific IgE. However, as it is possible that the IgE-anti-IL-
313 24 is multivalent, in which case only a fraction will be bound to IL-24 in the
314 microarray, this would cause an underestimation of the IU/ml level of our IgE-
315 anti-IL-24 results. One thing that is very clear from the Venn diagram (Fig. 2)
316 is that the majority of IgEs of CSU patients and IdA patients and normal
317 controls bind to different, group-specific AAs. We have explored further only
318 the IgE-AAbs in CSU sera.

319 For an IgE-AA to be involved in the pathogenesis of CSU it would need
320 to be both present in the skin and accessible to mast cell-bound IgE. Eight of
321 226 CSU IgE-AAs fulfil these criteria and are, therefore, likely candidates for
322 causing IgE-dependent skin mast cell activation in CSU. Of these, IL-24, is of
323 prime interest for three reasons: first, IgE against IL-24 is present in the serum
324 of most CSU patients; second IL-24 is present in a soluble form in the skin²⁰;
325 and, third IgE-anti-IL-24 had the highest serum levels of all IgE-AAbs
326 detected, with about 1% of the total IgE in CSU patients directed to this
327 cytokine. This 1% level suggests clinical relevance, as it is higher than the
328 clinically relevant 0.2% of IgE to beta-lactam antibiotic²¹ and similar to 1.4%
329 for cor a 14 and 3.2% for ara h2²² in patients with allergies to these allergens.

330 A further requisite for IgE-anti-IL-24 to be involved in the pathogenesis
331 of CSU would be its ability to activate mast cells to release histamine. This
332 was confirmed by loading cultured human mast cells with IgE derived from the
333 serum of CSU patients followed by challenge with IL-24.

334 Presently, we do not know and can only speculate whether the
335 biological effects of IL-24, other than being a target of IgE, are relevant in the
336 pathogenesis of CSU. IL-24, an IL-10-family cytokine²³, has been linked to
337 inflammation^{24, 25}, and it has been described to be upregulated in several
338 autoimmune diseases such as psoriasis and rheumatoid arthritis (RA)²⁶⁻²⁸. In
339 mouse models of psoriasis, IL-24 is induced by TNF in keratinocytes, and
340 inflammation is dependent on this IL-24-induction^{28, 29}. Unlike IL-10, IL-24 is
341 not immunosuppressive, but leads to a broad stimulation of various immune
342 cells and functions^{30, 31}.

343 In contrast to previous studies, our microarray screen did not identify
344 thyroid peroxidase (TPO) as a common IgE-AA^{5, 12, 32}. The reasons for this
345 are currently unclear but may include differences in the glycosylation, folding
346 or other features of the TPO used, quenching of the IgE-anti-TPO signal in
347 our assay by the presence of IgG-anti-TPO, or that IgE-anti-TPO has a higher
348 affinity to other autoantigens and therefore binds to a different spot on our
349 microarray.

350 Sir Bradford Hill's 6th criterion for causality states that demonstration of
351 a biological gradient may be associated with increased symptoms⁷. Our
352 finding that IgE-anti-IL-24 levels in CSU patients, assessed either by
353 microarray or ELISA, are linked to disease activity supports this criterion.
354 The negative association with blood basophil counts may be the consequence
355 of basophil activation by IgE-anti-IL-24 in the blood^{10, 33}.

356 The strength of this study is that it shows marked increase in IgE-anti-
357 IL-24 levels in patients with CSU as compared with normal donors. We used a
358 comprehensive array of AAs, included IgE-matched control samples as well
359 as normal donors to rule out effects that are based on higher total IgE levels

and confirmed our microarray results in large populations of patients and

normal controls by ELISA. A further strength of this study is that it provides *ex vivo* evidence for the bioactivity of autoreactive IgE and associations with disease activity, both of which suggest pathophysiologic relevance.

A weakness of the study is that we also identified IgE-anti-IL-24 in some normal donors, although with a markedly lower concentration. Other studies have shown that expression of a major anti-FcεRI antibody clone can also be found in healthy donors but does not correlate with any clinical finding^{14, 34}. A further important weakness of this study is that we cannot yet clarify whether IL-24 is the most specific and relevant AA in CSU as we screened only a fraction of the human proteome with only the major protein variants of each protein included. Also, we calculated the concentration of IgE-anti-IL-24 based on recombinant IgE-anti-GFP, which might have a much stronger affinity. If the IgE binding to IL-24 is only one hallmark of the autoreactive IgE and it is bound also to other AAs on the array, the concentration of the IgE-anti-IL-24 might be largely underestimated.

In conclusion, IL-24 has been identified as a common, specific, and functional autoantigen of IgE-antibodies in patients with CSU. Higher IgE-anti-IL-24 levels were associated with higher disease activity. This demonstration of autoreactive IgE in CSU may explain, at least in part, the clinical success of treatment of the disease with the anti-IgE agent omalizumab. New assays for measuring autoreactive IgE including IgE-anti-IL-24 may help in the clinical management and monitoring of treatment responses in CSU patients.

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387 diagnoses (MAD³) team of the Dermatological Allergy (DEAL) group at
388 Charité for help and support and Prof. Margitta Worm for providing sera of
389 patients with idiopathic anaphylaxis.

390

391 **Conflict of interest statement**

392 None of the authors have conflicts of interest to declare in relation to this
393 work.

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Table 1. CSU patients exhibit IgE-reactivity to many autoantigens (autoallergens) that are not detected by the IgE of patients with idiopathic anaphylaxis or healthy controls.

Rate (%) of CSU patients	1 of 7 (14%)	2 of 7 (29%)	3 of 7 (43%)	4 of 7 (57%)	5 of 7 (71%)	6 of 7 (86%)	7 of 7 (100%)
Number of AAs not detected by IgE in any of the control subjects	222	180	128	77	31	19	3

Abbreviations: CSU = chronic spontaneous urticaria, AAs = autoantigens. The table shows the numbers of specific IgE-AAs shared by all CSU patients (7 of 7), some CSU patients (2 to 6 of 7), and found in any of the CSU patients (1 of 7).

519 Table 2: The total number of autoantigens detected by IgE in the serum of CSU patients and control subjects.

520

	CSU patients							Idiopathic Anaphylaxis patients							Healthy controls						
Subject number	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Absolute number of IgE-AAs / subject	29	40	49	68	116	166	197	10	24	112	128	147	157	158	0	30	32	32	32	34	34
Mean $\pm$ SD number of IgE-AAs	95 $\pm$ 66							105 $\pm$ 63							28 $\pm$ 11						
Median (range) number of IgE-AAs	68 (29 – 197)							128 (10 – 158)							32 (0-34)						
Total number of IgE-AAs	226							161							34						

521

522 Abbreviations: CSU = chronic spontaneous urticaria, IgE-AAs = autoantigens detected by IgE, SD = standard deviation. The bottom line
523 shows the total number of autoantigens recognized within each group of 7 individuals.

Figure legends

Figure 1. Selection process of Interleukin-24 (IL-24) as a specific, accessible, and skin-expressed autoantigen detected by IgE of CSU patients. Accessible AAs are those that are secreted by cells or are membrane bound. IgE-anti-IL-24 was detected in all CSU patients, but none of the idiopathic anaphylaxis patients or healthy controls. Abbreviations: AAs: autoallergens, CSU: chronic spontaneous urticaria, total auto IgE: total detectable autoreactive IgE.

Figure 2. The number of autoantigens detected by IgE (autoallergens) in CSU patients, IdA patients and/or healthy control subjects.

Figure 3. IL-24 induces degranulation in mast cells sensitized with purified IgE or IgE-anti-IL-24. Peripheral stem cell derived mast cells were incubated with purified IgE (A) or IgE-anti-IL-24 (B) derived from the serum of CSU patients or (only in A) healthy control subjects, before stimulation with anti-IgE, coated IL-24, or vehicle as control. Data shown are mean values of absolute release and SEM. Spontaneous release was 12%. No specific mast cell degranulation was induced by IL-24 alone. Figure A shows pooled data from four experiments using the same IgE preparation but different cell donors. Data shown in B are from one of three experiments with similar results. *** $p < 0.005$; **** $p < 0.0001$.

Figure 4. IgE-anti-IL-24 levels are higher and more often elevated in patients with chronic spontaneous urticaria as compared to healthy controls. A) CSU patients show 2.2-fold higher serum levels of IgE-anti-IL-24 as determined by

ELISA. CSU: mean $\pm$ SEM = 0.5 IU/ml $\pm$ 0.2 (median 0.49, range 0.0-2.3); healthy: mean $\pm$ SEM = 0.3 IU/ml $\pm$ 0.1 (median 0.3, range 0.1-0.6). The box shows 10-90% percentiles. B) As compared to healthy controls, CSU patients exhibit a higher prevalence of elevated IgE-anti-IL-24 levels. C) ROC-analyses of ELISA-based measurements show that the cut-off for elevated IgE-anti-IL-24 is 0.33 IU/ml (dotted line) as determined by use of the Youden index, with a specificity of 80% and sensitivity of 80%.

All three panels show the same data from one representative measurement from 716 CSU patients and 332 healthy controls of four independent measurements, with a total of 1062 CSU patients and 482 healthy controls showing similar results. Dotted line indicates ***: $p < 0.001$

Figure 5. Higher IgE-anti-IL-24 serum levels are associated with higher disease activity and lower basophil counts. A) Higher IgE-anti-IL-24 levels are linked to higher levels of disease activity as assessed by use of the UAS7 in five of the seven CSU patients tested by microarray (for two CSU patients no UAS7 was available). B) Higher levels of relative (to total IgE) IgE-anti-IL-24 tested by ELISA are linked to higher disease activity as assessed by the UAS7, which reflects disease activity during the seven days prior to drawing of blood (N=52, averaged by pruning from N=580, i.e. each data point shown was averaged from 15 original data points for visualization purposes). C) Higher relative IgE-anti-IL-24 levels as determined by ELISA are linked to higher CSU disease activity on the day the blood was drawn as assessed by the UAS. D) Higher relative IgE-anti-IL-24 levels are associated with lower blood basophil counts. Linear (A-C) and sigmoidal (D) regression lines are shown with 95% confidence intervals as dotted lines.

9,374 human proteins screened
for IgE reactivity in CSU patients, patients
with anaphylaxis, and normal controls

↓ 8,957 are not
detected by IgE

417 human proteins are autoantigens
(AAs) detected by IgE

↓ 34 / 161 AAs detected by
IgE of normal controls /
anaphylaxis patients

222 AAs are detected by IgE of CSU
patients, but not control subjects

↓ 191 AAs are detected by
IgE in < 70% of CSU patients

31 AAs are detected by IgE
of >70% of CSU patients

↓ 23 AAs are not accessible
or expressed in the skin

8 AAs are accessible and
expressed in the skin

↓ 7 AAs are not detected by
IgE in all CSU patients

Interleukin 24 (IL-24)





