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Immunological effects and potential mechanisms of action of autologous serum therapy in chronic spontaneous urticaria

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Abstract

BACKGROUND Autoimmune processes are considered to play a major role in the pathogenesis of chronic spontaneous urticaria (CSU). Very recently, interleukin 24 (IL-24) has been identified as an IgE-autoantigen in CSU. Some studies revealed that notably autologous serum skin test (ASST)-positive CSU patients may benefit from autohemotherapy, however, the mechanisms of action remain unknown.

We aimed to investigate the immunological effects of autologous serum injections in ASST-positive CSU patients

METHODS 66 ASST-positive CSU patients were treated with weekly intramuscular autologous serum injections for 8 weeks and followed up for 12 weeks. Urticaria Activity Score (UAS7) and Dermatology Life Quality Index (DLQI) were assessed. The ASST was done at baseline, week 9 and week 21. Serum samples (baseline, week 9, 13 and/or 21) were analysed for the levels of IgE-anti-IL-24 and IgG-anti-IL-24 via ELISA and their ability to release histamine in basophils (BHRA).

RESULTS Autologous serum therapy resulted in a substantial improvement in disease activity and quality of life after 8 and 20 weeks. 28% and 34% of patients turned ASST-negative in week 9 and 21, respectively, but there was no link between their response to treatment and changes of ASST results. Also, no significant or relevant changes in BHRA were observed. In contrast, autologous serum therapy significantly decreased IgE-anti-IL-24 serum levels, but not IgG-anti-IL-24 serum levels, in responders but not in non-responders.

CONCLUSIONS Our findings suggest that the immunological effects of autologous serum therapy include a reduction in IgE-anti-IL24 autoantibodies, which may contribute to the pathogenesis of CSU.
Introduction

Chronic spontaneous urticaria (CSU) is a common skin disease characterized by the spontaneous occurrence of itching and short-lived wheals, angioedema or both for longer than 6 weeks\(^1,2\). The pathomechanisms of CSU have been studied for decades, but remain not fully understood. Nowadays, two types of autoimmune mechanisms are held to be relevant in many cases of CSU: Type I autoimmunity (also called autoallergy) with IgE autoantibodies to autoantigens and Type IIb autoimmunity with IgG autoantibodies to IgE or its high affinity receptor, FccRI\(^3-5\). The autologous serum skin test (ASST) is used as a nonspecific screening test to evaluate the presence of histamine-releasing factors in serum.

As stated in the EAACI/GA\(^2\)LEN/EDF/WAO guideline for urticaria\(^1\), modern non-sedating antihistamines are the first-line treatment. If symptoms persist, up dosing to the four-fold
dose is recommended. For antihistamine-resistant patients, omalizumab, a humanized anti-IgE antibody and, if ineffective, ciclosporin A are recommended treatment options\textsuperscript{1,6}. However, in some countries, such as China, Germany, India, Iran, South Korea, Turkey, Mexico and the USA, autohemotherapy is also commonly used to treat antihistamine-resistant patients with CSU\textsuperscript{7–12}. As of now, the level of evidence in support of the efficacy of autohemotherapy in the treatment of patients with CSU is limited and the mechanisms of action are currently not well understood, which is why autohemotherapy as a treatment approach in CSU is controversially discussed\textsuperscript{13}. (Supplemental Material)

Interleukin 24 (IL-24) is a member of the interleukin 10 family of cytokines. In humans, IL-24 is expressed in the skin and immune system, including the thymus, spleen and peripheral blood leukocytes. Very recently, IL-24 was identified as a common, specific and functional autoantigen of IgE autoantibodies in CSU\textsuperscript{14}.

Our study aimed to investigate the immunological effects and potential mechanisms of action of autologous serum injections in CSU patients. We analyzed serum reactivity before and after eight weeks of autohemotherapy by autologous serum skin testing and basophil histamine release assay. We also monitored the levels of IgE (and IgG) against IL-24. To assess changes in serum autoreactivity and IgE-anti-IL-24 levels for their clinical relevance, we correlated them with changes in clinical outcome measures, i.e. the weekly urticaria activity score (UAS7), the on demand use of antihistamines, and quality of life impairment (DLQI).

Methods

Patients and autohemotherapy protocol

ASST-positive patients (n=66, 18 years old or older) with CSU (duration > 6 weeks, UAS7 > 7 at baseline) were treated with autologous serum injections (Supplemental Material) at the GA\textsuperscript{2}LEN Urticaria Centers of Reference and Excellence\textsuperscript{2} in Berlin, Germany (n=21) and Istanbul, Turkey (n=33) as well as the Nashik Urticaria Clinic, India (n=12). The analyses of all available treatment outcomes, diaries, questionnaires and blood samples were approved by the ethics committee of the coordinating center of this study, the Department of Dermatology and Allergy at Charité – Universitätsmedizin Berlin.

Assessment of disease activity and impact

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Disease activity was assessed by the UAS7. The use of on demand antihistamines (AH-demand) was documented and served as a surrogate marker for disease activity and burden of disease. Disease impact on quality of life was assessed with the Dermatology Life Quality Index (DLQI). UAS7 scores, AH-demand data and DLQI values were obtained by use of patient diary documentation (Supplemental Table).

**Calculation of UAS7**

Disease activity was assessed by the UAS, a validated and widely accepted disease activity tool\textsuperscript{15} as recommended by the international urticaria guideline\textsuperscript{1,2,16}. The weekly UAS (UAS7, range 0-42) was calculated as sum of the daily values over 7 days.

Responses to autohemotherapy treatment were defined by the reduction of UAS7 compared to week 0. “Complete Response” (CR) was defined as a reduction of UAS7 equal or more than 90%, “Partial Response” (PR) as a reduction between 90%-30% and “Non Response” (NR) as equal or less than 30% reduction in UAS7\textsuperscript{17}. The minimal important difference (MID) is the smallest change that can be considered to be clinically relevant. A change in the score that is equal to or greater than 11 points has been estimated for the UAS7 in patients with CSU\textsuperscript{16}.

**Calculation of on-demand use of antihistamines**

A history of beneficial effects of antihistaminic treatment was part of the inclusion criteria. The use of antihistamines was restricted to on-demand intake (AH-demand) during weeks 0-8, 12, and 20. At all other times, patients were free to take antihistamines as on-demand or as prophylaxis. Cetirizine was recommended for treatment, however, patients could decide to use other non-sedating antihistamines according to their preference. The weekly AH-demand score was calculated as the sum of all antihistamine tablets (one point per tablet) used per week.

**Calculation of DLQI**

The DLQI consists of 10 questions across six domains: symptoms/feelings, daily activities, leisure, work/school, personal relationships and treatment\textsuperscript{18}. Each answer is scored from ‘very much’ (3) to ‘not at all’ (0), and an overall score (0–30) is calculated by summing the individual domain scores. Higher scores indicate higher impairment of quality of life.
The DLQI was applied in weeks 1, 9, 13, and 21 (Supplemental Table). A minimal clinically important difference (MCID) of 3-4 points has been estimated for the DLQI in patients with CSU\textsuperscript{19}. The MCID is the minimum change considered important by the patient and mandating a change in management. The proportion of patients whose change in DLQI from baseline reached an MCID of \( \geq 4 \) was assessed at week 9, 13, and 21.

**Autologous Serum Skin Test**

ASST was performed and assessed as reported earlier\textsuperscript{20}. (Supplemental Material)

**Blood samples**

Blood samples were taken in week 0, 9, 13 and 21 (Supplemental Table). Samples were centrifuged 15 minutes at room temperature and 1300g, and serum and plasma were portioned in 500\( \mu \)l Eppendorf microtubes and stored at -80°C.

**Basophil histamine release assay**

The BHRA was done as previously described\textsuperscript{21}. (Supplemental Material)

**IgE-anti-IL-24 and IgG-anti-IL-24 measurement**

IgE-anti-IL-24 and IgG-anti-IL-24 serum levels were assessed by a site-directed IgE or IgG capture ELISA as reported recently\textsuperscript{14}. (Supplemental Material)

**Statistical Analysis**

Statistical analysis of the data was performed using the software GraphPad Prism 6.0 and IBM SPSS Statistics 23. Data were analysed by calculating values for the mean and standard deviation (= SD). Paired and categorical data (e.g. data from the same patients obtained at different time points) were compared using the McNemar test. In the statistical tests, a p-value \( \leq 0.05 \) was considered as statistically significant.

**Results**

Autohemotherapy results in rapid, marked and sustained improvement in ASST-positive patients with CSU
Autohemotherapy significantly reduced the mean ± SD CSU disease activity as assessed by UAS7 from 23.3 ± 8.1 before treatment to 14.8 ± 11.3 at the end of the 8-week treatment period (-36 %, p ≤ 0.0001). Disease activity was first significantly reduced, to 17.9 ± 10.3, after 2 weeks of treatment (-23 %, p ≤ 0.001). UAS7 values continued to decrease after the end of treatment, to 12.8 ± 10.7 (-45 %, p ≤ 0.0001) and 12.6 ± 12.4 (-46 %, p ≤ 0.0001) at week 12 and 20, respectively (Figure 1a+b+c+d).

The use of on demand antihistamines was significantly decreased in week 8 of treatment (from 2.9 ± 3.9 to 1.5 ± 3.0 tablets per week, p ≤ 0.05) and continued to decrease thereafter, to 1.4 ± 3.1 tablets per week (p ≤ 0.01, in week 20). Quality of life was significantly improved from 8.8 ± 6.7 (at baseline) to 6.3 ± 6.1 (at week 9, p ≤ 0.05), 6.0 ± 6.7 (at week13, p ≤ 0.01) and 5.1 ± 6.4 (at week 21, p ≤ 0.001) after the start of treatment as assessed by the DLQI (Figure 1e+f). Furthermore, UAS7 in week 0, 8, 12 and 20 showed correlation with DLQI in week 1, 9, 13 and 21, respectively (Spearman Test, p = 0.047, 0.0071, 0.0006 and ≤ 0.0001).

At the end of the treatment phase (in week 8), the rate of complete response and partial response was 13 % and 48 %, respectively. At the end of the follow up, in week 20, nearly two thirds of patients (67.4 %) showed complete or partial response to autologous serum therapy (Figure 2a).

After 8 weeks of therapy with autologous serum, 35 % (15/43) of patients reached an MCID of ≥ 4 in total DLQI score compared to baseline. The percentage increased steadily to 42% (19/45) in week 13 and 48% (20/42) in week 21. However, there was no significant difference comparing these timepoints (McNemar Test, p > 0.05) (Figure 2b).

In ASST-positive CSU patients receiving treatment with autologous serum, there is no link between their change in disease activity in response to treatment and changes of ASST results

After 8 weeks of treatment with autologous serum, 28 % (14/50, p ≤ 0.001) of patients turned ASST-negative. In week 21, the ASST was negative in 34 % (17/50, p ≤ 0.0001) of patients (Figure 3a). There was no significant difference comparing complete responders (CRs), partial responders (PRs) and non-responders (NRs) for their rates of patients who became ASST negative (Chi-square Test, p > 0.05). 40 % (2/5) and 54.5 % (6/11) of CRs, 22.2 % (4/18) and 30.8 % (4/13) of PRs, and 26.7 % (4/15) and 21.4 % (3/14) of NRs were ASST-
negative at week 9 and week 21, respectively (Figure 3b). There was also no significant difference when comparing patients who became ASST negative with patients who remained ASST positive for their rates of CR, PR and NR (Figure 3c).

In addition, patients who experienced significant QoL (quality of life) improvement (MCID ≥ 4) did not show different rates of turning ASST-negative than those who did not improve in their QoL (MCID < 4). Also, patients who showed a change in their ASST status did not differ in their rates of showing marked QoL improvement as compared to patients who remained ASST positive.

*Serum autoreactivity and its changes during autohemotherapy as assessed by BHRA are not linked to the response to treatment*

Before the start of autohemotherapy, 5 of 41 patients were BHRA-positive, and these 5 patients were similar in their treatment responses as compared to BHRA-negative patients. After 8 weeks of weekly treatment with autologous serum injections, 4 of the 5 BHRA-positive remained BHRA-positive, and the one patient who became BHRA-negative was a non-responder and did not achieve a significant QoL improvement in week 9.

*In ASST-positive CSU patients, autohemotherapy reduces serum levels of IgE-anti-IL-24, but not IgG-anti-IL-24*

Therapy with autologous serum reduced the serum levels of IgE-anti-IL-24 by 18% and 22%, from 0.24 ± 0.02 IU/ml (before treatment) to 0.20 ± 0.03 IU/ml (at week 9, p ≤ 0.001) and 0.19 ± 0.02 IU/ml (at week 21, p ≤ 0.001), respectively (Figure 4a). In contrast, serum levels of IgG-anti-IL-24 did not change significantly (p > 0.05, Figure 4b).

*The reduction of IgE-anti-IL-24 in CSU patients treated with autologous serum is linked to the response to treatment, but not to changes in ASST status*

In complete and partial responders to autohemotherapy, but not in non-responders, the mean levels of IgE-anti-IL-24 declined significantly from week 0 to week 9 (p ≤ 0.05) (Figure 5). Moreover, the mean initial IgE-anti-IL-24 levels in complete and partial responders were

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higher compared to non-responders. There were no differences in serum levels of IgE-anti-IL-24 or IgG-anti-IL-24 in patients who became ASST negative or not at any timepoints after autohemotherapy (Figure 6).

Discussion

Our study is one of the first to explore the immunological effects and potential mechanisms of action of autohemotherapy in patients with CSU. Our findings suggest that autologous serum therapy has rapid, strong, and sustained beneficial clinical effects in ASST-positive CSU patients that are linked to the presence and changes of autoantibodies. Most importantly, high levels of IgE-anti-IL-24 before treatment and changes of IgE-anti-IL-24 in response to treatment are linked to the efficacy of autohemotherapy.

In our study, autohemotherapy reduced disease activity by 36 % after 8 weekly injections, lead to sustained benefit at week 20 (46% reduction), and improved disease activity as early as after the second injection of autologous serum. Also, the on demand antihistamine consumption was significantly decreased by more than 50% as compared to baseline. These results are in line with previous studies. For example, Majid et al.\textsuperscript{22} showed a marked decline of disease activity after the first few injections of autologous serum. Staubach et al.\textsuperscript{11} reported a persistent treatment effect of autologous whole blood injections in ASST-positive CSU patients and reduced disease activity as early as after four weekly treatments as assessed by the UAS7 and the use of on demand antihistamines. Our findings are also in agreement with previous studies by Chen et al.\textsuperscript{23}, Godse et al.\textsuperscript{24} and Abdallah et al.\textsuperscript{25}.

CSU is known to have a substantial impact on patients’ quality of life. In our study, quality of life of our patients was improved after the last autologous serum injection and further improved thereafter. One third of the patients and almost the half of patients experienced significant quality of life improvement, i.e. a reduction of the DLQI by the minimal clinically important difference of 4 points, by the end of the treatment and follow up phase, respectively. These results confirm the findings of an earlier study by Panchami et al.\textsuperscript{26}.

Taken together, our results and those of previous studies underline that autohemotherapy can have rapid, positive and persistent effects on disease activity and impact in patients with CSU, but they don’t provide an explanation for why this is\textsuperscript{8,9,25–27}.

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How does autohemotherapy work in CSU? CSU, in many patients is held to be an autoimmune disorder, linked to the presence of mast cell-degranulating autoantibodies, either IgE-autoantibodies directed to autoallergens (type I autoimmunity) or IgG autoantibodies to the high affinity IgE receptor or IgE itself (type IIb autoimmunity). Our hypothesis was that autohemotherapy, in patients who benefit from this treatment, reduces the levels and/or effects of these mast cell-degranulating IgE and IgG autoantibodies. Our results strongly suggest that autohemotherapy reduces the levels of IgE autoantibodies, but not the levels or effects of IgG autoantibodies.

Two of our findings independently suggest that autologous serum therapy in CSU does not work by reducing the levels or effects of mast cell-degranulating IgG autoantibodies. First, the ASST, a screening test for these autoantibodies, became negative in only one third of the treated patients at the end of the follow up phase, and this was not linked to the clinical response of patients. In other words, some patients (4/10 at week 9, 3/13 at week 21) without clinical benefit became ASST-negative, and some patients (17/28 at week 9, 14/25 at week 21) who had clinical benefit remained ASST-positive. The latter has been described previously by Mori and Hashimoto, who reported CSU remission in a ASST-positive patient treated with autologous whole blood injections, without changes in the patient’s ASST status. Second, the BHRA, a more sensitive and specific test than the ASST for mast cell-degranulating IgG autoantibodies to the high affinity IgE receptor, became negative in only one patient, and this patient was a non responder. We did not have access to direct assays for measuring the levels of IgG autoantibodies and their changes in our patients. But even if we had measured antibody levels and had found a reduction in response to autologous serum therapy, this would hardly be relevant pathomechanistically, as functional tests such as the ASST and the BHRA remained positive in the majority of patients and changes in the results of these functional tests were not linked to treatment responses.

Our results do suggest that autohemotherapy works in CSU, at least in part, because of its effects on IgE autoantibodies. First, we found that our patients treated with autohemotherapy showed a significant reduction of their IgE-anti-IL-24 levels after 8 weekly injections with autologous serum, and their levels continued to drop thereafter. Second, the drop in IgE-anti-IL-24 was only significant in responders, but not non responders to autohemotherapy although statistical comparisons in the analysis are based on small patient numbers.
numbers in each response group. Taken together, these findings support the idea that IgE-autoantibodies such as IgE-anti-IL-24 are one of the targets of the mechanisms of action of autohemotherapy in CSU. It would be interesting to extend analyses of autoreactive IgE to other autoallergens that have been described to be relevant targets of IgE in patients with CSU such as thyroperoxidase, tissue factor as well as dsDNA29,30.

Our study has several important strengths and limitations, and it points to interesting questions that need to be addressed by further studies. The limitations of our study include its uncontrolled design and relatively small sample size. In retrospect, we should not have limited inclusion into the study to ASST-positive patients. ASST positivity of our patient population, for sure, has biased our study group towards type IIb autoimmune CSU and against type I autoimmune CSU, in which autohemotherapy now has to be expected to be more effective than in type IIb autoimmune CSU. For sure, additional studies are required to investigate the effect of autohemotherapy in ASST-negative patients and to explore the role in type I autoimmunity. This is especially true, since our studies of IgE-anti-IL24 serum levels were only performed on 15 of the patients included in this study, due to limited volumes of sera available. On the plus side, our patient population was fairly homogeneous, our study was a multicenter study, and our key outcomes included major pathogenic drivers of type I and type IIb autoimmune CSU. Further studies are needed to better characterize the relevance and mechanisms of these effects of autohemotherapy. Are there any other functional IgE autoantibodies against autoantigens in autologous serum therapy? How is IgE-anti-IL-24 reduced by autohemotherapy, by effects on its production, its clearance, or both? Are other IgE autoantibodies and levels of total IgE also reduced by autohemotherapy in CSU patients, ASST-positive and ASST-negative patients? Are total IgE levels affected by autohemotherapy?

In summary, our study demonstrates that autohemotherapy can lead to clinical benefit in part by its effects on levels of IgE autoantibodies in ASST-positive CSU patients. This supports the notion that specific and functional IgE autoantibodies contribute to the pathogenesis of CSU and suggests that these IgE autoantibodies represent a potential therapeutic target of autohemotherapy.

Conflict of interest

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Prof. Per Stahl Skov is in the scientific board of RefLab.

Other authors have no conflicts of interest to declare.

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21. Platzer MH, Grattan CEH, Poulsen LK, Skov PS. Validation of basophil histamine release against the autologous serum skin test and outcome of serum-induced


Figure legends

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Figure 1. Autologous serum therapy results in rapid, marked and sustained improvement in ASST-positive patients with CSU

Effects of autologous serum therapy on disease activity (a, b, c), change of MID (d), use of on demand antihistamine medication (e) and quality of life impairment (f) in patients with chronic spontaneous urticaria (CSU) and a positive ASST. UAS7, on demand antihistamine use, and DLQI are shown for each patient and displayed as mean and standard deviation (SD). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001 (Wilcoxon Test) compared to week 0. N = 46, 46, 46, 46, 49, and 33 for a, b, c, d, e and f.

Figure 2. CSU patients treated with autologous serum therapy show high rates of responses and clinically important quality of life improvement

a) Distribution of patients with complete, partial and non response to treatment with autologous serum during the treatment phase (week 1-8) and the follow-up period (week 12 and week 20). Complete response = a reduction of UAS7 equal or more than 90 % compared to week 0. Partial response = a reduction of UAS7 between 90 %-30 % compared to week 0. Non response = a reduction of UAS7 equal or less than 30 % compared to week 0. N = 46.

b) Rates of patients who reached or did not reach MCID ≥ 4 in DLQI after autologous serum therapy. No significant difference was observed by McNemar Test (p > 0.05). MCID = Minimal clinically important difference. N = 43, 45 and 42, respectively.

Figure 3. In ASST-positive CSU patients receiving treatment with autologous serum, there is no link between their response to treatment and changes of ASST results.

Comparison of changes in ASST (a) and proportions of ASST positive and negative patients in complete, partial and non responders at week 9 and 21 (b and c). At baseline (week 0), all patients were ASST-positive. *** p ≤ 0.001, **** p ≤ 0.0001 (McNemar Test). ASST +/- = Autologous Serum Test Positive/ Negative. CR = Complete response, PR = Partial response, NR = Non response. N = 50, 38 and 38 in Figure 3a, 3b and 3c respectively.

Figure 4. In ASST-positive CSU patients, autologous serum therapy reduces serum levels of IgE-anti-IL-24, but not IgG-anti-IL-24

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Comparison of IgE-anti-IL-24 and IgG-anti-IL-24 serum levels before and after treatment with autologous serum. Data shown are from all patients with available data at the specific time points. The serum levels of IgE-anti-IL-24 (a) and IgG-anti-IL-24 (b) were measured via ELISA. Data are displayed as mean and standard deviation (SD). ** P ≤ 0.01, *** P ≤ 0.001 (Wilcoxon Test); IL-24 = Interleukin 24. N = 15;

**Figure 5. The reduction of IgE-anti-IL-24 in CSU patients treated with autologous serum is linked to the response to treatment**

Comparison of IgE-anti-IL-24 and IgG-anti-IL-24 serum levels in different response groups before and after treatment. Data are displayed as mean and standard deviation (SD). Data shown are from all patients with available data at the specific time points. Statistical comparisons were done only for paired data available at baseline (week 0) and post treatment (week 9 or week 21). * P ≤ 0.05 (Wilcoxon Test). N= 13,9,9 for 5a, 5c and 7,5,4 for 5b, 5d, respectively.

**Figure 6. The reduction of IgE-anti-IL-24 in CSU patients treated with autologous serum is not linked to changes in ASST status.**

Comparison of IgE-anti-IL-24 and IgG-anti-IL-24 serum levels patients who become ASST negative with patients who remain ASST positive after treatment. Data shown are from all patients with available data at the specific time points. Data are tested by Wilcoxon Test and displayed as mean and standard deviation (SD). N = 6, 10 for 6a, 6c and 14, 25 for 6b, 6d, respectively.

**Figures**
Figure 1
Figure 2

(a) Percent of patients over weeks 1 to 20, categorized by response: Complete Response, Partial Response, and Non Response.

(b) Percent of patients at weeks 9, 13, and 21, categorized by MCID: MCID<4 and MCID≥4.
Figure 3
Figure 4

Figure 5
Figure 6
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