
Autoantibodies to the high-affinity IgE receptor in children with chronic urticaria

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Background: Chronic urticaria (CU) in childhood remains a challenge for investigation, and its etiology is largely unknown. Autoantibodies to the high-affinity IgE receptor (FcεRI) are believed to play a role in the pathogenesis of this disease in adults.

Objective: To determine the prevalence of autoantibodies to FcεRIα on basophils in children with CU vs atopic eczema dermatitis syndrome (AEDS).

Methods: Eighty children with CU were compared with 38 children with AEDS. In addition to complete blood cell counts and total IgE measurements, CAP-RASTs to egg, codfish, soy, milk, and peanut were performed. Stool samples were examined for parasites, and autologous serum skin testing and a functional anti-FcεRIα assay were conducted to detect autoantibodies.

Results: No significant differences were observed between children with CU and controls in mean basophil or eosinophil counts. Twenty (26%) of 77 children with CU and 31 (82%) of 38 with AEDS had positive CAP-RAST results ($P < .001$). Only 2.5% of the children with CU and 0% with AEDS had stool samples positive for parasites ($P = .005$). Anti-FcεRIα autoantibodies were positive in 37 (47%) of 78 children with CU and in none of 33 with AEDS. Non-IgG histamine-releasing factors were found in 10 (13%) of 78 children with CU.

Conclusions: Children have a similar prevalence of autoantibodies to the FcεRIα as has been previously published for adults. Few have type I allergies, and parasite infestation is also uncommon. Further studies are required to investigate the predictive value of the autoantibodies in these children with respect to clinical profile, requirements for medications other than antihistamines, and remission rates.

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INTRODUCTION

Chronic urticaria (CU) in childhood remains a challenge in terms of its etiology, investigation, and management. Adult CU studies^{1–6} have demonstrated the presence of circulating functional autoantibodies directed against either the high-affinity IgE receptor (FcεRIα) or IgE in 35% to 60% of patients. Recently, FcεRIα autoantibodies were found to occur in as many as 30% of the children with CU in a northern hemisphere study.⁷ There is evidence to suggest that the findings of FcεRIα autoantibodies in children may affect the need for subsequent investigations (eg, concomitant autoimmunity, celiac disease, and *Helicobacter pylori* infection),^{7,8} the medication choice for patients who are poorly responsive to high-dose antihistamines, disease severity,⁶ and prognosis.⁹ However, the demonstration of FcεRIα autoan-

tibodies is laborious and expensive, and availability is limited to research centers. The autologous serum skin test (ASST) has been extensively investigated as a surrogate marker for the presence of autoantibodies, although the data are largely confined to adult studies.¹⁰ Recent studies^{7,11–14} have investigated its role in children with CU. There is conflicting evidence to support a role for viral, bacterial, and parasitic infection or food allergy in CU.^{14–16} The effects of these possible triggers on autoimmune-mediated CU are also unclear.

We investigated 80 children referred to the Allergy and Dermatology Clinic of the Red Cross Children's Hospital, Cape Town, South Africa, for evaluation of CU. The primary aim of the study was to investigate the frequency of functional autoantibodies, specific IgE to *Ascaris lumbricoides*, and sensitization to food allergens in these children. Secondary aims were to observe the natural history of the condition and medications required for symptomatic control.

METHODS

Participants

Chronic urticaria is defined as a condition in which wheals, with or without angioedema, occur continuously or on a frequently recurrent basis for more than 6 weeks.^{5,7,12} Eighty children referred to the Red Cross Children's Hospital during a 2-year period for the evaluation of urticaria met these criteria. Two age-matched control groups were enrolled: 38

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children with atopic eczema dermatitis syndrome (AEDS), who served as controls for specific IgE and autoimmune assays, and 50 healthy children awaiting elective eye surgery. A healthy laboratory control was used for the basophil release assays.

Children with urticaria required maintenance therapy and were given a weight-appropriate dose of cetirizine hydrochloride. Higher doses of cetirizine were given to children who had ongoing symptoms while using the weight-appropriate dose. The mean dose required during severe exacerbations was 0.38 mg/kg daily (range, 0.17–1.2 mg/kg daily). Informed parental consent and assent were obtained for all the participants. The University of Cape Town ethics and research committee approved the study.

Procedures

In addition to a clinical history and a physical examination, the following investigations were performed: complete blood cell count with manual differential cell count, erythrocyte sedimentation rate (ESR), total IgE, and CAP-RAST Fx5-6 food panel screen (Pharmacia, Uppsala, Sweden). Positive Fx5 test results were further evaluated for the presence of specific IgE to hen egg whites, codfish, soy, wheat, cow's milk, and peanuts. Stool microscopy, *A lumbricoides* specific IgE measurements, and serum eosinophil counts were performed to screen for intestinal parasitism. A standardized scratch test and an ice cube test were performed to detect the presence of dermatographism and cold-induced urticaria, respectively.¹⁷

The ASST

The ASST was conducted on children who had not been taking antihistamines for at least 7 days. The size of the wheal and flare was compared using a positive histamine control skin prick test. The ASST was performed using the technique described by Sabroe et al.¹⁰ The dermis of the volar surface of the arm was injected at 5-cm intervals with 50 μ L of autologous serum, histamine (10 mg/mL of histamine in 0.9% isotonic sodium chloride as positive control), and 0.9% isotonic sodium chloride (negative control). A positive skin test result was defined as the presence of a serum-induced wheal with a mean diameter at least 1.5 mm greater than that induced by 0.9% isotonic sodium chloride.¹⁰ All the ASSTs were performed by a single investigator (G.D.T.). In the absence of a positive response to histamine, the ASST was considered uninformative and was repeated after at least 24 hours. The ASST was performed while urticaria was active.

Functional Anti-Fc ϵ Receptor Assays

Functional circulating autoantibodies to the IgE receptor were determined using purified IgG-mediated histamine release from basophils of nonatopic donors. Patient IgG was extracted from an aliquot of serum, following caprylic acid treatment in the presence of acetate buffer; dialyzed in phosphate-buffered saline solution by ammonium sulfate precipitation at 50% concentration; reconstituted to the original volume; and dialyzed extensively with phosphate-buffered

saline solution. One nonallergenic adult, whose basophils were screened with anti-IgE for greater than 25% release, was used for the assay. Basophils in freshly collected whole blood samples were incubated with patient IgG diluted 50% in piperazine-1,4-bis(2-ethanesulphonic acid) (Sigma, St Louis, MO) containing 0.03 g/L human serum albumin and 2 ng/mL of recombinant interleukin 3 (PharMingen, Becton Dickinson, San Diego, CA) for 60 minutes at 37°C. The resulting histamine release was measured using a radioimmunoassay (Immuno-Biological Laboratories, Hamburg, Germany, and later Biosource, Nivelles, Belgium). A sample of IgG from a nonatopic individual was used in the histamine-release assay as a negative control. Spontaneous histamine release was measured and subtracted to determine specific release. Patient IgG-stimulated histamine release was defined as a percentage of the total cellular histamine content, and it was considered positive if it was at least 16.5%. Dilutions of serum were also studied in the basophil-release assay for the detection of histamine-releasing factors. The diagnosis of autoimmune urticaria was confirmed only if the histamine release–urticaria test result was positive. Spontaneous release in all experiments is less than 10%. The test result is considered positive only at a release of 16.5% or greater of the total cellular histamine content. This cutoff value was used for IgG- and serum factor-induced histamine release.

Statistical Analysis

The statistical evaluation was performed using a software program (SPSS; SPSS Inc, Chicago, IL). χ^2 Tests were used to compare the study and control groups.

RESULTS

Of 80 enrolled children (43 males and 37 females; mean age, 7.6 years; median age, 8.3 years; age range, 1.25–19 years), 32 (40%) reported rhinitis, 4 (5%) persistent asthma, and 6 (8%) AEDS. Disease remission occurred in 30 (36%) of 84 of the children during the 3-year study. The hematology results in 73 patients with CU were compared with those in 50 age-matched controls (mean age, 86.4 and 77.8 months, respectively). No significant differences were observed between the 2 groups with respect to mean basophil or eosinophil counts. The mean ESR was 11.87 mm/h (range, 1–60 mm/h), and 18 (30%) of the 61 patients had an increased ESR (>12 mm/h).

The Fx5e CAP-RAST result was positive in 20 (26%) of 77 children with CU compared with 31 (82%) of 38 children with AEDS ($P < .001$). In children with CU who had a positive Fx5 result, positive specific IgEs were found for peanuts in 4 (20%), hen eggs in 7 (35%), wheat in 7 (35%), milk in 5 (25%), codfish in 2 (10%), and soy in 4 (20%). None of the children with CU had specific IgE values that exceeded previously established 95% positive predictive values.¹⁸ In contrast, most of the children with moderate-to-severe AEDS had positive specific IgE results, some of which exceeded the 95% positive predictive range.

Dietary elimination of foods suspected of triggering events based on history or as a result of IgE test results was supervised by a dietician. The foods that were eliminated were highly processed, energy dense, and colored, based on parental suspicion of the food as a trigger. Dietician-supervised elimination diets of all candidate food allergens (as suspected by history or specific IgE results) did not result in any participants reducing or eliminating their requirement for ongoing antihistamine medication.

Results of stool microscopic examinations were positive for *A lumbricoides* in 1 (3%) of 39 children with CU and in none of 36 children with AEDS ($P < .005$). Twenty-one (29%) of 72 children with CU and 25 (66%) of 38 children with AEDS had specific IgE to *A lumbricoides* greater than 0.35 kU/L. Total eosinophil counts in the 2 groups were similar.

The results of the FcεR1α autoantibodies performed in 78 children are given in Table 1. In 2 patients, the serum samples obtained were insufficient for analysis. Thirty-seven (47%) of 78 children had positive autoantibody test results where histamine release from their basophils was at least 16.5% (range, 16.5%–60.0%). Mean histamine release in the positive group was 28.3%. In another 11 patients (14%), borderline histamine release (9.0%–16.4% of total basophil histamine) was induced by their IgG fractions. The basophils were also incubated with the patients' IgG-depleted serum where possible, and in 10 patients (13%), other factors released histamine (>16.5%). Only 1 of the 33 IgG samples from the children with AEDS had histamine-releasing activity, and it was below 16.5% of total histamine.

The ASST result was positive in 18 (41%) of the first 44 participants investigated (9 males and 9 females). The ASST was then discontinued owing to discomfort experienced by the children because topical anesthetic creams could not be applied. There was no association between a positive ASST result and disease remission, which was achieved in 30 (36%) of 84 children during the 3-year study ($P = .51$). There was no correlation between the presence of a positive ASST result and the presence of FcεR1α autoantibodies. There was no correlation between the presence of FcεR1α autoantibodies and positive specific IgE to the food allergens or positive *A lumbricoides* IgE. There was an equal distribution of males and females with positive FcεR1α autoantibodies and positive ASST results.

All the children were given a weight-appropriate dose of cetirizine. Higher doses of cetirizine were introduced for children with ongoing CU symptoms. The mean highest dose required during severe exacerbations was 0.38 mg/kg daily (range, 0.17–1.2 mg/kg daily). Adverse effects of this regimen included sedation ($n = 3$) and possible hyperactivity ($n = 1$). Only 2 children required a short course of oral prednisone pulses (2 mg/kg) to achieve adequate therapeutic control.

DISCUSSION

Autoantibodies to the IgE receptor were present in 47% of the children with CU and were absent in all the children with AEDS. This finding is similar to that of Brunetti et al⁷ for children (40%) and previous adult studies (35%–60% prevalence of anti-IgE or anti-FcεR1α autoantibodies).^{1–6} Ten of the autoantibody-negative patients (13%) demonstrated the presence of nonspecific histamine-releasing factors.

Brunetti et al⁷ demonstrated a positive correlation between a positive ASST result and histamine releasability (sensitivity, 78%; specificity, 85%) in 52 children. For the 44 children in the present study who underwent the ASST, we could not establish a significant correlation between the ASST result and the presence of FcεR1α autoantibodies or the presence of histamine-releasing factors ($P = .46$ by χ^2 test). The lack of correlation between the ASST result and the presence of FcεR1α autoantibodies in this study could be related to the fact that the ASST was performed in only 44 participants. Given the equivocal significance of the ASST in children with CU and its poor tolerability in young children, the role of ASST in the routine investigation of children with CU needs further study, particularly because the discomfort associated with the test was not acceptable to many of the children.

In the present study of histamine-releasing factors, our cohort revealed an equal sex distribution among the 37 children with positive FcεR1α autoantibodies; this differs from other pediatric data,⁷ which demonstrate a male bias, and from adult data, which demonstrate a female bias.¹¹ The children with CU revealed lower common food allergen sensitization rates than the control group with AEDS. Dietician-supervised elimination diets of all candidate food allergens (as suspected by history or specific IgE results) did not result in any participants reducing or eliminating their re-

Table 1. Presence of FcεR1α Autoantibodies*

	Children with CU (n = 78)	Children with AEDS (n = 33)
Positive IgG FcεR1α autoantibodies (histamine release, ≥16.5%)	37 (47)	0
Borderline IgG FcεR1α autoantibodies (histamine release, 9.0%–16.4%)	11 (14)	1 (3)
Negative IgG FcεR1 autoantibodies	30 (38)	32 (97)
Non-IgG serum histamine-releasing factors	10 (13)	ND

Abbreviations: AEDS, atopic eczema dermatitis syndrome; CU, chronic urticaria; FcεR1α, high-affinity IgE receptor; ND, not determined.

* Data are given as number (percentage) of participants.

quirement for ongoing antihistamine medication. Thus, the routine use of elimination diets is not recommended for children with CU.

Children residing in the Western Cape, which enjoys a temperate climate, are commonly infected with intestinal parasites. We did not establish a causal association between intestinal parasitemia and CU. However, children with suspected allergic conditions, such as CU and AEDS, routinely receive antihelminthic treatment before tertiary clinic referral. This may explain the negative stool microscopic results in all but 1 patient with CU and in all controls with AEDS. The positive *A lumbricoides* specific IgE antibody results (in participants with AEDS) indicate previous parasitic infection.

A paucity of circulating basophils was not detected in the CU group compared with the AEDS group as previously demonstrated in adult CU studies.¹⁹ Erythrocyte sedimentation rates were elevated (>12 mm/h) in 18 (30%) of the 61 patients. Given the high incidence of tuberculosis in Cape Town (589 cases per 100,000 population in 1998),²⁰ all the patients with an increased ESR underwent radiologic and Mantoux test screening for tuberculosis; none of the results were positive. In countries in which chronic infectious diseases such as tuberculosis, malaria, and schistosomiasis are prevalent, an ESR is useful to screen for the presence of "other" underlying diseases.

The natural history of CU in childhood is not clear. Twenty-five percent of the children with CU in this cohort experienced disease remission during the 3-year study. No clear predictors of disease remission were established. All the children with CU who met the strict clinical criteria for the diagnosis of CU in this study had a disease severity that required referral to a specialist center. The present study group (n = 80) and a previous large pediatric CU study group (n = 93)⁷ share similar median ages (8.3 and 7.8 years, respectively) and equivalent male-female ratios (1.1 and 1.2, respectively). The youngest child in each study was 16 and 9 months old, respectively.

We found no evidence that either a food allergy or intestinal helminthic infestation served as an ongoing stimulus for CU in children. Follow-up studies are required to determine whether the presence of FcεR1α autoantibodies affects disease severity, medication requirements, and disease remission rates in children with CU.

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