

Patients with chronic urticaria exhibit increased rates of sensitisation to *Candida albicans*, but not to common moulds

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Summary

Patients with chronic spontaneous urticaria (CU) frequently exhibit increased levels of total IgE (tIgE). However, sensitisations to relevant allergens are rarely detected in CU patients by standard prick testing suggesting that CU symptoms, at least in some patients, may represent hypersensitivity reactions to less common allergens. The objective of this study was to characterise skin reactivity and serum levels of IgE to *Candida albicans* (CA) and mould allergens in CU patients. A total of 112 CU patients and 35 healthy control subjects were subjected to intracutaneous skin testing for CA and seven ubiquitous moulds. Allergen specific and tIgE were determined using the Pharmacia CAP System. CU patients exhibited higher levels of tIgE than healthy subjects, but comparable rates of skin reactivity to CA and moulds and similar levels of specific IgE to mould allergens. However, increased IgE-anti-CA could only be detected in CU patients (13%). Almost all IgE-anti-CA positive CU patients showed high levels of tIgE. These data suggest that IgE-anti-CA expression contributes, at least in part, to increased tIgE levels in CU patients. Our findings call for further studies to characterise the relevance of CA hypersensitivity in CU, particularly in IgE-anti-CA positive CU patients that exhibit colonisation with *Candida albicans*.

Key words: Urticaria, mast cells, IgE, mould, *Candida*.

Introduction

Chronic urticaria (CU) is a frequent and disabling dermatosis characterised by pruritic wheal and flare type skin reactions and angio-oedema. A large number of underlying causes of CU have been identified, the most common of which include chronic infections, intolerance to food components and autoreactive processes. In contrast, CU is rarely found to be caused by a genuine allergy, i.e. IgE-mediated immediate hypersensitivity.¹ This notion is supported by the fact that few CU patients exhibit relevant positive reactions in routine skin prick testing for common food or aeroallergens.^{2,3}

However, CU patients frequently exhibit high levels of total IgE (tIgE),^{4,5} a hallmark feature of allergic diseases such as atopic dermatitis and other atopic conditions. This observation suggests that routine prick testing in CU patients may fail to identify sensitisations to less common allergens including some that may be relevant for the induction of urticaria symptoms.⁶

Moulds such as *Alternaria* and *Aspergillus* as well as *Candida albicans* (CA) are ubiquitous fungal species. Recently, sensitisations to mould and CA antigens have been shown to frequently occur in patients with respiratory and cutaneous allergic conditions including asthma and atopic dermatitis,^{7,8} in which hypersensitivity reactions to these allergens have been implicated to contribute to pathology.⁹ In contrast, the prevalence and relevance of sensitisations to mould or CA allergens in CU patients remain to be characterised in detail. As these allergens are frequently not included in standard prick testing of CU patients, sensitisations to moulds or CA allergens are rarely detected. Conse-

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quently, moulds and CA are widely regarded as irrelevant for the aetiopathogenesis of CU.

In this study, we investigated CU patients and healthy control subjects for skin reactivity and serum levels of specific IgE to mould and CA allergens and tIgE to answer two important questions: (i) Do CU patients exhibit increased rates of sensitisation to moulds and/or *Candida albicans*, and if so; and (ii) Could this account for the unexplained high rate of increased tIgE levels in CU patients.

Methods

Subjects

A total of 112 patients with chronic urticaria (69 female and 43 male; age: 37 ± 15 years) and 35 healthy volunteers (21 female and 14 male; age: 43 ± 11 years) were examined. The patients were recruited consecutively from the patients treated at the urticaria specialty clinic at the Department of Dermatology of the University Hospital Mainz. The patients were diagnosed by the physicians of the urticaria specialty clinic based on the patients' history and the clinical picture. We included only patients who presented with chronic spontaneous urticaria of 1-year duration or more. More than 90% of patients required continuous treatment with antihistamines, which were discontinued at least 1 week before skin testing (as were all other relevant medications including systemic steroids). In addition, we assessed the patients' atopy status, duration of the disease and prick test responses to common allergens (no sensitisations relevant for their urticaria were detected in any of the patients). Twenty-five of 111 (23%) CU patients and six of 35 (17%) healthy controls were atopics. Urticaria patients who did not suffer from CU (e.g. acute urticaria, physical urticaria, contact urticaria) were excluded from the analyses.

Skin testing

Skin tests were performed by intracutaneous injections of 50 μ l of commercially available, water soluble, highly purified extracts of *Alternaria alternata*, *Aspergillus fumigatus*, *Mucor mucedo*, *Fusarium roseum*, *Cladosporium herbarum*, *Penicillium notatum*, and *Aureobasidium pullulans* as well as *Candida albicans* (Allergopharma, Reinbek, Germany). Skin responses were recorded after 20 min by measuring the diameter of the wheal and the result was considered positive with a wheal ≥ 3 mm and if no reaction was observed to the control solution (saline 0.9%). Skin prick tests to common aeroallergens

(alder, birch, hazel, beech, mugwort, plantain, ragweed, grass mix, rye, house dust mites) were performed in 106 of 112 patients included in this study. We found positive results in seven of 14 CU patients (50%) showing IgE reactivity to CA and in 34 of 92 CU patients (37%) without CA-specific IgE. In line with earlier reports, we did not detect any sensitisations that were relevant for the onset and persistence of CU.

Detection of total and specific IgE

The levels of tIgE and the specific IgE antibodies to *Alternaria alternata*, *Aspergillus fumigatus*, *Mucor racemosus*, *Fusarium moniliforme*, *Cladosporium herbarum*, *Penicillium notatum* and *Aureobasidium pullulans* as well as *Candida albicans* were measured with Pharmacia CAP FEIA methods (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions. Results for specific IgE antibodies were expressed in CAP classes from 0 to 6: CAP classes 1 (0.35–0.69 kU/l) and higher were considered as positive. In addition, we examined the specific IgE antibodies of two different spices and mould cheese. We found that none of the 22 control subjects and only 4% of 111 CU patients exhibited IgE reactivity to these allergens.

Detection of intestinal *Candida* infections

Stool specimens were prepared by diluting 0.2 g of stool in 1.8 ml of sterile saline. After mixing by vortex for 5 min, a 100 μ l sample of the suspension was inoculated onto Sabouraud dextrose agar containing 300 μ g/ml chloramphenicol and 10 μ g/ml gentamicin. Yeast growth was assessed after 48 h of incubation at 37 °C as colony-forming units per gram.

Statistical analyses

Data were analysed using the Mann–Whitney *U*-test and Student's *t*-test for comparison of data between different subjects, and the χ^2 test to compare categorical data. Differences associated with probabilities (*P*) of less than 0.05 were considered significant.

Results

Levels of total IgE are increased in patients with chronic urticaria

Out of 112 CU patients tested, 55 (49%) exhibited increased levels of total serum IgE (>100 U/ml), which was found to be elevated in only four of 35 (11%)

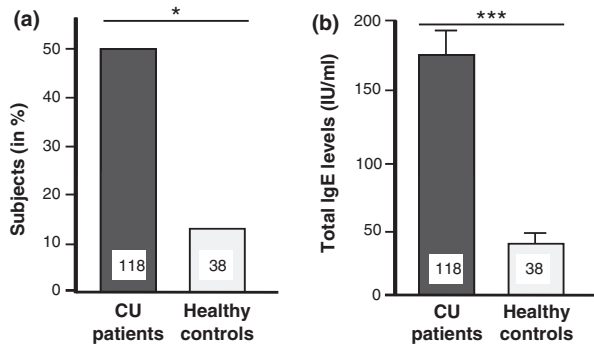


Figure 1 Increased total IgE (tIgE) levels are more frequent and tIgE levels are markedly increased in patients with chronic urticaria (CU). (a) Percentages of patients with chronic urticaria (CU patients, $n = 112$) or healthy control subjects ($n = 35$) with increased levels of tIgE (>100 IU/ml). (b) Levels of tIgE in CU patients and healthy controls. Data were presented as mean $\pm$ SEM. * $P < 0.05$, *** $P < 0.001$.

healthy control subjects (Fig. 1). In addition, CU patients showed significantly increased tIgE levels as compared with healthy controls (174 ± 22 vs. 42 ± 7 U/ml, $P < 0.001$).

Patients with chronic urticaria and healthy control subjects show comparable rates of sensitisations to common moulds

Positive skin reactions to intracutaneous injections of all seven common moulds tested were found in both CU patients and healthy controls (Fig. 2a). Rates of sensitisation varied depending on the moulds tested and ranged from 3 and 12% (*Alternaria*) to 34 and 24% (*Fusarium*) in healthy controls and CU patients, respectively. No significant differences of sensitisation rates were detected by intracutaneous testing or CAP-based quantification of specific IgE between CU patients and

healthy controls for any of the moulds tested (Fig. 2a). Rates of IgE-anti-mould expression were found to be lower than positive skin test reactions for all the moulds tested in both CU patients and healthy controls (Fig. 2a).

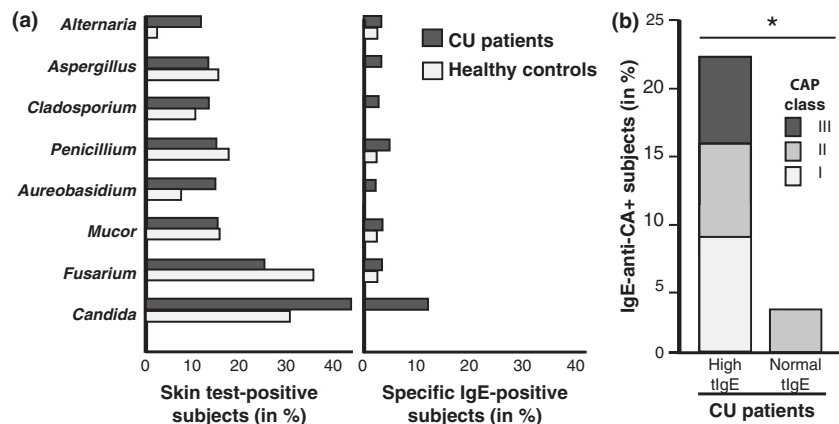
CU patients exhibit increased rates of sensitisation to *Candida albicans*

Intracutaneous (i.c.) testing with CA resulted in positive skin test reactions in 45% of 112 CU patients and 31% of 35 healthy control subjects (Fig. 2a). When we assessed IgE-anti-CA by CAP in these patients/subjects, we found that 13% of CU patients, but none of the healthy controls, expressed IgE-anti-CA (Fig. 2a). Interestingly, 12 of 14 IgE-anti-CA positive CU patients (CAP class $\geq$ II: nine of 14) showed increased levels of tIgE indicating that IgE-anti-CA could contribute to elevated tIgE levels in these CU patients (Fig. 2b). Sensitisation to CA in CU patients did not correlate with intestinal CA colonisation, i.e. CU patients with ($n = 30$) or without CA colonisation ($n = 64$) expressed similar prevalences of IgE-anti-CA (10% vs. 12.5%, $P > 0.05$). On the other hand, three of 11 IgE-anti-CA positive CU patients showed intestinal CA colonisation, suggesting that these patients are at the risk of experiencing allergic reactions to CA, as they carry both, specific IgE and the corresponding allergen (Table 1).

Discussion

Common causes of CU are thought to include chronic infections, autoreactivity and intolerance, but not immediate hypersensitivity, as relevant sensitisations to common allergens are rarely detected by routine testing in CU patients. This view is challenged by reports demonstrating that CU is frequently associated with

Figure 2 Patients with chronic urticaria do not show increased rates of sensitisation to common mould allergens but to *Candida albicans*. Relative numbers of chronic urticaria (CU) patients ($n = 112$) and healthy controls ($n = 35$) with positive skin test reactions to intracutaneous injections of *candida* or mould allergens and *candida* or mould specific IgE (a) None of the differences were statistically significant. (b) Numbers of CU patients with high total IgE (tIgE) levels ($n = 55$) and normal tIgE levels ($n = 57$) that produce IgE specific for *Candida albicans*. * $P < 0.05$.



IgE-anti-CA (CAP class)	Gender	Age (in years)	Total IgE (in U/ml)	Atopic (yes/no)	Stool for CA (+/-)
I	M	71	649	No	-
I	M	44	352	No	-
I	F	37	657	No	ND
I	M	63	647	No	+
I	M	45	1900	No	-
II	M	44	44	No	ND
II	M	34	239	No	ND
II	M	64	565	No	+
II	M	55	238	Yes	ND
II	M	37	559	No	+
II	M	60	48	No	-
III	M	55	225	Yes	ND
III	M	46	814	Yes	-
III	F	21	283	Yes	-

ND, not determined.

Table 1 Patients with chronic urticaria and positive IgE-anti-*Candida albicans* (CA) results

elevated tIgE levels, which we confirm with this study. In fact, we found that tIgE is increased in every second CU patient investigated and that tIgE levels in CU patients are markedly increased as compared with those of healthy controls ($P < 0.001$, Fig. 1).

This increase of tIgE in CU patients could be because of a number of possible mechanisms including atopic comorbidity or altered B-cell function resulting in increased production of irrelevant IgE. However, the number of atopics was comparable in both groups, CU patients and healthy controls.

The most likely explanation for these findings is that CU patients exhibit sensitisations and high levels of IgE to less common allergens that are simply not detected by routine skin prick testing. Indeed, Kaeser and coworkers reported that 29% (16 of 60) of CU patients exhibit specific IgE for one or more food allergens usually not included in routine testing.⁶

As disease activity in most CU patients does not show major seasonal changes, we tested CU patients for sensitisations to perennial antigens that are not included in routine skin prick testing, i.e. seven common moulds and CA. Rates of sensitisations to moulds assessed by skin testing or measuring IgE were found to vary considerably in CU patients and healthy controls, with both groups showing higher frequencies of positive skin test reactions than the production of specific IgE for all the moulds tested. Possible reasons for this previously reported discrepancy include IgE-independent inflammatory responses to intracutaneous allergen injections and differences in the allergens used in both tests (mould allergens are reportedly problematic to standardise).¹⁰ With respect to the hypothesis tested, the important result of this test series is that CU patients do not exhibit high or significantly increased

rates of sensitisation to common mould allergens as compared with healthy controls. This indicates that mould specific IgE production does not contribute to the high levels of tIgE found in CU patients.

In contrast, significant levels of CA-specific IgE were found in 13% of CU patients tested, but in none of the healthy control subjects, and all but two IgE-anti-CA positive CU patients, i.e. 12 of 14 IgE-anti-CA positive CU patients, showed increased levels of tIgE. Thus, elevated tIgE levels in some CU patients could be, at least in part, because of the production of IgE-anti-CA. Alternatively, elevated levels of tIgE could have led to false-positive increases of specific IgE against CA, even though this explanation seems less likely, because the majority of IgE-anti-CA-positive patients with elevated tIgE exhibited high levels, i.e. CAP classes $\geq$ II, of IgE-anti-CA and there is no relationship between IgE levels of the IgE and the CAP classes. Interestingly, we found that rates of sensitisations assessed by i.c. testing were similar in CU patients and healthy controls and more frequent than IgE-anti-CA production in both groups. Notably, 31% of healthy controls, all of which were negative for IgE-anti-CA, exhibited positive results in CA skin testing confirming the notion that the sensitivity of the skin test is higher and/or that skin testing is less specific than IgE measurements in detecting sensitisations to CA and stressing the need for continued development and standardisation of CA preparations for skin testing.¹¹ Serrano detected, in an older study, type I/III and IV skin reactions to CA antigen in 35 and 60% of patients with CU, respectively, and 71% of the type I/III reactions showed a positive urticaria provocation test result with CA antigen.¹² Unfortunately only IgA, IgG and IgM antibodies were investigated, and there were no differences in CU patients and controls.

Interestingly, Ergon and coworkers showed in patients with CU that intestinal and oral colonisation of CA spp. and serological evidence of CA infections were not significantly different to controls. They state that the possibility that CA triggers or causes CU should be explored in studies that measure CA-specific IgE and that include eradication therapy.¹³ This is inline with our findings.

Our data do not allow for the conclusion that immediate hypersensitivity to CA causes CU in sensitised patients. However, some IgE-anti-CA positive CU patients (three of 11) were found to exhibit intestinal CA colonisation. Whether or not the presence of CA in the gut of such patients can result in relevant CA antigen levels in the skin and the elicitation of CU symptoms remains to be clarified. On the other hand, intestinal CA colonisation does not appear to be the cause of sensitisation to CA in CU patients as similar rates of IgE-anti-CA expression are found in CU patients with and without intestinal CA colonisation (10% vs. 12.5%).

Taken together, our data show that CU patients exhibit elevated levels of tIgE and increased rates of sensitisation to CA. The fact that one in five CU patients with elevated tIgE levels exhibits IgE-anti-CA, as compared with <2% in CU patients with normal tIgE levels, demonstrates that the production of IgE-anti-CA could contribute to the high frequency of increased tIgE levels in CU. Ongoing and future investigations will have to determine the relevance of IgE-anti-CA expression for the induction of CU symptoms, especially in patients with intestinal CA colonisation.

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