

Thyroid autoimmunity in chronic urticaria

B.F. O'Donnell, D.M. Francis, G.T. Swana,* P.T. Seed,† A. Kobza Black and M.W. Greaves

St John's Institute of Dermatology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

*Department of Immunology, Guy's, King's and St Thomas' School of Medicine (GKT), St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

†Maternal & Fetal Research Unit, Department of Obstetrics & Gynaecology, GKT School of Medicine, King's College, London, U.K.

Summary

Correspondence

Dr B.F. O'Donnell, Department of Dermatology,
The Children's University Hospital, Temple Street,
Dublin 1, Ireland.

E-mail: amanda.mcgowan@tscch.ie

Accepted for publication

9 October 2004

Key words:

anti-FcεR1 autoantibodies, autoimmunity, chronic
urticaria, histamine release, thyroid autoantibodies,
thyroid disease

Conflicts of interest:

None declared.

Background Chronic urticaria (CU) is an autoimmune process in some patients. An association between CU and autoimmune thyroid disease has also previously been proposed. Our group has identified functionally significant histamine-releasing autoantibodies in one subset of CU patients (subset 1), predicted by positive autologous intradermal serum tests and positive histamine release from donor basophil leucocytes *in vitro*. Sera from a second subset of patients (subset 2), all of whom had positive autologous intradermal serum tests, failed to release histamine from donor basophils. A final disease subset (subset 3) has no identifiable skin reactivity (negative autologous serum skin test) or *in vitro* histamine releasing activity.

Objectives In order to examine further the possible relationships between thyroid autoimmunity, thyroid dysfunction and CU, we have examined thyroid autoantibodies and thyroid-stimulating hormone (TSH) levels (an indirect measure of thyroid dysfunction) in the three CU subsets.

Patients/methods We studied 182 patients (69% female), of whom 90 had a positive autologous intradermal serum test.

Results Eighteen skin test-positive and four skin test-negative patients had thyroid microsomal antibodies (TMA). TSH outside the normal range was found in 13 skin test-positive and one skin test-negative patient. These findings represent clustering of TMA positivity [risk ratio (RR) 4.06, 95% confidence interval (CI) 1.56–10.6] and of abnormal thyroid function (RR 15.5, CI 2.07–11.6) among the skin test-positive patients. However, in the overall study group an elevated TSH was present in seven patients (3.8%, CI 1.6–7.8) comparable to the 5% expected prevalence in the community. Thyroglobulin antibodies (TGA) were present in two of 182 patients.

Conclusions There were significant differences between skin test-positive and skin test-negative patients with regard to autoimmune thyroid disease. Evidence for autoimmune thyroid disease and abnormal thyroid function was largely found among the skin test-positive patients, supporting the theory of an autoimmune aetiology in this group.

Chronic urticaria (CU) describes a 6-week or longer history of widespread, transient, itchy cutaneous swellings with individual lesions lasting less than 24 h.¹ The condition, previously regarded largely as idiopathic,² is associated with a serological factor in approximately 60% of affected patients.³ The presence of circulating histamine-releasing factor(s) during an active phase of the disease may be predicted by the development of a weal-and-flare response following the intradermal injection of autologous serum (autologous serum skin test).^{4,5} Sera from about 50% of the skin test-positive patients can release

histamine from healthy donors' basophils *in vitro* due to the presence of anti-FcεR1α^{6,7} or less commonly anti-IgE autoantibodies (subset 1),^{6,8} suggesting that CU has an autoimmune basis in these patients. These autoantibodies also release histamine from human skin mast cells *in vitro*.^{3,9} The second subset (subset 2) of skin test-positive patients comprises those whose sera fail to release histamine from donor basophils *in vitro*. This subset includes some patients with a mast cell-specific factor (currently awaiting characterization⁹) that releases histamine from human mast cells but not basophils *in vitro* and

possibly patients with other types of histamine-releasing activity.⁹ A final subset of CU patients (subset 3) have no demonstrable histamine-releasing activity with negative autologous intradermal serum tests and also negative release from basophils *in vitro*. Thus, based on *in vivo* skin testing with autologous serum and on *in vitro* histamine-release studies using donor basophils, three pathogenetic subsets of CU may be recognized.

Reports of an increased prevalence of antithyroid autoantibodies among CU patients in different populations^{10–12} have led to recommendations for the testing of all CU patients for autoimmune thyroid disease.^{10–12} Leznoff and Sussman¹¹ reported 90 of 624 (14.4%) CU patients in Canada had thyroid autoimmunity and 49 of the patients (7% of total) had goitre or thyroid dysfunction. Their criteria^{10,11} for the diagnosis of 'thyroid autoimmunity' were a titre of thyroid microsomal antibodies (TMA) $\geq 1 : 1600$ or a titre of thyroglobulin antibodies (TGA) $\geq 1 : 160$ (standardized microtitration haemagglutination test), a biopsy specimen or needle aspirate of the thyroid gland demonstrating lymphocytic infiltration or a combination of idiopathic hypothyroidism longer than 5 years' duration with a TMA titre $\geq 1 : 400$. Thyroid disease was not a conspicuous finding in a large U.K. series of patients ($n = 2310$) with CU,¹³ but thyroid autoantibodies were not evaluated.

To elucidate the possible association of both autoimmune thyroid disease and thyroid dysfunction with CU, we have studied the frequency of thyroid autoantibodies and serum thyroid-stimulating hormone (TSH) levels in subsets of CU as defined by autologous serum skin test reactivity and the presence or absence of anti-Fc ϵ R1 α or anti-IgE autoantibodies.

Patients and methods

Patients, all unrelated, were recruited from the urticaria clinics at St John's Institute of Dermatology. All patients had active CU $\pm$ angio-oedema at the time of the study. Patients who had the common, concurrent association of delayed pressure urticaria or dermatographism with CU were included but those with other physical urticarias were excluded, as were patients with clinical or histological evidence of urticarial vasculitis.

The autologous intradermal serum test

All autologous intradermal serum tests were carried out by the same investigator (B.F.O'D.)¹⁴ with minor modifications compared with earlier work.^{3,8} Antihistamines were discontinued at least 48 h prior to skin testing and no patient was taking the long-acting antihistamine, astemizole. Aliquots (50 μ L) of undiluted autologous serum, sterile saline (negative control) and histamine (10 μ g mL⁻¹ in saline, positive control) were injected slowly at least 5 cm apart into the dermis of the volar forearm. Two orthogonal diameters of the resulting papules or weals were measured immediately and at 30 min post injection. A positive skin test response was defined as the development of a serum-induced weal-and-flare with the mean weal diameter at least 2 mm greater (mean $\pm$ 2 SD of normal

controls, $n = 30$) than the mean diameter of the papule induced by saline.

Laboratory studies

Histamine-release assays were performed using leucocytes prepared from the blood of the same two healthy donors as previously described.³ A net histamine release greater than 5% of the total histamine indicated a positive histamine-release test.

All sera were examined for abnormality of thyroid function by screening for abnormal levels of TSH. TSH was measured by a chemi-immunometric assay (Bayer Advia[®] ACS[®] 180; automated immunoassay analyser, Bayer, Newbury, U.K.). The results of TSH were expressed as low (< 0.3 mU L⁻¹), normal (0.3 – 5.5 mU L⁻¹), or high (> 5.5 mU L⁻¹). TMA and TGA antibodies were measured by a standardized microtitration haemagglutination test [Murex (Wellcome) Diagnostics Haemagglutination kit; Murex Biotech (UK) Ltd, Dartford, U.K.].¹⁵

Statistical analysis

TSH was expressed on a log scale throughout to achieve approximate normality. Five age groups of patients comprising approximately equal numbers were determined.

Comparisons of TSH were made between groups by age, sex, TMA and serum test and basophil categories using risk ratios (RR) with confidence intervals (CI) and χ^2 test or Fisher's exact test, as appropriate. Unpaired t-tests with unequal variance (Satterthwaite's approximation) were used to test for differences in the mean. Bartlett's test was used to compare variance. To determine whether serum test or basophil category was more important in determining variance change, Bartlett's test was repeated within subgroups.

Multiple logistic regression was used to find the determinants of TMA category, and to compare the importance of the categories determined by serum tests and basophil histamine release.

Results

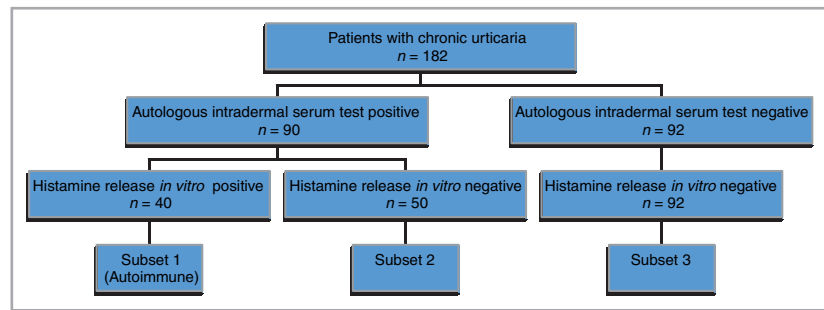
Patients

We studied 182 patients with active CU (mean age 39.2 years, range 12–74 years), of whom 126 (69%) were female. Almost 50% of the study group ($n = 90$) had a positive autologous intradermal serum test. Fewer males (41%) than females (53%) had a positive skin test, but this apparent difference in the sexes is not significant (RR = 0.66, CI 0.42–1.05).

Subsets of chronic urticaria

Sera from 40 of the 90 patients with a positive autologous intradermal serum test released histamine ($> 5\%$)³ from the basophil leucocytes of one or both healthy donor(s) (subset 1). Thirty-one of these 40 sera evoked histamine release ($29.3 \pm 18.5\%$, mean $\pm$ SD) from the basophil leucocytes of

Fig 1. Subsets of chronic urticaria identified by autologous intradermal serum tests and in vitro by serum-induced histamine release from the basophil leucocytes of healthy donors.



donor 1 (poorly IgE-sensitized) indicating the presence of anti-FcεR1α antibodies. Sera from nine skin test-positive patients induced histamine release ($9.5 \pm 3.4\%$, mean $\pm$ SD) only from the IgE-sensitized leucocytes of donor 2, indicative of the presence of anti-IgE antibodies. Sera from the remaining 50 skin test-positive patients failed to release histamine in vitro from donor basophils (subset 2). Sera from all 92 skin test-negative patients also failed to release histamine in vitro from basophil leucocytes (subset 3). The distribution of patients in the three subsets of CU is shown (Fig. 1).

Thyroid microsomal antibodies (TMA)

Eighteen autologous serum skin test-positive but only four skin test-negative patients had TMA antibodies, usually in low

titre (Table 1). The clustering of TMA positivity among the autologous serum skin test-positive patients is significant (RR = 4.06, CI 1.56–10.6). Within this skin test-positive group the frequency of TMA in subset 1 was nine of 40 (22.5%), and nine of 50 (18%) in subset 2 (Table 1). In the total study group of 182 CU patients moderate titres of TMA ($\geq 1 : 1600$) were present in only seven patients and a lower TMA in association with an abnormal TSH was present in five patients. Of these 12 patients, 10 had a positive autologous intradermal serum test.

There was no apparent effect of age or sex on TMA positivity ($P = 0.46$, $P = 0.91$, respectively, χ^2 test). Logistic regression techniques indicated that the key determinant of the presence of TMA was the serum test category (odds ratio 4.37, 95% CI 1.42–13.45).

Table 1 Results of antithyroid antibodies and thyroid-stimulating hormone (TSH) in serum test-positive and -negative patients and in the subsets of chronic urticaria

N	Age	Sex	TMA titre	TGA titre	TSH (mU L ⁻¹)	Serum test	Basophil release
Disease subset 1 (n = 40)							
1	36	F	1 : 100	neg	17.72	pos	pos
2	18	F	1 : 100	neg	2.3	pos	pos
3	14	M	1 : 100	neg	3.1	pos	pos
4	27	F	1 : 400	neg	8.82	pos	pos
5	13	M	1 : 400	neg	5.21	pos	pos
6	45	F	1 : 400	neg	2.27	pos	pos
7	36	M	1 : 400	neg	1	pos	pos
8	27	F	1 : 400	neg	0.05	pos	pos
9	22	F	1 : 6400	neg	2.9	pos	pos
Disease subset 2 (n = 50)							
10	41	F	1 : 100	neg	8.4	pos	neg
11	54	F	1 : 100	neg	1.03	pos	neg
12	38	M	1 : 400	neg	3.95	pos	neg
13	49	F	1 : 400	neg	2.53	pos	neg
14	42	M	1 : 400	neg	0.01	pos	neg
15	64	F	1 : 1600	1 : 1280	21.36	pos	neg
16	60	M	1 : 1600	neg	7.66	pos	neg
17	47	F	1 : 1600	neg	3.89	pos	neg
18	38	F	1 : 1600	neg	3.6	pos	neg
Disease subset 3 (n = 92)							
19	44	F	1 : 400	neg	2.11	neg	neg
20	36	M	1 : 400	neg	1.45	neg	neg
21	40	F	1 : 6400	1 : 640	1.7	neg	neg
22	43	F	1 : 6400	neg	1.12	neg	neg

TSH values outside the normal range (0.3–5.5 mU L⁻¹) are indicated in bold. TMA, Thyroid microsomal antibodies; TGA, thyroglobulin antibodies.

TSH (mU L ⁻¹)	Subset 1	Subset 2	Subset 3	Total
Low TSH (< 0.3)	4 (10%)	3 (6%)	0	7 (3.8%)
Normal TSH (0.3–5.5)	34 (85%)	43 (86%)	91 (99%)	168 (92.3%)
High TSH (> 5.5)	2 (5%)	4 (8%)	1 (1%)	7 (3.8%)
Total	40 (100%)	50 (100%)	92 (100%)	182 (100%)

Table 2 TSH (mU L⁻¹) in subsets of chronic urticaria

Thyroglobulin antibodies (TGA)

TGA was positive in two of 182 patients (Table 1).

Thyroid-stimulating hormone (TSH)

TSH values outside the normal range were largely confined to the autologous serum skin test-positive group (RR 15.5, CI 2.07–11.6), Table 2. Comparisons of the means of the TSH values in skin test-positive and -negative patients were not significantly different (ratio of geometric means = 0.97, CI 0.727–1.295, $P = 0.835$). However, the TSH values show a significantly wider range among the skin test-positive patients ($P = 0.000$, Bartlett's test for equal variances). TSH was elevated in seven of 182 patients (3.8%, CI 1.6–7.8) (Table 2) comparable to the 5% expected prevalence in the community.¹⁶

As was the case for TMA, the frequency of abnormal TSH was similar in subsets 1 (15%) and 2 (14%) (Tables 1 and 2). The major difference lies between these and the serum test-negative subset, only one in the latter subset having an abnormal value ($P = 0.001$, Fisher's exact test). Six patients had an abnormal TSH in the absence of antithyroid antibodies. There was no apparent effect of age or sex on TSH category ($P = 0.66$, $P = 0.38$, respectively, χ^2 test).

Discussion

The distribution of thyroid disorders in a cross-section of the community as studied in Whickham, County Durham, U.K. ($n = 2779$)¹⁶ showed that in a random survey, 5.3% had positive TMA $\geq 1:100$ (microhaemagglutination technique) and 5% had an elevated TSH ≥ 6 mU L⁻¹. The frequency of TMA and of elevated TSH is higher in females than in males at all ages and the percentage of females with a positive TMA peaks (10.8%) at age 45–54 years.¹⁶ CU also has a peak prevalence in the fourth decade and is commoner in women.

Leznoff and Sussman,¹¹ in their detailed study of 624 patients with CU, noted that 90 patients (86.6% female) had 'thyroid autoimmunity' (according to their definition). The titres of TMA are higher and more frequent (14%) than noted in our population. Applying Leznoff's definition to our study, 4.9% of our group have 'thyroid autoimmunity'. This is less than the 6% expected prevalence cited by Leznoff and Sussman. Comparison of Leznoff's group and our group indicates similarities in the two populations studied: the definition of CU as lasting more than 6 weeks, the inclusion of patients

with delayed pressure urticaria and dermographism, the age range and distribution, poor response to antihistamines and a requirement for prednisolone at some stage of their disease. However, Leznoff's group had a greater predominance of females (seven females to one male).

Comparison of TMA antibody titres between different studies are of limited value but the haemagglutination method is reportedly reproducible.¹⁷ Our results indicate a concentration of TMA and of abnormal TSH among the autologous serum skin test-positive patients. The abnormalities are distributed almost equally among subsets 1 and 2 and are not limited to the 'autoantibody' type (anti-Fc ϵ R1 α or anti-IgE) or subset 1 of CU. Subset 2 patients have a circulating histamine-releasing factor as evidenced by a positive autologous serum skin test. It is conceivable that because the cut-off point for positive histamine release from basophils was 5%, sera with weak histamine release from basophils, or those with a low level of antibody were misclassified. It is striking that the serum TSH level was normal in 99% of the skin test-negative patients.

HLA class II typing supports an autoimmune pathogenesis in a subset of CU patients.¹⁸ Thus DRB1*04 (DR4), DQB1*0301/4 and DQB1*0302 were each more frequent in subset 1 than in the control population ($P = 3.6 \times 10^{-6}$, $P = 2.4 \times 10^{-4}$ and $P = 1.6 \times 10^{-4}$). Subset 2 had a less striking but still highly significant association with DRB1*04 compared with controls ($P = 8.2 \times 10^{-4}$). In contrast, subset 3 showed no statistically significant difference from controls with respect to DRB1*04, DQB1*0301/4 and DQB1*0302.¹⁸ The current study also supports the concept of CU as a heterogeneous disease. In both studies subsets 1 and 2 are pathogenetically close, while patients in subset 3 are distinct. The clustering of TMA and abnormal thyroid function among the skin test-positive patients supports an autoimmune diathesis in this group.

Acknowledgments

We are grateful to the Eleanor Naylor Dana Charitable Foundation, the Dunhill Trust and the Kieckhefer Foundation for their support.

References

- Greaves MW. Chronic urticaria. *New Engl J Med* 1995; **332**:1767–72.
- Champion RH, Roberts SOB, Carpenter RG, Roger JH. Urticaria and angio-oedema. A review of 554 patients. *Br J Dermatol* 1969; **81**:588–97.

- 3 Niimi N, Francis DM, Kermani F *et al.* Dermal mast activation by autoantibodies against the high affinity IgE receptor in CU. *J Invest Dermatol* 1996; **106**:1001–6.
- 4 Grattan CEH, Wallington TB, Warin RP *et al.* A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation. *Br J Dermatol* 1986; **114**:583–90.
- 5 Grattan CEH, Hamon CGB, Cowan MA, Leeming RJ. Preliminary identification of a low molecular weight serological mediator in chronic idiopathic urticaria. *Br J Dermatol* 1988; **119**:179–84.
- 6 Hide M, Francis DM, Grattan CEH *et al.* Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in CU. *New Engl J Med* 1993; **328**:1599–604.
- 7 Fiebiger E, Maurer D, Holub H *et al.* Serum IgG autoantibodies directed against the α chain of Fc ϵ R1: a selective marker and pathogenetic factor for a distinct subset of CU patients? *J Clin Invest* 1995; **96**:2606–12.
- 8 Grattan CEH, Francis DM, Hide M, Greaves MW. Detection of histamine releasing autoantibodies with functional properties of anti-IgE in CU. *Clin Exp Allergy* 1991; **21**:695–704.
- 9 Kermani F, Niimi N, Francis DM *et al.* Characterization of a novel mast cell-specific histamine releasing activity in chronic idiopathic urticaria (CIU). *J Invest Dermatol* 1995; **105**:452 [Abstract].
- 10 Leznoff A, Josse RG, Denburg J, Dolovich J. Association of CU and angioedema with thyroid autoimmunity. *Arch Dermatol* 1983; **119**:636–40.
- 11 Leznoff A, Sussman GL. Syndrome of idiopathic CU and angioedema with thyroid autoimmunity: a study of 90 patients. *J Allergy Clin Immunol* 1989; **84**:66–71.
- 12 Turktas I, Gokcora N, Demirsoy S *et al.* The association of chronic urticaria and angioedema with autoimmune thyroiditis. *Int J Dermatol* 1997; **36**:187–90.
- 13 Champion RH. Urticaria: then and now. *Br J Dermatol* 1988; **119**:427–36.
- 14 O'Donnell BF, Barr RM, Kobza Black A *et al.* Intravenous immunoglobulin (IVIG) in autoimmune chronic urticaria. *Br J Dermatol* 1998; **138**:101–6.
- 15 Cayzer I, Chalmers SR, Doniach D, Swana G. An evaluation of two new haemagglutination tests for the rapid diagnosis of autoimmune thyroid disease. *J Clin Pathol* 1978; **31**:1147–51.
- 16 Tunbridge WMG, Evered DC, Hall R *et al.* The spectrum of thyroid disease in a community: the Whickham survey. *Clin Endocrinol* 1977; **7**:481–93.
- 17 Bird T, Stephenson J. Evaluation of a tanned red cell technique for thyroid microsomal antibodies. *J Clin Pathol* 1973; **26**:623–7.
- 18 O'Donnell BF, O'Neill CM, Francis DM *et al.* Human leucocyte antigen class II associations in chronic idiopathic urticaria. *Br J Dermatol* 1999; **140**:853–8.