

Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells

R. Asero*, M. Lorini†, S. U. Chong‡, T. Zuberbier‡ and A. Tedeschi†

*Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy, †Unità Operativa di Medicina Interna I, IRCCS Ospedale Maggiore Policlinico, Milano, Italy and ‡Department of Dermatology and Allergy, University Hospital Charité, Humboldt University, Berlin, Germany

Summary

Background All previous studies agree that only a proportion of sera from patients with chronic urticaria (CU) positive on the autologous serum skin test (ASST) are able to induce histamine release *in vitro*. A non-specific release of bradykinins during clotting of blood samples has been suggested; however, ASST seems rather specific and some data point to the existence of a mast cell-specific histamine-releasing factor.

Objective To assess whether, and to what extent, the use of both human basophils and mast cells increases the sensitivity of *in vitro* histamine release assays (HRAs) in ASST-positive patients with CU.

Methods The histamine-releasing activity of sera from 93 patients with CU selected on the basis of strong skin reactivity on ASST was assessed *in vitro* on basophils from 1 ($n = 86$), 2 ($n = 31$), or 3 ($n = 20$) normal donors, and on mast cells from 1 ($n = 3$), 2 ($n = 3$), or 3 ($n = 87$) normal donors.

Results Sera from 88/93 (95%) patients induced significant histamine release from mast cells or basophils on at least one HRA. 76/93 (82%), 45/90 (50%), 22/80 (28%), and 6/12 (50%) sera were able to induce significant histamine release from cells of 2/5, 3/5, 4/5 and 5/5 donors, respectively.

Conclusion Sera from nearly all ASST-positive patients with CU are able to induce histamine release *in vitro*. However, the serum from each single patient seems to show its maximal activity on autologous mast cells *in vivo*, and functional *in vitro* tests show much variability and seem less sensitive than ASST in the detection of patients with histamine-releasing factors in their blood.

Keywords basophils, chronic urticaria, histamine release, mast cells, skin tests

Submitted 7 May 2003; revised 24 November 2003; accepted 19 February 2004

Introduction

Chronic urticaria (CU) is characterized by the occurrence of transient cutaneous weals, with or without angioedema, for more than 6 weeks. During the last 15 years, some light on the pathogenesis of this disease was shed, first showing that the intradermal injection of autologous serum (ASST, autologous serum skin test) causes a weal-and-flare reaction in 30–60% of patients, particularly during the active phases of the disease, and subsequently demonstrating that sera from CU patients may induce histamine release from normal basophils *in vitro* (HRA, histamine release assay) [1–9]. Histamine-releasing activity has been ascribed to circulating IgG antibodies specific for the high-affinity IgE receptor (FcεRI) present on mast cells and basophils [4–8] or for IgE [2, 3]. However, several aspects remain controversial, particularly the marked differences between *in vivo* (ASST) and *in vitro* (HRA) findings. Histamine release induced by purified anti-FcεRI IgG *in vitro* is markedly enhanced by complement [9–12], a fact that has not been observed *in vivo* [13–15].

Moreover, although the prevalence of both positive ASST and positive HRA may show some differences in distinct series of patients depending on the severity of the disease in the study population, the technique of ASST, and the marked variability of histamine releasability between basophils from different healthy donors [16], all studies agree that only a proportion of sera from ASST-positive patients are able to induce histamine release *in vitro* [4, 5, 14, 17]. In a recent study, we found that only 20% of ASST-positive sera were HRA-positive as well [14]. Some authors suggested that this is due to 'falsely positive' ASST caused by non-specific release of bradykinins during clotting of blood samples [11]. However, ASST seems rather specific as it scores almost always negative in normal controls [14, 15]. Finally, the role of circulating autoantibodies has been recently questioned by Fagiolo et al. [13] who showed that IgG-depleted autologous sera from patients with CU maintain their ability to induce weal-and-flare reactions upon intradermal injection. On the basis of all these observations, the existence of a mast cell-specific histamine-releasing factor has been hypothesized [5, 7, 14, 15]. The aim of the present study was to assess whether, and to what extent, the use of both human basophils and mast cells increases the sensitivity of *in vitro* HRA in patients with CU.

Correspondence: Dr Riccardo Asero, Ambulatorio di Allergologia, Clinica San Carlo, Via Ospedale 21, 20037 Paderno Dugnano (MI), Italy.
E-mail: r.asero@libero.it

Patients and methods

Patients and ASST

Four hundred and eighty-seven consecutive adult patients (M/F 122/365) diagnosed as having CU at the allergy center of Bollate Hospital (Bollate, Milan, Italy) during the last 3 years were considered eligible. Patients with physical urticaria were excluded. CU was diagnosed on the basis of the appearance of continuous or recurrent hives with or without angioedema for more than 6 weeks. Five days after anti-histamine therapy (cetirizine, loratadine, or fexofenadine in all cases) was stopped, an intradermal test with 0.05 mL of fresh autologous serum (ASST) and with saline as negative control was carried out. ASST was performed and read at 30 min following the method by Greaves [17] and Sabroe et al. [18]. A skin prick test (SPT) with histamine 10 mg/mL was carried out to exclude any residual effect of anti-histamine drugs. Patients showing a pink weal with a diameter at least 1.5 mm greater than the control saline solution were considered positive [18]. Of 234 patients positive on ASST, 93 subjects showing strong skin reactivity (i.e. a weal diameter > 15 mm) were finally included. In all these subjects, the mean diameter of the weal-and-flare reaction induced by autologous serum was > 20 mm. In patients with CU, strong skin reactivity on ASST has been associated with the presence of circulating autoantibodies to FcεRI [18].

Basophil HRA

Leucocyte suspensions from normal blood donors were prepared by dextran sedimentation of peripheral venous blood, anticoagulated with 0.01 M EDTA, and mixed with 6% dextran in saline solution (Solplex 70, Sifra, Verona, Italy) and 30 mM dextrose (Sigma Chemical, St Louis, MO, USA). The cells were allowed to settle for 60–90 min at room temperature, according to Lichtenstein et al. [19]. The leucocyte-rich plasma was aspirated and centrifuged at 300g for 15 min at 4°C, and the cell button was washed twice in Tyrode's buffer, pH 7.4, containing (mM) 140 NaCl, 5.5 dextrose, 2.7 KCl, 0.36 NaH₂PO₄, and 12 NaHCO₃. Leucocytes (with about 7×10^4 basophils) were then resuspended in 100 µL volume of Tyrode's buffer with 1.8 mM CaCl₂ and 0.5 mM MgCl₂, and incubated with 100 µL of the serum under examination, making a final volume of 200 µL. After incubation for 40 min at 37°C, the reaction was stopped by addition of 800 µL of ice-cold buffer solution and centrifugation at 700g for 10 min at 4°C. After centrifugation, the supernatants were aspirated, mixed with an equal volume of 6% HClO₄ and centrifuged at 1000g for 10 min at 4°C. Histamine concentration in the supernatants was measured by an automated fluorometric method, according to Ruff et al. [20]. Spontaneous histamine release was evaluated by measuring histamine concentration in the supernatant of unstimulated cells, incubated for 40 min at 37°C. Total histamine content was obtained by adding 100 µL of 6% HClO₄ to 100 µL of cell suspension. Net histamine release was calculated as percentage of total histamine content, after subtraction of spontaneous release. A 5% release cut-off value was used. HRA was carried out using the same sera employed for ASST. The histamine-releasing activity of sera from 86 patients was assessed on

basophils from a normal donor (experiment no.1). Sera from 31 and 20 patients were tested with basophils from 2 other normal donors as well (experiments no. 2 and 3, respectively). Sera from 20 patients were tested on basophils from all 3 donors. Basophils from all three normal donors were stimulated with an optimal dose of polyclonal goat anti-human IgE antiserum (Sigma 10 µg/mL).

Mast cell HRA

Anti-IgE-responsive mast cells from breast skin, excised for cosmetic reasons after informed consent, were used as previously described [21]. Briefly, skin was cut into small slices and incubated overnight with 0.5 mg/mL dispase type I (Roche Diagnostics, Mannheim, Germany) in phosphate-buffered saline (PBS), in order to remove the epidermis. The minced tissue was digested in two cycles of incubation in PBS containing collagenase 1.5 mg/mL (CellSystems, St Katharinen, Germany), hyaluronidase type I S, 0.75 mg/mL (Sigma, Deisenhofen, Germany) and DNase type I, 10 ng/mL (Roche Diagnostics), lasting 1 h each. Mast cells were kept routinely at 5% CO₂ and 37°C in basal Iscove's containing 10% heat-inactivated fetal calf serum, 4 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin, 2.5 µg/mL amphotericin B (all from Biochrom, Berlin, Germany) and 0.002% α-monothioglycerol (Sigma). For stimulation, cell suspension was centrifuged and resuspended in 50 µL PAG-CM (25 mmol/L PIPES, 119 mmol/L NaCl, 5 mmol/L KCl, 0.0025% human serum albumin, 0.1% glucose, 3 mmol/L Ca²⁺, and 3 mmol/L Mg²⁺ adjusted to pH 7.4) for incubating 30 min at 37°C with 50 µL of either anti-IgE (1 : 10 000) or sera of CU patients. Stimulation was stopped by addition of 650 µL of ice-cold PAG-CM. After centrifugation 500 µL of supernatants were aspirated, mixed with 500 µL 4% HClO₄ and centrifuged for 10 min. Corresponding to basophils, histamine release was measured by an automated fluorometric method.

Three independent experiments with mast cells from different donors were performed. From the total of 93 sera, 87 sera were tested on mast cells from all 3 donors and sera from the remaining six patients were tested on mast cells from 2 donors ($n = 3$) and 1 donor ($n = 3$). Since histamine release induced by normal sera showed much variability, a different cut-off was used in each experiment. A cut-off corresponding to the mean histamine release induced by five normal sera +2 SD was used in all cases.

Results

Basophils (Table 1)

HRA using basophils of donors 1, 2, and 3 was positive in 38/86 (35%), 18/31 (58%), and 15/20 (75%) cases, respectively. The proportion of sera able to induce histamine release from basophils of at least two donors was rather low (Table 1), and only sera from 5/20 (25%) patients were able to induce histamine release from basophils of all three donors. Sera from 48/86 (56%) patients were able to induce histamine release from basophils of at least one donor.

Table 1. Results of *in vitro* HRAs

Basophils	
Positive on donor 1 cells	38/86 (35%)
Positive on donor 2 cells	18/31 (58%)
Positive on donor 3 cells	15/20 (75%)
Positive on both donors 1 and 2 cells	10/31 (32%)
Positive on both donors 2 and 3 cells	10/20 (50%)
Positive on both donors 1 and 3 cells	9/20 (45%)
Positive on cells from all three donors	5/20 (25%)
Positive either on cells from donors 1, 2, or 3	48/86 (56%)
Mast cells	
Positive on donor 1 cells	55/91 (60%)
Positive on donor 2 cells	56/89 (63%)
Positive on donor 3 cells	70/90 (78%)
Positive on both donors 1 and 2 cells	37/87 (43%)
Positive on both donors 2 and 3 cells	45/88 (51%)
Positive on both donors 1 and 3 cells	46/89 (52%)
Positive on cells from all three donors	32/87 (37%)
Positive either on cells from donors 1, 2, or 3	86/93 (92%)
Basophils + mast cells	
No. positive on basophils only	2/86 (2%)
No. positive on mast cells only	32/86 (37%)
Positive either on basophils or mast cells	88/93 (95%)

HRAs, histamine release assays.

Mast cells (Table 1)

Histamine release from mast cells of donors 1–3 was induced by 60–78% of patients' sera. As with basophils, sera from most patients (86/93 [92%]) were able to induce histamine release from mast cells of a single donor, but only a minority of them (32/87 [37%]) were able to do so on cells from all three donors.

Basophils and mast cells (Table 1)

HRAs with both mast cells and basophils showed marked variability, as in many cases the same serum behaved differently on basophils or mast cells from different donors. Sera from 88/93 (95%) patients induced histamine release from mast cells or basophils on at least one HRA. Sera from 74/93 (79%), 48/93 (52%), 25/84 (30%), 11/28 (39%), and 3/14 (21%) sera were able to induce significant histamine release in assays using basophils or mast cells from 2, 3, 4, 5, and 6 donors, respectively. The main quantitative results of HRAs are summarized in Table 2.

Table 2. Quantitative aspects of HRAs

	Anti-IgE (%)	Cut-off (%)	No. positive	Histamine release	
				Range (%)	Mean (SD)
Basophils donor 1	25	5	38	5.2–36.8	11.7% (7.3)
Basophils donor 2	35	5	18	5.7–64.6	15.6% (15.3)
Basophils donor 3	22	5	15	7.1–58.3	16.3% (12.4)
Mast cells donor 1	28.5	5	55	5.5–37.9	12.0% (7.9)
Mast cells donor 2	18.7	20	56	22.2–77.8	30.9% (11.8)
Mast cells donor 3	26.4	5	70	5.1–51.1	13.8% (10.1)

HRAs, histamine release assays; SD, standard deviation.

Discussion

This study investigated the histamine-releasing activity of sera from a large number of patients with CU on both human basophils and mast cells from different donors. In our knowledge, only two previous studies compared the histamine-releasing activity of chronic urticaria sera on both mast cells and basophils. Niimi et al. [22] found that sera that released histamine from basophils induced histamine release from mast cells as well; however, these represented only a proportion of sera from ASST-positive patients. Also Ferrer et al. [23] found an excellent correlation between histamine release from basophils and mast cells suggesting a common mechanism of activation, but in that study ASST was not considered. Our population included only subjects scoring strongly positive on ASST, a condition that has been associated with circulating autoantibodies specific for FcεRI or IgE [18]. Therefore, theoretically, all sera were expected to show histamine-releasing activity *in vitro*. Although a large majority of sera (94%) induced histamine release either from basophils or mast cells in at least one experiment, significant histamine release from both basophils and mast cells in all experiments was observed only in a minority of cases. In most cases several experiments with cells from different donors were needed to observe significant histamine-releasing activity. Previous studies showed that histamine release induced by anti-FcεRI antibodies is strongly influenced by the density of surface IgE on basophils [24]. However, if the cells from a single donor were fully IgE sensitized, histamine release would be induced mostly by sera containing anti-IgE antibodies but not from sera containing functionally active anti-FcεRI antibodies, whereas, conversely, poorly IgE-sensitized cells from another donor would respond to sera containing anti-FcεRI antibodies but not to sera containing anti-IgE. This was not the case in our study where each single serum showed a variable activity on cells from single donors. Another possibility is that sera from patients with CU that react in IgE-sensitized basophils and mast cells may either behave like anti-IgE or non-competitive anti-FcεRI autoantibodies. Several reasons may be advocated to explain the variability of basophil and mast cell response to CU sera. One important reason is the heterogeneity of the histamine-releasing activity contained in CU sera, which may be related to an anti-FcεRI antibody, an anti-IgE antibody, a mast cell-specific factor or an as yet unidentified factor [13, 15, 24]. Other factors conditioning basophil and mast cell response are the density and the saturation of membrane IgE receptors, and the different releasability of cells obtained from different donors. Finally, C5a availability and C5a receptors represent critical factors influencing basophil and mast cell response to the activating signals contained in CU sera [12]. Complement activation and C5a generation seem to play a critical role in augmentation of basophil and mast cell histamine release particularly with low concentrations of IgG autoantibodies [11].

In summary, the results of this study suggest that although in some experiments individual factors regulate releasability, sera from most ASST-positive patients with CU are able to induce histamine release *in vitro* and therefore are likely to contain functionally active anti-FcεRI (or anti-IgE) antibodies. In conclusion, currently available functional *in vitro* tests seem less sensitive than ASST in the detection of patients

with autoimmune CU and show much variability. Apparently, factors other than the density of surface IgE and the availability of free IgE receptors (e.g. releasability and availability of complement factors) play a role in serum-induced histamine release, and the serum from each single patient seems to show its maximal activity on autologous mast cells in the presence of additional conditions only. This could also explain previous findings of autoantibody-positive patients with chronic continuous urticaria who were free of symptoms after treatment of HP infection or after a pseudoallergen-free diet [25].

References

- Grattan CE, Wallington TB, Warin RP, Kennedy CT, Bradfield JW. A serological mediator in chronic idiopathic urticaria – a clinical, immunological and histological evaluation. *Br J Dermatol* 1986; 114:583–90.
- Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol* 1988; 90:213–7.
- Grattan CEH, Francis DM, Hide M, Greaves MW. Detection of circulating histamine releasing autoantibodies with functional properties of anti IgE in chronic urticaria. *Clin Exp Allergy* 1991; 21:695–704.
- Greaves MW. Chronic urticaria. *N Engl J Med* 1995; 332:1767–72.
- Greaves MW, O'Donnell B. Urticaria causes and treatment. *Int J Immunopathol Pharmacol* 1997; 99:461–5.
- Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993; 328:1599–604.
- Tong LJ, Balakrishnan G, Kochan JP, Kinet J-P, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 1997; 99:461–5.
- Fiebiger E, Maurer D, Holub H et al. Serum IgG autoantibodies directed against the alpha chain of FcεRI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995; 96:2606–12.
- Zweiman B, Valenzano M, Atkins PC, Tanus T, Getsy JA. Characteristics of histamine releasing activity in patients with chronic idiopathic urticaria. *J Allergy Clin Immunol* 1996; 98:89–98.
- Ferrer M, Nakazawa K, Kaplan AP. Complement-dependence of histamine release in chronic urticaria. *J Allergy Clin Immunol* 1999; 104:169–72.
- Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001; 107:1056–62.
- Kikuchi Y, Kaplan AP. A role for C5a in augmenting IgG-dependent histamine release from basophils in chronic urticaria. *J Allergy Clin Immunol* 2002; 109:114–8.
- Fagiolo U, Krichek F, Ruf C, Peserico A, Amadori A, Cancian M. Effects of complement inactivation and IgG depletion on skin reactivity to autologous serum in chronic idiopathic urticaria. *J Allergy Clin Immunol* 2000; 106:567–72.
- Asero R, Tedeschi A, Lorini M, Salimbeni R, Zanoletti T, Miadonna A. Chronic urticaria: novel clinical and serological aspects. *Clin Exp Allergy* 2001; 31:1105–10.
- Grattan CE, Hamon CG, Cowan MA, Leeming RJ. Preliminary identification of a low molecular weight serological mediator in chronic idiopathic urticaria. *Br J Dermatol* 1988; 119:179–83.
- Lichtenstein LM, MacGlashan DW. The concept of basophil releasability. *J Allergy Clin Immunol* 1986; 77:291–4.
- Greaves MW. Chronic urticaria. *J Allergy Clin Immunol* 2000; 105:664–72.
- Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140:446–52.
- Lichtenstein LM, Osler AG. Study on the mechanism of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen. *J Exp Med* 1964; 120:507–30.
- Ruff F, Saindelle A, Dutripon E, Parrot JL. Continuous automatic fluorometric evaluation of total blood histamine. *Nature* 1967; 214:279–81.
- Grützkau A, Henz BM, Kirchhof L, Luger T, Artuc M. α-melanocyte stimulating hormone acts as a selective inducer of secretory functions in human mast cells. *Biochem Biophys Res Commun* 2000; 278:14–9.
- Niimi N, Francis DM, Kermani F et al. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol* 1996; 106:1001–6.
- Ferrer M, Kinet J-P, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-FcεRI (alpha subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998; 101:672–6.
- Sabroe RA, Fiebiger E, Francis DM, Maurer D et al. Classification of anti-FcεRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002; 110:492–9.
- Zuberbier T, Henz BM, Fiebiger E, Maurer D, Stingl G. Anti-FcεpsilonRIalpha serum autoantibodies in different subtypes of urticaria. *Allergy* 2000; 55:951–4.