Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum

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Background: Several aspects of the pathogenesis of chronic urticaria (CU) remain contradictory. Autologous serum skin tests (ASSTs) and *in vitro* histamine release assays seem to look into distinct aspects of the disease, and the specificity of ASST has been questioned.

Objective: We compared the autologous plasma skin test (APST) with ASST to detect autoreactivity in patients with CU. The clotting process was investigated as well by measuring *in vivo* thrombin generation.

Methods: A total of 96 adults with CU underwent ASST; 71 of them underwent APST with Na citrate–anticoagulated plasma. Prothrombin fragment 1+2 plasma levels were measured by a sandwich ELISA in Na citrate–anticoagulated plasmas from 28 patients and 27 controls.

Results: Fifty-one of 96 (53%) patients scored positive on ASST, whereas 61 of 71 (86%) patients scored positive on APST (21/30 [70%] ASST-negative and 40/41 [98%] ASST-positive). Plasma prothrombin fragment 1+2 was higher in patients than controls (3.06 [SD 3.36] vs 0.80 [0.34]; $P < .001$) and in ASST-positive/APST-positive than in ASST-negative/APST-positive patients (3.89 [SD 3.68] vs 1.33 [1.64]; $P = 0.058$) and was directly related to urticaria severity ($r = 0.37$; $P < .05$).

Conclusion: Most patients with CU are positive on APST–Na citrate. CU is associated with the generation of thrombin, a serine protease able to activate mast cells and to cause relevant increase in permeability of endothelium. APST and ASST only partially depend on the presence of circulating antibodies to FcεRI or to IgE.

Clinical implications: These findings provide new insights into the pathogenesis of CU and suggest new therapeutic opportunities for treating this disease. (J Allergy Clin Immunol 2006;117:1113-7.)

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Chronic urticaria (CU) is a rather common skin disorder characterized by the recurrent eruption of short-lived wheals accompanied by redness and itching for at least 6 weeks.[1] The pathogenetic mechanisms of CU are still poorly understood. In a variable proportion (30-60%) of patients with active disease the intradermal injection of autologous serum (ASST, autologous serum skin test) causes a wheal-and-flare reaction[1-5] and the serum from some CU patients is able to induce histamine release from cultured basophils of healthy subjects (HRA, histamine release assay). Both phenomena have been ascribed to circulating IgG specific for the high affinity IgE receptor FcεRI present on mast cells and basophils or for IgE.[1-9] However, several aspects remain contradictory, and some evidence suggests that ASST and HRA look into distinct aspects of the disease and do not invariably indicate the presence of autoantibodies.

About 50% of patients with CU are positive on ASST,[2-5,10] but sera from only ½ of them are able to induce histamine release from cultured basophils *in vitro*.[1,5,10-13] We recently observed that the sensitivity of *in vitro* assays is much increased if both mast cells and basophils from several donors are used,[14] suggesting a wide variability in the response of cells from different donors.

Serum from patients negative on ASST does not induce any histamine release *in vitro*,[10] although these subjects do not show any clinical and/or pathological difference from ASST-positive/HRA-positive patients.

Histamine release induced by purified anti-FcεRI IgG *in vitro* is markedly enhanced by complement,[9,15-17] whereas decomplementation does not influence the result of the ASST.[10,13,18]

Finally, IgG-depleted autologous sera from patients with CU maintain their ability to induce wheal-and-flare reactions on intradermal injection,[13] because ASST was detecting factors other than anti-FcεRI IgG.

Autologous serum skin test has become the easiest way to diagnose autoreactive CU in the clinical practice,[19] but some authors have questioned the specificity of this test, suggesting that it might produce false-positive results because of the generation of large quantities of bradykinin

Abbreviations used
CU: Chronic urticaria
ASST: Autologous serum skin test
APST: Autologous plasma skin test
F_{1+2}: Prothrombin fragment 1+2
HRA: Histamine release assay

during the clotting process[20] and the direct cleavage of C5 by tryptaselike plasma proteases secreted by neutrophils.[21] If this were the case, intradermal testing with autologous plasma (APST, autologous plasma skin test) instead of autologous serum would be expected to result in a better specificity of *in vivo* assays (ie, in a reduced frequency of positive tests). Previous studies showed that heparin-anticoagulated plasma cannot be used because heparin inhibits degranulation of mast cells and basophils both *in vivo*[10,22] and *in vitro*.[10]

The first aim of the current study was to investigate skin autoreactivity by using plasma anticoagulated with substances other than heparin (APST) and compare it with ASST in patients with CU. Second, because experimental and clinical data indicate that (1) thrombin, the final enzyme of the coagulation cascade, increases vascular permeability,[23,24] (2) thrombin generation occurs during attacks of angioedema in patients with C1-inhibitor deficiency,[25] (3) thrombin injection can induce urticarial reactions,[26] and (4) both heparin (which highly increases the antithrombin activity in plasma) and oral anticoagulant therapy can be effective in the treatment of CU,[27,28] we evaluated thrombin generation in plasma of our patients with CU by measuring prothrombin fragment 1+2 (F_{1+2}), inactive fragments considered to be a reliable marker of thrombin generation *in vivo*.[29]

METHODS

Patients

Ninety-six consecutive adult patients (male:female, 24:72; age range, 18-70 years) with CU seen at the allergy department of the Clinica San Carlo (Paderno Dugnano, Italy) were studied. CU was diagnosed on the basis of the appearance of continuous or recurrent hives with or without angioedema for more than 6 weeks.[1] Patients with physical urticaria were excluded. Urticarial activity was estimated according to the number of wheals present at the time when blood samples from these 28 patients were collected as previously described[30]: 1 to 10 small (<3 cm in diameter) wheals = grade 1 (slight); 10 to 50 small wheals or 1 to 10 large wheals = grade 2 (moderate); >50 small wheals or >10 large wheals = grade 3 (severe); virtually covered with wheals = grade 4 (very severe).

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and all subjects gave their informed consent before participation.

Skin tests

After antihistamine treatment (cetirizine 10 mg, loratadine 10 mg, or ebastine 20 mg daily in all cases) had been stopped for at least 5 days, all patients underwent intradermal testing with 0.05 mL of both sterile autologous serum (ASST) and saline as negative control. Seventy-one patients underwent intradermal test with Na citrate–anticoagulated plasma (APST–Na citrate, 0.125 mol/L of Na citrate). Serum and plasma samples were centrifuged after 15 minutes at 1250*g* for 3 minutes and immediately used for intradermal tests. All intradermal tests (serum, plasma, and negative control) were performed and read at the same time in all patients. A skin prick test with histamine 10 mg/mL was used as positive control. Readings were taken at 30 minutes; only an unequivocal wheal-and-flare reaction with a wheal diameter of at least 3 mm in the absence of any reaction to saline was considered a positive response. Ten atopic subjects and 5 subjects with contact dermatitis, all without a history of chronic urticaria, underwent APST–Na citrate as control.

F_{1+2} measurement

Na citrate–anticoagulated plasmas from 28 patients with CU and from 27 age-matched and sex-matched normal subjects were stored into plastic cones at −20°C until prothrombin fragment F_{1+2}[31,32] was measured by a sandwich ELISA (Enzygnost F_{1+2}; Behring Diagnostics GmbH, Frankfurt, Germany). Intra-assay and interassay coefficients of variation were 5% and 8%, respectively.

Statistics

Means were compared by 2-tailed Student *t* test. Probability (*P*) values less than 5% were considered statistically significant. Correlation between urticaria severity and F_{1+2} plasma levels was assessed; an *r* value corresponding to a probability <5% was considered significant.

RESULTS

Skin tests

No patient reacted on the intradermal injection of saline, and skin prick test with histamine scored positive in all cases. Altogether, 51 of 96 (53%) patients scored positive on ASST.

We first used K_2EDTA as anticoagulant to test plasma skin reactivity. Of 25 patients submitted to intradermal testing with K_2EDTA-anticoagulated plasma, 24 (96%) scored positive. To assess the specificity of skin reactions induced by autologous plasma, 11 patients (4 negative and 7 positive on ASST) underwent further intradermal tests using saline containing 1 mg/mL K_2EDTA; this solution induced a wheal-and-flare reaction in all 11 cases. In addition, in 1 patient, the intradermal tests performed with different concentrations of K_2EDTA in saline (obtained adding different amounts of saline to sterile tubes containing K_2EDTA) showed that the intensity of the wheal-and-flare reaction was directly related to the concentration of K_2EDTA. Thus K_2EDTA induced nonspecific reactions and was no longer used to detect patients' autoreactivity.

Sixty-one of 71 (86%) patients scored positive on APST–Na citrate. The skin test with APST–Na citrate was positive in 21 of 30 (70%) patients who scored negative on ASST and in 40 of 41 (98%) ASST-positive patients. In ASST-positive patients, the mean diameter of the wheal-and-flare area induced by autologous plasma exceeded that induced by autologous serum in 21 of 41 cases; in 19 cases, the wheal-and-flare area induced by autologous serum and plasma was equivalent, whereas in 1 case, the