
Circulating stem cell factor in patients with chronic idiopathic urticaria

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Background: The nature of histamine-releasing factors involved in the pathogenesis of chronic idiopathic urticaria (CIU) is still controversial, since functional IgG autoantibodies specific for the high-affinity IgE receptor, FcεRI, can be detected in only 20% of patients showing a strong skin reactivity on the autologous serum skin test. The absence of systemic eosinophilia in CIU patients, along with the increase in mast cells in skin biopsy specimens, suggests a possible role for stem cell factor (SCF), the only cytokine/growth factor known to induce mediator release from human mast cells.

Objective: To investigate the possible role of SCF as a histamine-releasing factor in patients with CIU.

Methods: The SCF levels were measured in serum samples from 65 patients with CIU who scored strongly positive on the autologous serum skin test; of these patients, 32 had negative results and 33 had positive results on in vitro histamine release assay by a quantitative commercial sandwich immunoassay technique. Serum samples from 40 healthy subjects were used as controls.

Results: Serum SCF levels in all 65 CIU patients did not differ from those found in healthy controls. No difference in SCF levels was found between patients with positive and negative results on histamine release assay.

Conclusions: An increase in serum SCF levels does not play a pathogenic role in CIU.

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INTRODUCTION

Chronic urticaria is characterized by the recurrence of transitory, itchy wheals for more than 6 weeks. In most cases no cause can be detected, and the disease is termed *idiopathic*. Recently, some light has been shed on the origin of chronic idiopathic urticaria (CIU) by studies demonstrating that the intradermal injection of autologous serum induces a wheal-and-flare reaction in a relevant proportion of patients.^{1–5} In a recent study⁶ performed with more than 300 consecutive patients with CIU, approximately 60% of patients tested positive on the autologous serum skin test (ASST). The nature of the histamine-releasing factors involved in the pathogenesis of CIU is still controversial. In serum samples from a small proportion of patients (approximately 20% of those with positive ASST results⁶), functional IgG autoantibodies specific for the high-affinity IgE receptor, FcεRI, can be detected.^{1–10} However, hitherto uncharacterized histamine-releasing factors are possibly involved in most patients with positive ASST results whose sera do not contain circulating anti-FcεRI antibodies.⁶ Some previous studies reported a preliminary, partial characterization of a histamine-releasing factor as a protein with a molecular weight of more than 30 kD; however, its exact nature has not been defined yet.

Histologically, CIU is characterized by a nonnecrotizing vasculopathy with perivascular infiltration of lymphocytes (mostly CD4+), monocytes, and variable numbers of neutrophils and eosinophils.¹¹ The absence of systemic eosinophilia in CIU patients seems to rule out a possible role for some β-chemokines that show histamine-releasing properties, such as RANTES and MIP-1α. Such chemokines induce histamine release from basophils, but on cutaneous challenge they did not show a similar effect on mast cells¹²; furthermore, they induce tissue eosinophilia, which is normally absent in CIU.^{12,13} Stem cell factor (SCF) produced by endothelial, mast, and other stromal cells plays a central role in the development and maturation of mast cells^{14–16} and is the only cytokine/growth factor known to induce mediator release from human mast cells.^{17,18} Although a significant increase in total body mast cell load has not been observed in CIU, histopathologic studies performed on biopsy specimens from patients with urticaria showed an increased number of mast cells in the dermis of both lesional and uninvolved skin, particularly in those with CIU.^{19,20} Therefore, SCF was a natural candidate in an investigation that looked for a possible pathogenic factor in CIU. We investigated the possible role of SCF as a histamine-releasing factor in patients with CIU.

PATIENTS AND METHODS

Patients

All 188 patients diagnosed as having CIU seen at the Allergy Department of the Bollate Hospital (Bollate, Milan, Italy) during the last 12 months were considered eligible for the study. In all cases, known causes of chronic or recurrent

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urticaria had been ruled out by appropriate investigations. All patients underwent ASST as previously described.⁶ Eighty patients who showed a marked wheal-and-flare reaction on injection of autologous serum (graded 4+ by comparison with the reaction induced by the positive control)⁶ were included in the serologic part of the study.

Histamine Release Assay

Leukocyte suspensions from healthy 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 of dextrose (Sigma Chemical Company, St. Louis, MO). The cells were allowed to settle for 60 to 90 minutes at room temperature, according to Lichtenstein and Osler.²¹ The leukocyte-rich plasma was aspirated and centrifuged at 300 × *g* for 15 minutes at 4° C, and the cell button was washed twice in Tyrode buffer, pH 7.4, containing 140 mM sodium chloride, 5.5 mM dextrose, 2.7 mM potassium chloride, 0.36 mM NaH₂PO₄, and 12 mM NaHCO₃. Leukocytes (with approximately 7 × 10⁴ basophils) were then resuspended in 100-μL volumes of Tyrode buffer with 1.8 mM calcium chloride and 0.5 mM magnesium chloride and incubated with 100 μL of the serum samples, making a final volume of 200 μL. After incubation for 40 minutes at 37° C, the reaction was stopped by addition of 800 μL of ice-cold buffer solution and centrifugation at 1000 × *g* for 10 minutes at 4° C. After centrifugation, the supernatants were aspirated, mixed with an equal volume of 6% perchloric acid, and centrifuged at 1000 × *g* for 10 minutes at 4° C. Histamine concentration in the supernatants was measured by radioimmunoassay using a commercially available kit (Immunotech, Marseille, France). Spontaneous histamine release was evaluated by measuring histamine concentration in the supernatant of unstimulated cells incubated for 40 minutes at 37° C. Total histamine content was obtained by heating the cells at 85° C for 40 minutes. Net histamine release was calculated as a percentage of total histamine content after subtraction of spontaneous release. The histamine-releasing activity of serum samples from all 80 patients who scored strongly positive on the ASST was evaluated on basophils from 2 healthy donors. A 5% cutoff was chosen.

Serum SCF Measurement

The SCF levels were measured in serum samples from 65 patients (32 who tested negative and 33 who tested positive on histamine release assay [HRA]) and from 40 healthy subjects by a quantitative commercial sandwich immunoassay technique (Quantikine R&D Systems, Minneapolis, MN). The microplate is precoated with a monoclonal antibody specific for SCF. Any SCF present in the serum is revealed by an enzyme-linked polyclonal antibody specific for SCF. The minimum dose of SCF detectable by this kit is typically less than 9.0 pg/mL. All detections were performed in duplicate.

RESULTS

Results of the SCF immunoassay are summarized in Table 1. Circulating SCF levels in all 65 ASST-positive patients with CIU did not significantly differ from those found in serum samples from 40 healthy controls (*P* = 0.08). Furthermore, the subsets of patients who tested positive or negative on HRA did not show any difference in circulating SCF levels (*P* = 0.37). No significant difference was found comparing healthy controls and HRA-positive patients (*P* = 0.28) or HRA-negative patients (*P* = 0.06). The range of SCF in the serum of our control population was similar to that reported in the data sheet available with the Quantikine kit.

DISCUSSION

On the basis of ASST and in vitro HRA results, different subsets of patients with CIU can be distinguished. Some patients who show both a negative ASST result and no histamine-releasing activity in vitro appear to have a truly idiopathic disease. In contrast, reactivity apparent on the ASST suggests the presence of circulating histamine-releasing factors.⁴ Serum samples from a few ASST-positive patients are able to induce significant histamine release from basophils of healthy donors; these patients probably have an autoimmune disorder caused by circulating anti-FcεRI antibodies or anti-IgE autoantibodies,¹⁻¹⁰ although the pathogenic role of such autoantibodies has been questioned by a recent study²² that showed that serum samples containing FcεRI antibodies maintain their in vivo ability to induce histamine release (by ASST) also after depletion of IgG. Another subgroup of CIU patients with positive ASST results but negative HRA results exists.²³ These patients probably represent more than 50% of patients with CIU.⁶ It was particularly in this latter group that SCF represented a candidate as a possible pathogenic histamine-releasing factor other than an immunoglobulin. In fact, SCF induces mast cell degranulation but has no significant histamine-releasing activity on basophils.^{17,18} Our preliminary study excluded the presence of increased serum levels of SCF in patients with CIU. Further investigations will be needed to look at the functional role of SCF in CIU, for instance, by the use of indolinone derivatives as a means to inhibit the activity of c-Kit from patients with CIU in vitro.²⁴

Table 1. SCF Levels in ASST-Positive Patients With CIU and in Healthy Controls Detected by Immunoassay

Patients	SCF, pg/mL	
	Range	Mean ± 2 SDs
Healthy (n = 40)	260-868	636 ± 280
CIU patients who tested ASST positive (n = 65)		
HRA positive (n = 33)	368-814	604 ± 256
HRA negative (n = 32)	158-936	570 ± 332
Total	158-936	588 ± 296

Abbreviations: ASST, autologous serum skin test; CIU, chronic idiopathic urticaria; HRA, histamine release assay; SCF, stem cell factor.

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