

Detection of Low-Molecular-Weight Mast Cell–Activating Factors in Serum From Patients With Chronic Spontaneous Urticaria

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■ Abstract

Background: Functionally active autoantibodies to IgE and to the high-affinity IgE receptor (FcεRI) can be detected in serum in about 40% of patients with chronic spontaneous urticaria (CSU). Recent studies showed that serum from patients with CSU can induce activation of mast cells, irrespective of whether they carry high-affinity IgE receptors.

Objective: To evaluate mast cell activation induced by factors in the serum of CSU patients with a molecular weight lower than that of autoantibodies.

Methods: Eight CSU patients and 5 healthy controls were evaluated. Whole serum and serum fractionated at 100, 50, and 30 kDa were used to stimulate in vitro LAD2 mast cells. The enzymatic activity of β-hexosaminidase was evaluated in supernatants and cell pellets as a measure of mast cell degranulation.

Results: Mean (SEM) release of mast cell β-hexosaminidase induced by whole serum from CSU patients was higher than that induced by serum from the healthy controls (14.4 [2.7%] vs 5.1 [2.4%]; $P=.027$). In addition, serum fractions below 100 kDa and below 50 kDa from CSU patients induced mast cell degranulation that was significantly higher than that induced by the corresponding fractions in sera from healthy controls (10.2% [1.4%] vs 3.8% [1.9%] [$P=.024$] and 10.1% [1.2%] vs 3.9% [1.7%] [$P=.012$], respectively). In 4 CSU patients, we evaluated serum fractions <30 kDa, which retained their capacity to activate mast cells (11.0% [0.7%]).

Conclusions: This study shows that sera from CSU patients may contain low-molecular-weight mast cell–activating factors other than autoantibodies. These factors could be an additional mechanism contributing to the pathogenesis of CSU.

Key words: Chronic urticaria. Pathogenesis. Histamine-releasing factors. Mast cells.

■ Resumen

Introducción: Los autoanticuerpos IgE funcionalmente activos y los receptores de alta afinidad para la IgE (FcεRI) pueden ser detectados en el suero de aproximadamente un 40% de los pacientes con urticaria crónica espontánea (UCE). Estudios recientes muestran que el suero de estos pacientes puede inducir activación de mastocitos con o sin receptores de alta afinidad para la IgE.

Objetivo: El objetivo de este estudio fue evaluar la actividad de los factores séricos de los sueros de pacientes con UCE con un peso molecular inferior al de los autoanticuerpos.

Métodos: Para ello se estudiaron 8 pacientes con UCE y 5 controles sanos. El suero completo de cada uno de ellos y el fraccionado a 100, 50 y 30 kDa se utilizó para estimular in vitro mastocitos LAD2. La actividad enzimática de la β-hexosaminidasa se determinó en los sobrenadantes y en el botón celular con el fin de cuantificar la degranulación mastocitaria.

Resultados: En cuanto a los resultados obtenidos se observó una liberación de β -hexosaminidasa mastocitaria inducida por los sueros completos de los pacientes con UCE ($14,4 \pm 2,7\%$, media \pm EE de la media) significativamente mayor que la inducida por sueros de controles sanos ($5,1 \pm 2,4\%$; $p=0,027$). Así mismo, las fracciones séricas inferiores a 100 kDa e inferiores a 50 kDa de los pacientes con UCE indujeron degranulación mastocitaria significativamente superior a la inducida por las fracciones correspondientes de sueros controles ($10,2 \pm 1,4\%$ vs $3,8 \pm 1,9\%$ [$p=0,024$] and $10,1 \pm 1,2\%$ vs $3,9 \pm 1,7\%$ [$p=0,012$], respectivamente). En 4 pacientes con UCE observamos que las fracciones inferiores a 30 kDa mantenían la capacidad de activar a los mastocitos ($11,0 \pm 0,7\%$).

Conclusiones: En conclusión, este estudio muestra que el suero de los pacientes con UCE puede contener factores de bajo peso molecular diferentes a los autoanticuerpos que son capaces de activar a los mastocitos. Este hallazgo podría contribuir a conocer los mecanismos de la patogénesis de la UCE.

Palabras clave: Urticaria crónica. Patogénesis. Factores liberadores de histamina. Mastocitos.

Introduction

Chronic spontaneous urticaria (CSU) is characterized by recurrent eruption of itchy wheals with or without angioedema for more than 6 weeks. More than 20 years ago, functionally active autoantibodies to IgE and to the high-affinity IgE receptor (Fc ϵ RI) were first detected in the sera of CSU patients [1-3], and this appeared to be the most reasonable explanation for the pathogenesis of CSU. However, functional autoantibodies can be detected in approximately 40% of cases of CSU [2,3], and some sera from CSU patients lacking autoantibodies to Fc ϵ RI are able to induce histamine release from basophils cultured in vitro [4]. Furthermore, in recent years, Eckman et al [5] demonstrated that there is no strict association between Fc ϵ RI autoantibodies and the histamine-releasing activity of serum from CSU patients. About 50% of CSU patients, a proportion that includes all those with functionally active circulating autoantibodies to Fc ϵ RI and IgE, are "autoreactive," that is, intradermal injection of autologous serum (autologous serum skin test [ASST]) elicits a wheal-and-flare reaction [6]. However, the result of the ASST does not always correlate with the result of the in vitro assay with histamine-releasing autoantibodies. Fagiolo et al [7] found that serum from CSU patients retains the ability to induce a wheal-and-flare reaction upon intradermal injection of autologous serum even after depletion of IgG. Although isolated IgG anti-IgE receptor was shown to be responsible for the induction of histamine release from basophils, and the residual IgG-depleted serum was shown to be negative [8], overall experimental findings suggest the possible involvement of factors other than autoantibodies, both in the autoreactive state detected by ASST and in the pathogenesis of CSU. Recently, we found that serum from CSU patients induced degranulation of mast cells lacking the high-affinity IgE receptor, thus showing that degranulating factors may also act through different pathways [9]. In the present study, we tried to further characterize mast cell-activating factors by fractionation of sera from CSU patients. The rationale of our approach is that serum fractionation makes it possible to detect the molecular weight range of mast cell-activating factors and to investigate whether or not these factors are autoantibodies.

Methods

We studied sera from 8 patients with CSU (5 women and 3 men; age range, 23-55 years) diagnosed according to generally accepted criteria [10] and from 5 healthy controls (3 women and 2 men; age range, 25-50 years). All patients underwent the ASST as previously described [6]. All patients and controls gave their informed consent to blood collection for research purposes, and the study was conducted in accordance with the Declaration of Helsinki. Whole sera were filtered at 4°C through membranes with a cutoff of 100 and 50 kDa (Amicon Inc). Samples of whole sera as well as of fractions <100 kDa and <50 kDa were frozen until use. In subsequent experiments, sera from 4 of the CSU patients (2 ASST-positive, 2 ASST-negative) were also filtered with a cutoff of 30 kDa. Whole serum and serum fractions were used for in vitro stimulation of mast cells from the Laboratory of Allergic Diseases 2 cell line (LAD2, kindly provided by Dr A Kirshenbaum, NIH, Bethesda, Maryland, USA), which closely

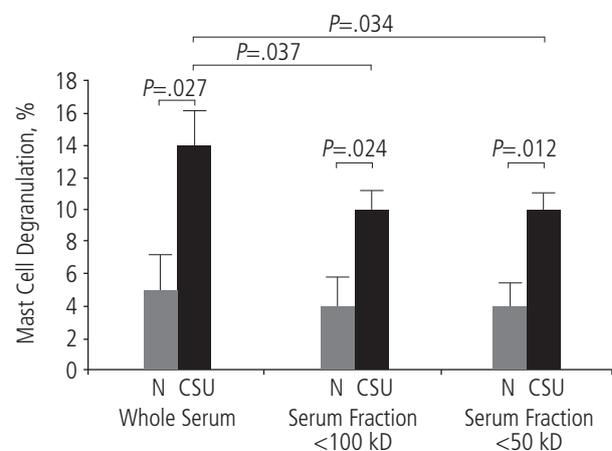


Figure 1. Mast cell degranulation induced by serum and serum fractions (<100 kDa and <50 kDa) from 8 patients with chronic spontaneous urticaria (CSU) and 5 normal controls (N). Results are expressed as mean (bars) and standard error of the mean (whiskers) of the percentage of β -hexosaminidase release over total β -hexosaminidase content. The statistical analysis was based on an analysis of variance and t test for unpaired data.

resemble CD34⁺-derived human mast cells. LAD2 cells were suspended at $2 \times 10^6/\text{mL}$ in Tyrode/BSA 0.05% solution and incubated with whole serum and serum fractions from patients or with serum from healthy donors (1:100) for 30 minutes. The enzymatic activity of β -hexosaminidase in supernatants and in the cell pellets after solubilization was evaluated using a chromogenic method described elsewhere [9]. The result was expressed as the proportion of released β -hexosaminidase over total β -hexosaminidase and indicated the percentage of mast cell degranulation. A positive control was obtained by cell stimulation with $1 \mu\text{M}$ ionomycin for 30 minutes. The t test for unpaired values was used to assess the statistical significance of the differences between groups. Data were analyzed using SPSS Statistics for Windows, version 22.00 (IBM Corp). Statistical significance was set at $P < .05$.

Results

The results of the degranulation experiments are shown in Figure 1. Mean (SEM) degranulation induced by whole serum from the 8 CSU patients was significantly higher than that induced by the 5 control sera (14.4% [2.7%] vs 5.1% [2.4%]; $P = .027$). In addition, serum fractions $<100 \text{ kDa}$ and $<50 \text{ kDa}$ from CSU patients induced mast cell degranulation that was significantly higher than that induced by the corresponding fractions from control sera (10.2% [1.4%] vs 3.8% [1.9%] [$P = .024$] and 10.1% [1.2%] vs 3.9 [1.7%] [$P = .012$], respectively). The average β -hexosaminidase-releasing activity of CSU serum fractions was lower than that of whole serum (Figure 1). Owing to limited sample supply, we were only able to test mast cell degranulation of serum samples undergoing 30-kDa fractionation in a subgroup of 4 CSU patients. As shown in Figure 2, these serum fractions were constantly able to induce LAD2 mast cell degranulation (11% [0.7%]) compared with those of the negative and positive controls, ie, phosphate-buffered saline (4.0% [0.6%])

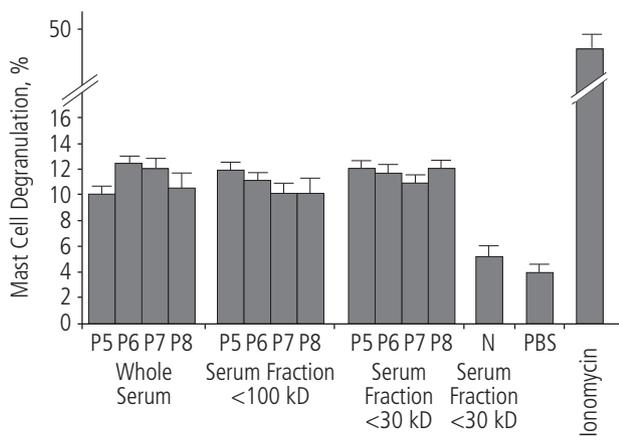


Figure 2. Mast cell degranulation induced by serum fractions $<30 \text{ kDa}$ from 4 patients with chronic spontaneous urticaria (patients [P] 5, 6, 7, and 8) and from 5 normal controls (N), phosphate-buffered saline (PBS) and ionomycin. Results are expressed as mean (bars) and standard error of the mean (whiskers) for patients (3 replicates), PBS (5 replicates), ionomycin (5 replicates), and 5 healthy controls.

and ionomycin (47.3% [2.7%]). No significant differences were observed between ASST-positive patients ($n=4$) and ASST-negative patients ($n=4$). The Table shows the results of the ASST and β -hexosaminidase release from LAD2 cells stimulated with whole serum and serum fractions with a molecular weight $>100 \text{ kDa}$ and $<50 \text{ kDa}$ from 8 patients with CSU and 5 healthy controls.

Discussion

Some years ago we demonstrated that serum from patients with CSU can degranulate mast cells through a mechanism that is independent of $\text{Fc}\epsilon\text{RI}$ and, hence, of both IgE and IgG [9]. This finding was in keeping with the observation that most patients with CSU lack autoantibodies to the mast cell $\text{Fc}\epsilon\text{RI}$ and to IgE. In the present study, we aimed to confirm this finding and tried to better characterize the factor(s) involved in mast cell degranulation. On average, whole serum was able to induce more intense degranulation than serum fractions, probably owing to the presence of autoantibodies to the high-affinity IgE receptor or to IgE in the $>100\text{-kDa}$ serum fraction of some patients (1-4, 6). However, interestingly, all the sera that we examined also contained low-molecular-weight circulating mast cell-activating factor(s). In some cases, such as the 4 sera for whom the $<30\text{-kDa}$ fraction was available, β -hexosaminidase release from LAD2 cells was induced to the same extent by low-molecular-weight fractions as by whole serum, thus suggesting the presence of low-molecular-weight mast cell-activating factors. These observations might explain the occurrence of histamine release, and hence of CSU, in patients whose serum lacks functional autoantibodies specific for the high-affinity IgE receptor or for IgE. Moreover, these findings are in keeping with those of Eckman et al [5], who found that the presence of autoantibodies was not correlated with histamine release from cultured cells. Another interesting finding is that circulating

Table. Results of Autologous Serum Skin Test (ASST) and Mast Cell Degranulation Induced by Sera and Serum fractions (Mw, $>100 \text{ kDa}$ and $<50 \text{ kDa}$) From 8 Patients With Chronic Spontaneous Urticaria and 5 Healthy Controls^a

| Patient | ASST, mm | Serum | $>100 \text{ kDa}$ | $<50 \text{ kDa}$ |
|-----------------------|----------|-----------|--------------------|-------------------|
| 1 | Negative | 5.53 | 2.92 | 5.00 |
| 2 | 8 mm | 11.86 | 8.30 | 6.00 |
| 3 | 8 mm | 24.51 | 9.70 | 13.00 |
| 4 | Negative | 28.07 | 8.20 | 15.00 |
| 5 | Negative | 10.09 | 3.90 | 11.00 |
| 6 | 4 mm | 12.54 | 5.30 | 9.00 |
| 7 | 4 mm | 12.20 | 6.20 | 11.00 |
| 8 | Negative | 10.30 | 4.60 | 11.00 |
| Normal controls (n=5) | Negative | 5.1 (2.4) | 1.1 (0.1) | 3.9 (1.7) |

^aExpressed as percentage of β -hexosaminidase release over total β -hexosaminidase content.

low-molecular-weight β -hexosaminidase-releasing factor(s) is present in sera from both ASST-positive and ASST-negative CSU patients. In this sense, our findings differ from those of a very old study in which Grattan et al [11] were able to detect a low-molecular-weight serum factor causing a wheal-and-flare skin reaction upon intradermal injection. In the light of our findings and those of other studies [5], factors unrelated to autoantibodies to the high-affinity IgE receptor or to IgE and apparently unrelated to "autoreactivity" (ie, positive ASST result) also seem to be involved in the pathogenesis of CSU. The nature of the low-molecular-weight factor(s) inducing mast cell degranulation that we detected remains unclear, although we intend to characterize these factors in the near future. We cannot exclude a possible relationship with C5a or other cell-activating chemokines. However, the possibility that this factor corresponds to C5a in all cases seems questionable, as the complement fraction is formed following complement activation by anti-Fc ϵ RI autoantibodies (12), whose presence is generally associated with a clearly positive ASST result (6). We also detected mast cell-activating fractions in ASST-negative patients.

It could prove interesting to evaluate the effect of anti-C5a on mast cell degranulation induced by sera from ASST-positive CSU patients. In conclusion, circulating mast cell-activating factor(s) with a molecular weight <30 kD can be detected in patients with CSU. The presence of these factors is independent of autoantibodies and autoreactivity and might contribute to the pathogenesis of CSU.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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