

DETECTION OF LOW MOLECULAR WEIGHT MAST CELL ACTIVATING FACTORS IN SERA FROM PATIENTS WITH CHRONIC SPONTANEOUS URTICARIA

Short Title: Low m.w. mast cell activating factors in CSU

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ABSTRACT

BACKGROUND: Functionally active autoantibodies to IgE and to the high affinity IgE receptor (FcεRI) can be detected in sera from about 40% of patients with chronic spontaneous urticaria (CSU). Recent studies showed that CSU sera can induce the activation of mast cells bearing or not the high affinity IgE receptors.

OBJECTIVE: This study aimed at evaluating mast cell activation induced by CSU serum factors with a molecular weight lower than that of autoantibodies.

METHODS: Eight CSU patients and 5 normal controls were evaluated. Whole sera and sera 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: Mast cell β-hexosaminidase release induced by whole sera from CSU patients ($14.4 \pm 2.7\%$, mean \pm SEM) was higher than that induced by sera from normal controls ($5.1 \pm 2.4\%$; $p=0.027$). Also serum fractions below 100 kDa and below 50 kDa from CSU patients induced a mast cell degranulation that was significantly higher than that induced by the corresponding fractions from normal control sera ($10.2 \pm 1.4\%$ vs $3.8 \pm 1.9\%$ [$p=0.024$] and $10.1 \pm 1.2\%$ vs $3.9 \pm 1.7\%$ [$p=0.012$], respectively). In 4 CSU patients we evaluated serum fractions below 30 kDa which retained the same capacity to activate mast cells ($11.0 \pm 0.7\%$).

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

Key Words: Chronic urticaria; pathogenesis; histamine releasing factors; mast cells.

Resumen:

Los autoanticuerpos IgE funcionalmente activos y los receptores de alta afinidad para la IgE (FceRI) 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.

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.

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.

En cuanto a los resultados obtenidos se observó una liberación de β -hexosaminidasa mastocitaria inducida por los sueros completos de los pacientes con UCE ($14.4 \pm 2.7\%$, mean \pm SEM) 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 ($11.0 \pm 0.7\%$).

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 the 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 (FcεRI) were detected in sera from CSU patients [1-3]. This appeared as the most reasonable explanation of the pathogenesis of CSU. However, functional autoantibodies can be detected in no more than 40% of cases of CSU [2,3] and some sera from CSU patients lacking FcεRI autoantibodies are able to induce histamine release from cultured basophils in-vitro [4]. Furthermore, in recent years, Eckman et al. were able to demonstrate that there is not a strict association between FcεRI autoantibodies and histamine-releasing activity of CSU sera [5]. About 50% of CSU patients, a proportion that includes all those with functionally active circulating autoantibodies to FcεRI and IgE, are characterized by an “autoreactive” state; in these patients, the intradermal injection of autologous serum (ASST, autologous serum skin test) elicits a wheal-and-flare skin reaction [6]. However, ASST does not always correlate with the in-vitro assay of histamine-releasing autoantibodies. Fagiolo et al. found that sera from CSU patients retain the ability to induce a wheal-and-flare reaction upon intradermal injection of autologous serum even after depletion of IgG [7]. Although in another study isolated IgG anti-IgE receptor was responsible for the induction of histamine release from basophils, and the residual IgG-depleted serum was 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 sera from CSU patients are able to induce the degranulation of mast cells lacking the high affinity IgE receptors, thus showing that the degranulating factors may act also through different pathways [9]. In the present study, we tried to further characterize such mast cell activating factors by fractioning the sera from CSU patients. The rationale of our approach is that serum fractioning allows to detect the molecular weight range of mast cell activating factors, and to investigate the nature of such factors, i.e. autoantibodies or not.

METHODS

Sera from 8 patients with CSU (5 women and 3 men, age range 23-55 years) diagnosed by generally accepted criteria [10] and from 5 normal controls (3 women and 2 men, age range 25-50 years) were studied. All patients underwent the autologous serum skin test as previously described (6). All patients and controls gave an 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 cut-off of 100 and 50 kDa (Amicon Inc., Beverly, MA, USA), respectively. Samples of whole sera as well as of the fractions < 100 kDa and < 50 kDa were frozen until use. In subsequent experiments, filtration with a cut-off of 30 kDa was performed on sera from four of the CSU patients, two positive and two negative on autologous serum skin test, respectively, as well. Whole sera and serum fractions were used to stimulate in vitro mast cells of the cell line LAD2 (Laboratory of Allergic Diseases 2 - kindly provided by Dr. A. Kirshenbaum, NIH, Bethesda, MD, USA). These cells closely resemble CD34+-derived human MCs. LAD2 cells were suspended at 2×10^6 /ml in Tyrode's/BSA 0.05% and incubated with patients' whole sera and serum fractions or with healthy donor sera (1: 100) for 30 min. The enzymatic activities of β -hexosaminidase in supernatants and in the cell pellets, after solubilization were evaluated with a chromogenic method as previously described [9]. The results were expressed as percentage of released β -hexosaminidase over total β -hexosaminidase content and referred as percentage of mast cell degranulation. Positive control was obtained by cell stimulation with 1 μ M ionomycin for 30 min. Student's t test for unpaired values was used to assess the statistical significance of the differences between groups. The data were analyzed using the SPSS statistical package, version 22.00 (SPSS INC., Chicago, IL, US), and a p value of <0.05 was considered statistically significant.

RESULTS

The results of degranulation experiments are shown in Figure 1. The degranulation induced by the whole sera from the 8 CSU patients ($14.4 \pm 2.7\%$, mean \pm SEM) was significantly higher than that induced by the 5 control sera ($5.1 \pm 2.4\%$; $p=0.027$). In addition, also serum fractions below 100 kDa and below 50 kDa from CSU patients induced a mast cell degranulation that was significantly higher than that induced by the corresponding fractions from normal control sera ($10.2 \pm 1.4\%$ vs $3.8 \pm 1.9\%$ [$p=0.024$] and $10.1 \pm 1.2\%$ vs $3.9 \pm 1.7\%$ [$p=0.012$], respectively). The average β -hexosaminidase releasing activity of CSU serum fractions was lower than that of whole sera (Figure 1). Due to limited sample supply, we could test mast cell degranulation of sera submitted to 30 kDa fractioning only in a subgroup of 4 CSU patients. As shown in figure 2, these serum fractions showed a consistent capacity to induce LAD2 mast cell degranulation ($11 \pm 0.7\%$) compared to negative and positive controls, i.e. phosphate buffered saline (PBS) ($4.0 \pm 0.6\%$) and Ionomycine ($47.3 \pm 2.7\%$). No significant difference was observed between patients positive ($n=4$) or negative ($n=4$) on autologous serum skin test. In table 1, we reported the results of autologous serum skin test and β -hexosaminidase release from LAD2 cells stimulated with whole sera and serum fractions with $M_w > 100$ kDa and < 50 kDa from 8 patients with chronic spontaneous urticaria and 5 normal controls.

DISCUSSION

Some years ago [9] we demonstrated that sera from patients with CSU are able to degranulate mast cells through a mechanism that is independent of FcεRI receptor and, hence, of both IgE and IgG. This finding was in keeping with the observation that most patients with CSU lack autoantibodies to the mast cell high affinity IgE receptor or to IgE. In the present study we aimed at confirming this finding and tried to better characterize the factor(s) involved in mast cells degranulation. On average, whole sera were able to induce a more intense degranulation than serum fractions; this is possibly due to the presence of autoantibodies to the high affinity IgE receptor or to IgE in the serum fraction > 100 kDa of some patients (1-4, 6). However, interestingly, all the sera that we examined contained also low molecular weight circulating mast cell activating factor(s). In some cases, such as the 4 sera for whom the fraction < 30 kDa was available, the 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 those patients whose sera lack functional autoantibodies specific for the high affinity IgE receptor or for IgE. Moreover, these findings are in keeping with those by Eckman and co-workers that the presence of autoantibodies is not correlated with histamine release from cultured cells [5]. Another interesting finding is that the circulating low molecular weight β-hexosaminidase releasing factor(s) is/are present in sera from CSU patients either positive or negative on the autologous serum skin test. In this sense, our findings differ from those of a very old study (11) in which Grattan and co-workers 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 as well as of those of other studies (5) factors unrelated to autoantibodies to the high affinity IgE receptor or to IgE, and apparently unrelated to “autoreactivity” (i.e., positive autologous serum skin test) 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 have detected is still unclear and it is our objective to characterize them in the next future. We cannot exclude its possible relationship to C5a, or to other cell-activating chemokines. However, the possibility that this factor corresponds to C5a in all cases seems questionable, as such complement fraction is formed following complement activation by anti FcεRI autoantibodies (12) whose presence is generally associated with a frankly positive ASST (6). In our hands, mast-cell activating fractions were detected also in patients negative on ASST. However, it could be interesting to evaluate the effect of anti C5a on mast cell degranulation induced by sera from ASST-positive CSU patients. This could be the matter of future investigation.

In conclusion, circulating mast cell activating factor(s) showing a molecular weight < 30 kD can be detected in patients with CSU; their presence is independent of autoantibodies and autoreactivity, and might contribute to the pathogenesis of CSU.

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Table 1. Results of autologous serum skin test (ASST - expressed as mm of skin reaction) and mast cell degranulation (expressed as percentage of β -hexosaminidase release over total β -hexosaminidase content) induced by sera and serum fractions ($M_w > 100$ kDa and $M_w < 50$ kDa) from 8 patients with chronic spontaneous urticaria and 5 normal controls.

Patient	ASST	Serum	>100 kDa	<50 kDa
1	Neg	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	Neg	28.07	8.20	15.00
5	Neg	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	Neg	10.30	4.60	11.00
Normal controls (n=5)	Neg	5.1±2.4	1.1±0.1	3.9±1.7

FIGURE LEGENDS

Figure 1. Mast cell degranulation induced by serum and serum fractions (<100 kD and <50 kD) 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. Statistical analysis was made by analysis of variance and Student's t test for unpaired data.

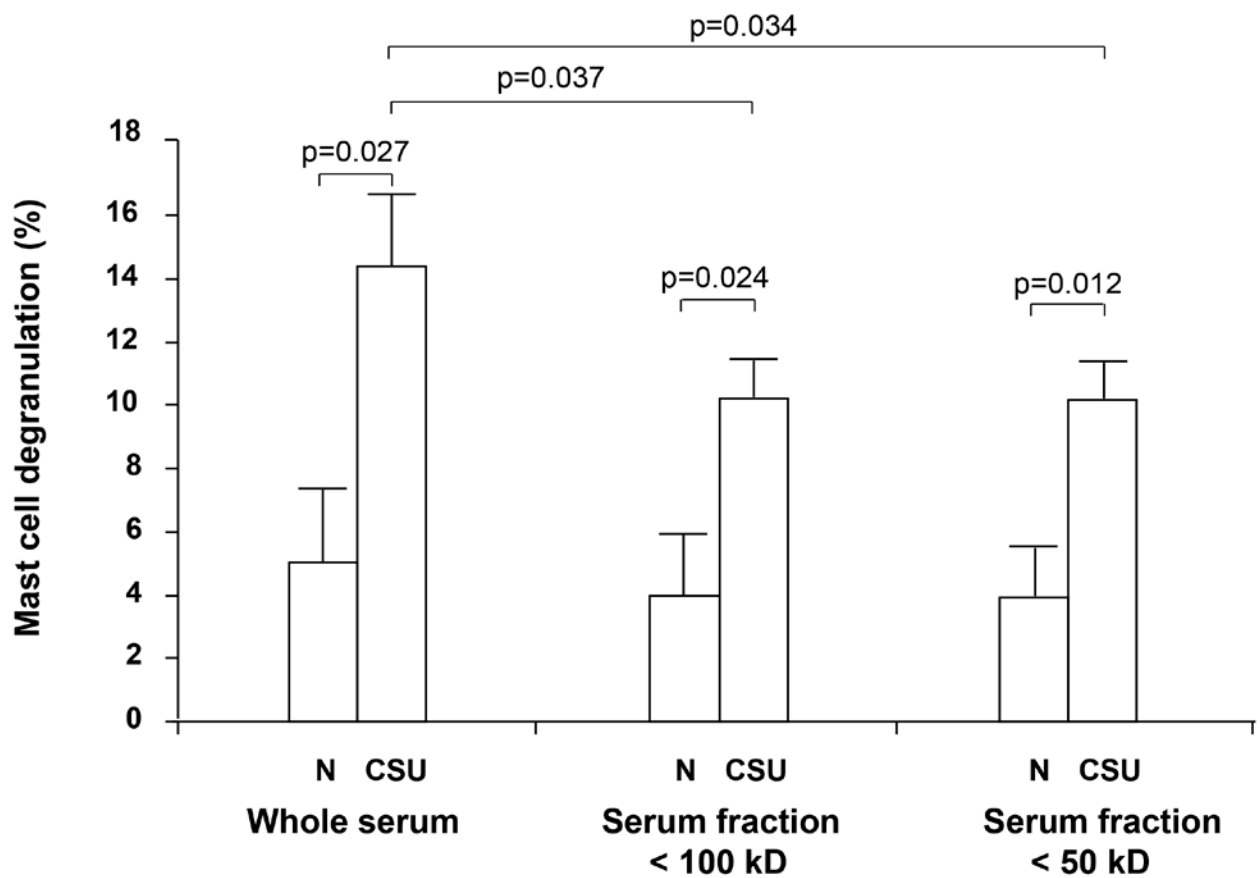


Figure 2. Mast cell degranulation induced by the serum fractions <30 kD from 4 patients with chronic spontaneous urticaria (patient 5, 6, 7 and 8) and from 5 normal controls, phosphate buffered saline (PBS) and Ionomycin. Results are expressed as mean (bars) and standard error of the mean (whiskers) for patients (3 replicates), PBS (5 replicates), Ionomycin (5 replicates) and 5 normal controls.

