

Chronic urticaria sera increase basophil CD203c expression

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Background: Approximately 40% of patients with chronic idiopathic urticaria have antibodies to the α subunit of the high-affinity IgE receptor. CD203c is a basophil activation marker known to be upregulated by cross-linking of the Fc ϵ RI α receptor and may serve as a useful marker to identify these patients.

Objective: The primary objective was to assess the affect of sera from patients with chronic idiopathic urticaria on basophil CD203c expression. Secondary objectives were to correlate CD203c expression with basophil histamine release and size of the autologous serum skin test and to determine whether the mechanism is mediated by an IgG antibody.

Methods: Sera were obtained from patients with chronic idiopathic urticaria and positive autologous serum skin test or negative autologous serum skin test and normal controls. Sera were incubated with donor whole blood. Activated basophils from whole blood were identified by flow cytometry on the basis of the presence of CD203c on high-expressing IgE positive cells.

Results: Incubation of donor basophils with sera from patients with chronic idiopathic urticaria and positive autologous serum skin test demonstrated significant upregulation of CD203c. IgG depletion of representative sera from patients with chronic idiopathic urticaria resulted in significant decrease in CD203c expression on donor basophils. CD203c expression correlated with basophil histamine release and the size of the autologous serum skin test.

Conclusion: Sera from patients with chronic idiopathic urticaria and positive autologous serum skin test significantly upregulate basophil CD203c and correlate with basophil histamine release.

Clinical implications: This article describes an activation marker on basophils whose expression is increased by sera from patients with chronic idiopathic urticaria. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Chronic idiopathic urticaria, autoimmunity, auto-antibodies, CD203c, basophils, autologous serum skin test, flow cytometry, basophil histamine release

Chronic idiopathic urticaria (CU) is defined as recurrent hives occurring for at least 6 weeks. In the majority of cases, there is no identifiable trigger despite extensive evaluation for an underlying etiology. A subset of these patients is classified as having autoimmune urticaria defined by the presence of a functional IgG antibody to the α subunit of the high-affinity IgE receptor (Fc ϵ RI α) or to IgE.¹ These antibodies trigger mast cell and basophil degranulation by the engagement of this receptor. Functional IgG antibody to the receptor has been identified in approximately 30% to 40% of patients with CU, and anti-IgE antibody has been identified in another 5% to 10% of patients. Nonfunctional antibodies to Fc ϵ RI may be found in other autoimmune conditions.²

Techniques to detect the autoantibody to Fc ϵ RI α include Western blot and ELISA, which are technically time-consuming and fail to identify antibodies with histamine releasing properties. Detection methods for functional antibodies include the autologous serum skin test (ASST) and basophil histamine release (BHR). The ASST involves an intradermal injection of the patient's serum into the skin with observation for a wheal and flare reaction. ASST is approximately 70% sensitive and 80% specific compared with the BHR assay.³

Recently, flow cytometry has been used to identify activated basophils in both allergic disease and CU. Two studies have been published demonstrating that sera of patients with CU and positive ASST induce higher expression of CD63 compared with skin test-negative chronic urticaria sera.^{4,5} CD63, a member of the transmembrane-4 superfamily, is a basophil and mast cell activation marker expressed as a result of the fusion between intracytoplasmic granules and the plasma membrane. It has been proposed that it rapidly appears on the basophil surface on the addition of anti-IgE, allergen, or IL-3.^{6,7} However, CD63 is not specific to basophils and mast cells but can be expressed on other cells present in the peripheral blood, such as monocytes and platelets.⁸

CD203c (ectonucleotide pyrophosphatase/phosphodiesterase) is an ectoenzyme expressed only on basophils, mast cells, and their CD34⁺ progenitor cells in the peripheral blood.⁸ It has been described that CD203c may be

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Abbreviations used

ANA: Antinuclear antibody
 ASST: Autologous serum skin test
 BHR: Basophil histamine release
 CU: Chronic idiopathic urticaria
 FITC: Fluorescein isothiocyanate
 fMLP: f-Met-Leu-Phe
 PerCP: Peridinin-chlorophyll-protein complex

a better basophil activation marker because of its specificity and potentially increased sensitivity in detecting IgE-mediated hypersensitivity.⁹ CD203c is expressed on both resting and activated basophils and is upregulated in response to cross-linking of the FcεRIα receptor.¹⁰ CD203c has been used to evaluate IgE-mediated hypersensitivity to latex and aeroallergens.^{9,11} Upregulation of CD203c on peripheral blood basophils from patients with CU has also been described.¹²

In the current study, we sought to compare the effect of sera from ASST⁺ and ASST[−] patients with CU on basophil CD203c expression to normal control sera and to determine the correlation with BHR. In addition, IgG depletion of sera from ASST⁺ individuals was performed to determine whether the mechanism of CD203c upregulation is IgG-mediated.

METHODS

Patients and controls

Chronic idiopathic urticaria was defined as recurrent wheals occurring at least 3 times per week for more than 6 weeks without an identifiable cause. Patients were excluded if they had evidence of a known trigger or cause including physical urticaria, urticarial vasculitis, or allergic cause of their hives. Sera were obtained from 32 patients with CU at the time of ASST and stored at −80°C. Antihistamines were held for at least 48 hours before collection of sera. Eleven sera from healthy adult individuals were used as normal controls. Four sera with a high titer antinuclear antibody (ANA) ≥ 1:360, 3 with a history of active connective tissue disease, and the other with eosinophilic gastroenteritis were used for disease controls. The study was approved by the Institutional Review Board at the National Jewish Medical and Research Center.

ASST

The test was performed by injecting 0.05 cc of the patient's own serum intradermally into the volar aspect of the forearm. Sterile saline and histamine were used as negative and positive controls, respectively. Wheal and flare reactions were measured at 30 minutes. A mean wheal diameter of 2 mm greater than or equal to the control was considered to be a positive ASST.

Basophil donor

Whole blood from 7 normal individuals was screened, and 1 donor was identified on the basis of the marked upregulation of CD203c on basophils (CD203c/IgE positive cells) in response to f-Met-Leu-Phe (fMLP) and anti-FcεRIα antibody. The basophil donor was atopic and had a serum IgE level of 154 kU/L. The basophil donor provided informed consent and was bled ≤ 1 time per week.

IgG depletion

Selected patients' and normal control patients' sera were depleted of IgG using a protein G-Sepharose column (Sigma, St Louis, Mo). Pre and post IgG depletion levels were measured by nephelometry (Image Immunochemistry System; Beckman Coulter, Fullerton, Calif). All sera had postdepletion levels of <33.3 mg/dL (the lowest level of detection by nephelometry). Dilution of sera passing through the column was accounted for by measuring serum albumin levels predepletion and postdepletion.

Antibodies

The following mAbs were used: phycoerythrin (PE)-conjugated antihuman CD203c (Beckman Coulter), peridinin-chlorophyll-protein complex (PerCP)-conjugated antihuman CD45 (BD Bioscience, San Jose, Calif), and fluorescein isothiocyanate (FITC)-conjugated antihuman IgE (Caltag, Burlingame, Calif).

Measurement of CD203c surface expression

The test was performed on heparinized blood within 3 to 4 hours after drawing the donor's blood. Aliquots of the donor's heparinized whole blood (200 μL) were incubated for 10 minutes at 37°C with 40 μL sera from patients with CU or normal controls, or IgG-depleted sera. For controls, 40 μL basophil stimulation buffer (20 mM HEPES, 125 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, 0.5 mM glucose, and 0.1% BSA, pH 7.4) was added to 200 μL of donor's blood and used as the negative control, and 40 μL 2-μM fMLP (Sigma) and 40 μL of a 1:50 dilution of anti-FcεRIα receptor antibody (Upstate, Charlottesville, Va) in Ca⁺⁺ and Mg⁺⁺ free PBS were used as positive controls. The reactions were stopped by placing the tubes on ice. Cells were then stained with PE-antihuman CD45, PerCP-antihuman CD203c, and FITC-antihuman IgE at 4°C in the dark for 30 minutes. Red cells were lysed with FACS Lysing Solution (Becton Dickinson, San Jose, Calif). The cells were then washed once with 2 mL PBS and fixed in 1% formaldehyde. The cells were then analyzed on a FACSCalibur flow cytometer (Becton Dickinson).

Flow cytometry

Basophils from whole blood were identified by the presence of CD203c on high-expressing IgE⁺ cells (Fig 1, A and B). The data are expressed as the mean fluorescence intensity or the percentage of CD203c expression. Percent CD203c expression is defined as the percent of basophils expressing more CD203c than ≥99% of basophils incubated with buffer only. This was determined by defining an M1 region on the histogram analysis (Fig 1, C).

BHR

Whole blood from the same donor for the CD203c assay was collected in a heparinized tube, and BHR was performed according to the manufacturer's instructions (Beckman Coulter). For the BHR, whole blood was diluted 1:7 in the histamine release buffer. Stimuli (50 μL) including patient sera, anti-FcεRI (as a positive control), or histamine release buffer (as a negative control) were added to 100 μL blood and incubated at 37°C for 30 minutes. To determine total histamine, whole blood was diluted 1:20 in distilled water followed by 2 rapid freeze-thaw cycles. Histamine concentrations in the supernatants of centrifuged blood were performed by ELISA (Beckman Coulter) according to the manufacturer's instructions. Spontaneous histamine release was determined by the amount of histamine released by cells incubated with the histamine release buffer. Spontaneous release was <5% of total release. Positive histamine release was defined as >15% of total release.¹³

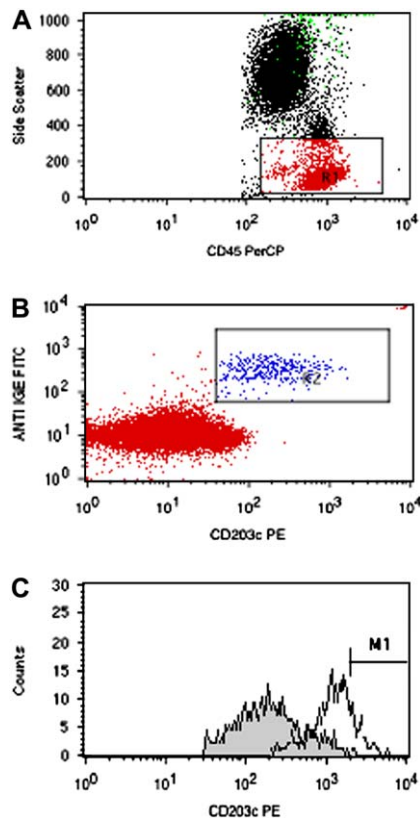


FIG 1. Basophil identification and expression of CD203c. **A**, Side scatter and CD45 staining. Gating region (*boxed area*) contained the basophils located between the monocytes and lymphocytes. **B**, Gated cells were selected by high IgE and high CD203c expressing cells. **C**, Histogram of CD203c expression after addition of buffer (*shaded area*) and after addition of serum from patient with CU and a positive ASST. M1 defines a region where $\leq 1\%$ of the basophils express CD203c after incubation with buffer. PE, Phycoerythrin.

ANA determination

Antinuclear antibodies were detected by indirect immunofluorescence with HEp-2 cells (Bio-Rad Laboratories, Redmond, Wash).

Statistics

Significant differences between groups were analyzed by using a 2-tailed unpaired *t* test. Correlation was determined by using Pearson product moment correlation. Sensitivity, specificity, and negative and positive predictive value were also calculated.

RESULTS

Basophil CD203c expression

CD203c expression was higher in basophils incubated with sera from patients with CU compared with normal control individuals (Fig 2). The mean percent basophil CD203c expression of 11 normal control individuals was $-0.6\% \pm 0.1\%$ ($\pm$ SEM) versus $20\% \pm 4\%$ in 32 patients with CU ($P = .002$). Sera from patients with high-titer ANAs (disease controls) did not induce basophil CD203c expression. The mean values of the positive

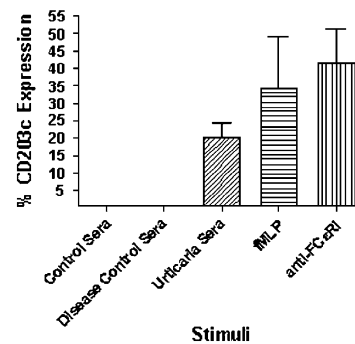


FIG 2. The mean percent ($\pm$ SEM) CD203c expression above baseline by sera from normal controls ($N = 11$), disease controls ($N = 4$), all patients with CU ($N = 32$), and positive controls, ie, fMLP and anti-Fc ϵ RI.

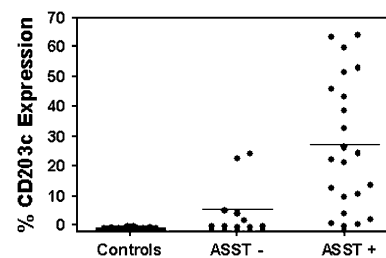


FIG 3. Percent CD203c expression by normal individuals, patients with CU and negative ASST, and patients with CU and positive ASST. Horizontal bars represent means.

controls fMLP and anti-Fc ϵ RI α were $34.5\% \pm 14.6\%$ and $41.9\% \pm 9.4\%$ ($n = 4$), respectively.

CD203c expression was significantly higher after incubation of basophils with sera from ASST⁺ patients with CU than normal control sera ($P < .001$; Fig 3). The mean percent CD203c expression was $30\% \pm 5$ (SEM; $n = 22$) in basophils incubated with ASST⁺ CU sera versus $-0.6\% \pm 0.1\%$ for normal control sera ($n = 11$). The range of percent CD203c expression in cells incubated with ASST⁺ serum was -0.1% to 63.3% . Seventeen of 22 ASST⁺ sera (77%) resulted in basophil activation of greater than 5%. The range of percent basophil activation in cells incubated with normal control serum was -1.2% to 0.0% compared with buffer control. CD203c expression was also significantly higher in basophils incubated with ASST⁺ sera than with ASST⁻ sera ($P = .006$). The mean percent CD203c expression was $30\% \pm 5\%$ versus $6\% \pm 3\%$, respectively. The range of CD203c expression for basophils incubated with ASST⁻ sera was 0.1% to 24% . Eight of 10 ASST⁻ sera resulted in CD203c expression of $\leq 5\%$, and the other 2 resulted in 22% and 24% expression. Incubation with ASST⁻ sera also resulted in significantly higher CD203c expression than control sera ($P = .04$).

BHR

Basophil histamine release was performed in 10 patients with negative ASSTs and in 18 patients with positive ASST. All sera were not analyzed because of

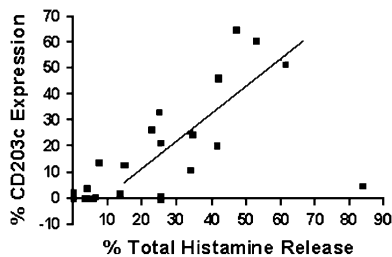


FIG 4. Correlation of CD203c expression with % total histamine release.

insufficient quantity. In patients with negative ASST, 1 of 10 (10%) had a positive BHR, and 4 of 18 (22%) patients with positive ASSTs had a negative BHR. The percent CD203c expression correlated with the percent histamine release ($R = 0.6$; $P = .001$; Fig 4). The sensitivity, specificity, positive predictive value, and negative predictive value were calculated by using the BHR assay as the gold standard to identify functional antibodies to the FcεRIα receptor and by using >5% CD203c expression as the cutoff value for a positive test. With these criteria, CD203c expression demonstrated a sensitivity of 77%, a specificity of 82%, a positive predictive value of 83%, and a negative predictive value of 75%.

Correlation between ASST and CD203c expression

The mean diameter of the serum autologous skin test was compared with percent CD203c expression. Results demonstrated a significant correlation between the size of the ASST and CD203c expression ($R = 0.5$; $P = .02$; Fig 5).

IgG depletion

To determine if the mechanism of CD203c expression was mediated by an IgG antibody, 3 selected sera that induced basophil CD203c expression were depleted of IgG using a protein G Sepharose column. The mean percent CD203c expression before IgG depletion was $30\% \pm 9\%$. The mean percent CD203c expression after IgG depletion was $5\% \pm 1\%$, suggesting that in these sera, CD203c expression may be triggered by an IgG antibody to FcεRIα. There was no change from pre and post IgG depletion with 2 control sera: $0.2\% \pm 0.4\%$ (pre) and $-0.1\% \pm 0.02\%$ (post; $P = .5$).

DISCUSSION

Results from this study demonstrate that the sera from patients with CU upregulate basophil CD203c expression. Sera from patients with positive ASST induced significantly higher CD203c expression compared with the sera from patients with negative ASST and from normal controls. Twenty percent of patients' sera with negative ASSTs induced $\geq 5\%$ basophil activation compared with buffer control. Seventy-seven percent of patients with positive ASSTs had $\geq 5\%$ basophil activation. It is an

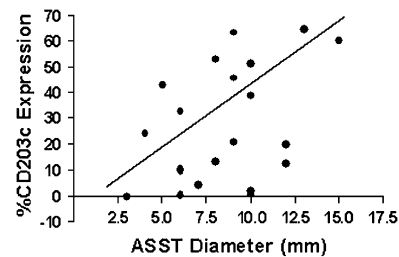


FIG 5. Correlation of percent CD203c expression and ASST mean wheal diameter.

interesting observation that the sera from ASST[−] patients induced significantly higher CD203c expression than normal controls, although still significantly lower than ASST⁺ patients. The 2 patients with negative autologous serum skin tests and sera inducing an elevation of CD203c of more than 5% may represent those patients with false-negative ASST. However, on reanalyzing the data without the 2 outlying sera, ASST[−] CU sera still induces CD203c expression, more so than controls. This finding suggests that there are other undefined serum factors present in CU sera that can upregulate CD203c.

CD203c is known to be upregulated by cross-linking of FcεRIα by transport of preformed CD203c molecules to the surface.¹⁴ The decline in CD203c expression after IgG depletion of representative sera is consistent with an IgG-mediated mechanism. This experiment did not remove IgE from donor basophils, so the results do not differentiate between an IgG antibody to the FcεRIα subunit or to IgE.

In comparison with our results, Wedi et al⁴ found increased CD63 expression in 70% of ASST⁺ patients with CU and 45% of ASST[−] patients with CU, and in 35% of controls. Gyimesi et al⁵ found elevated CD63 expression induced by 11 of 12 ASST⁺ sera (92%) and 3 of 18 (17%) ASST[−] sera. These results using CD63 are similar to our findings with CD203c, although Wedi et al⁴ had a higher percentage of normal controls inducing CD63 expression and Gyimesi et al⁵ had a higher percentage of ASST⁺ sera inducing CD63 expression.

CD203c belongs to the type II transmembrane protein family and is a multifunctional ectoenzyme called ectonucleotide pyrophosphatase/phosphodiesterase 3 that catalyzes the cleavage of several molecules, including deoxynucleotides and nucleotide sugars. It also contains a somatomedin B-like domain and a cell adhesive motif, but their potential functions are unknown. Among leukocytes, CD203c appears to be selectively expressed on the basophil/mastocyte lineage. Boumiza et al⁹ compared basophil activation tests using either CD63 or CD203c in the diagnosis of latex allergy and found that the sensitivity was considerably higher with CD203c (75%, compared with 50% with CD63). The authors speculate that the improved sensitivity may be a result of 2 factors. First, the recognition of basophils is superior with CD203c. Second, CD203c is expressed at a higher level in activated basophils compared with CD63. In sensitized patients, basophils increased their CD203c levels as much as 350%

above control values in response to allergens, whereas the increase in CD63 was below 100%. However, it should be pointed out that few comparative studies have been performed, and further studies are needed to establish the validity and superiority of these tests. The difference in response between CD203c and CD63 can be explained by their different mechanism of upregulation. For example, PGD₂ is a strong inducer of CD203c expression. However, it does not stimulate CD63 expression.¹⁵ The kinetics of upregulation of CD203c is fast and peaks after 5 to 15 minutes, whereas that of CD63 is slow and peaks after 20 to 40 minutes.¹⁶

It is also interesting to note that some recently identified surface markers follow the upregulation kinetics of either CD203c or CD63. Thus, the upregulation of CD13 and CD164 is fast and similar to that of CD203c. On the other hand, the surface increase of CD107a is slow and follows the kinetics of CD63.¹⁵

Our results indicate that donor basophil expression of CD203c is increased by incubation of donor basophils with sera from patients with chronic urticaria and is consistent with an IgG-mediated mechanism. Assessing basophil activation with CD203c may be a useful way to identify individuals with autoimmune urticaria.

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