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 FcεRIα 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 2006;117:1430-4.)

Key words: *Chronic idiopathic urticaria, autoimmunity, autoantibodies, 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 (FcεRIα) 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 FcεRI may be found in other autoimmune conditions.[2]

Techniques to detect the autoantibody to FcεRIα 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 a better basophil activation marker because of its

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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

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 (Immage 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 $CaCl_2$, 1 mM $MgCl_2$, 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]