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Autoimmune Mechanisms in Chronic Idiopathic Urticaria

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To the Editor:

Chronic idiopathic urticaria (CIU) is defined as repeated episodes of hives occurring over a period of 6 weeks without an identifiable cause¹. CIU is a common disorder with a prevalence of 0.5% in the general population². In the 1980s, the presence of a serum factor that induced a wheal and flare response following intradermal injection of autologous serum was reported³ and has since been regarded as a helpful surrogate for autoimmunity in CIU¹. IgG autoantibodies directed against the α-subunit of the high affinity IgE receptor (FcεRI) are thought to be the primary autoimmune mechanism of CIU and these are reportedly present in 30–60% of CIU patients^{4–7}.

We have developed an immortalized human mast cell line (LUVA) displaying FcεRI that both degranulates and releases arachidonic acid-metabolites in an FcεRI-dependent fashion⁸. These cells express high levels of chymase and tryptase (CT-positive) and therefore display characteristics of connective tissue mast cells. We investigated mast cell prostaglandin D₂ (PgD₂) production and Ca²⁺ influxes by LUVA cells in response to incubation with sera derived from CIU patients.

Patients with CIU and matched control subjects were recruited under a protocol approved by our human investigations committee⁹. Autologous serum skin tests (ASSTs) were performed using standard methodology¹. Serum (diluted 1:1) was incubated with LUVA cells (5×10⁴) at 37°C for 30 minutes and activation was determined via enzyme immunoassay (EIA) as PgD₂ secretion (sensitivity >55 pg/ml; Cayman Chemical Co., Ann Arbor, MI). To properly assess LUVA activation, we also measured PgD₂ concentrations in comparably diluted serum samples and subtracted this from the LUVA supernatant values. Additional PgD₂ assays were performed after incubating LUVA cells overnight with monoclonal IgE (100 ng/mL; Millipore, Temecula, CA) to upregulate FcεRI expression⁸. As an alternative measure of mast cell activation by CIU serum, we investigated induction of Ca²⁺ fluxes

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using the Fluo-4NW calcium assay kit (Molecular Devices, Sunnyvale, CA). Briefly, loading buffer with 5 mM probenecid (Sigma, St. Louis, MO) was added to each well and incubated at 37°C for 30 minutes. Cells were stimulated with CIU serum and cytoplasmic $[Ca^{+2}]_i$ was determined at an extinction wavelength of 494 nm and an emission wavelength of 516 nm using a Flexstation spectrophotometer (Molecular Devices). ATP (300 mM) and monoclonal anti-FcεRI antibodies (Upstate, Lake Placid, NY) were used as positive controls and data are expressed as arbitrary relative fluorescence units (RFU). To evaluate the role of FcεRI-dependent and -independent pathways in cellular activation^{10–14}, LUVA cells were incubated with the syk inhibitor, ER27319 (10–60 μM, Tocris, Ellisville, MO) or pertussis toxin (100ng, Tocris) for 2 hrs at 37° C. Unless otherwise specified, data are expressed as the mean±SD and the differences analyzed by paired t-test.

Fourteen patients with CIU were enrolled in the study. Characteristics of patients enrolled are presented in the Online Supplement (Tables EI and EII). Our CIU population was predominantly comprised of females with an average age of 40±14 years, similar to what other investigators have found^{15, 16}. In addition, the majority of our patients were variously noted to have well-recognized markers of autoimmunity in CIU, including anti-FcεRI antibodies (9/14), positive ASSTs (9/14), and/or anti-thyroid antibodies (6/14).

As the most prominent activation pathway triggered by LUVA mast cells⁸, we initially focused on PgD₂ secretion. We observed increased *de novo* PgD₂ secretion after incubation of CIU-derived serum with LUVA cells in comparison to control serum (363.3± 125.9 v. 243.5± 94.18 pg/ml; p<0.01; figure 1). PgD₂ secretion was modestly (18%) but consistently (14/14 subjects) increased after LUVA cells were incubated overnight with IgE (768.0±177.3 to 909.0±203.1 pg/ml; p<0.001)(online supplement figure E1). To dissect the role of FcεRI-dependent and -independent pathways in CIU, PgD₂ secretion using serum samples obtained from the 6 subjects displaying the highest induction was measured after exposing LUVA cells to either a syk inhibitor or pertussis toxin, as well as after overnight incubation of serum with recombinant human FcεRI (rhFcεRI) as described⁹. Pre-incubation with the syk inhibitor decreased PgD₂ production in all 6 subjects (mean percent decrease was 27.3±4.7%; p<0.05)(figure 2). Similarly, partial inhibition of PgD₂ secretion was observed with pertussis toxin (18.5±3.9% decrease; p<0.05). No synergy was observed when the syk inhibitor and pertussis toxin were combined (30±8.17% decrease). Better inhibition of activation was observed after overnight “capture” of anti-FcεRI antibodies with excess rhFcεRI (53±28% (n=7); data not shown). We also established the ability of CIU serum to induce Ca⁺² fluxes (online supplement figure E2). Induction of Ca⁺² influxes was consistently observed across the entire cohort of subjects (p<0.0001). As with the PgD₂ data, pre-treatment of LUVA cells with the syk inhibitor produced consistent, but incomplete inhibition of Ca⁺² influx, comparable to the extent of inhibition observed using anti-FcεRI antibodies (figure E2).

Previously, basophil activation tests have been considered the benchmark diagnostic test to identify autoimmune causes of CIU^{17–19}. However, this approach is limited by the inherent variability of basophils, which requires rigorous screening of basophils from numerous donors to identify “releaser” basophils capable of FcεRI-dependent activation and, even amongst donors displaying this phenotype, there is day-to-day variability in this capacity²⁰. Stringent screening is also required to ensure against high spontaneous secretion of histamine. While basophils provide useful diagnostic information, CIU is a disease targeting cutaneous, largely connective tissue, mast cells. As basophils are not considered the primary cellular target of CIU, a mast cell model would be the more ideal framework to study CIU pathophysiology. The concept that CIU involves autoimmune mechanisms that include anti-FcεRI antibodies is strongly supported by our current studies. A putative contributing role for anti-FcεRI antibodies is supported by the increase in PgD₂ secretion after overnight

incubation with IgE, an intervention that increases cell-surface expression of these receptors and also as the inhibition of secretion after overnight “capture” of putative anti-FcεRI antibodies with excess recombinant FcεRI protein^{8, 21}. It should be noted, however, that the upregulation of PgD₂ secretion induced by IgE incubation can not categorically distinguish FcεRI-dependent effects from non-specific priming of the LUVA cells. In further support of a role for FcεRI-mediated cell activation, application of a syk inhibitor significantly reduced PgD₂ production and Ca⁺² fluxes. The extent of this inhibition of Ca⁺² fluxes was comparable to that produced when cells were stimulated by anti-FcεRI antibodies. Of note, however, pertussis toxin also reduced PgD₂ production, presumably reflecting additional complementary or collateral activation pathways¹¹. This mast cell line may also prove useful in dissecting additional mast cell targeting autoantibodies or other complementary pathways such as those mediated by complement, bradykinin, or the clotting cascade that might contribute to the development of CIU.

In summary, we utilized a unique connective tissue mast cell line to study effects of CIU patient sera on activation, demonstrating PgD₂ secretion and Ca⁺² influxes. Upregulation after overnight incubation with IgE and inhibition of PgD₂ secretion and Ca⁺² fluxes after pre-incubation with a syk inhibitor or rhFcεRI points to a role for FcεRI-dependent autoimmune mechanisms, although additional complementary pathways appear to be involved. Future studies will be required to determine the relative validity of diagnostic testing for autoimmune mechanisms in CIU using basophil activation as opposed to assays based upon what is arguably the more relevant cellular target, the connective tissue mast cell.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ASST	Autologous serum skin test:
CIU	Chronic idiopathic urticaria
EIA	Enzyme-linked immunoassay
PgD₂	Prostaglandin D ₂
rhFcεRI	Recombinant human FcεRI

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Figure 1. PgD₂ Production after Incubation with Control- and CIU-Derived Serum
 Serum was incubated with LUVA cells at 37°C for 30 minutes and secreted PgD₂ assayed by EIA. Data represent net production of PgD₂ after subtracting out PgD₂ present in the applied serum sample (p<0.01).

Figure 2. Inhibition of PgD₂ Secretion by Syk Inhibitor and Pertussis Toxin

LUVA mast cells were incubated with the syk inhibitor, ER27319 (60 μ M) or pertussis toxin (100 ng) for 2 hrs at 37° C. LUVA cells were subsequently challenged with CIU patient sera and PgD₂ secretion determined.