

Comparative studies of functional and binding assays for IgG anti-Fc ϵ RI α (α -subunit) in chronic urticaria

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Background: Recent data suggest that a subpopulation of patients with chronic urticaria have an autoimmune disorder that is caused by the presence of antibodies to the IgE receptor. The actual incidence of these antibodies is uncertain.

Objective: We sought to assess the incidence of autoimmunity to the IgE receptor in patients with chronic urticaria and to compare functional and binding assays.

Methods: We isolated skin mast cells and studied a large number of patient sera (68) for their ability to activate these cells and isolated basophils. We then compared the results with those obtained by immunoblotting using cloned α -subunit of the IgE receptor.

Results: Sera from patients with chronic urticaria released significant histamine (>15% of basal) on incubation with basophils (48%) and mast cells (46%). By immunoblotting we obtained positive results in 64% of subjects tested and also identified a small subpopulation that is active on cells but does not bind Fc ϵ RI α .

Conclusion: Our data suggest that approximately 45% to 50% of patients with chronic urticaria have a cutaneous autoimmune disorder. Immunoblotting may provide a rapid screening method for anti-Fc ϵ RI α detection in such patients.

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Key words: Urticaria, mast cells, histamine release, IgE receptor, autoimmunity

Urticaria is a common complaint that affects as much as 20% of the population.¹ It is often transient but can be chronic, and the causes of acute and chronic urticaria differ substantially. In most cases of chronic urticaria, there is no identifiable exogenous allergen to explain patients' symptoms. The disorder also occurs in substantial numbers of nonatopic patients.

In recent years several authors have reported data that suggest an autoimmune mechanism as the precipitating event in chronic urticaria. In 1983, Leznoff et al.^{2,3} reported a high incidence of Hashimoto thyroiditis

among patients with chronic urticaria (particularly women), and many patients had antimicrobial antibodies present in their serum, even if they were clinically euthyroid. More recent studies suggest the presence of IgG antibodies reactive with the IgE receptor as assessed by histamine release from human basophils,⁴ β -hexosaminidase release from rat basophil leukemia cells that had been transfected with the α -subunit of the IgE receptor, and immunoblotting with the purified receptor subunit.⁵ The incidence of these antibodies is uncertain, but appears to be at least 30%.

The pathogenic lesion of chronic urticaria consists of a nonnecrotizing^{6,7} perivascular infiltration with mononuclear cells (primarily CD4⁺), T lymphocytes, and monocytes, as well as variable numbers of eosinophils and, in some instances, neutrophils. Mast cells are considered to be the major primary effector cell in chronic urticaria⁸ by releasing histamine, as well other vasoactive substances, but new evidence suggests that mast cells may also contribute to cytokine-production during allergic and nonallergic processes.⁹⁻¹¹ Thus mast cells have the potential to initiate a sustained inflammatory response in the skin. In this study we have isolated human skin mast cells and assessed the capability of sera from patients with chronic urticaria to release histamine from those cells. For comparison, we performed the same test with normal basophils. Finally, in order to assess whether this activity is due to the presence of antibodies against the high-affinity receptor for IgE, we developed a sensitive and specific immunoblotting method with cloned α -subunit of the IgE receptor and tested the same sera for binding activity. Our data indicate a greater degree of positivity by immunoblot than is seen with basophils or mast cells, such that over 60% of patients have identifiable abnormalities. We also defined a subpopulation of patients whose sera activates basophils, mast cells, or both but are negative by immunoblot.

METHODS

Patients and control subjects

Sixty-eight sera from patients with chronic urticaria were tested. We included patients with recurrent hives of greater than 12 weeks duration, occurring at least four times a week, with individual lesions lasting over 4 hours. Patients with clinical evidence of urticarial vasculitis or physically-induced urticaria (e.g., cold urticaria, cholinergic urticaria, or dermatographism) were excluded.

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Antihistamine treatment was stopped at least 48 hours before serum samples were collected, and none of the patients were taking corticosteroids or immunosuppressive drugs at the time of venipuncture. Sera from normal subjects served as controls.

All sera were separated by centrifugation at 300 g for 25 minutes and stored at -80°C .

Histamine release from basophils

Basophils from a single, responsive normal donor were tested with the sera of patients with chronic urticaria. These persons' basophils released 35% histamine on incubation with our monoclonal antireceptor antibody. Leukocytes for use in histamine-release assays were prepared as described previously.⁵

Histamine release was measured with enzyme immunoassay (Immunotech International, Marseille, France) according to the manufacturer's instructions. All histamine-release experiments were done in duplicate, averaged, and the results were expressed as a percentage of total histamine content. Spontaneous histamine was less than 5% of total histamine, and replicate samples agreed within 10 percent.

Mast cell isolation

Foreskins from newborns were processed with a modification of the technique described by Benyon et al.¹² and Rein et al.¹³ Dispersed mast cells from three foreskins were used in each individual experiment. This provided approximately 1 gm of tissue from which 2×10^5 mast cells/ml were obtained. The yield is 2 to 4 ml.

Samples were collected in RPMI-1640 medium containing 10% fetal bovine serum plus 150 IU/ml penicillin and 150 $\mu\text{g}/\text{ml}$ streptomycin. The skin was cut into 2 mm fragments and incubated for 2 hours with RPMI-1640 medium plus 10% fetal bovine serum and 1.5 mg/ml collagenase (Sigma, St. Louis, Mo.) and 0.5 mg/ml hyaluronidase (Sigma). Dispersed cells were harvested by filtration through nylon gauze (150 mm) (Tetko) and washed once with erythrocyte-lysing buffer (0.1 mol/L NH_4Cl , 0.001 mol/L KHCO_3 , and 2×10^{-6} mol/L ethylenediamine tetraacetic acid) and twice with RPMI-1640. Once washed, the cells were resuspended in RPMI-1640 containing 10% fetal bovine serum, 150 IU/ml penicillin and 150 $\mu\text{g}/\text{ml}$ streptomycin and incubated overnight. After incubation, they were washed twice with N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid-buffered saline containing 0.3% human serum albumin with 2 mmol/L CaCl_2 and 0.2 mmol/L MgCl_2 . One hundred microliters of serum or buffer were incubated with 100 μl suspension containing 2×10^5 mast cells for 20 minutes at 37°C . After centrifugation (700 g), histamine was determined in the supernatants. Replicate cell samples were boiled to determine total histamine content.

Immunoblotting

Purified recombinant $\text{Fc}\epsilon\text{RI}\alpha$ was prepared as previously described¹⁴ (provided by Sandoz Research Institute, Vienna, Austria). Once boiled and reduced with 2-mercaptoethanol (Kodak, Rochester, N.Y.), the sample was electrophoresed with 10% sodium dodecylsulfate gels and blotted onto nitrocellulose membranes (Costar Scientific Co., Cambridge, Mass.). The membranes were blocked with 5% dry milk for 90 minutes and reacted with serum (1:500 dilution) or monoclonal mouse anti- $\text{Fc}\epsilon\text{RI}\alpha$ (monoclonal antibody 22E7, dilution 1:2000). Bound IgG was detected with a goat anti-human IgG (Fc fragment-specific alkaline phosphatase conjugate, dilution 1:5000) (Jackson ImmunoResearch Laboratories Inc., West Grove, Pa.) or a goat anti-mouse IgG alkaline-phosphatase

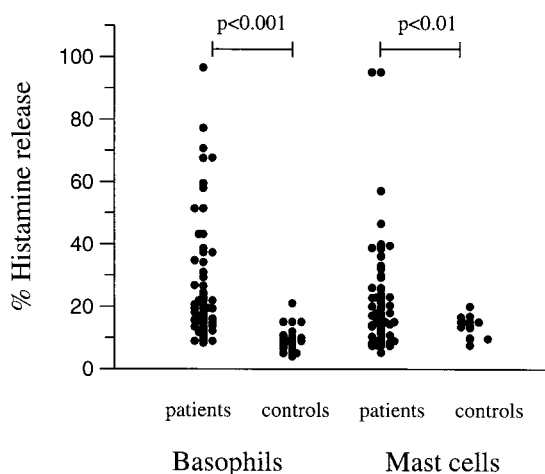


FIG. 1. Percentage of histamine release in basophils and mast cells. Human basophils from healthy donor and mast cells from newborn foreskins were challenged with sera from patients with chronic urticaria (68 sera tested on basophils and 65 sera tested on mast cells) or normal control subjects (24 sera tested on basophils and 14 sera tested on mast cells), and percentage of histamine release was determined.

conjugate (1:5000) (Jackson ImmunoResearch Laboratories Inc.). Antibody binding was visualized by incubating the membrane with BCIP/NBT phosphatase substrate (Kierkegard & Perry Laboratories Inc., Gaithersburg, Md.).

Statistical analysis

For correlation studies, Pearson's coefficient was used. We used the Mann-Whitney U test for comparing unpaired, nonparametric data. For more than two independent groups, we used one-way analysis of variance followed by Student-Newman-Keuls tests.

RESULTS

Histamine release from basophils obtained from one chosen donor was examined with 24 control sera from normal subjects. The percentage histamine release varied from 2% to 20% with a mean of 9.7% and a standard deviation (SD) of 3.6%. Sera from chronic urticaria patients were then examined and a response 2SD from the mean was considered positive (i.e., when release exceeded 16.9%). Sixty-eight sera were tested, and 31 of them (48%) released more than 17% histamine. The range for all samples was 5% to 100% histamine release. Thirteen of 14 normal sera tested elicited histamine release of 15% or less (Fig. 1). The ability of 65 sera to stimulate histamine release from cutaneous mast cells was assessed as well. Twenty-eight of the sera (46%) had histamine release greater than 19%, with a range of 8% to 97%. The mean $\pm$ SD for histamine release from mast cells by 14 control sera was $12.7\% \pm 3.1\%$. A response 2SD from the mean was again considered to be positive (i.e., 18.8% histamine release). As was observed with basophils, only one of the controls released more than 15% histamine (Fig. 1). There was an excellent correlation between histamine release from basophils

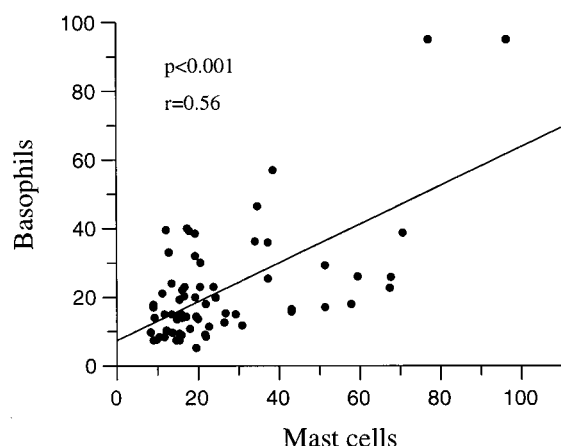


FIG. 2. Correlation between basophils and mast cell histamine release. Once percentage was determined, we found significant correlation between histamine release from basophils and mast cells.

and mast cells ($r = 0.56$ and $p < 0.001$), suggesting a common mechanism of cell activation (Fig. 2).

We performed immunoblots of 53 sera, and we observed a positive blot in 34 (64%) of them. A representative sample is shown in Fig. 3 at three serum dilutions. The samples reported herein were assayed at a dilution of 1:500 or greater because we predetermined that none of the control sera were positive at this dilution. Some normal sera were positive when tested between 1:50 and 1:250 dilution and were considered to have insignificant titers. We obtained a single protein band at 30 to 35 kd in all instances.

When all three assays are compared, basophil histamine release results were positive in 48% of patients, cutaneous mast cell histamine release results were positive in 46% of the patients, and the yield by immunoblotting was positive in 64%. We also found that sera from nine patients that tested negative by immunoblot had positive basophil histamine release responses, and 10 patients' sera with negative immunoblot results were positive with cutaneous mast cells.

DISCUSSION

Gruber et al.¹⁵ first sought evidence that chronic urticaria might be autoimmune in origin, and reported an increased incidence of anti-IgE antibodies in patient sera. The percentage was only 10% (as assessed by ELISA), and of these, only half appeared to be functional and cause basophil histamine release. More recently Hide et al.¹⁶ reported the presence of IgG antibodies directed to $Fc\epsilon RI_\alpha$ in patients with chronic urticaria who had a positive autologous skin test response. Of 26 such patients selected, 17 had positive results for basophil histamine release; 12 had IgG anti- $Fc\epsilon RI_\alpha$; and five had either IgG anti-IgE or both. However, most patients with chronic urticaria do not have a positive autologous skin test response, thus the incidence of these autoantibodies in the general popu-

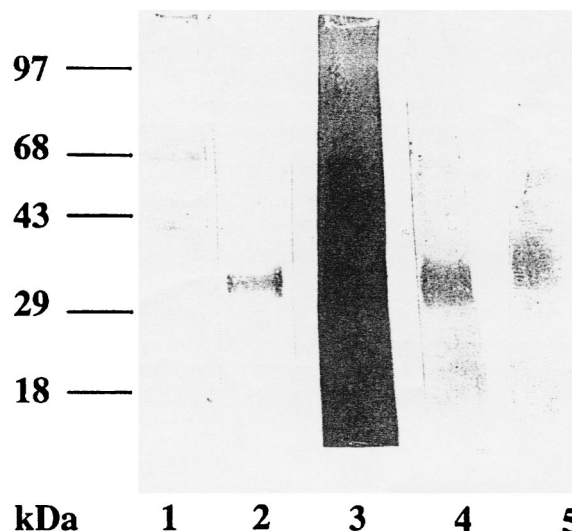


FIG. 3. Immunoblotting to ascertain presence of IgG anti- $Fc\epsilon RI_\alpha$. Soluble recombinant $Fc\epsilon RI_\alpha$ was subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose. Membranes were reacted with monoclonal antibody or whole sera from patients with chronic urticaria or normal control subjects. Antibody binding was detected with alkaline phosphatase-conjugated goat anti-mouse IgG antibody or goat anti-human IgG fragment-specific antibody, respectively. Lane 1, Molecular marker; lane 2, monoclonal antibody; lane 3, patient serum at 1:50 dilution; lane 4, serum at 1:500 dilution; lane 5, serum at 1:1000 dilution.

lation of patients with chronic urticaria is unclear. Fiebiger et al.¹⁷ confirmed the presence of IgG anti- $Fc\epsilon RI_\alpha$ by immunoblot. In this series 37% of patients with chronic urticaria had positive responses, and 60% of these sera were functionally active (i.e., released histamine from human basophils). Our most recent data, reported herein, found positive histamine release assays of 48% and 46% by using basophils and cutaneous mast cells, respectively. Previous studies have assessed these autoantibodies by one or more of the aforementioned methods, but none have compared all three. It should be noted that the response of different basophil donors to patients' sera are highly variable, as reported by others previously,¹⁸ and we have therefore used a single responsive donor; such individuals may have more available IgE receptor for interaction with immunoglobulin. There are no data comparing responsiveness of cutaneous mast cells of individual subjects, and cell number requirements necessitate our use of cells derived from multiple donors. Thus one can question the validity of any comparison of histamine release between basophils and mast cells, and hence our focus on the immunoblot as a method that can be standardized, although it is a nonfunctional approach. Nevertheless, here too we may miss antibodies directed to the α -subunit that bind to conformational structures that are unavailable once the α -protein is blotted. Eventually, ELISA methods may allow quantitation so that more precise limits for *normal* versus *abnormal* can be defined. It should also be noted

that other immunoglobulins that might be directed to the α -subunit are not detected by this method, although our preliminary data suggest that IgM and IgA antibodies are not present (unpublished observations). Potential reactivity of sera with the β - or γ -subunit of the IgE receptor has not been reported.

Fewer sera (53) were available for assay by immunoblot compared with the number tested functionally (basophils, 68; mast cells, 65). Nevertheless, we obtained an even higher incidence of positive sera (64%) by using immunoblots of improved sensitivity. Earlier studies reporting the incidence of functional IgG anti-Fc ϵ RI α in patients with chronic urticaria found approximately 30% to 40% that had positive results.^{4,5} However, the data herein suggest that the incidence of such antibodies may actually be higher.

We are in agreement with the results of Niimi et al.¹⁹ that sera from patients with chronic urticaria can activate cutaneous mast cells in addition to basophils; however, our incidence of positivity of sera is approximately 10% higher. This may relate to our use of dispersed cutaneous mast cells rather than ultrathin skin slices and study of a large population of subjects. Mast cells are clearly the initiating effector cell of the urticaria process. However, basophils can infiltrate skin as part of the late-phase response and may be activated by antigens or by cytokines such as the β -chemokines.²⁰ Thus basophil secretion could contribute to an inflammatory process at a later time. There is a good correlation between the results obtained with both cell types (Fig. 2) consistent with a common activation mechanism (i.e., anti-Fc ϵ RI α).

We observed heterogeneity among the different assays used and also defined a subpopulation of patients whose sera released histamine but did so in the absence of antibodies directed to the α -subunit of the IgE receptor or to IgE (data not shown). Thus a small percentage of patients may have circulating factors capable of activating basophils, mast cells, or both whose identity is as yet unknown. There were also patients with sera containing antireceptor antibodies, which do not release histamine from mast cells, indicating that these antibodies are not always functional,²¹ as is true for anti-IgE. This has been observed previously,¹⁷ and may depend on the particular epitopes with which it is reactive. There is also heterogeneity of responsiveness of basophils,¹⁵ and perhaps cutaneous mast cells, and we did not test sera using a panel of donors of each cell type. We do not know whether the presence of IgG anti Fc ϵ RI α is truly pathogenic or whether it is a disease marker. Clearly it is more specific for chronic urticaria than anti-IgE. The latter is also found in patients with atopic dermatitis and some healthy patients, whereas anti-Fc ϵ RI α is not.¹⁷ Another observation that remains to be explained is why such antibodies stimulate only cutaneous mast cells (in vivo) so that the patients have no respiratory symptoms or show signs of systemic anaphylaxis.

The data presented demonstrate that a large fraction of patients with chronic urticaria have antibodies di-

rected to the α -subunit of the high-affinity IgE receptor, which is functionally consistent with an autoimmune origin of this disease. These data also suggest that the perivascular cellular infiltrate observed may represent a late-phase type reaction as a result of ongoing cutaneous mast cell degranulation, which could explain the histology of the lesions characteristic of chronic urticaria. These data are also consistent with the observation that antimediator drugs, such as antihistaminics, are not effective if the urticaria is severe, and such patients require drugs that interfere with the recruitment of cells,²² such as alternate-day steroids.²³ These observations may lead eventually to novel therapeutic approaches based on competition with the reactive epitope or epitopes on the α -subunit, use of other antiinflammatory agents (or plasmapheresis), or administration of cytokines or anticytokines to dampen the inflammatory response.

We thank Sandoz Laboratories for providing α -subunit and Dr. Richard Chizzonite for preparation of monoclonal antibody 22E7.

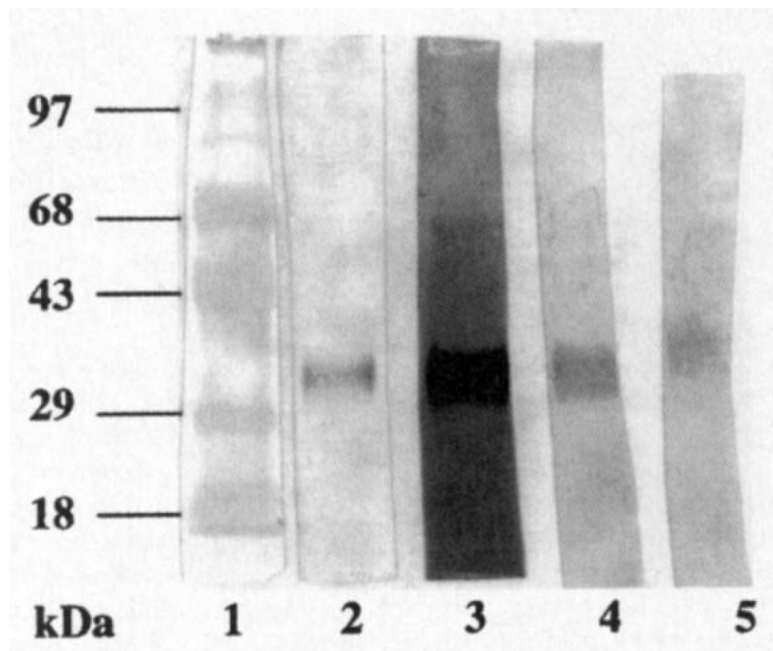
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Fig. 3. Immunoblotting to ascertain presence of IgG anti-FcRI. Soluble recombinant FCRI was subjected to sodium dodecylsulfate–polyacrylamide gel electrophoresis and blotted onto nitrocellulose. Membranes were reacted with monoclonal antibody or whole sera from patients with chronic urticaria or normal control subjects. Antibody binding was detected with alkaline phosphatase–conjugated goat anti-mouse IgG antibody or goat anti-human IgG fragment–specific antibody, respectively. Lane 1, Molecular marker; lane 2, monoclonal antibody; lane 3, patient serum at 1:50 dilution; lane 4, serum at 1:500 dilution; lane 5, serum at 1:1000 dilution.



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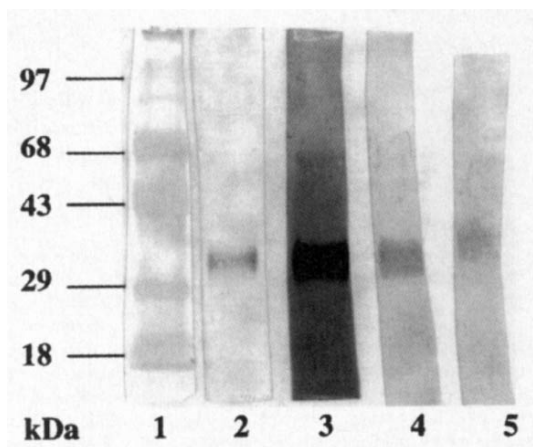
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Correction

The following correction applies to the article by Ferrer et al. entitled “[Comparative studies of functional and binding assays for IgG anti-FcεR1α \(α-subunit\) in chronic urticaria](#),” which appeared in volume 101, pp. 672-676, 1998, of The Journal.

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Fig. 3. Immunoblotting to ascertain presence of IgG anti-FcεR1α. Soluble recombinant FcεR1α was subjected to sodium dodecylsulfate–polyacrylamide gel electrophoresis and blotted onto nitrocellulose. Membranes were reacted with monoclonal antibody or whole sera from patients with chronic urticaria or normal control subjects. Antibody binding was detected with alkaline phosphatase–conjugated goat anti-mouse IgG antibody or goat anti-human IgG fragment–specific antibody, respectively. *Lane 1*, Molecular marker; *lane 2*, monoclonal antibody; *lane 3*, patient serum at 1:50 dilution; *lane 4*, serum at 1:500 dilution; *lane 5*, serum at 1:1000 dilution.



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