

Complement dependence of histamine release in chronic urticaria

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Background: IgG autoantibodies directed to the α -subunit of the IgE receptor have been identified in 30% to 45% of patients with chronic urticaria. However, the exact mechanism by which histamine secretion is initiated is uncertain.

Objective: Histamine release from cutaneous mast cells may occur by cross-linking the IgE receptor or by activation of complement. Our goal is to distinguish these 2 possibilities.

Methods: We incubated human cutaneous mast cells with patient sera, decomplexed sera, or purified patient IgG. The IgG was also added to pooled normal serum or to sera deficient in either C2 or C5, and its ability to activate mast cells was assessed. Mast cells were incubated with human IgE myeloma to saturate α -subunits to determine the effect on histamine release.

Results: Patient sera released histamine ($18.26\% \pm 4.39\%$), but purified IgG from patients ($5.5\% \pm 4.3\%$) did not. Addition of the patient IgG to normal sera rendered the sera positive for histamine-releasing activity ($18.4\% \pm 4.3\%$), whereas control IgG or patient IgG added to C2- or C5-deficient sera did not release histamine. Histamine release with decomplexed patient sera was also diminished ($8.34\% \pm 4.3\%$). Preincubation of mast cells with a C5-blocking peptide decreased histamine release but was not statistically significant; there was a significant decrease after preincubating mast cells with IgE myeloma.

Conclusion: The degranulation of mast cells by IgG autoantibodies in patients with chronic urticaria requires binding to the IgE receptor and activation of the classical complement cascade. Saturation of the IgE receptor with IgE inhibits such degranulation, presumably by preventing binding of the requisite IgG. (*J Allergy Clin Immunol* 1999;104:169-72.)

Key words: Chronic urticaria, complement, C5a, autoantibodies

A large subgroup of patients with chronic urticaria (30% to 45%) possess an IgG antibody directed to the α -subunit of the Fc ϵ RI.¹⁻⁴ This antibody has been shown to directly degranulate rat basophil leukemia cells to release either histamine or β -hexosaminidase,⁵ presumably by cross-linking the IgE receptor. However, it has been reported that the purified IgG of such patients, which clearly binds to the α -subunit by immunoblot,^{2,5} does not necessarily degranulate human basophils and that complement activation (C5a anaphylatoxin in particular) may be required.⁶ However, the IgE receptor-positive cell of

primary interest is clearly the cutaneous mast cell, and basophils of patients are in fact hyporesponsive to stimuli that activate by means of the IgE receptor.^{7,8}

In this article we distinguish the ability of patient sera to degranulate cutaneous mast cells by direct cross-linking of the IgE receptor from cell activation by complement and also demonstrate that saturation of the IgE receptor with IgE inhibits cell activation.

METHODS

Patients and control subjects

We selected 11 histamine-releasing sera (range, 15% to 40% release) and 7 normal control sera ($5.5\% \pm 4.3\%$ release). We included patients with recurrent hives of greater than 12 weeks duration, occurring at least 4 times a week, with individual lesions lasting over 4 hours. Patients with clinical evidence of urticarial vasculitis or physically induced urticaria (eg, cold urticaria, cholinergic urticaria, or dermatographism) were excluded.

Antihistamine treatment was stopped at least 48 hours before serum samples were collected, and none of the patients were taking tricyclic antidepressants, corticosteroids, or immunosuppressive drugs at the time of venipuncture or in the recent past (3 months). Sera from normal subjects served as controls.

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

Materials

Human C3a and C5a and human IgE myeloma were provided by Calbiochem (La Jolla, Calif). The IgE myeloma was used at a final concentration of 100 $\mu\text{g/mL}$.

Mast cell isolation

Foreskins from newborns were processed by using the technique described by Benyon⁹ and Rein¹⁰ with the addition of erythrocyte lysis with hypotonic buffer consisting of 0.1 mol/L NH_4Cl , 0.001 mol/L KHCO_3 , and 2×10^{-6} mol/L EDTA. Dispersed mast cells from 3 foreskins were used in each individual experiment. This provided approximately 1 g of tissue from which 2×10^5 mast cells/mL were obtained.

Samples were collected in RPMI-1640 medium, 150 IU/mL penicillin, and 150 $\mu\text{g/mL}$ streptomycin. The skin was cut into 2-mm fragments and incubated for 2 hours with RPMI-1640 medium, 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, NY) 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 EDTA) and twice with RPMI-1640. Once washed, the cells were resuspended in RPMI-1640 containing 10% FBS, 150 IU/mL penicillin, and 150 $\mu\text{g/mL}$ streptomycin and incubated overnight. After incubation, they were washed twice with N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid-buffered saline containing 0.3% human serum albumin with 2 mmol/L CaCl_2 and 0.2 mmol/L MgCl_2 .

We then incubated 75 μL of the cell suspension containing $2 \times$

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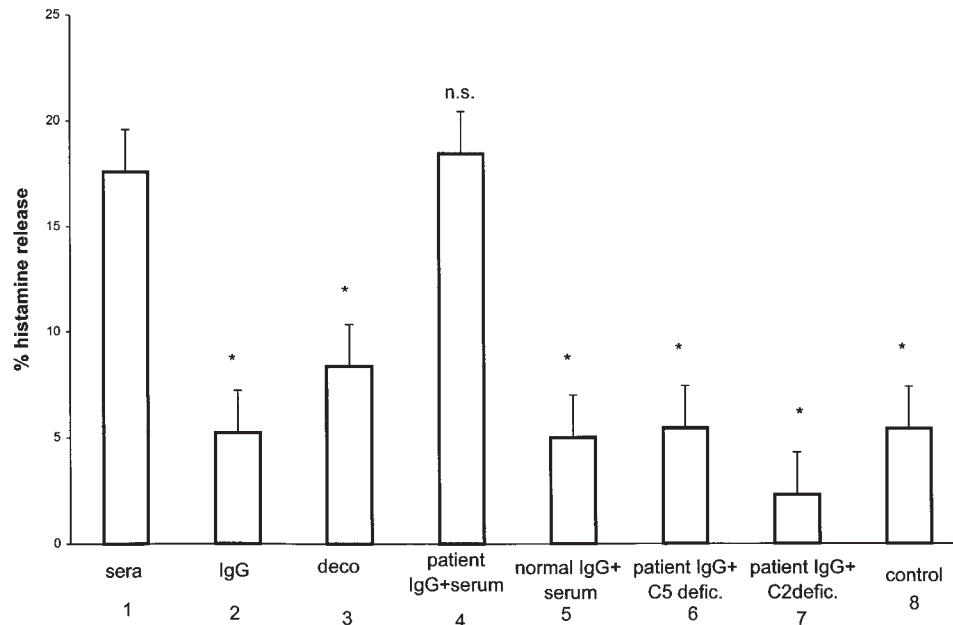


FIG 1. Average of mast cell histamine release. Seventy-five microliters of a cell suspension containing 2×10^5 mast cells were incubated for 20 minutes at 37°C with $75 \mu\text{L}$ of patient or control serum, $75 \mu\text{L}$ of decomplexed patient serum (for 1 hour at 56°C), $75 \mu\text{L}$ of 1 to 10 mg/mL protein G-eluted IgG, $15 \mu\text{L}$ of purified IgG plus $60 \mu\text{L}$ of pooled normal serum, $15 \mu\text{L}$ of pure IgG plus $60 \mu\text{L}$ of C5- or C2-deficient serum, or $75 \mu\text{L}$ of buffer. Results are expressed as a percentage of histamine release from 2 cell suspension replicates. Cells were boiled to determine total histamine content. Significant differences ($P < .05$, as determined by the Student *t* test) for paired samples, each of which was compared with the histamine release for patient sera (far left bar), are indicated by asterisks. Each bar represents an average of histamine release by using 5 different patient sera, each of which was assayed 5 times with duplicates ($n = 50$).

10^5 mast cells (stained by the Kimura method¹¹) for 20 minutes at 37°C with $75 \mu\text{L}$ of sera from patients with urticaria, sera from control subjects, or decomplexed sera (1 hour at 56°C)¹² to determine the effect of each on histamine release. Two cell suspension replicates were boiled to determine total histamine content. We also incubated mast cell suspensions with C5a- and C3a-blocking octapeptides (Bachem Bioscience, King of Prussia, Pa) at 10^{-4} mol/L concentration for 1 hour at 4°C to test for anaphylatoxin-independent histamine release.

Purification of IgG

IgG was isolated by protein G (Pharmacia Biotech, Piscataway, NJ) affinity chromatography and concentrated back to a normal plasma level of 10 mg/mL. Seventy-five microliters of IgG was incubated with either basophils or mast cells to determine whether histamine-releasing activity is present in patient versus control IgG. To test for a requirement of complement in the process, $15 \mu\text{L}$ of IgG was mixed with $60 \mu\text{L}$ of either pooled normal human serum or sera deficient in either C2 or C5 (Quidel, San Diego, Calif).

Statistical analysis

We used the Student *t* test for comparing paired means. A *P* value of less than .05 was considered statistically significant.

RESULTS

Requirement for complement in autoimmune mast cell activation

IgG was isolated from the sera of 5 patients with chronic urticaria and 2 normal control subjects. The

patients had strikingly positive immunoblots for IgG anti- α and were among our most plentiful sera. The normal subjects were random choices. The patient sera and the purified IgG (tested at 10 mg/mL to approximate normal plasma IgG) were incubated with cutaneous mast cells, and the percent histamine release was determined. As shown in Fig 1, patient sera released between 15% and 40% histamine (18.26 ± 4.39 ; column 1), whereas the IgG released was only $5.2\% \pm 2.3\%$ (column 2); the latter value was not significantly different from the release with 7 pooled control sera (column 8). We next decomplexed the patient sera by incubation for 1 hour at 56°C , and histamine release was diminished as shown in column 3 ($8.34\% \pm 4.3\%$). We then prepared a pool of normal serum from 7 subjects to which IgG of patients or normal control subjects was added (final concentration, 2 mg/mL). The samples were then assayed. When patient IgG was added to normal serum (column 4) and the mixture was tested for activation of cutaneous mast cells (column 4), histamine release was $18.4\% \pm 4.3\%$, which was the same as that observed with patient sera (column 1). When normal IgG was added to normal pooled serum and tested for activation of cutaneous mast cells, there was no release of histamine (column 5). Each patient IgG was then added to either C2-deficient (column 7) or C5-deficient (column 6) sera and similarly examined. In each case histamine release was no longer observed, indicating a requirement for the classical path-

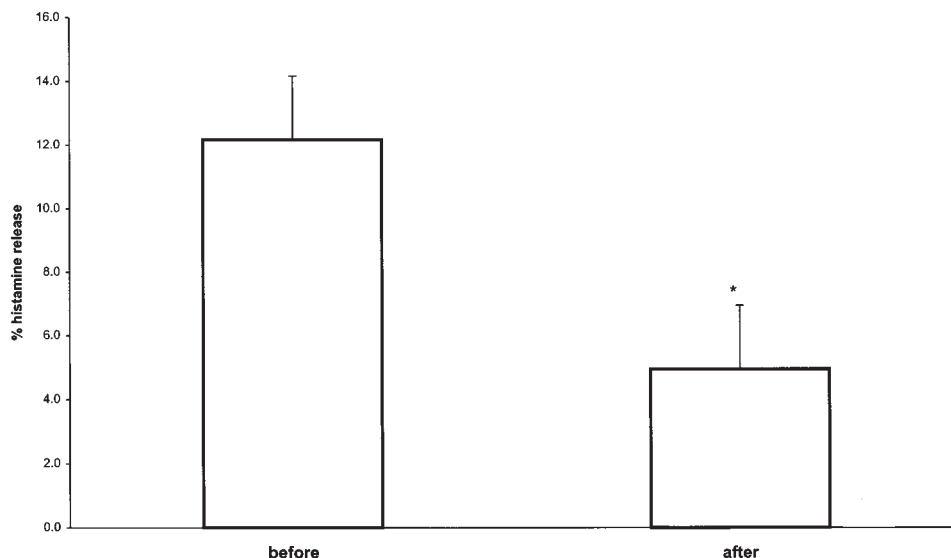


FIG 2. Mast cell histamine release with patient sera after preincubation with IgE myeloma. The mast cell suspension (2×10^5 cells) was incubated for 20 minutes at 37°C with 75 μ L of patient sera or was preincubated with 500 ng/mL IgE myeloma protein and then incubated with patient sera. The percent histamine release before and after IgE myeloma preincubation were compared. Four patient sera were assayed, and a statistically significant decrease in histamine release (59.6%) is observed when the IgE myeloma was present. Cells incubated with buffer alone had 5% histamine release.

way because C2-deficient sera did not support activation. The data also suggest C5a as the likely histamine-releasing product because C5-deficient serum did not support histamine release.

Effect of IgE on histamine release by IgG anti-Fc ϵ RI

To determine whether activation of mast cells by the sera from patients with chronic urticaria is perturbed by IgE bound to the α -subunit, we preincubated mast cells with IgE myeloma to saturate the receptor and then retested the cells for responsiveness to patient sera. The presence of the myeloma protein decreased histamine secretion to control values (Fig 2).

Cutaneous mast cell reactivity with C5a

To assess the ability of mast cells to be activated by human C3a and C5a, we performed a dose-response curve with both complement components and attempted to inhibit each with the octapeptides. A maximal response with human C5a was observed at a concentration of 100 ng/mL, which caused 37% histamine release. After preincubation of the cells with the inhibitory peptide, histamine release was reduced to 14%. Histamine release provoked by similar concentrations of C3a did not exceed 10% (data not shown).

We then used the octapeptides to attempt to inhibit complement-dependent histamine release of patient sera. We studied 8 different sera, and we observed a 17% reduction in histamine release when we preincubated the mast cells with the C5-inhibiting peptide (data not shown). This represents a downward trend, but a statistically significant difference was not obtained.

DISCUSSION

The mechanism by which cutaneous mast cell activation occurs in chronic urticaria is unknown. However, there is an accumulating body of data to indicate that an IgG antibody directed to the α -subunit of the IgE receptor is the initiator in a substantial fraction of patients. The first data demonstrating that this antibody is functional used rat basophil leukemia cells, which were transfected with the human α -subunit. These cells, which lack surface IgE and patient sera, as well as their isolated IgG, led to degranulation and release of histamine and β -hexosaminidase.⁵ It was assumed that cross-linking of the α -subunit by IgG occurred to activate the cells. Activation of human basophils and human cutaneous mast cells by such sera was demonstrated soon thereafter.^{1,13} However, in one study with basophils the IgG fraction isolated from positive sera did not activate the cells, and a source of complement was required.⁶ Inhibition of activation by a peptide analog of C5a, which blocks the C5a receptor, suggested that complement might be required for basophil activation. It is clear, however, that cutaneous mast cells are the cells of interest in chronic urticaria and that basophils of patients are in fact desensitized so that activation by means of the IgE receptor is abnormally low.^{7,8}

Therefore we have also assessed activation by using cutaneous mast cells, and our data all point to a critical role of complement in cell activation. The most striking observation, shown in Fig 1, is that IgG isolated from patient sera that are known to be positive did not activate cutaneous mast cells. Because binding to the α -subunit by isolated IgG has been demonstrated for those particu-

lar sera by immunoblot,¹³ there is binding, but effective cross-linking has not taken place. Thus the density or distribution of IgE receptor on the surface of the cells may not be sufficient for cross-linking and cell activation to occur. Patient IgG, but not control IgG, when added to normal serum can activate cutaneous mast cells, as well as the patient sera, but the IgG is ineffective if added to C2-deficient serum. Thus activation of the classical complement pathway appears to be requisite. To initiate cell activation, the Fc portion of 2 IgG molecules in proximity must be able to bind to 2 of the 6 globular heads of C1q. To achieve this, only one fragment antibody (Fab) of each of 2 adjacent IgG molecules needs to bind to adjacent α -subunits. Another consideration is the possibility that receptors occupied by IgE antibody are not available to the IgG and that cross-linking might be possible, although limited by the percent saturation of receptors. Evidence to suggest this possibility has been presented by using normal basophils.¹⁴ As shown in Fig 2, preincubation of cutaneous mast cells with an IgE myeloma in concentrations in excess of that needed to saturate IgE receptors inhibits activation completely. These data suggest that binding of the pathogenic IgG does not take place, and therefore complement activation cannot proceed. It is important to note that IgE myeloma-treated mast cells release histamine normally with C5a, and therefore C5a receptors remain available (data not shown).

Although C5a is the likely mast cell activator under these conditions, more work will be needed to demonstrate its role unequivocally. First, C5a is known to be a more effective activator of cutaneous mast cells than is C3a,^{15,16} and we have confirmed these observations. We examined C5-deficient sera and also attempted to inhibit activation by using a C5a-blocking peptide. The data with C5-deficient sera are clear. There was no release above background by using any of the 5 sera tested. Because activation can proceed through the C3 step, a role for C3a appears unlikely. On the other hand, our data obtained by using the C5a-blocking peptide, although inhibiting, were not statistically significant, even though the peptide blocked activation of the cutaneous mast cells with synthesized C5a. Thus activation by any complement factor between C5 and C9 would remain a possibility. There are no data to suggest cytotoxicity of mast cells by the C5b678poly C9-terminal complex. Another consideration is that C5 in serum (or plasma) is rapidly cleaved to C5a des-arginine,^{17,18} which then binds to C5a cochemotaxin (Vit D binding protein),¹⁹ and inhibition of the activity of the complex by the blocking peptide may differ from that of C5a itself, even though it is a less potent activator.

Our data suggest a need for further assessment of patient sera for activation of complement-using sensitive assays of cleaved components or release of phlogistic

peptides because significant depletion of serum complement is not seen. Skin biopsy specimens also need to be reassessed because complement deposition such as that associated with immune complex-mediated reactions is not observed, but more subtle evidence of complement activation might be present.

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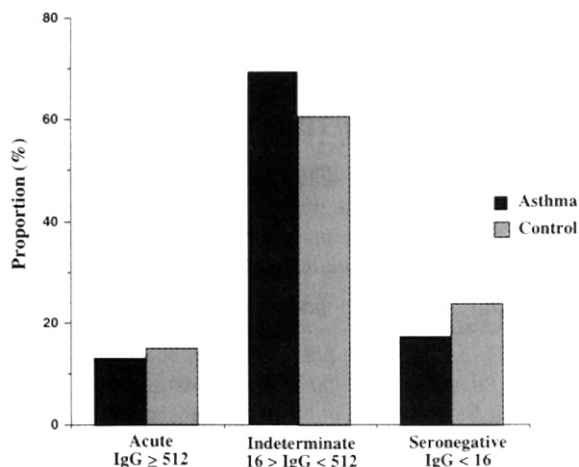


FIG 1. IgG antibody titers in asthmatic and control subjects. Levels of IgG *C pneumoniae* antibody titers in asthmatic and control subjects indicating acute infection, prior exposure (indeterminate), or no prior exposure.⁵

there was no correlation between the magnitude of IgG *C pneumoniae* antibody titers and asthma. In fact, the majority of control and adult asthmatic subjects had random IgG titers $\geq 1:64$ (Fig 1). Seroreactivity (IgG titers $\geq 1:16$) to *C pneumoniae* in our control group was similar to that of prior community-based epidemiologic studies.⁵

The reasons our results contrast with previously published results correlating seropositivity to *C pneumoniae* and asthma are not readily apparent. However, methodologic differences in the measurement of IgG and IgM specific *C pneumoniae* antibodies are unlikely to account for the discrepancy. Our and prior studies used the same method of microimmunofluorescence developed by

Grayston and Wang to measure IgG and IgM specific *C pneumoniae* antibodies. Furthermore, control sera and the strains of *C pneumoniae* and *Chlamydia trachomatis* used in these microimmunofluorescence assays were provided by a common source (Washington Research Foundation, Seattle, Wash).

Our data suggest that random IgG or IgM *C pneumoniae* titers would not be clinically useful in selecting asthmatic patients for antimicrobial therapy effective against *C pneumoniae*. Seroreactivity to *C pneumoniae* in older adults was common and did not correlate with asthma (Fig 1). This study does not exclude the possibility that *C pneumoniae* respiratory tract infections may exacerbate or even cause the development of asthma in a subset of individuals. However, future studies that investigate the possible link between *C pneumoniae* infection and asthma should use methods (eg, culture, PCR) that are more specific for continuing *C pneumoniae* infection than serology.

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