Anti-FcεRI autoantibodies and basophil histamine releasability in chronic idiopathic urticaria

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Background: Circulating functional autoantibodies to the high-affinity IgE receptor (FcεRI) or to IgE have been found in approximately one third of patients with chronic idiopathic urticaria (CIU).
Objective: We sought to compare basophil histamine release and basophil numbers in patients with CIU with and without autoantibodies.
Methods: Basophil histamine release to the anti-FcεRI mAb 22E7, anti-IgE, and formyl-methionyl-leucyl-phenylalanine (fMLP); basophil numbers; and total cellular histamine were measured in 26 patients with CIU and 18 healthy control subjects. Twelve patients were classified as having functional anti-FcεRI and/or anti-IgE autoantibodies on the basis of their serum-evoked histamine release from the basophils of 2 healthy donors.
Results: 22E7 and anti-IgE, but not fMLP, released less histamine from basophils of patients with CIU than from those of control subjects. Mean ± SEM maximum histamine release to 22E7 from basophils of control subjects and patients with CIU with and without autoantibodies was 38.5% ± 5.0%, 17.9% ± 6.0% ($P = .01$), and 1.0% ± 0.3% ($P < .0001$), respectively. Similar results were obtained with anti-IgE, which is dependent on and cross-links cell bound IgE, and 22E7, which directly cross-links the IgE receptor. The mean ± SEM basophil counts for control subjects and patients with CIU without and with autoantibodies were 52 ± 7, 34 ± 9 ($P = .04$), and 5 ± 1 ($P < .0001$) $\times 10^6$ cells/L, respectively, and similar changes were found in measurements of total cellular histamine.
Conclusion: Patients with autoantibodies have both markedly reduced basophil numbers and basophil histamine release to factors acting through FcεRI, which indicates either a residual pool of functionally distinct basophils or may be a consequence of desensitization of the FcεRI pathway. (J Allergy Clin Immunol 1998;102:651-8.)

Key words: *Basophil releasability, basophils, FcεRI, chronic idiopathic urticaria, histamine, IgE, autoantibody*

Abbreviations used
CIU: Chronic idiopathic urticaria
FcεRI: High-affinity IgE receptor
fMLP: Formyl-methionyl-leucyl-phenylalanine

Releasability is a term used to describe the response of basophils or mast cells to stimulation, and it is usually applied in the context of stimulation by cross-linking of receptor-bound IgE.[1,2] Basophil releasability is normally expressed as the maximum histamine release obtained under optimum stimulation, but the term has also been applied to the release of other mediators, including those synthesized de novo, such as the leukotrienes.[1-3] There is a wide variation in IgE-dependent basophil releasability in the normal population. Basophils from between 2% and 22% of normal subjects are unresponsive to IgE-mediated stimulation, although degranulation occurs in response to non-IgE-mediated stimulation, such as C5a and fMLP.[1,4-6] Releasability appears to be an intrinsic property of basophils and is not a reflection of differences in the density of receptor-bound IgE.[5] IL-3 is capable of enhancing release of histamine and other mediators from responsive basophils, but there is conflicting evidence for its effects on nonreleasing basophils.[5,7,8]

IgE-dependent basophil histamine release is reduced in patients with chronic idiopathic urticaria (CIU) compared with the normal population.[4,9] CIU is characterized by widespread transient cutaneous wheals that occur daily, or almost daily, for at least 6 weeks.[10] There is evidence for mast cell degranulation and histamine release within the wheals, but the pathogenesis of the disease remains unclear.[11] We have recently shown that approximately one third of patients with CIU have circulating autoantibodies, which are directed against the high-affinity IgE receptor (FcεRI) in 25% to 30% of patients or against IgE in 5% to 7% of patients.[12-14] The autoantibodies are functionally active, causing histamine release from basophils of healthy donors and from dermal mast cells in vitro.[14] The presence of anti-FcεRI autoantibodies in CIU has been confirmed by Western blot analysis and ELISA.[15-17] Thus in a subset of patients with CIU, basophils are continually exposed to concentrations of autoantibodies in the patient's serum, which could be expected to modify basophil function

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and/or cause degranulation in vivo, but there is little direct evidence for this. In 1962, Rorsman[18] reported basophilic leukopenia in CIU, and this was recently confirmed by Grattan et al,[19] who also showed that patients with CIU with circulating autoantibodies had the most pronounced reduction in basophils. Basophil leukopenia was not observed in the earlier studies on decreased basophil histamine releasability in CIU.[4,9]

In this study we aimed to determine whether the reduced basophil releasability found in patients with CIU was associated with the subset of patients with autoantibodies and to relate histamine releasability to circulating basophil numbers. In addition, we stimulated basophils from patients with CIU and healthy control subjects with a recently developed mouse anti-human FcεRIα mAb, 22E7, which directly cross-links FcεRI, thus excluding any effects of differences in structure or function of IgE on basophil releasability. We found that the subset of patients with CIU with autoantibodies had the most pronounced reduction in basophil histamine releasability and the fewest identifiable circulating basophil leukocytes.

METHODS

Subjects

Patients with CIU and healthy volunteers were recruited from specialist urticaria clinics at St. John's Institute of Dermatology and from the staff at St. Thomas's Hospital. Ethical approval was obtained from the St. Thomas's Hospital Research Ethics Committee. All subjects gave written consent after verbal and written explanation of the study.

Twenty-six patients with CIU were included. They had a mean age of 48 years (range, 20 to 77 years), and 8 were men. The duration of the urticaria ranged from 10 weeks to 18 years, and all patients had daily or almost daily wheals at the time of the study. The severity of urticaria on the day of testing was scored as follows: 0 = no wheals; 1 = 1 to 10 small (<3 cm in diameter) wheals; 2 = 10 to 50 small wheals or 1 to 10 large wheals; 3 = more than 50 small wheals or 10 to 50 large wheals; and 4 = virtually covered with wheals.[20] Ten patients with CIU had a history of eczema, asthma, or hay fever. Antihistamines were withdrawn 72 hours before the study, and astemizole was withdrawn at least 6 weeks beforehand. No patient had been taking doxepin or other tricyclic antidepressant for at least 2 months, nor had any patient taken steroids or immunosuppressant drugs (cyclosporin or azathioprine) for at least 3 months before testing.

Eighteen healthy control subjects were recruited, with a mean age of 41 years (range, 23 to 78 years), and 5 were men. None had eczema or asthma, but 2 had a current or previous history of allergic rhinitis.

Reagents

22E7 is a mouse anti-human mAb that directly cross-links FcεRI. The binding of 22E7 to FcεRI is not inhibited by bound IgE.[21] The 22E7 was a kind gift from Dr Richard Chizzonite and Dr Jarema Kochan from Hoffman LaRoche (Nutley, NJ). Goat polyclonal anti-IgE antibody and formyl-methionyl-leucyl-phenylalanine (fMLP) were obtained from Sigma Chemicals (Poole, UK).

Basophil releasability assays

Histamine release assays were performed essentially as previously described.[12,14] Briefly, 30 mL of heparinized blood from patients with CIU and control subjects was mixed with 10 mL of 1% methyl cellulose in 0.9% saline and allowed to sediment for 30 minutes. Mixed leukocytes were recovered from the supernatant by centrifugation, washed twice in calcium/magnesium free assay buffer,[14] and resuspended in complete assay buffer containing 2 mmol/L Ca^{++} and 1 mmol/L Mg^{++}. The cells were dispensed into duplicate assay tubes containing the histamine-releasing stimuli 22E7 (dose response 3.2-20,000 ng/mL final concentration), polyclonal goat anti-IgE (1/3000 dilution or 15.5 μg/mL) and fMLP (10^{-6} mol/L), and 0.9% saline control, giving a final volume of 0.1 mL. The samples were incubated for 40 minutes at 37° C, and reactions were then stopped by cooling the tubes on ice, adding 0.8 mL of ice-cold assay buffer, and separating the cells by centrifugation at 500 *g* for 5 minutes. Histamine in the supernatant and the residual histamine in the cell pellet were measured spectrofluorometrically[22] after precipitation of protein with perchloric acid, as described previously.[12] The minimum detection limit of histamine was 0.1 ng/mL. Histamine release (histamine in the supernatant) was expressed as the percentage of the total histamine content in each assay tube. Values were corrected by subtraction of the spontaneous histamine release found when basophil leukocytes were incubated with buffer alone.

Identification of patients with circulating autoantibodies

Serum-evoked histamine release from basophils of healthy donors was used to identify patients with CIU and control subjects with circulating functional anti-FcεRIα and/or anti-IgE autoantibodies. The methods (using 2-fold dilution of sera from each subject) and basophil donors were the same as previously described.[12,14] However, because the serum IgE level in donor 1 had increased from 1 to 2 kU/L, with a consequent increase in basophil reactivity to polyclonal goat anti-IgE, the mixed leukocyte preparation of donor 1 was additionally treated with 10 mmol/L lactic acid (pH 3.9) for 3.5 minutes at room temperature to dissociate FcεRI-bound IgE.[13,23] Polyclonal goat anti-IgE (1/3000 dilution) evoked a mean ± SEM histamine release from IgE-stripped basophils of donor 1 of 1.8% ± 0.3% (n = 3) compared with 58.7% ± 4.1% (n = 6) from donor 2, whose basophils were endogenously sensitized with IgE (serum IgE = 104 kU/L). Stimulation with the mAb to FcεRI, 22E7, at a suboptimal concentration of 80 ng/mL, induced a similar percentage histamine release from the basophils of both donors (donor 1: 23.1%, n = 3; donor 2: 26.0%, n = 4).

Histamine release by the subject's serum was considered positive if 5% or greater of the total histamine was released after correction for spontaneous histamine release.

Basophil counts and histamine content

In 14 control subjects and 25 patients, a sample of the mixed leukocyte cell suspension prepared for the basophil releasability assay was diluted 1:10 in a toluidene blue–based metachromatic stain.[24] Basophils and the total number of white cells were counted with an Improved Neubauer hemocytometer to give a differential basophil count. If no basophils were present in a field of at least 2000 cells, the result was expressed as the maximum likely value of 1 basophil per 2000 cells. To obtain an absolute value for basophils in blood and to correct for cells lost during the washes in the basophil releasability assays, the differential basophil counts were corrected to a total white cell count obtained from an automated full blood count performed on K_2EDTA anticoagulated whole blood taken at the same time as blood for the releasability assay.

The histamine content of the mixed leukocyte suspension was calculated from the sum of the histamine levels measured in the supernatants and pellets in each basophil releasability experiment.

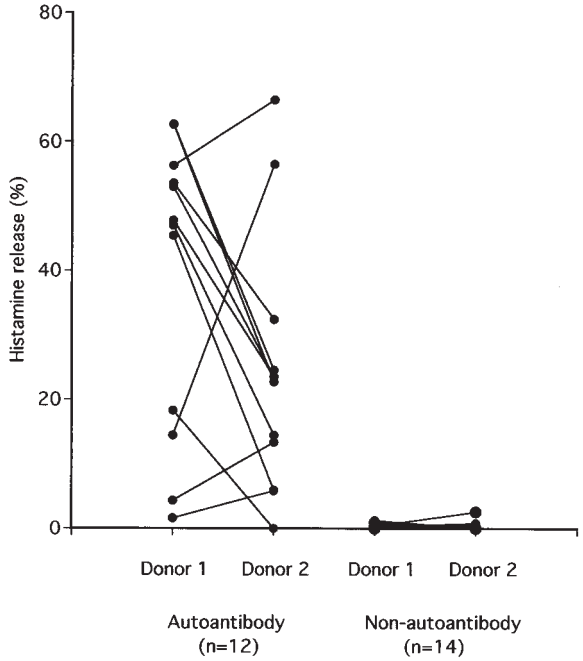

FIG 1. Percentage histamine release from basophils of 2 healthy donors induced by serum from 26 patients with CIU. Graph indicates how patients with CIU can be divided into 2 groups: those with serum histamine–releasing activity on basophils of 1 or both donors and those without. On the basis of previous work, serum histamine–releasing activity can be taken to indicate the presence of functional autoantibodies.[14]

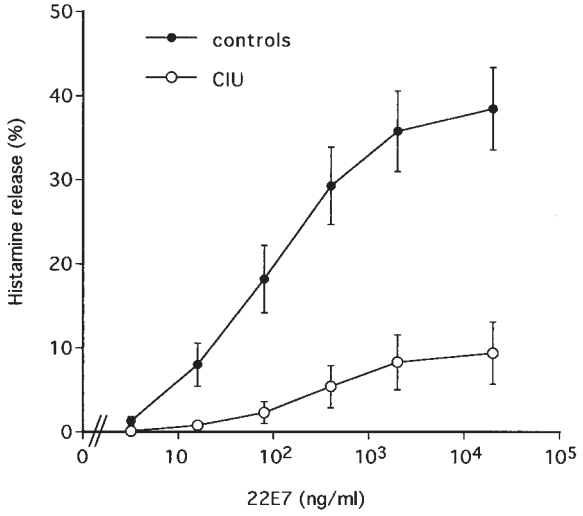

FIG 2. Mean percentage histamine release to mAb to FcεRI, 22E7 (3.2 to 20,000 ng/mL) from the basophils of 18 control subjects and all 26 patients with CIU. *Error bars* indicate 1 SEM.

Serum IgE levels

Total IgE levels were measured in serum samples from all subjects by radioimmunoassay (Pharmacia, Milton Keynes, UK). The manufacturer's normal range was 1 to 80 kU/L.

Statistical analysis

Statistical analysis was performed by using the Mann-Whitney U test for unpaired data.

RESULTS

Identification and characterization of subjects with autoantibodies

Subjects were identified as having functional autoantibodies by the magnitude of their serum-evoked histamine release from the basophils of 2 healthy donors in vitro. Serum from 12 of the 26 patients with CIU released 5%

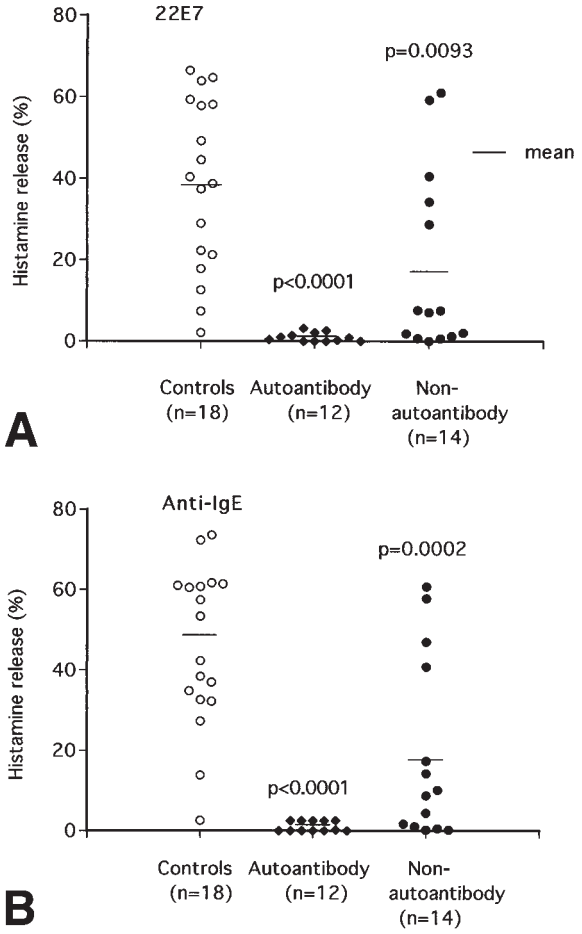

FIG 3. A, Maximum percentage histamine release to 22E7 from basophils of control subjects and patients with and without autoantibodies. **B,** Percentage histamine release to anti-IgE (1/3000) from basophils of control subjects and patients with and without autoantibodies.

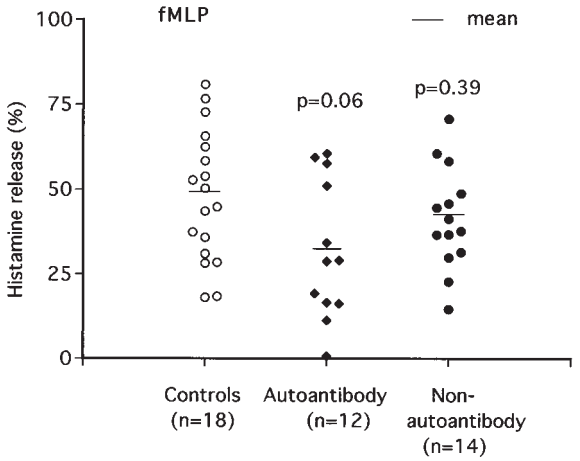

FIG 4. Percentage histamine release to fMLP (10^{-6} mol/L) from basophils of control subjects and patients with and without autoantibodies.

or greater histamine from the basophils of 1 or both of the healthy donors, indicating the presence of functional anti-FcεRI and/or anti-IgE autoantibodies (Fig 1). Serum from the remaining 14 patients with CIU and all 18 control subjects did not release histamine from the basophils of either donor.

Patients with autoantibodies had significantly higher urticaria scores than those without, the mean ± SEM urticaria severity score being 3.1 ± 0.2 and 2.0 ± 0.3, respectively (P = .0062).

In patients with CIU, the mean ± SEM serum IgE concentration was 76.0 ± 14.5 kU/L, which was significantly higher than that of the control subjects (45.6 ± 20.7 kU/L; P = .014). The higher levels in patients with CIU was largely due to patients without autoantibodies (101.7 ± 17.7 kU/L) who had significantly higher levels than patients with autoantibodies (46.1 ± 21.3 kU/L; P = .0047).

Basophil releasability

Comparison of all patients with CIU with control subjects. The mAb, 22E7, induced concentration-dependent histamine release from the basophils of patients with CIU and control subjects. Histamine release was significantly reduced in the 26 patients with CIU compared with the 18 healthy control subjects at all concentrations of 22E7 16 ng/mL or greater (P < .01; Fig 2). The mean ± SEM percentage histamine release to the maximum dose of 22E7 (20,000 ng/mL) from the basophils of control subjects and patients was 38.5% ± 5.0% and 9.4% ± 3.7%, respectively (P < .0001).

The responses to goat polyclonal anti-IgE antibodies were very similar to those of 22E7. The mean ± SEM percentage histamine release to anti-IgE (1/3000) in control subjects and all 26 patients with CIU was 45.7% ± 4.7% and 10.3% ± 3.7 %, respectively (P < .0001).

Comparison of patients with CIU with and without autoantibodies and control subjects. The reduction in 22E7-evoked histamine release was most marked in the 12 patients with CIU with autoantibodies who had a significantly lower percentage histamine release to 22E7 than the 14 patients without autoantibodies. The mean ± SEM maximum histamine release values for patients with and without autoantibodies were 1.0% ± 0.3% and 17.9% ± 6.0%, respectively (P = .011; Fig 3, *A*). All patients with autoantibodies and 6 of 14 of the patients without autoantibodies, but only 1 of 18 control subjects, released less than 5% of their total histamine content to 20,000 ng/mL 22E7.

The basophil responses to anti-IgE stimulation were similar to those to 22E7 (Fig 3, *B*). The mean ± SEM percentage histamine release to anti-IgE (1/3000) in control subjects, patients with autoantibodies, and patients without autoantibodies was 45.7% ± 4.7%, 0.2% ± 0.2%, and 18.9% ± 6.0%, respectively. Basophils that were nonreleasers to 22E7 also failed to release histamine in response to anti-IgE.

To estimate whether there was any difference between patients with CIU and control subjects in the sensitivity of their basophils to stimulation by 22E7, the concentration of 22E7 required to give 50% of the maximum histamine release was also calculated, excluding subjects with basophils that released less than 5% of their total histamine content. The mean ± SEM concentration of 22E7 required for control subjects (n = 17) and patients (n = 8, all without antibodies) was 222 ± 49 and 564 ± 380 ng/mL, respectively (P = .025).

fMLP-induced histamine release. There was no significant difference between the control subjects and patients with CIU in basophil histamine release to fMLP (Fig 4). The mean ± SEM percentage histamine release to fMLP in control subjects, the 12 patients with autoantibodies, and the 14 patients without autoantibodies was 47.7% ± 4.6%, 32.1% ± 5.9%, and 41.4% ± 4.0%, respectively.

Basophil numbers and total cellular histamine in the mixed leukocyte suspension

The mean ± SEM stainable absolute basophil count was significantly reduced in patients with CIU (21 ± 6 × 10^6 cells/L blood, n = 23) compared with control subjects (52 ± 7 × 10^6 cells/L, n = 14; P = .0005). This reduction was largely due to depletion of basophils in the patients with autoantibodies (5 ± 1 × 10^6 cells/L, n = 10; P < .0001), although there was also a significant reduction in patients without autoantibodies (34 ± 9 × 10^6 cells/L, n = 13; P = .04; Fig 5, *A*).

In agreement with basophil numbers, the mean total cellular histamine in the mixed leukocyte suspension, as estimated from the sum of the histamine levels in each pellet and supernatant in basophil releasability assays, was significantly lower in patients with CIU than in control subjects (P < .0001), patients with autoantibodies having significantly lower values than those without autoantibodies (P < .0001; Fig 5, *B*). The mean ± SEM estimated total cellular histamine (ng/mL of mixed leukocyte suspension) in control subjects, patients with autoantibodies, and patients without autoantibodies was 47.7 ± 5.3, 5.5 ± 1.3 (P < .0001) and 30.0 ± 5.6 (P = .0083), respectively.

Basophil histamine content

There was no significant difference between the amount of histamine per basophil in patients with CIU with or without autoantibodies or control subjects, the mean ± SEM histamine per basophil (pg/cell) being 1.5 ± 0.4 (n = 11), 1.3 ± 0.1 (n = 14), and 1.4 ± 0.1 (n = 14), respectively.

DISCUSSION

Previous studies have shown that patients with CIU have reduced basophil histamine release to anti-IgE stimulation compared with healthy subjects,[4,9] but the mechanisms for the low releasability have not been fully elucidated. Greaves et al[9] proposed a qualitative abnormality of cell-bound IgE, but Kern and Lichtenstein[4] suggested an early postreceptor defect in basophils, which occurred after cross-linking of receptor-bound IgE but before degranulation, and suggested that this was similar to desensitization. We compared basophil stimulation evoked by cross-linking FcεRI, either indirectly by using anti-IgE antibody or directly with the mAb 22E7, which binds to FcεRI independently of bound IgE, in patients with CIU and control subjects. Our findings of similar reductions in histamine release to 22E7 and anti-IgE in patients with CIU is conclusive evidence for a defect arising in the basophil and not in the structure or function of receptor-bound IgE.

Previous studies have suggested that the defect in basophil histamine release in CIU is an acquired rather than an innate property of basophils.[4] There is now substantial evidence for an autoimmune pathogenesis in some patients with CIU. The recent identification of histamine-releasing autoantibodies directed against the α-chain of FcεRI and/or IgE in 30% to 50% of patients with CIU[12-16,25] provides a possible explanation for

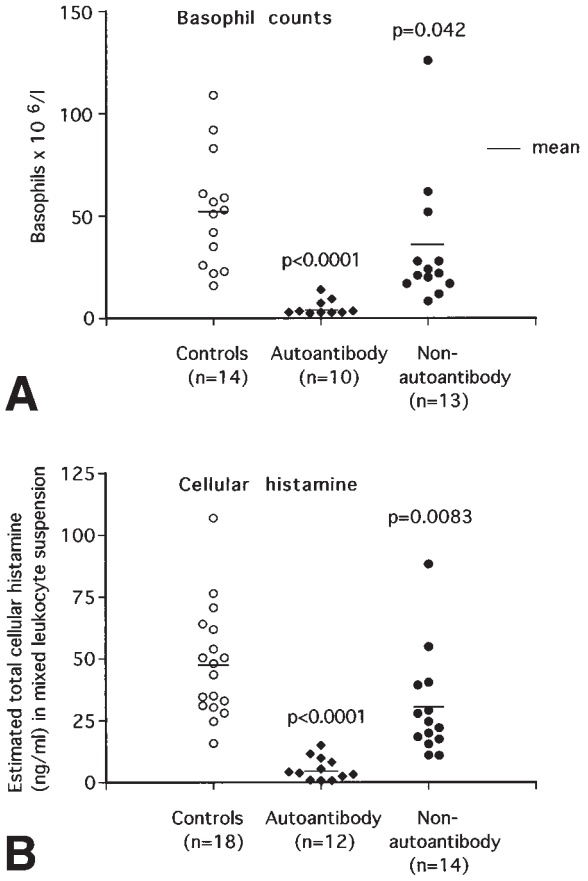

FIG 5. A, Absolute basophil counts in control subjects and patients with and without autoantibodies. **B,** Total cellular histamine in mixed leukocyte suspension, as estimated from sum of histamine levels detected in supernatants and pellets in control subjects and patients with and without autoantibodies.

reduced basophil releasability in CIU. Twelve (46%) of the patients with CIU included in this study had serum histamine–releasing activity indicative of anti-FcεRI antibodies and/or anti-IgE autoantibodies. Our finding that all patients with CIU in this subset were nonresponders to stimulation with 22E7 and anti-IgE suggests that continual exposure of basophils to circulating anti-FcεRI antibodies and/or anti-IgE autoantibodies may lead to desensitization of FcεRI-dependant histamine release. The ability of fMLP, which acts through an independent receptor,[6] to evoke similar histamine release from basophils of control subjects and patients with CIU with and without autoantibodies is consistent with specific desensitization of FcεRI-dependant basophil activation by anti-FcεRI and/or anti-IgE autoantibodies.

In agreement with Rorsman,[18] we found reduced basophil numbers in patients with CIU compared with control subjects. The most pronounced decrease was in patients with CIU with autoantibodies, in whom basophil counts were usually at the lower limit of detection for a differential count (1 basophil per 2000 leukocytes). This finding suggests that histamine-releasing autoantibodies not only desensitize basophils but may also enhance their removal from the circulation. Failure to identify degranulated or partially degranulated basophils by metachromatic staining is unlikely to account for the basopenia because it has been shown previously that basophil counts obtained after staining correlate with flow cytometric basophil counts in CIU.[19] Additionally, in this study there was agreement between basophil numbers obtained by counting and the total cellular histamine content estimated from the mixed leukocyte preparations and also with the total blood histamine levels (data not shown).

Assuming that our cell counts accurately reflect the true number of circulating basophils in CIU, basophils from patients with and without autoantibodies and control subjects contained similar amounts of histamine per cell. Thus patients with CIU with autoantibodies appear to have a small residual pool of basophils with a normal histamine content but which are unresponsive to FcεRI-dependent stimulation, although normally responsive to FcεRI-independent (fMLP-induced) activation. It is unclear why the residual pool of basophils in patients with CIU with autoantibodies is unresponsive to stimuli acting through FcεRI. It could be speculated that these are immature basophils prematurely released from the bone marrow, or alternatively the basophils could be desensitized either by repetitive stimulation of FcεRI or possibly by a mechanism involving negative signaling through the FcγRII subtype of IgG receptors.[26,27] Because normal basophil numbers are seen in the bone marrow in urticaria,[28] the basopenia would appear to be due to increased basophil removal from the circulation rather than to decreased production. Additionally, basophil counts have been reported to return to expected levels after recovery from urticaria,[29] and total cellular histamine levels can return to normal after plasmapheresis in severe CIU,[30] indicating that basopenia is disease related and not a preexisting property of basophils in patients with CIU.

Reduced basophil releasability and basophil numbers were also found in some of the autoantibody-negative patients with CIU, although fewer patients were involved, and reductions in basophil numbers and basophil histamine release were less pronounced. Additionally, basophils from patients with CIU that released greater than 5% of their histamine to 22E7 (8 patients without autoantibodies only) appeared to be less sensitive to stimulation by 22E7 than histamine-releasing basophils from healthy subjects (17 of 18), as indicated by the higher concentration of 22E7 required to produce 50% of the maximum basophil histamine release in the 8 patients with CIU. One possible explanation for these observations is that some patients have sufficient circulating autoantibody activity in vivo to affect basophil releasability and numbers but insufficient activity at a 2-fold serum dilution in vitro to evoke histamine release from the basophils of donor 1 or 2. Alternatively, some patients may have anti-FcεRI autoantibodies that do not release histamine from basophils[15] but that can downregulate FcεRI expression. Basophil histamine release may also be influenced by disease activity. Indeed, in this study autoantibody-negative patients with CIU had less active urticaria than those with autoantibodies. This may explain why Kern and Lichtenstein[4] did not find basopenia in their patients with CIU, although the distributions of the histamine release values for anti-IgE–stimulated basophils of both patients with CIU and healthy control subjects were similar to values obtained in this study. Finally, basophil histamine release in response to anti-IgE varies widely in healthy subjects in the general population, ranging from no histamine release in 2% to 22% of subjects to release of up to approximately 80% of the total histamine content in others.[1,4,5]

It seems unlikely that atopy influenced basophil releasability or basophil counts in this study. Ten patients with CIU had a personal history of atopy, which was usually mild and not active at the time of study. These patients were almost equally divided between the autoantibody (4 of 12 patients were atopic) and nonautoantibody (6 of 14 patients were atopic) groups. There was no significant difference between atopic and nonatopic patients in their basophil histamine release to 22E7 or fMLP or their basophil counts. Overall, the mean serum levels of IgE were slightly higher in patients without autoantibodies, but the maximum IgE levels were similar at 272 and 255 kU/L in patients with and without autoantibodies, respectively.

Anti-IgE autoantibodies can only be functionally effective in patients with IgE-sensitized basophils. Additionally, although not essential for anti-FcεRI autoantibody activity, receptor-bound IgE may modulate anti-FcεRI autoantibody-induced basophil down regulation. The pattern of serum-induced histamine release from the basophils of donor 1 and donor 2 is indicative of the type of autoantibody present. The basophils of donor 1 were not sensitized to IgE and did not release histamine to

anti-IgE, whereas those of donor 2 were sensitized to IgE and released histamine in response to anti-IgE and only those anti-FcεRI antibodies that could bind FcεRI in the presence of bound IgE. Serum from 10 of 12 patients with CIU with autoantibodies released histamine from the basophils of donor 1, indicating the presence of anti-FcεRI autoantibodies. Four patients had serum that induced higher histamine release from the basophils of donor 2 than from those of donor 1, suggesting anti-IgE activity. Two of these 4 patients also had anti-FcεRI autoantibodies. All except 1 of the 12 patients with CIU had a serum IgE level of greater than 2 kU/L, which in our experience is sufficient to sensitize basophils for anti-IgE–evoked histamine release. Serum from this single patient caused 18% histamine release from donor 1, but no histamine release from donor 2, which indicates the presence of anti-FcεRI autoantibodies, which are inhibited by receptor-bound IgE. Because the patient had low levels of serum IgE, it is likely that their basophils were not sensitized to IgE and could therefore still show autoantibody-mediated downregulation. Serum from the other 11 patients caused histamine release from the basophils of donor 2 (IgE sensitized), indicating the functional activity of their anti-FcεRI autoantibodies in the presence of receptor-bound IgE.

Two patients with autoantibodies had very low levels of histamine in the pellets (0.3 to 0.6 ng/mL) and undetectable levels of histamine (<0.1 ng/mL) in the supernatants of almost all samples. These were included as the maximum percentage histamine release possible assuming 0.1 ng/mL histamine in the supernatant. Because histamine concentrations were at the lower limit of detection in some assays, particularly in those for patients with autoantibodies, it was difficult to accurately determine spontaneous histamine release. However, the mean spontaneous histamine release for patients with and without autoantibodies and controls was 6.1%, 2.1%, and 1.7%, respectively.

We conclude from our findings that anti-FcεRI and/or anti-IgE autoantibodies are likely initiators of reduced histamine releasability and basopenia in many patients with CIU, with induction of basophil downregulation being slightly more sensitive to the presence of autoantibodies than basopenia. The altered basophil releasability and basopenia are likely to be acquired features in CIU, rather than a preexisting property of the basophil. Mechanistically, bound autoantibodies may lead to FcεRI internalization and/or desensitization. Bound IgG autoantibodies may also increase removal of basophils from the circulation by the mononuclear phagocyte system, leaving a small population of basophils that are inherently unresponsive to FcεRI-mediated activation. Mast cells are the most important source of histamine in the skin and the primary effector cell in CIU; however, there is controversy as to whether cutaneous mast cell releasability[7,31] or numbers[32,33] are altered in CIU, although there is evidence that rat peritoneal mast cells can be desensitized to antigen.[34] Thus the relationship between basophil and mast cell numbers and releasability remains to be determined in CIU.

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