Classification of anti-FcεRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity

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Background: Circulating autoantibodies against FcεRI, IgE, or both occur in approximately one third of patients with chronic idiopathic urticaria (CIU), but not all autoantibodies initiate histamine release.

Objective: We sought to classify patients with CIU into subsets on the basis of serum bioactivity and immunoreactivity and to examine the relationship between newly defined subtype and disease severity.

Methods: Sera from patients with CIU (n = 78), dermographism (n = 15), and cholinergic urticaria (n = 10) and sera from healthy subjects (n = 39) were analyzed by means of Western blot analysis for anti-FcεRI autoantibodies and for histamine release from basophils and dermal mast cells. In vivo reactivity of autologous serum was tested by means of intradermal injection, and CIU severity was determined on the basis of clinical interview.

Results: We classified sera from patients with CIU into 5 subsets: immunoreactive histamine-releasing anti-FcεRI autoantibodies (n = 20 [26%]); immunoreactive anti-FcεRI autoantibodies without histamine-releasing activity (n = 12 [15%]); anti-IgE–like autoantibodies (n = 7 [9%]); serum containing a mast cell–specific histamine-releasing factor (n = 7 [9%]); and sera with no identifiable factor (n = 32 [41%]). Patients with serum histamine-releasing activity had more severe urticaria than patients without such activity. Positive skin test responses to autologous sera were associated with histamine-releasing anti-FcεRI autoantibodies but not with non–histamine-releasing anti-FcεRI autoantibodies. Neither healthy subjects nor patients with dermographism or cholinergic urticaria had histamine-releasing anti-FcεRI autoantibodies.

Conclusion: These data support the specificity of functional anti-FcεRI autoantibodies to CIU. The identification of distinctive subsets of patients suggests that other pathogenic mechanisms occur in CIU in addition to direct ligation of FcεRI by autoantibodies causing dermal mast cell degranulation. Elucidating these mechanisms might lead to new treatments for CIU. (J Allergy Clin Immunol 2002;110:492-9.)

Key words: *Chronic idiopathic urticaria, FcεRI, autoantibodies, histamine, basophils, human dermal mast cells*

Chronic idiopathic urticaria (CIU) is characterized by transient cutaneous wheals occurring most days for at least 6 weeks.[1] Approximately one third of patients with CIU have circulating autoantibodies against FcεRI.[2-7] These were initially identified on the basis of the release of histamine from basophils and dermal mast cells in vitro,[2,3] and their presence subsequently was confirmed by means of Western blot analysis,[4,6,7] ELISA,[5] and β-hexosaminidase release from rat basophil leukemia cells.[6] About 10% of patients with CIU have histamine-releasing anti-IgE autoantibodies.[3] The pathogenic mechanism in the residual 50% to 60% of patients with CIU remains unknown. Patients with circulating histamine-releasing anti-FcεRI autoantibodies, anti-IgE autoantibodies, or both have more severe urticaria.[8]

Functional anti-FcεRI autoantibodies were thought initially to cause basophil and mast cell degranulation by means of direct ligation of FcεRI.[2] Subsequently, anti-FcεRI autoantibodies in CIU were identified as predominantly IgG1 and IgG3 complement-activating isotypes.[5] Serum complement inactivation and complement receptor blockade inhibit basophil histamine release by some CIU sera,[5,9,10] suggesting interaction of FcεRI and complement receptor signal transduction pathways, at least in some patients. Mast cells from different sites differ in their expression of complement receptors; for example, mast cells in the skin express C5a receptor, but those in the lung do not.[11] Therefore involvement of complement might explain the localization of mast cell activation mainly to the skin in CIU.

We now report the classification of patients with CIU into 5 groups on the basis of the detection of anti-FcεRI antibodies by means of Western blot analysis and serum-induced histamine release from basophils and cutaneous mast cells. For the first time we correlate the classification with in vivo skin reactivity to intradermal injection of

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Abbreviations used
cIgE: Chimeric human IgE antinitrophenylacetyl
CIU: Chronic idiopathic urticaria
HSA: Human serum albumin

autologous serum and to disease severity. We found minimal evidence for anti-FcεRI autoantibodies in patients with cholinergic urticaria and dermographism, confirming the specificity of functional autoantibodies to CIU.

METHODS

Subjects

Patients with CIU, dermographism, and cholinergic urticaria were recruited at St John's Institute of Dermatology. Dermographism and cholinergic urticaria were confirmed by means of standard challenge testing.[12] If there was any reason to suspect urticarial vasculitis from the history, a skin biopsy was performed, and if there were any features suggestive of urticarial vasculitis, such as leukocytoclasia, patients were excluded. Healthy control subjects were recruited from the staff at St Thomas's Hospital. All subjects were aged 15 years or older and provided written consent after oral and written explanation. The study was approved by the St Thomas' Hospital Research Ethics Committee. Relevant drugs were withdrawn before the study: antihistamines by 72 hours (except astemizole by 6 weeks); doxepin and other tricyclic antidepressants by 10 days; and corticosteroids, cyclosporine, and azathioprine by 4 weeks beforehand.

Materials

The mouse anti-human FcεRI mAb 22E7, which directly cross-links FcεRI and is not inhibited by receptor-bound IgE,[13] was a gift from Dr R. Chizzonite (Hoffman LaRoche, Nutley, NJ). Soluble recombinant FcεRI α-chain genetically fused to human serum albumin (rHSA-FcεRIα) for Western blot analysis was a gift from Dr F. Kricek (Novartis Research Institute, Vienna, Austria). Rabbit anti-human IgG $F(ab')_2$ and rabbit anti-human IgE horseradish peroxidase $F(ab')_2$ conjugates were obtained from Jackson ImmunoResearch Laboratories (Richmond, Calif), and chimeric human IgE antinitrophenylacetyl (cIgE) was obtained from Serotec (Oxford, United Kingdom).

Assays and skin tests

Western blot analysis for the detection of IgG anti-FcεRIα autoantibodies was as described previously.[4] The reactivity of cIgE with rHSA-FcεRIα served as a positive control and was used for semiquantitation of IgG anti-FcεRIα autoantibodies. The relative blot intensities of IgG to cIgE were used to scale autoantibody serum reactivity in each experiment: 3, IgG more intense than cIgE; 2, IgG equivalent to cIgE; 1, IgG less intense than cIgE; and 0, no detectable IgG signal. An IgG2a isotype mAb was used as a negative control.

Basophil histamine release assays were as previously described, using the same basophil donors.[2,3] The poorly IgE-sensitized basophils of donor 1 (serum IgE level, 1-2 kU/L) were additionally treated with lactic acid to strip residual receptor-bound IgE to ensure minimal responsiveness to anti-IgE receptor ligation.[14] The basophils of donor 2 were fully IgE sensitized. Mast cell histamine release assays from skin slices were as previously described,[3] with each serum assayed in 2 experiments using skin from different donors. Histamine release was considered positive if 5% or more of the total histamine was released after correction for spontaneous histamine release.

Autologous serum skin tests were as previously described,[15] with 50 μL of serum injected intradermally into the volar aspect of the forearm. A positive test result was a red wheal with a diameter of 1.5 mm or greater than that produced by a saline control at 30 minutes.[15]

Urticaria severity scores

Urticarial activity was estimated according to the number of wheals present at the assessment time and 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. Severity of itching at its worst was scored on a visual analogue scale (0-10).[8] The distribution of the wheals was scored one each (maximum, 7) for involvement of the face, mouth or tongue, scalp, trunk, limbs, palms, and soles. Associated symptoms were scored one each (maximum, 12) for nausea, diarrhea, abdominal pain, indigestion, wheeze or breathlessness, palpitations, flushing, joint pain, joint swelling, headache, malaise, and lassitude.[8]

Statistical analysis

Data were analyzed by using the statistical package Stata, version 6.0 (StataCorp, College Station, Tex). Histamine release and Western blot scores were compared by using nonparametric tests and trend analysis.[16] Autologous serum skin test data were analyzed with logit estimates of the odds ratio for a positive test result and calculation of sensitivities and specificities.[15] Mean clinical activity scores were compared by means of linear regression with robust standard errors to correct estimates, sensitivities and specificities with exact CIs, and significance tests for any nonnormality and unequal variance.

RESULTS

Identification of subjects with serum activity

The frequencies of positive skin test responses, serum-induced histamine release assays, and immunoreactive anti-FcεRI autoantibodies detected by means of Western blot analysis are compared in Table I. Eleven (14%) of 78 sera from patients with CIU were positive in all 5 assays, whereas 26 (33%) sera were consistently negative. Thirty-nine healthy control subjects, with 2 exceptions, had negative results in all assays: serum from one released 6% histamine from mast cells, and the second had a positive skin test response. Sera from 3 (20%) of 15 patients with dermographism had anti-FcεRIα autoantibodies, as determined by means of Western blot analysis (2 scored 1 and 1 scored 2), but none released histamine from basophils, and all had negative skin test responses. Sera from patients with cholinergic urticaria failed to release histamine from basophils of either donor and were negative on Western blot analysis and skin testing, except for that from one patient with a positive skin test response and a Western blot score of 1.

Classification of patients with CIU according to histamine release and Western blot reactivity

Sera from the 78 patients with CIU were classified into 5 subsets according to their predominant histamine-releasing activity and Western blot identification of anti-FcεRI autoantibodies (Fig 1 and Table II).

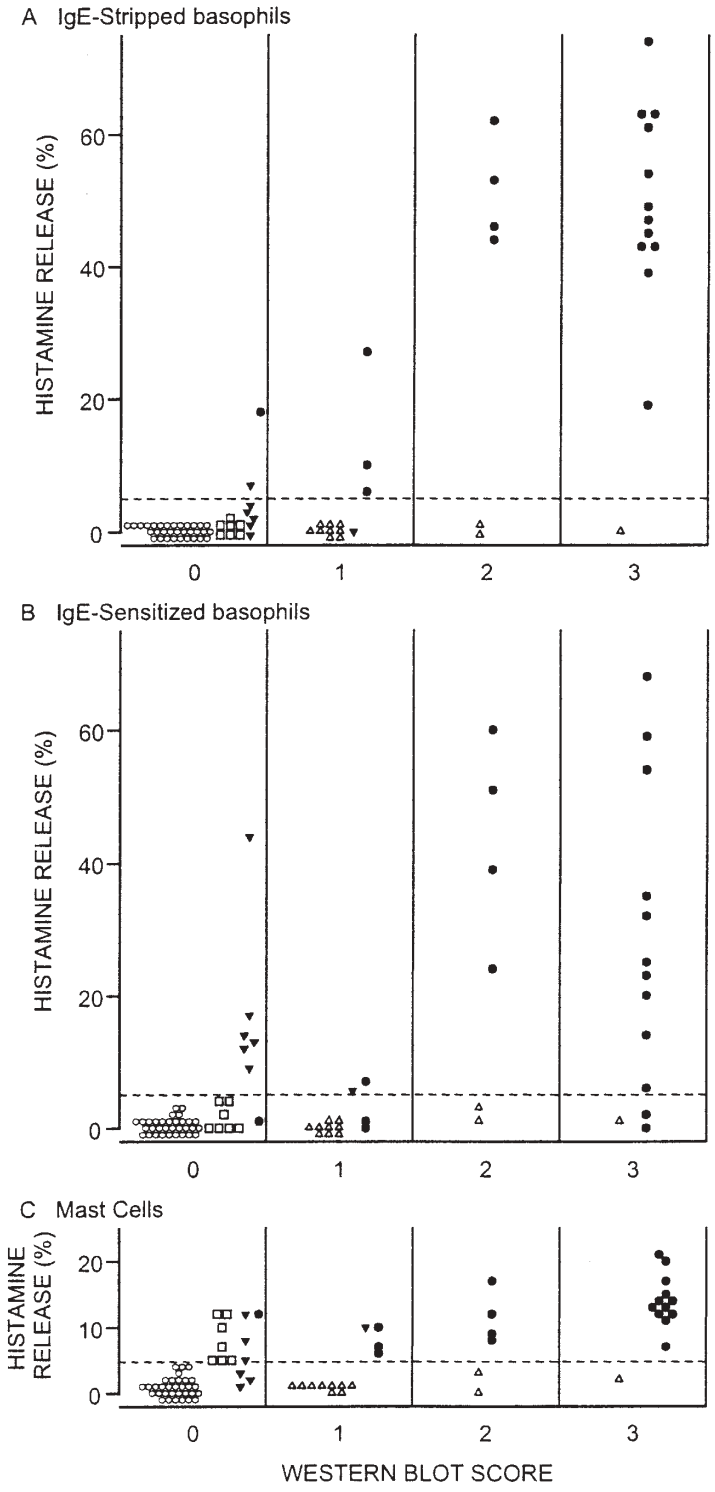

FIG 1. Patients can be subdivided into 5 categories depending on histamine release assays and Western blot analysis. Western blot analysis score (0-3) for anti-FcεRI autoantibodies in sera from 78 patients with CIU is plotted against histamine release from IgE-stripped basophils **(A)**, IgE-sensitized basophils **(B)**, and mast cells in skin slices **(C)**. Histamine release was considered positive if 5% or more of the total histamine was released, as indicated by the *dashed line. Filled circles,* IgE-stripped basophils (FcεRI histamine release positive); *filled inverted triangles,* IgE-sensitized basophils (anti-IgE); *open squares,* mast cell–specific histamine release; *open triangles,* no histamine-releasing activity, positive Western blot result (anti-FcεRI histamine release negative); *open circles,* no histamine-releasing activity, negative Western blot result.

TABLE I. Frequencies of positive test responses in sera from healthy control subjects and patients

Subjects	No. of subjects	No. of positive sera				
		Skin test	Histamine release			Western blot analysis
			IgE-stripped basophils	IgE-sensitized basophils	Mast cells	
Control subjects	39	1	0	0	1	0
Patients with CIU	78	27	21	22	31	32
Dermographic patients	15	0	0	0	NA	3
Cholinergic patients	10	1	0	0	NA	1

NA, Not analyzed.

TABLE II. Classification of 78 sera from patients with CIU by means of histamine release and Western blot reactivity into putative pathologic groups

CIU group	No. of positive sera				
	Histamine release			Western blot analysis	No. (%) of patients
	IgE-stripped basophils	IgE-sensitized basophils	Mast cells		
Anti-FcεRI HR$^+$	20	15	20	19	20 (26.9)
Anti-FcεRI HR$^-$	0	0	0	12	12 (16.6)
Anti-IgE	1	7	4	1	7 (9)
Mast cell specific	0	0	7	0	7 (9)
No identified factor	0	0	0	0	32 (41)

HR, Histamine release.

Twenty-one sera from patients with CIU released histamine from IgE-stripped basophils, of which 19 contained anti-FcεRI autoantibodies, as determined by means of Western blot analysis (Fig 1, *A*). The trend for higher histamine release with more intense blot scores was significant ($P < .01$, n = 21). One serum in this group showed substantially higher histamine release from IgE-sensitized than IgE-stripped basophils (see below) and had a negative Western blot result. The remaining 20 sera were classified as a histamine-releasing anti-FcεRI autoantibody subset (Table II).

Twenty-two sera, including 15 in the anti-FcεRI autoantibody subset, released histamine from IgE-sensitized basophils (Fig 1, *B*). In total, 27 sera caused histamine release from IgE-stripped basophils, IgE-sensitized basophils, or both. The ratio of histamine release from IgE-sensitized to that from IgE-stripped basophils for each sera was compared with that induced by the control anti-FcεRI mAb 22E7, the binding of which to FcεRI is not influenced by receptor-bound IgE (Fig 2). Eleven of the 27 sera had significantly lower histamine release ratios than 22E7, indicating the presence of anti-FcεRI autoantibodies, the activity of which was inhibited by receptor-bound IgE.

Conversely, 7 of the 27 sera had a significantly higher IgE-sensitized to IgE-stripped basophil histamine release ratio than 22E7 (Fig 2), and all released more than 5% histamine from IgE-sensitized basophils, indicating anti-IgE autoantibody activity (Fig 1, *B*). All 7 sera except one released less than 5% histamine from the IgE-stripped basophils (Fig 1, *A*) and, with one other exception, were negative, as determined by means of Western blotting, for immunoreactive anti-FcεRI autoantibodies (Fig 1, *B*). These 7 sera have been classified as an anti-IgE autoantibody subset (Table II). The 2 exceptional sera showed more than a 4-fold higher histamine release from IgE-sensitized basophils than from IgE-stripped basophils and, because of this, have been included in the anti-IgE antibody subset, although both might also contain anti-FcεRI autoantibodies.

Twelve sera contained anti-FcεRI autoantibodies, as determined by means of Western blot analysis, but failed to release histamine from either IgE-stripped basophils, IgE-sensitized basophils, or skin mast cells (Fig 1). These sera have been classified as the anti-FcεRI histamine release–negative autoantibody subset (Table II).

The magnitude of serum-induced histamine release from mast cells correlated with histamine release from the IgE-stripped ($r = 0.81$, $P < .01$, n = 78) and IgE-sensitized ($r = 0.71$, $P < .01$, n = 78) basophils. All 20 sera classified as having histamine-releasing anti-FcεRI autoantibodies and 4 of the 7 sera classified as having anti-IgE autoantibodies induced histamine release from skin mast cells (Fig 1, *C*). Of the 12 sera with immunoreactive anti-FcεRI autoantibodies that failed to induce basophil histamine release, none caused histamine release from mast cells. Seven sera released 5% or more histamine from mast cells but without histamine release from IgE-stripped or IgE-sensitized basophils or evidence of anti-FcεRIα autoantibodies, as determined by means of Western blot analysis, and have been classified as a mast cell–specific histamine-releasing subset. Thirty-two sera had no identifiable activity (Fig 1 and Table II).

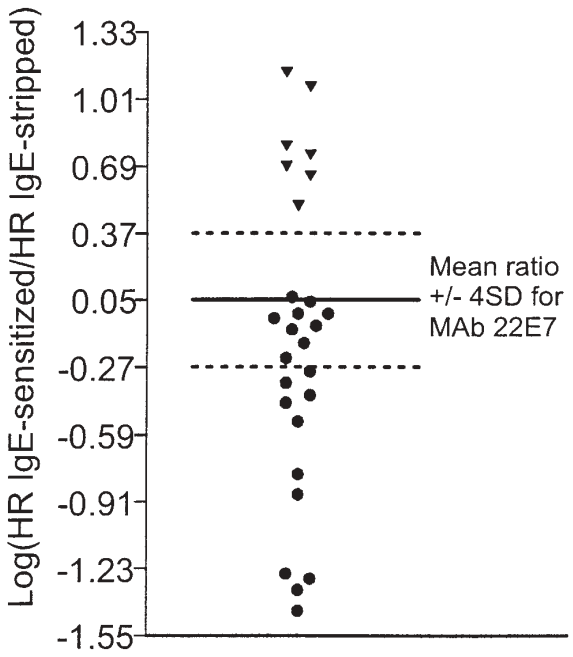

FIG 2. Identification of patients with predominant anti-IgE autoantibodies. Variation in the log ratio of histamine release *(HR)* from IgE-stripped and IgE-sensitized basophils is plotted for 27 patients with CIU with serum histamine-releasing activity. The mean log ratio (±4 SDs) for mAb 22E7 used as a control secretagogue, which is not competitive with IgE for binding to FcεRI, are shown by *solid lines* (mean) and *dashed lines* (±4 SDs; n = 4 experiments for IgE-stripped and IgE-sensitized basophils). Histamine release with mAb 22E7 from IgE-stripped and IgE-sensitized basophils was 23.1% ± 3.4% and 26.1% ± 3.0% (mean ± SD), respectively. As in Fig 1, *filled circles,* IgE-stripped basophils (FcεRI histamine release positive); *filled inverted triangles,* IgE-sensitized basophils (anti-IgE).

Autologous serum skin tests

Overall, 27 (35%) of 78 patients with CIU had a positive autologous serum skin test response (Table I). The odds ratio for a positive skin test response relative to the patient subset with no identifiable serum factor showed a strong effect for the histamine-releasing anti-FcεRI autoantibodies patient subset ($P > .001$) and the anti-IgE subset ($P > .048$) but not for the anti-FcεRI autoantibody histamine release–negative or mast cell–specific subsets (Table III). The specificities and sensitivities of the skin test for identification of histamine-releasing anti-FcεRI and anti-IgE autoantibodies for the 2 subsets analyzed individually and combined are shown in Table III. Sera from control subjects, with one exception, and from patients with dermographism and patients with cholinergic urticaria, with one exception, did not result in positive skin test responses.

Clinical features

The relationships between a patient's serum autoimmune activity and measures of CIU severity are shown in Fig 3. The differences between the mean scores of the 5 serum activity subsets were small for each of the 4 clinical measures of severity (Table IV). The histamine-releasing anti-FcεRI, anti-IgE autoantibody, and mast cell–specific histamine-releasing factor subsets showed consistently and significantly more severe clinical measures, both individually and in total, than patients with histamine release–negative anti-FcεRI autoantibodies or no identifiable serum activity (Table IV). Also, comparison of histamine-releasing and histamine release–negative anti-FcεRI autoantibody subsets showed significantly higher disease severity for 2 of 4 clinical measures and for the overall total score.

DISCUSSION

Differences in immunoreactivity and histamine-releasing activity enabled us to classify sera from patients with CIU into 5 subsets, implying that there is variation between patients in the pathogenesis of their CIU. Not all sera fall neatly into the classification, and 2 sera with properties suggestive of dual anti-IgE and anti-FcεRI reactivity were included in the anti-IgE subset. Alternate classification of these sera (in the histamine release–positive anti-FcεRI antibody subset for one serum and the histamine release–negative anti-FcεRI antibody subset for the second) did not affect interpretation of either skin test or clinical data. We cannot exclude other dual reactivities in serum, such as histamine-releasing anti-FcεRI autoantibodies coexisting with histamine release–negative anti-FcεRI autoantibodies or with the mast cell–specific histamine-releasing factor. The underlying pathogenesis for patients with no identifiable factor might coexist with autoimmunity.

The proportion of sera containing anti-FcεRI autoantibodies, as identified by means of Western blot analysis, but inactive for basophil degranulation was similar to that found previously by means of ELISA.[5] We now demonstrate that these autoantibodies also fail to degranulate mast cells, indicating that their lack of activity is not caused by unresponsive basophils but might be due to the autoantibodies recognizing epitopes on recombinant FcεRI used in immunoassays that are inaccessible on cell-surface receptors. This might provide one explanation for the lack of correlation between basophil histamine release and Western blot analysis found in a recent publication, a discrepancy that the authors found difficult to explain.[10] In the current study Western blot analysis shows that the mast cell–specific histamine-releasing factor is not an IgG anti-FcεRI autoantibody. This substantiates our preliminary findings that the factor was not an IgG species, although the kinetics for mast cell histamine release were comparable with those associated with FcεRI activation (unpublished data).

Serum histamine-releasing activity is an important determinant for urticarial severity. All patients in this study were recruited from a tertiary referral center, and therefore most had severe urticaria. This might partly account for the overlap in the severity of clinical features among the groups. However, mean severity scores ranked consistently higher for patients with functional autoanti-

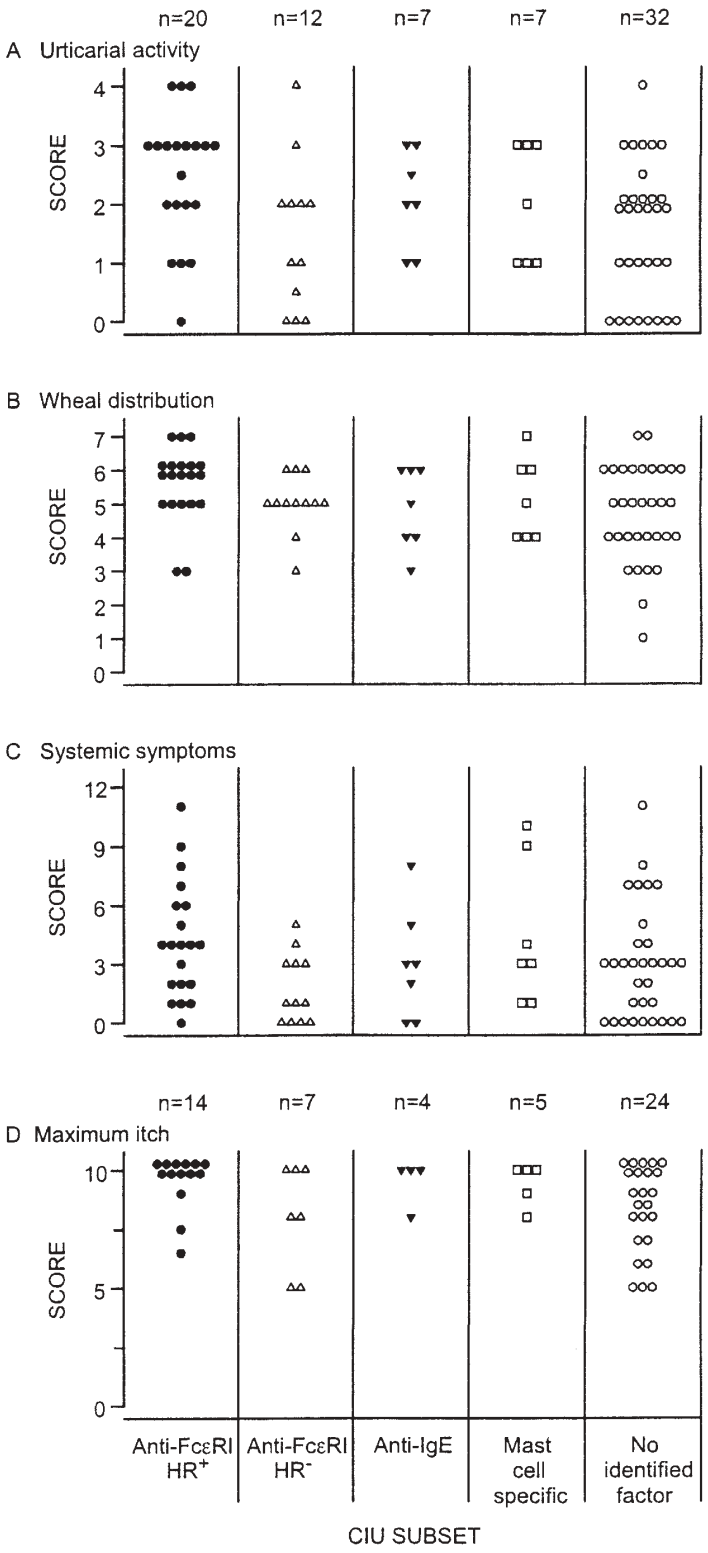

FIG 3. Relationship between CIU subset and the severity of clinical features. The CIU subset is plotted against urticaria activity score **(A)**, wheal distribution score **(B)**, number of associated systemic symptoms **(C)**, and worst episodes of itching **(D)**. Statistical analysis of data is given in Table IV. As in Fig 1, *filled circles,* IgE-stripped basophils (FcεRI histamine release positive); *filled inverted triangles,* IgE-sensitized basophils (anti-IgE); *open squares,* mast cell–specific histamine release; *open triangles,* no histamine-releasing activity, positive Western blot result (anti-FcεRI histamine release negative); *open circles,* no histamine-releasing activity, negative Western blot result.

TABLE III. Autologous serum skin test results in the 5 putative CIU subsets

	n	Positive skin test response	Odds ratio (CI) relative to group with no identified factor	Versus No identified factor		Versus No identified factor, mast cell specific and anti-FcεRI HR⁻ combined	
				Sensitivity, % (CI)	Specificity, % (CI)	Sensitivity, % (CI)	Specificity, % (CI)
Anti-FcεRI HR⁺	20	14	10.1 (2.7-31.3)	70 (46-88)	81 (64-93)	61 (39-80)	88 (75-95)
Anti-FcεRI HR⁻	12	2	0.9 (0.1-5.0)				
Anti-IgE	7	4	5.8 (1.0-33.0)	40 (12-74)	90 (73-98)	31 (9-61)	93 (82-99)
Mast cell specific	7	1	0.7 (0.1-7.2)				
No identified factor	32	6	1				
Anti-FcεRI⁺ and anti-IgE	27	18		75 (53-90)	74 (56-87)	67 (46-83)	82 (69-92)

Relationship is shown between in vivo skin response to autologous serum and the classification of sera from patients with CIU into 5 putative pathologic groups, as described in Fig 1 and Table II. The sensitivities and specificities are shown for the anti-FcεRI histamine release–positive and anti-IgE subsets combined, which was the classification used in our original study to determine the optimum criteria for skin testing.

TABLE IV. Clinical activity scores

	Mean clinical activity scores						
	Total patients (n = 78)				Total patients (n = 54)		
	n	Urticaria activity (0-4)	Wheal distribution (0-7)	Systemic symptoms (0-12)	n	Itching (0-10)	Total score (maximum, 4)
A							
Anti-FcεRI HR⁺	20	2.5	5.6	4.2	14	9.5	2.8
Anti-FcεRI HR⁻	12	1.5	5.0	1.8	7	8.0	2.1
Anti-IgE	7	2.1	4.9	3.0	4	9.5	2.6
Mast cell specific	7	2.0	5.1	4.4	5	9.4	2.5
No activity	32	1.5	4.7	2.9	24	8.3	2.1
B							
All HR⁺ sera	34	2.3	5.4	4.0	23	9.5	2.7
All HR⁻ sera	44	1.5	4.8	2.6	31	8.2	2.1
C							
Statistical comparisons*							
Anti-FcεRI HR⁺ vs anti-FcεRI HR⁻		1.0† (0.1 to 1.9)	0.6 (–0.1 to 1.3)	2.4† (0.8 to 4.1)		1.5 (–0.3 to 3.3)	0.7† (0.4 to 1.0)
All HR⁺ vs all HR⁻		0.8† (0.3 to 1.3)	0.6† (0.03 to 1.1)	1.4† (0.1 to 2.7)		1.3† (0.5 to 2.1)	0.6† (0.4 to 0.9)

Mean clinical activity scores for patients with CIU are classified in part A as defined in Table II and in part B on the basis of any serum histamine-releasing activity (IgE-sensitized and/or IgE-stripped basophils and/or mast cells) versus patients with no circulating histamine-releasing activity. Each clinical parameter was assessed in 78 patients, except for maximum itching, which was assessed in 54 patients. The total score is the sum of 4 normalized mean values (mean score/possible maximum score). Statistical data shown (part C) has been confined to the 2 most important comparisons.
*Values in part C are given as the difference between means (95% CI).
†Significant difference in subset means.

bodies and those with a mast cell–specific factor than for patients with non–histamine-releasing serum. The similar clinical scores for mast cell–specific factor and the anti-FcεRI and anti-IgE autoimmune patients suggest that it is mast cell degranulation, rather than basophil degranulation, that is important in disease severity. The autologous serum skin test provides evidence for cutaneous activity of serum factors. The criteria for a positive test response were defined to provide the optimum sensitivity and specificity for clinical identification of patients with basophil histamine-releasing anti-FcεRI autoantibodies, anti-IgE autoantibodies, or both.[15] Mast cell histamine-releasing activity of the serum mast cell–specific factor was not sufficient to elicit a positive skin test response in this study, although it was initially identified on the basis of a positive skin test response. The discrepancy might be due to the stricter criteria now used for the test.

Basophil histamine release by some sera is inhibited when cell-surface FcεRI is saturated with IgE, presumably because epitopes for the anti-FcεRI autoantibodies are at or close to the IgE binding site. This implies that such anti-FcεRI autoantibodies would be inactive in CIU pathology if mast cell anti-FcεRI was saturated with IgE. Dermal mast cells, however, rarely seem to be fully sensitized with IgE. Mast cells in 90% of skin samples release similar amounts of histamine when challenged with the IgE-inhibited anti-FcεRI mAb 6F7 as with the noninhibited mAb 29C6 (17.2% ± 8.9% and 15.9% ±

6.5% [mean ± SD], respectively; n = 23; unpublished data). In the present study all sera containing immunoreactive anti-FcεRI autoantibodies that degranulated basophils also caused mast cell histamine release. Furthermore, there was no association between disease severity and the ratio of histamine release from IgE-stripped and IgE-sensitized basophils. It seems unlikely that modulation of anti-FcεRI activity by mast cell receptor–bound IgE is a major regulatory factor in vivo. However, the failure of 3 sera with putative anti-IgE activity, on the basis of IgE-sensitized basophil histamine release, to release histamine from mast cells might be due to inadequate IgE sensitization of mast cell FcεRI.

We did not confirm the presence of anti-IgE antibodies by means of Western blot analysis in this study. However, we have previously published data supporting the identity of this histamine-releasing factor.[2,17,18] We have also independently shown, by means of Western blot analysis, that anti-IgE antibodies are found in approximately two thirds of patients with atopy or CIU and in about 20% of the healthy population.[4] Thus it is likely that if we screened the current serum samples for anti-IgE antibodies by using this method, we would identify a high percentage of patients and some of the healthy control subjects with non–histamine-releasing anti-IgE antibodies. We believe that it is the ability of these antibodies to release histamine that is of importance in CIU.

No identifiable circulating factor was found in 41% of the patients with CIU, a result similar to those of other studies on autoimmunity in CIU.[2-7] Pathogenic mechanisms in these patients remain unclear but might involve local generation of mast cell–activating factors or mast cell dysfunction. Indeed, similar mechanisms might be responsible for wheal formation in patients (16.6%) with non–histamine-releasing anti-FcεRI autoantibodies. The failure to detect histamine-releasing anti-FcεRI autoantibodies in cholinergic urticaria and dermographism is consistent with the findings of a recent study[19] and supports the hypothesis that functional anti-FcεRI antibodies are specific to CIU. It is of interest that autoantibodies were detected by means of Western blot analysis in some patients with physical urticarias and previously in other autoimmune diseases,[5] but these autoantibodies did not release histamine from basophils.

Treatment of patients with CIU is often variable and unpredictable, and for some patients, the disease is refractory to currently available therapies. The efficacy of different treatments might reflect the underlying pathologic mechanisms. The ability to identify the subtype of a patient with CIU would help to study and optimize the selection of therapy. This, at present, is difficult to achieve outside a specialist research clinic. Autologous serum skin testing is a simple clinical procedure. Its limitations are illustrated in our current and previous studies,[15] but it might provide useful information if laboratory tests are not available. Of the laboratory tests, Western blot analysis is probably the easier technique for identification of autoimmune patients, particularly if the specialist reagents required become commercially available. Histamine release assays provide important functional data and have been used as a gold standard to date. However, they require experience and careful standardization to provide comparable data in different assays, particularly if different donors are used. Results of all tests provide helpful information, and choice is likely to depend on local availability.

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