

Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria

C. E. H. GRATTAN, D. M. FRANCIS, M. HIDE and M. W. GREAVES

Institute of Dermatology, St Thomas's Hospital, London SE1 7EH, U.K.

Summary

Circulating histamine releasing factor(s) have been demonstrated previously in chronic urticaria by an immediate weal-and-flare response to intradermal autologous serum injection. We have studied 25 chronic urticaria patients by *in vivo* skin testing with autologous sera and an *in vitro* histamine release assay using mixed leukocytes of healthy donors, to define the nature and functional properties of the serum factor(s). Twenty showed a weal response to autologous serum (mean $\pm$ s.e.m., 37.3 ± 6.8 mm³). Weal formation was confined to ultrafiltered serum fractions >100 kD in nine of nine patients. There was no response in 10 healthy controls or five patients with symptomatic dermographism. Fourteen chronic urticaria sera elicited histamine release $>10\%$ (mean $\pm$ s.e.m., $44.3\% \pm 6.7$) above basal levels from leukocytes of at least one of seven healthy donors. This *in vitro* response was also confined to ultrafiltered serum fractions >100 kD in seven of seven sera and was present in IgG fractions of six of seven chronic urticaria sera that showed histamine releasing activity. Functional studies indicated that this histamine releasing autoantibody had the properties of anti-IgE: chronic urticaria sera 'desensitized' basophil leukocytes to subsequent challenge with other chronic urticaria sera and to goat anti-human IgE antibody; human myeloma IgE inhibited histamine release from leukocytes in response to chronic urticaria sera; removal of surface-bound IgE by lactic acid 'stripping' reduced histamine release in response to chronic urticaria sera and anti-IgE and subsequent passive sensitization with IgE myeloma serum partially restored it. Stainable peripheral blood basophils/mm³ in chronic urticaria patients were significantly reduced (mean $\pm$ s.e.m., 7.9 ± 2.0) when compared to healthy controls (39.6 ± 4.4), $P < 0.001$. These results suggest that histamine releasing autoantibodies are important in the pathogenesis of chronic urticaria by stimulating or facilitating degranulation of basophils and cutaneous mast cells through cross-linking cell surface IgE receptors.

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Introduction

Chronic urticaria (CU) is characterized by recurrent weals lasting up to 24 hr and may run a course of months or years. Investigations are usually unhelpful and a cause can only be identified in a minority of patients [1]. An increased incidence of thyroid autoantibodies offers indirect evidence of an autoimmune basis [2]. Skin testing with autologous serum has been shown to elicit an

immediate weal-and-flare response in the majority of CU patients during disease activity but not during clinical remission [3]. This cutaneous response provides an *in vivo* marker of histamine releasing activity in serum [4]. Histology of autologous serum skin test biopsies from CU patients showed mixed neutrophil, mononuclear and eosinophil infiltrates, resembling the late phase response of immediate hypersensitivity reactions in atopics and electron microscopy showed mast cell degranulation [5]. The nature of the histamine releasing factor has remained uncertain.

Correspondence: Dr C. E. H. Grattan, Dermatology Department, Norfolk & Norwich Hospital, Norwich, Norfolk NR1 3SR, U.K.

We aimed to define the functional activity and molecular weight range(s) of the histamine releasing factor(s) in CU sera by *in vivo* skin testing and *in vitro* histamine release studies using mixed leukocytes.

Methods

Patients and control subjects

Twenty-five patients with CU, defined as recurrent weals lasting less than 24 hr and occurring at least twice a week for over 2 months, were recruited [18 female; mean age $\pm$ s.e.m., 43.3 years $\pm$ 2.9]. Patients with clinical evidence of urticarial vasculitis or predominant physical urticaria were excluded. All were negative for hepatitis B antigen. Antinuclear antibody assayed on rat liver substrate was 1/40 or less in all but one patient (1/160). Mean duration of CU was 16.9 months $\pm$ 5.2. Sixteen patients gave a history of exacerbation of weals at sites of pressure which is a well documented association [6], 10 had had a previous episode of CU, 21 had angio-oedema of eyelids, mouth or genitalia and six a personal history of atopic eczema, asthma or hayfever. Eighteen of 24 patients who were questioned admitted to systemic symptoms during severe attacks, including depression, lassitude, fatigue, aching, shivering and flushing. None were taking steroids or immunosuppressive therapy at the time of investigation. Control subjects were 10 healthy laboratory staff [six female, mean age 34.6 years $\pm$ 6.1] without urticaria and five patients with symptomatic dermographism [three male, mean age 43.2 years $\pm$ 5.1, mean duration of disease, 21.4 months $\pm$ 4.3]. Two healthy controls and one dermographic patient gave a personal history of atopy.

Skin tests

Patients discontinued antihistamines at least 48 hr before skin testing; none were taking the long-acting antihistamine, astemizole. Venous blood was clotted for 30 min at room temperature in sterile glass tubes or anticoagulated with 100 U preservative-free heparin in disposable Universal® tubes (Sterilin, Hounslow, U.K.). Sera and plasma were separated by centrifugation. Fifty microlitre aliquots were injected intradermally into volar forearm skin with 27 G needles after measuring baseline skin fold thickness (mm) with a low tension spring-loaded caliper (Mitutoyo, Japan). Serum and plasma skin tests were accompanied by an adjacent control injection of phosphate buffered saline (PBS), pH 7.2. Skin fold thickness and the perpendicular diameters (mm) of any palpable oedema were measured at 60 min because it had been shown in a previous study that the weal response to autologous serum increased up to 2 hr in contrast to the histamine response which diminished after 30 min [4].

Weal volumes (mm³) were calculated as the product of weal area and depth [(60 min skin fold thickness – baseline)/2]. Autologous serum and plasma skin test weal volumes were corrected for non-specific effects of injection by subtracting PBS-injected weal volumes.

Laboratory analysis

Ultrafiltration. Sera were filtered at 4°C under nitrogen through YM100 membranes (Amicon Corporation, Gloucester, U.K.) with a 100 kD cut off until at least 2/3 of the sample had passed through, then the filtrates were passed sequentially through C30 (30 kD cut off) and C10 (10 kD cut off) Centricon® microconcentrators (Amicon). Filtrates and retentates were made up to the applied volume with phosphate-buffered saline (PBS) after each filtration. Fractions > 100 kD, 100–30 kD, 30–10 kD and < 10 kD were sterilized through 0.22 µm disposable filters (Millex-GV®, Millipore, Watford, U.K.) and stored at –70°C.

Preparation of peripheral blood leukocytes. Peripheral blood leukocytes containing about 1% basophils were obtained from seven healthy donors, four of whom were atopic. Sixty to 120 ml venous blood were mixed in 30 ml volumes with 10 ml 1% methyl cellulose in saline and 300 U preservative-free heparin and the red cells allowed to sediment at room temperature for 30 min. The leukocyte rich supernatant was centrifuged at 450 g for 15 min, the pelleted cells washed twice by resuspension in HEPES buffered salt solution (HBSS) [10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH₂PO₄, pH 7.4] with 0.03% human serum albumin and 5 mM glucose (HAG) followed by centrifugation for 15 min at 300 g and finally resuspended in HAG with 2 mM calcium chloride and 1 mM magnesium chloride immediately prior to incubation. Basophil leukocytes (BL) were counted in an improved Neubauer counting chamber using a toluidine blue-based stain [7].

BL histamine release assay. Leukocyte suspensions were warmed in a waterbath at 37°C for 5 min and aliquoted in 100 µl volumes (containing about 10⁵ BL) into paired 2 ml conical-bottomed polypropylene tubes (Sarstedt, Leicester, U.K.) containing the challenge stimuli, making a final reaction volume of 200 µl. Challenge stimuli were used at the following final concentrations unless stated otherwise; goat anti-human IgE (anti-IgE): 1/1000 (46.5 µg protein/ml), calcium ionophore A23187 (a mast cell and BL secretagogue independent of IgE cross-linking, used as a positive control in all assays): 5 µM and sera: 1/2. After incubation at 37°C for 40 min the reaction was terminated by addition of 800 µl ice-cold HBSS (HBSS with 5 mM

Table 1. Corrected percentage histamine release from BL of five donors and three CU patients showing the variability of response to the same stimuli amongst healthy donor BL and the generally lower response to anti-IgE and CU sera of BL from CU patients

	Healthy BL donors					CU BL donors		
	1	2	3	4	5	1	2	3
Anti-IgE (1/1000)	49.4	0.5	10.4	73.0	57.5	6.2	10.4	-1.0
Ca ionophore (5 μ M)	70.1	65.3	35.0	76.0	66.3	33.3	27.0	31.6
CU serum A (1:2)	21.7	0.0	13.0	68.2	49.5	0.0	6.2	-3.9
CU serum B (1:2)	5.8	0.0	59.7	34.3	21.7	0.0	8.0	2.0*
CU serum C (1:2)	42.1	0.9	66.3	82.9	72.1	0.0	15.1	-2.5
Spontaneous release	2.6	1.5	1.2	0.9	1.8	3.6	0.0	5.0
Histamine/sample (ng)	100	136	73	198	93	7	27	15
IgE (IU/ml)	600	180	<20	220	180	20	310	40

* Autologous serum.

EDTA was used to stop the reaction in the time course experiments). Cells were pelleted by centrifugation at 500 *g* for 5 min at 4°C, supernatants decanted and 1.0 ml HBSS added to the pellets. Perchloric acid (60%) was added to cell and supernatant samples to a final concentration of 0.4 M, tubes were vortex mixed, centrifuged at 8800 *g* for 10 min and samples stored at 4°C for histamine analysis using the automated fluorimetric method of Siraganian [8]. Supernatants were corrected for non-specific fluorescence due to histamine and other amines in serum by subtracting the corresponding blank value of 100 μ l of serum or plasma made up to 1 ml with HBSS (mean 1.3 ng/ml $\pm$ 0.1, *n* = 24). Total histamine/assay tube ranged from 70–200 ng. Spontaneous release was <3% except in the 'cross-desensitization' studies.

Net percentage histamine release was calculated as the mean of duplicate supernatant values divided by the total histamine in supernatant and cell samples $\times$ 100, minus the spontaneous histamine release from cells incubated with buffer. Significant histamine release from BL was taken as the mean + 2 s.d. of 10 control sera tested on BL from the same donor. When data from several BL donors were compiled the highest histamine release value was used. An arbitrary threshold of 10% was taken to be significant because the panel of 10 control sera was not tested on each BL donor.

'Cross-desensitization' of BL. Incubation of BL at 37°C with one IgE cross-linking stimulus in the absence of calcium renders them unresponsive to subsequent challenge with another [9]. Leukocytes were divided into three equal aliquots and incubated with 3 ml anti-IgE (1/1000),

CU sera (1/2) in HAG with 5 mM EDTA or buffer control at 37°C for 90 min. The three cell aliquots were washed twice in HAG at room temperature to remove unbound stimuli, resuspended in HAG with calcium and magnesium and then incubated with the challenge stimuli as described above.

Inhibition of histamine release by IgE. Challenge stimuli were preincubated for 30 min at 37°C with myeloma IgE serum or purified myeloma IgE protein. Polyclonal IgG or buffer were used as controls. The preincubated challenge stimuli were then used in the histamine release assay, as described.

IgE 'stripping' and passive sensitization of BL. IgE was 'stripped' from basophils using lactic acid by the method of Pruzansky [10] before challenge with anti-IgE, calcium ionophore or CU sera to see whether histamine release by IgE-dependent stimuli could be reduced. The 'stripped' cells were then passively sensitized with myeloma IgE serum to see if the response could be restored. Mixed leukocytes from donor 4 (Table 1), prepared in the standard manner, were divided into two aliquots. One was washed first with saline then mixed with 5 ml 10 mM lactic acid (pH 3.9) for 3.5 min at room temperature, washed twice, then resuspended with HAG with 5 mM EDTA and subdivided into two further aliquots to one of which was added myeloma IgE serum (20 μ g IgE/ml). The other unstripped control aliquot was also suspended in HAG with EDTA. All three aliquots were incubated at 37°C for 90 min, washed three times, then exposed to the challenge stimuli in the presence of calcium, as described previously.

Peripheral blood cellular histamine analysis. Heparinized peripheral blood was centrifuged at 600 *g* and plasma removed. Cells were washed three times in PBS, resuspended in HBSS to the original volume and 5 ml of 0.8 M perchloric acid added to 5 ml of cells. Aliquots of the lysed cells were centrifuged at 8800 *g* and histamine in the supernatants was analysed by automated fluorimetric assay.

Affinity chromatography. IgG was purified from sera with a Mabtrap[®] kit (Pharmacia, Milton Keynes, U.K.) containing a 3 ml protein G-sepharose column, 0.02 M sodium phosphate buffer (pH 7.0) for washing, 0.1 M glycine-HCl (pH 2.7) for elution and 1.0 M Tris-HCl (pH 9.0) for neutralization. IgG-containing eluates and the column washes (including other immunoglobulins) were concentrated and washed with HBSS on YM30 membranes (30 000 molecular weight cut off) and made up to the original volume of applied serum with HAG containing calcium and magnesium. IgM containing eluates were prepared on an anti-IgM affinity column (3 mg of μ -chain specific anti-human IgM coupled to 3.5 ml of cyanogen bromide activated sepharose 4B gel) using the same protocol as above. The purity of IgG in the protein G eluate was confirmed by SDS PAGE. No other protein was detected except for a trace of albumin. IgG was not detected in the protein G column washes.

Immunoglobulin assays. IgG, A and M were quantified in whole sera and protein G fractions by immunoturbidimetry calibrated against SPS-01 standards. Recovery was expressed as the percentage of immunoglobulin in serum. IgE was measured with a sandwich ELISA technique [11] and results expressed in IU/ml.

Reagents. Lyophilized human IgG, calcium ionophore A23187, human serum albumin (essentially globulin-free), goat anti-human IgE (ϵ -chain specific) and goat anti-human IgM (μ -chain specific) were obtained from Sigma Chemical Company, Poole, U.K. Human IgE- κ myeloma serum (25 g/l IgE) was a gift from The Binding Site, Birmingham, U.K. who also supplied the human IgE- κ myeloma protein at 95% purity.

Statistical analysis

Results are expressed as means $\pm$ s.e.m. Peripheral blood cellular histamine concentrations, BL counts and serum IgE concentrations in patients and controls were analysed by Student's *t*-test. Correlations between plasma versus serum skin tests and peripheral blood cellular histamine versus BL counts were determined by linear regression analysis.

Results

Skin tests

Mean PBS-corrected autologous serum skin test weal volumes (mm^3) were 37.3 ± 6.8 in CU patients, -0.4 ± 1.4 in healthy controls and 0.8 ± 1.5 in dermatographic patients (Fig. 1a). The response to serum correlated well with plasma ($r=0.79$, $P<0.001$). Twenty of 25 CU patients showed a positive skin test response greater than 2 s.d. above the mean of the control group ($>10 \text{ mm}^3$). Nine CU patients showing a strong weal response to autologous serum (50.3 ± 4.4) were selected for studies of the response to ultrafiltered serum fractions. Nine of nine responded to $>100 \text{ kD}$ fractions (mean weal volume $53.8 \pm 12.8 \text{ mm}^3$), but not to fractions between $100\text{--}30 \text{ kD}$ (mean 5.3 ± 3.6); $30\text{--}10 \text{ kD}$ (mean 1.4 ± 1.8) or $<10 \text{ kD}$, (mean -1.4 ± 1.4). There were no responses to fractions $>100 \text{ kD}$ in healthy controls ($n=5$) or dermatographic patients ($n=3$).

BL histamine release studies

Fourteen of 25 CU sera elicited histamine release $>10\%$ (mean $44.3\% \pm 6.7$) above basal levels from BL of at least one of seven healthy donors. To study heterogeneity of BL in respect of histamine release the response to anti-IgE, three CU sera and calcium ionophore A23187 were compared on BL from five healthy donors (Table 1). One of them (donor 4) responded well to all stimuli whereas BL from donor 2 were completely unresponsive to anti-IgE and the three CU sera. By comparison, BL of three CU patients showed little or no response to anti-IgE (mean $5.2\% \pm 3.3$) or heterologous CU sera (Table 1). Total histamine per assay tube (range 7–27 ng) was substantially less than controls probably due to low numbers of circulating BL.

All sera and ultrafiltrates from patients and controls were therefore screened on BL from one anti-IgE responsive donor (donor 4, Table 1). Histamine release $>2.6\%$ was significant for this donor, as defined previously. The range of histamine release from BL of donor 4 in response to sera from patients and controls is shown in Fig. 1b. Histamine releasing activity of CU sera was confined to ultrafiltered fractions $>100 \text{ kD}$ (mean $31.6\% \pm 9.8$ before ultrafiltration, $20.5\% \pm 6.9$ after, $n=7$). Serum and the $>100 \text{ kD}$ fraction of one dermatographic patient, showed weak histamine releasing activity (3.8% and 3.4%, respectively) of unknown biological significance but there were otherwise no reactions in controls. Histamine release in response to CU sera followed the same time course as that to anti-IgE (Fig. 2a). The dose response curves of three CU sera and a protein G eluate derived from one of them showed similar but slightly steeper slopes than anti-

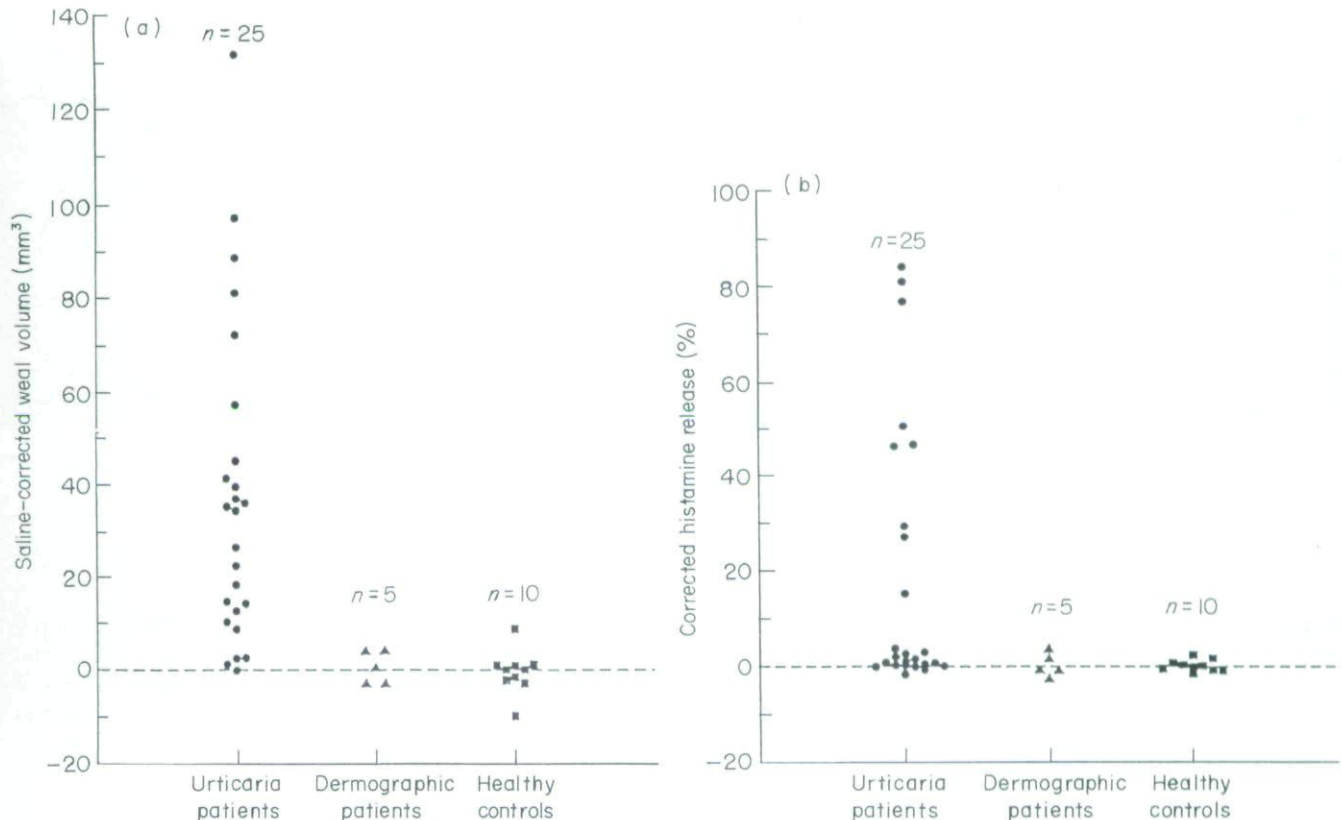


Fig. 1. (a) Autologous serum skin test weal volumes (mm^3) in patients with CU, symptomatic dermographism and healthy controls. (b) Histamine release from basophil leukocytes of a healthy donor (donor 4, Table 1) challenged with sera from patients with CU, symptomatic dermographism and controls.

IgE (Fig. 2b). Histamine releasing activity of CU sera was stable to freezing ($\times 4$ freeze/thaw cycles, $n=1$) and heat (56°C , 2 hr, $n=9$) (results not shown).

Six of seven IgG fractions purified from CU sera by protein G immunoadsorption (mean IgG concentration $6.6 \text{ g/l} \pm 1.0$) elicited significant histamine release (mean $13.8\% \pm 6.9$) from the BL of donor 4 at 1:2 final dilution. Mean histamine release of the corresponding whole sera was $37.6\% \pm 9.8$. This loss of activity was equivalent to 2–8 fold dilutions, as derived from serum dilution curves on the same BL donor. Mean recovery of IgG in the protein G eluates of patients and controls was 71% ($n=9$) measured by immunoturbidimetry, suggesting that the loss of functional activity could have been due to the relatively harsh elution conditions or removal of a cofactor required for full activity. Five of seven column washes containing $< 3\%$ serum IgG stimulated histamine release (mean $9.1\% \pm 4.3$) suggesting that histamine releasing activity was not confined to IgG. Immunoadsorption of three protein G column washes with histamine releasing activity on anti-IgM sepharose almost abolished histamine release (mean 16.5% before, 0.8%

after) although very little histamine releasing activity was recovered in the IgM-containing eluates (mean 0.6%).

'Cross desensitization' between anti-IgE and CU sera was demonstrated in three experiments using sera or plasma from three CU patients and BL from three healthy donors (Fig. 3). Preincubation of BL with anti-IgE in the absence of calcium almost abolished subsequent histamine release following incubation with anti-IgE or CU serum in the presence of calcium. Similarly, preincubation with CU serum substantially reduced the response to CU serum itself and to anti-IgE suggesting that anti-IgE and the CU sera stimulate histamine release from BL by a common mechanism. Similar results were obtained using a protein G eluate of serum A (concentrated $\times 2$) on BL of donor 4 (Table 1).

Histamine release was inhibited in a concentration dependent manner by preincubation of serum or plasma from three CU patients, or anti-IgE, with IgE myeloma serum (up to $50 \mu\text{g/ml}$ IgE) using BL from donor 4 (Table 1) but there was no inhibition with human polyclonal IgG ($500 \mu\text{g/ml}$ and $50 \mu\text{g/ml}$) suggesting that the CU serum factor binds IgE, thereby inhibiting subsequent histamine

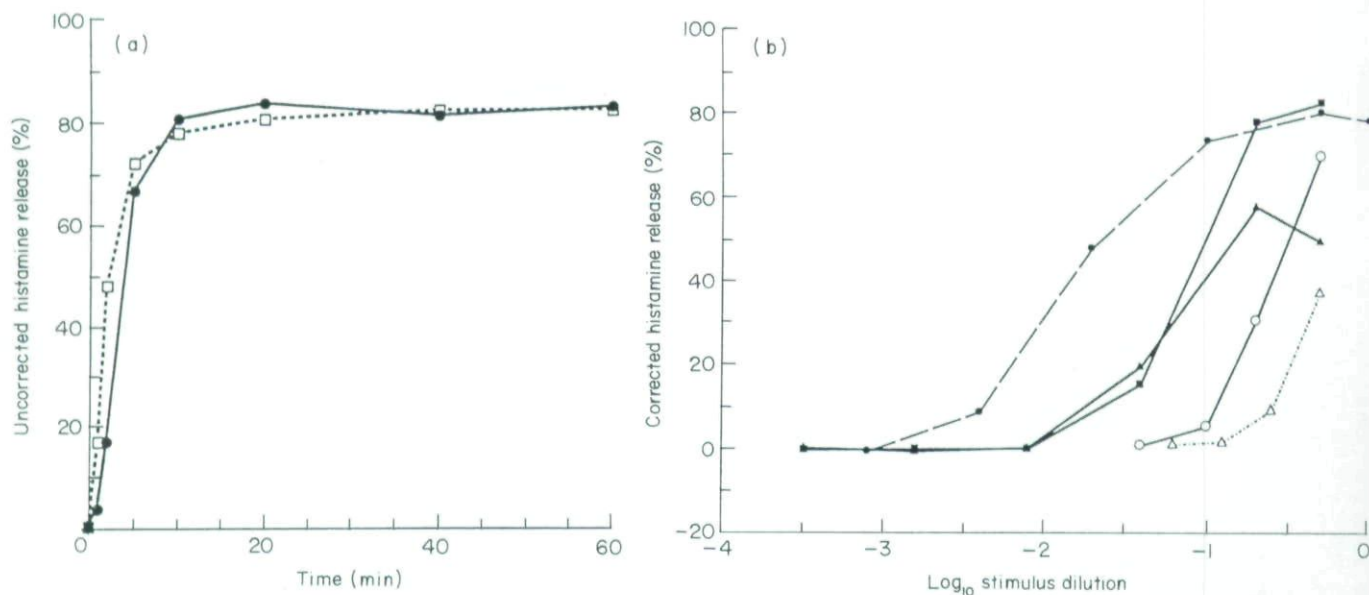


Fig. 2. (a) Time course of histamine release from basophil leukocytes of a healthy donor (donor 4, Table 1) in response to anti-IgE (□) and a CU (●) serum. Representative data from one of three experiments. (b) Individual dose-responses of histamine release from basophil leukocytes of a healthy donor (donor 4, Table 1) challenged with sera from three CU patients. Protein G eluate from one of them (CU serum 3) shows an identical slope to the parent serum. Anti-IgE shows a similar but shallower slope. For convenience of comparison, the highest concentration of anti-IgE (1/500) corresponds to 0 on the log₁₀ scale. ●, anti-IgE; ■, CU serum 1; ▲, CU serum 2; ○, CU serum 3; △, protein G eluate (from CU serum 3).

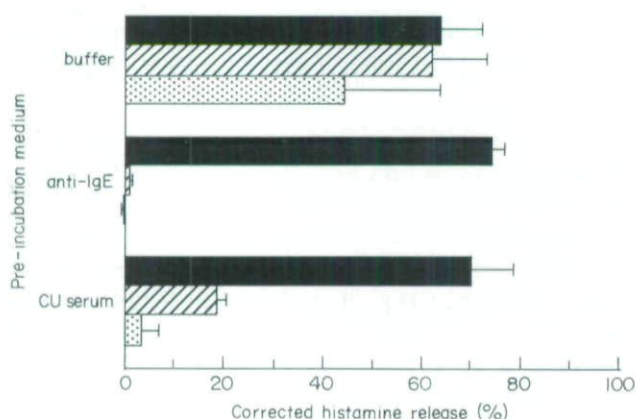


Fig. 3. 'Cross-desensitization' of basophil leukocytes to challenge with CU sera and anti-IgE but not calcium ionophore A23187 (■) following preincubation with anti-IgE (■) or CU serum (▨) in the absence of calcium. Values are means $\pm$ s.e.m. of three experiments using sera from three CU patients and basophils from three anti-IgE responsive donors.

release from BL (Fig. 4). Preincubation of the protein G eluate of serum A (Table 1) (concentrated $\times 2$) with purified myeloma IgE (10 $\mu\text{g}/\text{ml}$) inhibited histamine release by 51% in one experiment (results not shown).

Mean histamine release in response to five CU sera and

anti-IgE fell substantially following lactic acid 'stripping' of IgE from BL of donor 4 and recovered partially after passive sensitization with myeloma IgE serum but there was no appreciable effect on calcium ionophore-induced histamine release (Fig. 5a). The individually plotted results for CU sera shown in Fig. 5b illustrate that histamine release recovered completely after passive sensitization with myeloma IgE serum in response to one CU serum, showed partial recovery in response to three others and little change with another that elicited the lowest levels of histamine release before and after 'stripping'.

Total serum IgE

Total serum IgE in CU patients (mean 167.8 IU/ml $\pm$ 34.5, $n=23$) was similar to controls (mean 160.0 $\pm$ 61.0, $n=9$). Although total IgE was lower in CU sera which elicited HR (mean 113.8 IU/ml $\pm$ 22.3, $n=13$) than those that did not (mean 238.0 $\pm$ 69.8, $n=10$) the difference was not significant.

Peripheral blood basophil counts and cellular histamine

Mean basophils/mm³ in peripheral blood of CU patients (7.9 $\pm$ 2.0, $n=14$, nine female, mean age 37.6 yr $\pm$ 3.7) were significantly reduced compared to controls

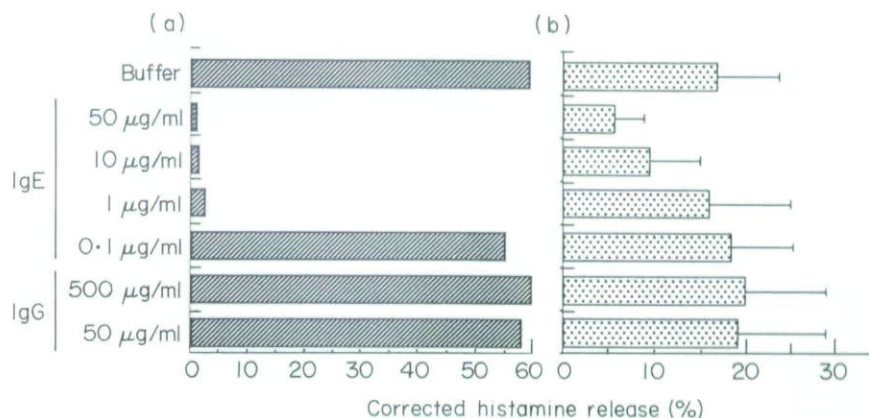


Fig. 4. Concentration dependent inhibition of histamine release in response to (a) anti-IgE and (b) three CU sera (mean $\pm$ s.e.m.) using basophil leukocytes from a healthy donor (donor 4, Table 1). Stimuli were preincubated with dilutions of myeloma IgE serum (IgE 0.1–50 μ g/ml) or IgG for 30 min prior to challenge.

(39.6 ± 4.4 , $n = 25$, 17 female, mean age $30.8 \text{ yr} \pm 1.6$), $P < 0.001$. Mean peripheral blood cellular histamine was also reduced in CU patients ($21.4 \text{ ng/ml} \pm 3.2$) compared to controls (69.8 ± 4.9), $P < 0.001$. There was a significant correlation between peripheral blood cellular histamine and BL numbers in controls ($r = 0.82$, $P < 0.001$) but not CU patients ($r = 0.32$, $P = \text{NS}$).

Discussion

Histamine releasing activity was detected in the sera of most CU patients but not in dermatographic patients or healthy controls indicating that it may be specific to chronic urticaria. Five CU patients who showed little or no response to autologous serum skin testing had marked angioedema and pressure urticaria and only one of them showed *in vitro* histamine releasing activity which suggests that they may belong to a distinct subgroup.

The 'cross-desensitization', inhibition and IgE stripping/passive sensitization studies indicate that histamine release is initiated by cross-linking of IgE on basophils. The similarity in dose-response and time course of histamine release induced by anti-IgE supports this interpretation. Furthermore, the demonstration of *in vitro* histamine releasing activity in IgG fractions of six of seven CU sera tested at physiological concentrations, together with the *in vitro* and *in vivo* activity found in all $> 100 \text{ kD}$ ultrafiltered fractions, indicates that most, if not all, histamine releasing activity can be attributed to autoantibodies against IgE. Detection of some histamine releasing activity in the protein G column washes, however, suggests that it is not restricted to IgG and may be of other immunoglobulin isotype(s) against IgE or possibly due to circulating cytokines. In fact, this activity could be removed by an anti-IgM affinity column although it was not possible to recover sufficient IgM in

the eluate to demonstrate histamine releasing activity in the basophil assay.

The greatly reduced response of chronic urticaria leukocytes to stimulation by anti-IgE, autologous serum and sera of other CU patients may reflect *in vivo* 'desensitization' or selection for the population of basophils that are relatively resistant to degranulation by circulating anti-IgE. Previous studies have shown that basophils of CU patients release less histamine on stimulation with anti-IgE than basophils from controls [12,13] and that stainable basophils are reduced in active CU [14] as we have demonstrated in this study. The low peripheral blood cellular histamine levels in CU patients reflects the reduction in basophils.

Anti-IgE autoantibodies have been detected by immunoassay in serum or plasma of many individuals including those with CU, urticarial vasculitis and cold urticaria [15], atopic dermatitis [16], asthma [17], allergic rhinitis and the hyper IgE syndrome [18] and even non-allergic healthy individuals [19]. However, most workers have found great difficulty in demonstrating a functional role for anti-IgE autoantibodies and histamine releasing activity has only been described in a few cases. One of the cold urticaria sera examined by Gruber *et al.* [15], containing high titres of IgM anti-IgE antibodies, was found to have histamine releasing activity and induced cold sensitivity in a normal recipient on passive transfer. IgG from two other patients with cold urticaria induced successful passive transfer but had no *in vitro* histamine releasing activity. Marone *et al.* [9] reported that IgG anti-IgE from only one of six atopic dermatitis patients with high levels of the antibodies was capable of releasing histamine from basophils and mast cells. The reason for this functional heterogeneity of human anti-IgE is not clearly defined. However, it is feasible that functional activity depends upon the balance between anti-IgE and IgE levels in serum, as there is evidence that they form

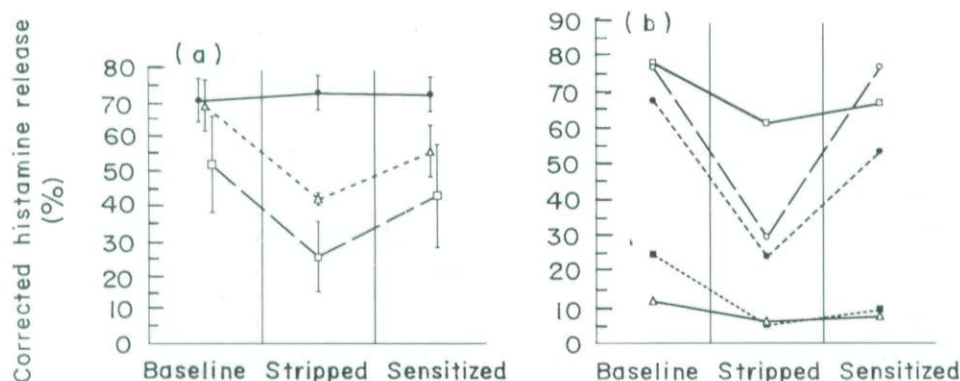


Fig. 5. Effect of removal of cell surface IgE by lactic acid 'stripping' and passive sensitization of 'stripped' BL with myeloma IgE serum on the percentage histamine release from BL (donor 4, Table 1). (a) Mean percentage histamine release ($\pm$ s.e.m.) in response to CU sera ($n=5$), anti-IgE ($n=3$) and calcium ionophore A23187 ($n=4$). $\bullet$, A23187; Δ , anti-IgE; $\square$, CU sera. Individual data for five CU sera are shown in (b). $\square$, CU serum 1; $\circ$, CU serum 2; $\bullet$, CU serum 3; $\blacksquare$, CU serum 4; Δ , CU serum 5.

complexes *in vivo* [20], or on the location of the epitope on the IgE antibodies. Stadler *et al.* have produced a set of murine monoclonal antibodies, one of which, binding to the D_E2 region of human IgE, induced histamine release, and the other, binding to the D_E1 region, did not [21].

Differential sensitivity of mast cell populations in different tissues to anti-IgE [22] might explain why patients with chronic urticaria develop skin and oral mucosal lesions, but rarely bowel symptoms and are without wheezing or anaphylaxis. Anti-IgE autoantibodies induced in rats by immunization with rat myeloma IgE [23] reduced safranin staining mast cells in skin, tongue and bone marrow (connective tissue type), but mast cell numbers in gut and lung, greater than 99% of which stained with alcian blue (mucosal type), were either unaffected or increased [24]. Local changes in sensitivity of cutaneous blood vessels to mast cell derived mediators and of cutaneous mast cell 'releasability' in response to degranulating stimuli may also be important in the pathogenesis of chronic urticaria. Skin test reactivity to kallikrein and bradykinin has been shown to be increased and the weal response to histamine prolonged compared to control subjects [25]. However, this could be due to increased vascular permeability to histamine releasing autoantibodies leading to subsequent mast cell degranulation. Spontaneous histamine release from skin slices of three urticaria patients was increased suggesting an underlying 'instability' of the cutaneous mast cell [26].

Human recombinant interleukin-3 and granulocyte-macrophage colony-stimulating-factor release histamine from normal basophils *in vitro* [27] and have been shown to potentiate the effects of various secretagogues, including anti-IgE antibody, on histamine release from basophils [28] and may therefore exercise a powerful local pro-inflammatory function. Naturally occurring histamine releasing cytokines have been demonstrated in the culture

supernatant of human lung macrophages [29], nasal washings [30] and monocytes of atopic dermatitis patients with food allergies [31]. It was demonstrated that ultrafiltered serum fractions < 30 kD elicited a skin test response in some CU patients in a previous study [32]. Failure to demonstrate histamine releasing activity in ultrafiltered CU serum fractions < 30 kD in this series may have been due to technical factors such as incomplete serial filtration through the membranes and does not totally rule out the occurrence of histamine releasing cytokines.

The CU patients studied were all referred to dermatology clinics because their urticaria was controlled poorly or not at all by antihistamines. They therefore represent a relatively selected group. The occurrence of systemic symptoms in 18 of them reflects the severity of their disease. Although skin biopsies and complement studies were not performed it is unlikely that patients with urticarial vasculitis were included in the study group because patients were excluded if weals lasted more than 24 hr.

Our study suggests that a substantial proportion of CU sera contain histamine releasing anti-IgE autoantibodies and that serum skin testing may be a good strategy for screening other diseases, such as atopic dermatitis and asthma, in which histamine releasing antibodies may also be present. Detection of histamine releasing autoantibodies in CU not only offers a model for understanding the pathogenesis of the condition but also provides a rational basis for novel approaches to treatment, such as plasma exchange and immunosuppressive regimens, in patients with severe refractory disease.

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