

Flow cytometric analysis of basophil numbers in chronic urticaria: basopenia is related to serum histamine releasing activity

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Summary

Background Peripheral blood basophils are reduced in some chronic urticaria patients when counted with granule stains. Approximately 30% of patients with severe chronic urticaria have functional autoantibodies which release histamine from healthy donor basophils *in vitro* but the relationship between basophil numbers *in vivo* and serum histamine releasing activity has not been studied.

Objective To determine the relationship between basophil numbers and serum basophil histamine releasing activity and to assess whether basophils are present, but undetectable, in peripheral blood with granule stains by using a new flow cytometric method based on surface immunophenotype.

Methods Basophils were counted manually by a chamber method using a granule stain and by flow cytometry using dual staining with anti-IgE and anti-FcεRI in 25 chronic idiopathic urticaria patients and 25 healthy controls. Serum histamine releasing activity was assessed on healthy donor basophils *in vitro* and by the weal response to autologous serum skin testing *in vivo* (patients only).

Results Basophils were significantly reduced in chronic urticaria by manual counting and flow cytometry. A subgroup of seven patients with *in vitro* histamine releasing activity showed a marked reduction or absence of basophils by both methods. There were no obvious distinguishing clinical characteristics between these patients and the others; six of them showed positive autologous serum skin-test responses. On comparing the two methods, the manual basophil counts were generally lower than flow cytometric counts. Agreement over the full range of values was not strong and therefore counts obtained by the two methods are not directly interchangeable.

Conclusion Basopenia in chronic idiopathic urticaria is associated with serum basophil histamine releasing activity in a subgroup of patients. The lack of granule and surface immunophenotype staining suggests a reduction in numbers rather than an inability to detect circulating degranulated cells by conventional counting methods.

Keywords: basophils, chronic urticaria, histamine releasing activity, flow cytometry

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Introduction

The reduction in stainable peripheral blood basophils of

chronic urticaria patients first described by Rorsman [1] in 1962 was confirmed in a later study [2], when it was also noted that peripheral blood cellular histamine was reduced. The apparent basopenia may be due to spontaneous degranulation induced by histamine releasing autoantibodies or

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removal from the circulation. The evidence for an autoimmune aetiology in some chronic idiopathic urticaria patients has been reviewed [3]. Most chronic urticaria patients with serum histamine releasing activity have functional autoantibodies which bind the high affinity IgE receptor (FcεRI) [4–6] on basophils and mast cells although others appear to have affinity for cytophilic IgE itself [2]. Basophils are normally present in small numbers, representing only about 1% of circulating leucocytes. Counting methods may be based on metachromatic granule stains [7], size and granularity (as in automated differentials) or surface immunophenotype [8–10]. Manual counting used in the previous studies of basophil numbers in chronic urticaria [1,2] carries several uncertainties, including a possible loss of staining after degranulation, relatively subjective colour differences and statistical errors associated with low circulating basophil numbers. We have therefore developed a flow cytometric counting method based on surface immunophenotype using dual staining of IgE and FcεRI to determine the relationship between basophil numbers in chronic urticaria and serum histamine releasing activity, using autologous serum skin testing as an *in vivo* measure of it [11] and an *in vitro* histamine release assay using heterologous basophils of two well-characterized healthy donors shown previously to respond to anti-FcεRI or anti-IgE [5].

Subjects and methods

Subjects

Twenty-five chronic idiopathic urticaria patients referred to the Dermatology Department of the Norfolk and Norwich Health Care National Health Service (NHS) Trust by primary care practitioners, aged 14–72 years (median 36, 17 female) were studied. All had urticaria of at least 2 months duration (range 2–216, median 6). Most had daily or almost daily weals with or without angio-oedema at the time of testing although one had weekly episodes and two appeared to be going into remission with less frequent sporadic weals. Patients less than 14 years, those with predominantly physical urticaria, C1 inhibitor deficiency, systemic lupus erythematosus or who had taken immunosuppressive treatments or the long-acting antihistamine, astemizole, within 1 month were excluded. Patients were asked about previous episodes of urticaria, maximum and minimum weal duration, occurrence of angio-oedema, frequency, pattern, systemic symptoms including fatigue and aching, aggravating factors including pressure, past and family histories of atopy and autoimmune disorders.

Twenty-five healthy controls, aged 19–54 years (median 40.5, 16 female), were recruited from technical, medical and nursing staff. The same exclusions applied. The study protocol was approved by the Norwich Health

District Ethics Committee and all subjects gave informed consent.

Blood samples

Venous blood was drawn from the antecubital fossa into plain tubes (Vacutainer®, Becton Dickinson, New Jersey, USA) for serum skin testing and total IgE assay. Excess sera were stored at –70°C for basophil histamine release assays. Automated full blood count (FBC) and basophil counts were performed on K₃EDTA anticoagulated blood. Blood for erythrocyte sedimentation rate (ESR) was collected into tubes containing trisodium citrate.

Skin tests

Patients but not healthy controls were skin tested with 50 µL aliquots of fresh autologous serum and sterile normal saline injected intradermally into volar forearm skin at least 2 cm apart after stopping antihistamines for 48 h, as described previously [2]. A positive skin test was defined as a serum skin test weal 2 mm or larger in diameter than the saline test at 30 min.

IgE

Total IgE levels in sera were measured by fluorescence enzyme immunoassay (Kabi Pharmacia Diagnostics, Uppsala, Sweden).

Basophil histamine release assay

Peripheral blood leucocytes from two healthy donors were prepared from 60–120 mL blood as described previously [5]. The basophils of donor 1 were poorly sensitized with endogenous IgE (<1.0 kU/L serum) and released little histamine in response to anti-IgE [5] but responded well to the mouse monoclonal antibodies 6F7 and 29C6 against the alpha subunit of the high affinity IgE receptor (FcεRIα) [12]. Monoclonal 6F7 antibody binding is inhibited by the presence of receptor-bound IgE whereas 29C6 binding is unaffected [5]. Basophils of donor 2 were endogenously sensitized to IgE (serum level >145 kU/L) and responded well to anti-IgE and 29C6 but not 6F7. Dose–response curves to monoclonal antibodies 29C6 and 6F7 for basophil donors 1 and 2 have been published [5]. It was found from these curves that a concentration of 50 ng/mL of 29C6 stimulated substantial histamine release from the basophils of all donors, whereas 6F7 at this concentration only released from poorly sensitized basophils (Table 1).

Prepared leucocytes were washed twice in calcium and magnesium free assay buffer (10 mM HEPES, 137 mM NaCl, 2.7 mM KCL, 0.4 mM NaH₂PO₄, 0.03% human

Table 1. Dual staining flow cytometry basophil numbers/ μL , skin-test weal response and % basophil histamine release on two healthy donor basophils for chronic urticaria patients showing positive *in vitro* histamine release or a positive autologous serum skin-test response. Responses to the control stimuli of basophil donors 1 and 2 are also shown. The two basophil donors were not used as controls for basophil enumeration

	Flow cytometry basophils/ μL	Skin-test weal response (mm)	% basophil histamine release	
			Donor 1	Donor 2
Skin-test positive sera				
1	0	12	3	13
2	0	9	30	27
3	0	11	ND	ND
4	0	10	68	29
5	4	5	56	23
6	4	4	40	6
7	8	14	68	41
8	12	12	1	0
9	30	2	2	1
10	45	2	0	0
11	46	8	0	0
Skin-test negative sera				
12	0	1	20	50
Control stimuli				
anti-IgE (1/1000)			4	47
29C6 (not inhibited by IgE: 50 ng/mL)			32	41
6F7 (inhibited by IgE: 50 ng/mL)			51	2

ND, not done.

serum albumin (HSA) and 5 mM glucose, pH 7.4). The leucocyte suspensions were resuspended in the complete assay buffer (containing 2 mM calcium and 1 mM magnesium) warmed at 37°C for 5 min and 50 μL aliquots added to duplicate 2 mL polypropylene tubes containing the challenge stimuli to a final volume of 100 μL . The stimuli were used at the following final concentrations; sera: twofold dilution, goat anti-human IgE (anti-IgE) at an optimal concentration for histamine release: 1/1000 (46.5 μg protein/mL), mouse monoclonal anti-human Fc ϵ RI α antibodies 29C6 and 6F7, both at 50 ng/mL. After incubation at 37°C for 40 min the reaction was terminated by cooling on ice and the addition of 800 μL ice-cold buffer without HSA. Cells were pelleted by centrifugation at 500 *g* for 5 min at 4°C and supernatants decanted. Cell pellets were resuspended in assay buffer and 100 μL of 3M perchloric acid was added to cell and supernatant samples to give a final concentration of 0.3M. Histamine in the samples was measured by an automated fluorometric assay [13]. Histamine release was calculated as the percentage of the total histamine content in

each sample released from the cells into the supernatant by the stimulus, after correction for any serum fluorescence blank value. The net histamine release was obtained by subtraction of the spontaneous release (<2%) found when leucocytes were incubated with buffer alone. Histamine release of >5% was regarded as positive.

Manual basophil counts

Manual counts were performed in duplicate at a 1/5 dilution of K₃EDTA blood in a toluidine blue-based stain [6] using modified Fuchs-Rosenthal (Manners) chambers, as described previously [14]. Results were expressed as basophils per μL of whole blood.

Flow cytometric counts

Flow cytometry was performed with a Coulter Epics Profile II (Coulter Electronics, Luton, UK) on two 100 μL aliquots of K₃EDTA blood, containing about 1×10^6 leucocytes. Erythrocytes were haemolysed using a Q-prep workstation by brief exposure to formic acid (reagent A), followed immediately by addition of 1.8% saline (reagent B) to restore osmolality, but leucocytes were left unfixed by preventing the addition of paraformaldehyde (reagent C). The cells were washed immediately with cold PBSAA [0.01 M phosphate buffered saline (9 mM Na₂HPO₄, 2.6 mM NaH₂PO₄, 135 mM NaCl), pH 7.2 with 20 mM sodium azide and 0.1% bovine serum albumin] and pelleted at 300 *g* for 5 min at 4°C.)

The supernatant was removed, the cells washed once more in cold PBSAA to remove unbound IgE and soluble Fc ϵ RI complexes, and the cells pelleted as before. Cells were resuspended in 90 μL buffer. Ten microlitre of normal mouse IgG1 (100 $\mu\text{g}/\text{mL}$) and 10 μL biotinylated sheep IgG (1 $\mu\text{g}/\text{mL}$) were added to the control tube to final concentrations of 10 $\mu\text{g}/\text{mL}$ and 0.1 $\mu\text{g}/\text{mL}$ respectively. Similarly, 10 μL mouse anti-Fc ϵ RI monoclonal antibody 29C6 (100 $\mu\text{g}/\text{mL}$) and biotinylated sheep anti-IgE (1 $\mu\text{g}/\text{mL}$) were added to the test. The tubes were incubated for 30 min in crushed ice to minimise histamine release, the cells washed once with cold PBSAA and pelleted. After removal of the supernatant and addition of 10 μL FITC-conjugated sheep anti-mouse IgG (SAM-FITC) at 100 $\mu\text{g}/\text{mL}$ and 10 μL streptavidin R phycoerythrin (SA-RPE) both tubes were incubated in crushed ice for a further 30 min in the dark. The cells were washed, pelleted and resuspended in 500 μL of cold PBSAA for immediate flow cytometry. Fifty thousand viable cells were analysed. Cells showing dual fluorescence with anti-IgE and anti-Fc ϵ RI were counted after gating out cells showing non-specific fluorescence in the control tube. The number of anti-IgE and anti-Fc ϵ RI positive basophils was calculated per μL whole blood.

Reagents

The mouse monoclonal anti-FcεRI antibodies (29C6 and 6F7) were a generous gift of Dr J. Hakimi and Dr R. Chizzonite (Hoffmann LaRoche, New Jersey, USA). The biotinylated polyclonal sheep anti-IgE (AB014), biotinylated normal sheep IgG (CUS276) and SAM-FITC (PF272) were obtained from the Binding Site, Birmingham, UK. Normal mouse IgG1 (X931) and SA-RPE (R438) were supplied by Dako Ltd, High Wycombe, UK. The goat anti-IgE (ε-chain specific) was obtained from Sigma Chemical Company, Poole, UK.

Statistical analysis

Results are expressed as means $\pm$ SD. Basophil numbers and IgE (corrected for skewness by converting to natural logarithms) were compared between patients and control subjects for each method by two-tailed Student's *t*-tests. A probability of <0.05 was regarded as significant. Analysis by agreement of methods was used to compare manual and flow cytometric counts instead of correlation which is an inappropriate test in this situation [14].

Results

Patients

Eleven patients showed a positive autologous serum response. There were no important differences in clinical or laboratory characteristics between responders and non-responders although a slightly higher proportion of responders had had previous episodes of urticaria (5/11 vs 4/14), angio-oedema (8/11 vs 9/14) and aching or fatigue (6/11 vs 6/14).

Identification of basophils on flow cytometry

The degree of non-specific fluorescence was determined using the isotype control and gating parameters set accordingly (data not shown). A population of dual stained leucocytes was identified when compared with the isotype control (box 2, Fig. 1a; representative data from 1 experiment). By reanalysing the data and backgating the dual labelled cells into a size and granularity histogram it was found that these cells lay between the lymphocyte and mononuclear cell populations (Fig. 1b; representative data of 1 experiment) in keeping with a previous estimate of cell volumes [16].

Basophil numbers

The difference between manual basophil counts/ μ L for patients (20.1 ± 17.2) and healthy controls (30.8 ± 11.4)

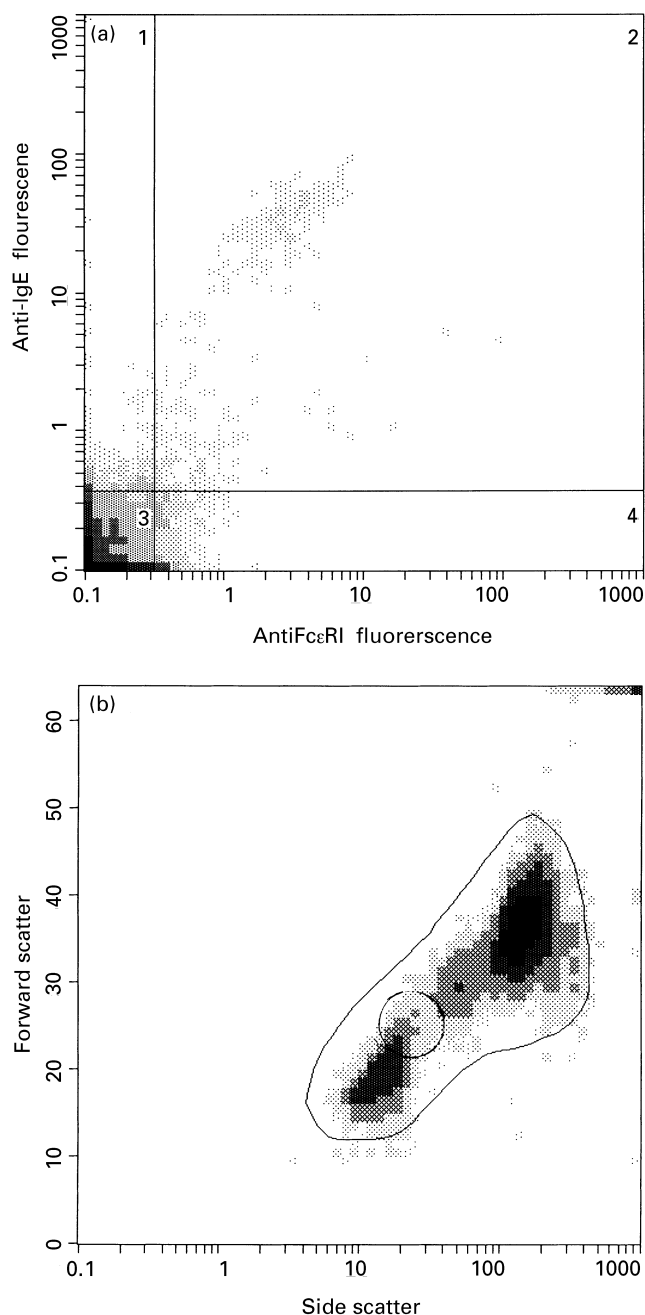


Fig. 1. (a) Dual fluorescence scattergram of anti-IgE and anti-FcεRI stained leucocytes showing a clearly defined population of dual stained cells in box 2 when compared with the isotype control (data not shown). (b) Size and granularity flow cytometry bitmap of peripheral blood leucocytes corresponding to forwards and sideways light scatter. The outer circle is the gate applied to exclude non-viable leucocytes. The inner circle shows the location of anti-IgE and anti-FcεRI dual stained basophils backgated onto the original scatter histogram.

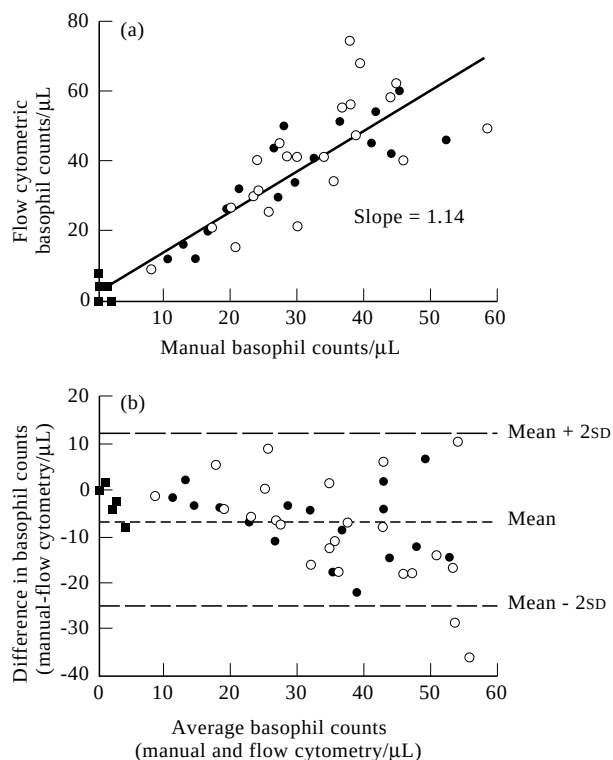


Fig. 2. (a) Scatterplot of basophil numbers in all subjects counted manually and by dual staining flow cytometry showing an approximately linear relationship. Chronic urticaria patients not showing serum histamine releasing activity (●); those showing histamine releasing activity (■) (there is some overlap of datapoints). Healthy controls (○). (b) Difference between blood basophil numbers in all subjects counted manually and by flow cytometry plotted against their means, showing relatively wide limits of agreement. Chronic urticaria patients not showing serum histamine releasing activity, (●); those showing histamine releasing activity. (■) (there is some overlap of datapoints). Healthy controls (○).

was significant ($P < 0.02$). Basophil counts by flow cytometry were also significantly lower in patients (25.2 ± 20.4) than controls (39.8 ± 16.8 , $P < 0.01$).

A scatterplot of basophil numbers for all subjects measured by both methods showed an approximately linear relationship (Fig. 2a, slope = 1.14 not significantly different from unity; urticaria patients alone 1.12; controls alone 1.11). Comparison of methods analysis revealed that agreement was not strong over the full range of values for all subjects, especially at the higher counts (Fig. 2b). The manual counts ranged from 25 below to 12 per μL of blood above the corresponding flow cytometer values, taking 2 SD either side of the mean difference. The limits of agreement were slightly narrower for chronic urticaria patients alone (range 19 per μL below to 9 per μL above the mean difference) but still not good enough to assume that manual and flow cytometric analyses of peripheral blood

basophils numbers are directly comparable in clinical practice.

Basophil histamine releasing activity

Seven of 24 chronic urticaria patients' sera that were available for testing showed $>5\%$ histamine release (Table 1), similar to the incidence in a larger study [5]. Of these, six released $>5\%$ histamine from the basophils of donor 1 ($46\% \pm 20$) and seven released from the basophils of donor 2 ($27\% \pm 25$). Five sera (patients 2 and 4, 5, 7 and 12, Table 1) gave substantial histamine release from the basophils of both donors consistent with the presence of anti-Fc ϵ RI α autoantibodies whose binding is not dependent on or inhibited by receptor-bound IgE. This pattern of release was seen with monoclonal antibody 29C6 (Table 1). One serum (patient 6) showed substantially less histamine release from basophils of donor 2 compared with donor 1, similar to 6F7, suggesting anti-receptor activity inhibited by receptor bound IgE. Serum from patient 1 released only from IgE-sensitized basophils of donor 2 consistent with the presence of anti-IgE autoantibodies. None of the sera from 24 healthy controls released $>5\%$ histamine from the basophils of donor 1 (mean $0.6\% \pm 0.3$) or donor 2 (mean $0.4\% \pm 0.7$). Four of the control sera tested on the basophils of donor 2 were of a later date than the blood used for basophil counts.

Relationship between basophil numbers and histamine releasing activity

In vitro The seven chronic urticaria patients with $>5\%$ *in vitro* serum histamine releasing activity had the lowest basophil counts by flow cytometry (Table 1) and formed a clearly defined subgroup (Fig. 3). Six of them were skin test positive. A similar distribution was shown with manually counted basophils (data not shown). Basophil counts in the subgroup of seven chronic urticaria patients showing *in vitro* histamine releasing activity in their sera were significantly lower than those without (2.3 ± 3.1 vs 34.2 ± 16.8 flow cytometry basophils/ μL , $t = 4.91$, $P < 0.001$). The difference between basophil counts of chronic urticaria patients without *in vitro* histamine releasing activity and healthy controls was not significant.

In vivo The relationship between basophil numbers and the autologous serum skin test weal response was less clearly defined (Fig. 4). Of the 11 patients showing a weal response to autologous serum skin testing at 30 min there was a subgroup of seven showing ≤ 8 basophils/ μL and a weal response ≥ 2 mm larger than an adjacent control saline skin test. Six of these stimulated $>5\%$ histamine release from basophils *in vitro*. The very small weal response in one

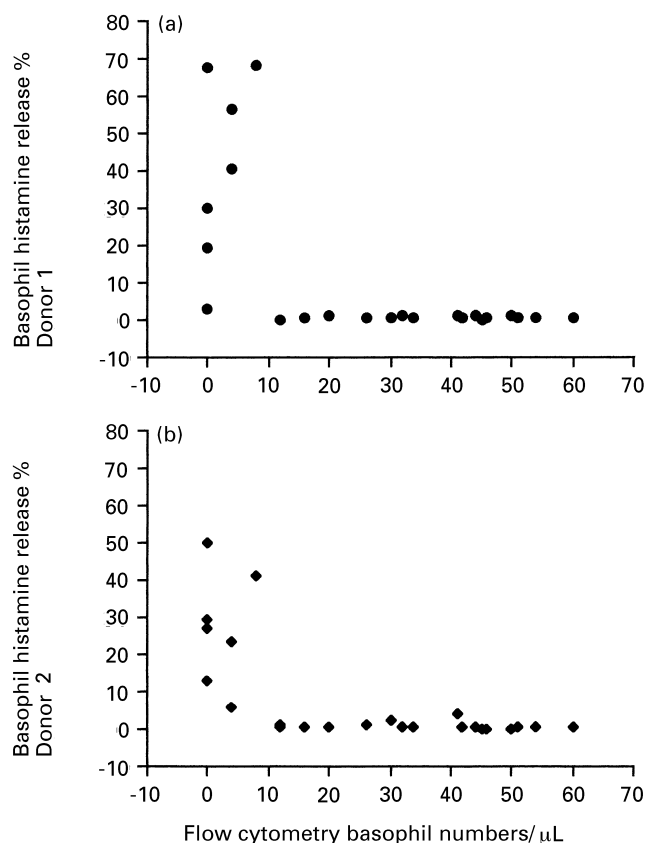


Fig. 3. Basophil numbers in chronic urticaria patients' blood counted by dual staining flow cytometry against the *in vitro* histamine release induced by the patients' sera from the basophils of two healthy donors (a) basophils of donor 1 (non-IgE sensitized) (b) basophils of donor 2 (IgE-sensitized). A clearly defined subgroup of patients have serum basophil histamine releasing activity and markedly reduced circulating basophil numbers.

patient showing significant *in vitro* histamine release (number 12, Table 1) may have been due to the skin test being performed over an area previously affected by a spontaneous weal since she had active widespread urticaria at the time and no detectable basophils by manual or flow cytometric counting. Four patients showed basophil numbers within the range of normal controls but still met the criteria for a positive autologous serum skin test response. As none of these showed *in vitro* histamine releasing activity on basophils of donor 1 and 2 it is possible their sera contained a mast cell-specific factor which appears to differ from either anti-Fc ϵ RI α or anti-IgE autoantibodies [17]. Basophil counts of the 11 chronic urticaria patients with positive autologous serum skin tests were lower than those without (13.5 ± 18.1 vs 34.4 ± 17.7 flow cytometry basophils/ μL , $t = 2.90$, $P = 0.008$). The difference between skin-test negative patients and controls (39.8 ± 16.8) was not significant.

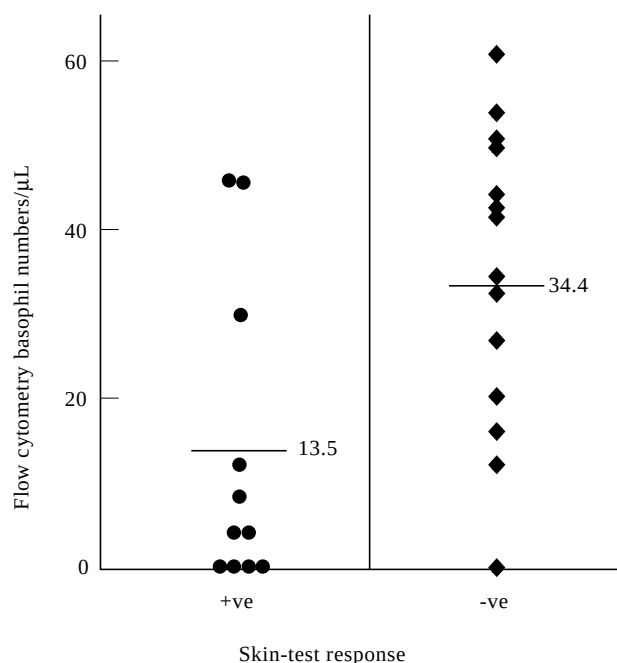


Fig. 4. The skin-test response, assessed by the weal response to autologous serum injection at 30 min, shows a similar but less clearly defined relationship with circulating peripheral blood basophil numbers than does *in vitro* basophil histamine release. Mean basophil numbers are shown for skin test positive and negative patients ($t = 2.90$, $P = 0.008$). Seven of 11 patients with a positive skin test have 8 or less basophils/ μL whereas all but one of those with a negative skin test have 12 or more. The one patient with a negative skin test and no basophils nevertheless showed significant *in vitro* serum basophil histamine releasing activity and probably had a false negative skin test. The four patients with positive skin tests and 12 or more basophils/ μL without significant basophil serum histamine releasing activity may have had a mast cell specific factor. ●, positive skin test; ◆, negative skin test.

IgE

There was no significant difference between total serum IgE in patients (180 ± 407 kU/L) and healthy controls (166 ± 353 kU/L). Two patients with serum IgE < 2 kU/L (numbers 5 and 9, Table 1) had similar basophil counts with manual staining and flow cytometry (1.5 vs 4; 27 vs 30 basophils/ μL) demonstrating that sufficient receptor-bound IgE was present on their basophils for detection with anti-IgE in the dual-staining method.

Discussion

These data extend earlier studies [1,2] by showing that a clearly defined subgroup of chronic idiopathic urticaria patients have basopenia associated with serum histamine releasing activity. The primary purpose of this study was to

examine circulating basophil numbers in chronic urticaria patients with histamine releasing activity in their blood rather than the nature of the histamine releasing factors. Nevertheless, the profile of release on basophils of two well characterised donors [5] was consistent with autoantibodies against Fc ϵ RI α in six of the seven patients and anti-IgE in one. The possibility that other factors, such as interleukin-3, might have been present in the sera and had a permissive effect on histamine release can not be completely excluded since the *in vitro* studies were not repeated with IgG purified from them. A higher proportion of the patients had evidence of *in vivo* serum histamine releasing activity, as indicated by their serum skin test response. The four patient's sera eliciting a skin-test weal response without *in vitro* histamine release from basophils could have had a circulating mast cell specific factor [17] which remains to be identified. There was no obvious clinical difference between the skin test positive and negative patients. It is possible that longitudinal studies would have shown basopenia and serum histamine releasing activity in more of the patients over the full course of their disease since clinical improvement was associated with reduced *in vitro* histamine releasing activity after plasmapheresis [18] and Rorsman showed that peripheral blood basophil numbers increased in urticaria patients within 3 days of their symptoms remitting [1].

In the present study basophils were undetectable in some patients by both granule staining and flow cytometry using surface immunophenotype so it is probable there is an absolute basopenia rather than a failure of detection of degranulated basophils. Bochner *et al.* [19] found that degranulation of partially purified basophils by anti-IgE, FMLP or calcium ionophore did not significantly affect the number of scatter characteristics of IgE-staining cells on flow cytometry and there was an inconsistent association between histamine release and reduction in basophils on light microscopy. Removal of activated basophils from the circulation by migration into spontaneous weals or removal by the reticuloendothelial system could explain the basopenia. An effect on basophil production in the bone marrow due to autoantibody binding Fc ϵ RI α or IgE can not be ruled out. Little is known of the fate of basophils in the circulation but adherence to activated human vascular endothelial cells has been demonstrated [20]. Activation of human basophils with anti-IgE has been shown to upregulate adhesion molecule expression [21] and to increase basophil adherence to cultured endothelial cells at lower concentrations of anti-IgE than required for optimal histamine release. Such a mechanism could facilitate removal from the circulation of basophils activated by autoantibodies in chronic urticaria patients. Basophil numbers were increased 14-fold over control sites in late phase reactions after antigen challenge in atopic skin [22] suggesting that a similar recruitment could occur in chronic urticaria weals.

Double-staining with anti-IgE and anti-Fc ϵ RI antibodies offers a new and simple method for quantifying basophils by flow cytometry. The relatively poor agreement with manual counting found in this study and the lower counts obtained with the chamber method could reflect subjective interpretation of colour differences between leucocytes, loss of staining in degranulated basophils and statistical errors associated with the relatively low basophil numbers counted in each chamber (ranging from 0 to 115). In contrast, flow cytometric analysis counted basophils in 50 000 peripheral blood leucocytes. Dual staining flow cytometry may be of value in monitoring longitudinal studies of chronic urticaria patients, the effects of urticaria treatments and other disorders associated with basophil histamine release including atopic eczema [23] and acute allergic reactions.

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References

- 1 Rorsman H. Basophilic leucopenia in different forms of urticaria. *Acta Allergologica* 1962; 17:168–84.
- 2 Grattan CEH, Francis DM, Hide M, Greaves MW. Detection of circulation histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991; 21:695–704.
- 3 Hide M, Francis DM, Grattan CEH, Barr RM, Winkelmann RK, Greaves MW. The pathogenesis of chronic idiopathic urticaria: new evidence suggests an auto-immune basis and implications for treatment. *Clin Exp Allergy* 1994; 24:624–7.
- 4 Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993; 328:1599–604.
- 5 Niimi N, Francis DM, Kermani F *et al.* Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol* 1966; 106:1001–6.
- 6 Fiebiger E, Maurer D, Holub H *et al.* Serum IgG autoantibodies directed against the α -chain of Fc ϵ RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995; 96:2602–12.
- 7 Kimura I, Moritani Y, Tanizaki Y. Basophils in bronchial asthma with reference to reagin-type allergy. *Clin Allergy* 1973; 3:195–202.
- 8 Bodger MP, Mounsey GL, Nelson J, Fitzgerald PH. A monoclonal antibody reacting with human basophils. *Blood* 1987; 69:1414–8.
- 9 Takahashi K, Takata M, Suwaki T *et al.* New flow cytometric method for surface phenotyping basophils from peripheral blood. *J Immunol Methods* 1993; 162:17–21.

- 10 Gane P, Pecquet C, Lambin P, Abuaf N, Leynadier F, Rouger P. Flow cytometric evaluation of human basophils. *Cytometry* 1993; 14:344–8.
- 11 Grattan CEH, Wallington TB, Warin RP, Kennedy CTC, Bradfield JW. A serological mediator in chronic idiopathic urticaria – a clinical, immunological and histological evaluation. *Br J Dermatol* 1986; 114:583–90.
- 12 Riske F, Hakimi J, Mallamaci M et al. High affinity human IgE receptor (FcεRI). Analysis of functional domains of the α-subunit with monoclonal antibodies. *J Biol Chem* 1991; 266:11245–51.
- 13 Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975; 7:283–90.
- 14 Moore JE, James GW. A simple direct method for absolute basophil leucocyte count. *Proc Soc Exp Biol Med* 1953; 82:601–3.
- 15 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; i:307–10.
- 16 De Boer M, Roos D. Metabolic comparison between basophils and other leukocytes from human blood. *J Immunol* 1986; 136:3447–54.
- 17 Kermani F, Niimi N, Francis DM et al. Characterisation of a novel mast cell-specific histamine releasing activity in chronic idiopathic urticaria. *J Invest Dermatol* 1995; 105: 452 (abstract).
- 18 Grattan CEH, Francis DM, Slater NGP, Barlow RJ, Greaves MW. Plasmapheresis for severe unremitting chronic urticaria. *Lancet* 1992; 339:1078–80.
- 19 Bochner BS, Sterbinsky SA. Altered surface expression of CD11 and Leu 8 during human basophil degranulation. *J Immunol* 1991; 146:2367–73.
- 20 Bochner BS, Luscinskas FW, Gimbrone MA Jr et al. Adhesion of human basophils, eosinophils and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991; 173:1553–6.
- 21 Bochner BS, MacGlashan DW, Marcotte GV, Schleimer RP. IgE-dependent regulation of human basophil adherence to vascular endothelium. *J Immunol* 1989; 142:3180–6.
- 22 Charlesworth EN, Hood AF, Soter NA et al. Cutaneous late phase response to allergen. Mediator release and inflammatory cell infiltrates. *J Clin Invest* 1989; 83:1519–26.
- 23 James JM, Kagey-Sobotka A, Sampson HA. Patients with severe atopic dermatitis have activated circulating basophil leucocytes. *J Allergy Clin Immunol* 1993; 91:1155–62.