

Increased responsiveness of basophils of patients with chronic urticaria to sera but hypo-responsiveness to other stimuli

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Summary

Background Although it has been shown that basophils from patients with chronic ordinary urticaria (CU) are less responsive than normal basophils when stimulated with anti-IgE, very few studies have examined the response of those cells to alternative stimuli.

Objective To compare releasability between basophils from healthy donors and patients with CU.

Methods We examined the response of IL-3-treated basophils from healthy donors, atopic controls and CU patients to anti-IgE, monocyte chemoattractant protein-1 (MCP-1), bradykinin, C5a and to sera obtained from other urticaria patients and normal controls. We also compared the response of basophils from CU patients whose sera activate normal basophils (autoimmune CU) from those who do not (idiopathic CU).

Results Basophils of CU patients release significantly less histamine than basophils of normal controls when stimulated with anti-IgE, and to a lesser degree with C5a. No differences were observed when basophils from patients were incubated with Bradykinin or MCP-1. However, when basophils of CU patients were incubated with sera from other CU patients or even normal sera, we found significantly higher histamine release compared with the response of basophils from normal donors. We could not distinguish responsiveness of basophils of patients with chronic autoimmune urticaria from patients with chronic idiopathic urticaria.

Conclusion Basophils of patients with chronic idiopathic urticaria and chronic autoimmune urticaria are hypo-responsive to anti-IgE and C5a, normally responsive to MCP-1 or bradykinin, and hyper-responsive to serum. The serum factor to which a response has not yet been identified; however, basophils of patients with chronic urticaria, in general, appear to have abnormal regulation of signaling pathways.

Keywords anti-IgE, autoimmunity, basophils, chronic urticaria, histamine, releasability

Submitted 29 March 2004; revised 21 January 2005; accepted 24 January 2005

Introduction

Urticaria is considered chronic when it is present daily or almost daily for 6 weeks or more. Apart from physical urticarias and urticarial vasculitis, in the majority of cases we cannot establish a cause. It has been suggested that this entity be named chronic ordinary urticaria (CU) [1]. An autoimmune origin is found in a subpopulation of such patients based on the ability of patients' sera to activate normal donor basophils and induce histamine release. This is because of the presence of anti-IgE receptor antibody (35–40%) and/or anti-IgE (5–10%) [2–4]. CU patients lacking any detectable autoantibody are designated 'idiopathic' [5] because no alternative etiology has been found.

Most studies have focused on the properties of chronic urticaria sera, but few studies have compared basophils (or mast cells) derived from chronic urticaria patients to basophils derived from normal donors. Prior reports have demonstrated that basophils of patients have a diminished response to anti-IgE [6–8] but no differences in releasability was found with stimuli such as fMLP, A23187 and PAF [9].

In this study of basophil releasability, we examined the response of basophils of healthy donors, atopic donors and patients with chronic urticaria to a variety of stimuli including anti-IgE, bradykinin [10], monocyte chemoattractant protein-1 (MCP-1) [11], C5a [12–14], and serum.

Furthermore, we divided chronic urticaria sera in two groups, one whose serum was able to activate normal basophils and therefore believed to be autoimmune, and the group lacking this ability, designated chronic idiopathic urticaria. We compared histamine releasability when these sera are incubated with basophils derived from patients with chronic urticaria.

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Materials and methods

We studied basophils from 30 patients with chronic urticaria (21 female, age: median: 47(37; 57)), 21 healthy control subjects (17 female, age: median: 27(25; 35)) and 12 atopic donors (six female, age: median: 28(20; 45)). The number of basophils employed for each experiment is included in Table 1. These basophils were incubated with sera from another 20 healthy donors (15 female, age: mean: 38.08(14.44)) and 45 patients with chronic urticaria (31 female, age: 45.54(12.9)).

We defined chronic urticaria patients as having recurrent hives of greater than 6 weeks duration, occurring at least four times a week, with individual lesions lasting less than 24 h. Antihistamine treatment was stopped at least 48 h before serum samples were collected, and none of the patients were taking corticosteroids or immunosuppressive drugs at the time of veinpuncture. Patients with clinical evidence of urticarial vasculitis or physically induced urticaria (e.g. cold urticaria, cholinergic urticaria, dermatographism) were excluded.

We considered atopic controls as those patients with rhinitis and/or asthma, or eczema, and a positive skin test to at least one allergen, and specific IgE (CAP-FEIA, Pharmacia, Upsala, Sweden) against that allergen. We included as healthy donors those with negative skin prick tests to a standard pool of common allergens, no history of hives, rhinitis, asthma, or eczema, and normal IgE levels.

All sera were separated by centrifugation at 1000 g for 10 min and stored at -80°C .

Leucocyte isolation

Because the aim of our study was to compare basophils, we only included those CU patients with no basopenia in order to be able to adjust basophil number. We found that 25% of our CU patients had basopenia and so were not included in this work.

10 mL of whole blood was collected in EDTA coated venepuncture tubes and were mixed with 2 mL of 3% dextran-3% glucose-0.15 M NaCl saline solution. The mixture was sedimented for 90 min, the supernatant was removed, and the cells were placed in 15 mL conical tubes, which were washed twice with HBSS-HSA at pH 7.4 (NaCl, KCl, Hepes 10 mM, HSA, Glucose, NaH_2PO_4 , double distilled water). The cells were then resuspended in HBSS-HSA containing 2 mM

CaCl_2 , 1 mM MgCl_2 . For all the experiments the basophil number was adjusted to $1 \times 10^4/\text{mL}$. Manual basophil counts were performed at a 1/2 dilution of an alcian blue-based stain [15] using a modified Fuchs–Rosenthal (Manners) chamber, as described previously [16] and expressed as counts/ μL of whole blood.

Once adjusted for cell number, we incubated basophils with a buffer containing IL3 for 15 min at 37°C (Hepes 20 mM, NaCl 133 mM, KCl 5 mM, CaCl_2 7 mM, MgCl_2 3.5 mM, BSA 1 mg/mL, 2 ng/mL of IL3) and then incubated this cell suspension with the different stimuli for 30 min at 37°C . After incubation, the supernatants were separated by centrifugation at 1000 g for 10 min at 4°C , and histamine release was determined. Two replicate aliquots of cells were boiled to determine total basophil histamine content.

Histamine release from basophils

We employed two techniques to determine histamine release. We utilized an ELISA assay for the study of the histamine release by serum. To compare histamine release from basophils of healthy subjects and chronic urticaria patients stimulated with bradykinin, MCP-1, C5a, anti-IgE, spontaneous release and total histamine content, we utilized a fluorometric technique. This was because of practical considerations e.g. amount of sera needed and cost of determinations. Both techniques were performed in our laboratory in the same experiments with the same cell suspension. The only variation between the two techniques is the amount of sample employed.

For the ELISA technique, 75 μL of serum or buffer was incubated with 75 μL of basophil suspension for 30 min at 37°C .

Histamine release was measured by ELISA (Immunotech International, Marseille, France) according to the manufacturer's instructions and replicate samples agreed within 10%. For the fluorometric technique, 250 μL of buffer or stimulus was incubated for 30 min at 37°C with 250 μL of cell suspension and histamine was assayed by fluorometric method [17] as previously described [18].

We calculated the percentage release using the following formula: $(\text{Sample-Basal})/(\text{Total-Basal}) \times 100$. Spontaneous histamine was less than 5% of total histamine.

The sensitivity of both methods is 0.5 ng/mL.

Subjects were identified as having functional autoantibody to the IgE receptor when sera released 15% or more when incubated with basophils of healthy donors. This percentage was calculated based upon the results obtained with 24 control sera from healthy subjects [3]. The percentage histamine release in this study varied from 2–20% with a mean of 9.7% and a standard deviation of 3.6%. A response of 2SD from the mean was considered positive.

Statistical analysis

To compare two independent samples (Basophils of patients vs. healthy with stimuli: bradykinin, MCP-1 and C5a) we used the Students' *t* test when the distribution was normal (MCP-1) and the Mann–Whitney *U*-test for a non-normal distribution (bradykinin and C5a). We used the Mann–

Table 1. Number of basophil donors included in each experiment

Experiment	Number of basophil donors	
	Control	Urticaria
Spontaneous histamine release	21	21
Total histamine content	21	21
Anti-IgE	21	21
Bradykinin	16	13
C5a	16	13
MCP-1 100 ng/mL	18	18
MCP-1 10 ng/mL, 1 ng/mL	16	13

MCP, monocyte chemoattractant protein-1.

Whitney *U*-test to compare the histamine released upon stimulation with sera.

To compare the spontaneous and total histamine release between healthy controls, atopic controls and chronic urticaria patients, we utilized a nonparametric test for K-independent samples (Kruskal–Wallis) followed by a Mann–Whitney *U*-test. We used the same test to compare the histamine released upon stimulation with anti-IgE.

To compare basophil histamine release from patients with and without the anti-IgE receptor autoantibody, we employed a Mann–Whitney *U*-test.

Results

Anti-IgE histamine release

When we stimulated basophils of chronic urticaria patients (CU basophils) with anti-IgE and compared the results with histamine release upon stimulation of basophils from healthy controls with anti-IgE, we observed a significantly decreased histamine release in CU basophils (median: healthy control, 51.04%; atopic, 50.91; CU, 10; $P = 0.000$). There were no differences in histamine release between healthy controls and atopic subjects (Fig. 1).

Spontaneous and total histamine content

We then compared spontaneous and total histamine release between controls, atopic subjects and CU basophils in order to assess whether these differences were because of differences in histamine content. We found that in spite of a much lower histamine release when stimulating with anti-IgE, CU basophils release more histamine at basal conditions with no stimulus (Fig. 2a). Spontaneous histamine release was significantly higher in atopic donors (Median: healthy controls, 1.58 ng/mL; atopic donors, 5.65; CU patients, 3.08 ng/mL; healthy controls vs. urticaria patients $P = 0.03$, atopic donors vs. urticaria patients $P > 0.05$, healthy donors vs. atopic subjects $P = 0.002$).

In contrast, significant differences were not found when comparing total histamine content between control and CU basophils, although there was a significantly higher total histamine content in atopic basophils vs. healthy controls

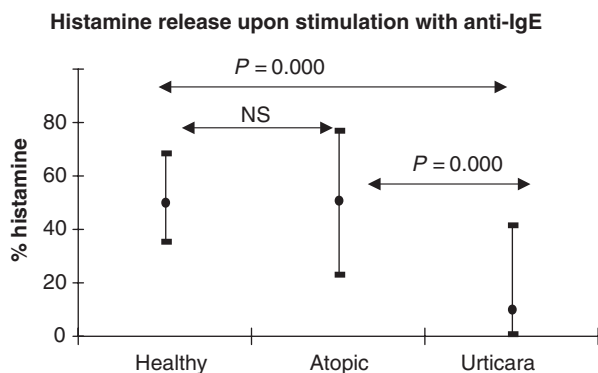


Fig. 1. Histamine release from basophils of healthy donors, atopic subjects and chronic urticaria patients is compared upon incubation with anti-IgE (median interquartile range).

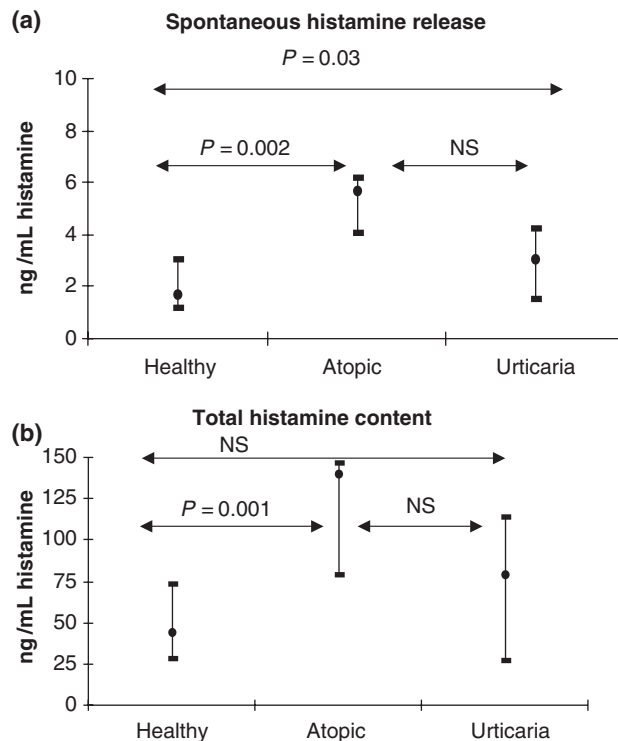


Fig. 2. (a) Spontaneous histamine release from basophils of healthy donors, atopic subjects and chronic urticaria patients is compared (median interquartile range (IQR)). (b) Total histamine content in basophils from healthy donors, atopic subjects and chronic urticaria patients is compared. Two replicate aliquots of cells were boiled to determine total basophil histamine content (median IQR).

(Median: healthy controls, 47.49 ng/mL; atopic, 139.58; $P = 0.001$) as shown in Fig. 2b.

Histamine release when stimulating with bradykinin, C5a and MCP-1

We then compared histamine release with stimuli that act upon different receptors, each of which signals via G proteins such as bradykinin, C5a and MCP-1.

We found a significantly decreased response of CU basophils compared with normal basophils at the lower C5a concentration employed i.e. at 10 and 100 ng/mL, but not with 1 µg/mL (Fig. 3) although the trend with 1 µg suggested a diminished response of CU basophils as well.

With bradykinin, CU basophils had a slightly diminished response compared with basophils of healthy controls, but the differences did not reach statistical significance.

There were also no differences upon stimulation of basophils of chronic urticaria patients and healthy controls with MCP-1.

Histamine release when incubating with sera

Surprisingly, we observed a much greater histamine release when basophils of CU patients were stimulated with other chronic urticaria patients sera compared with healthy basophils stimulated with CU sera. Moreover, even those sera that were not able to activate healthy basophils were highly active on CU basophils compared with healthy basophils. Finally, control sera were also able to activate CU

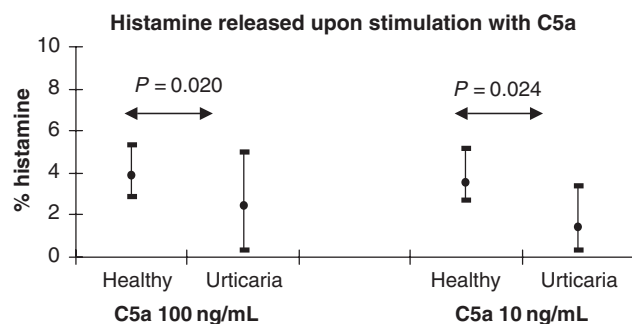


Fig. 3. Comparison of histamine release between basophils from control donors and chronic urticaria patients upon stimulation with two concentrations of C5a (median interquartile range).

basophils more readily than healthy basophils. The results indicate that basophils of chronic urticaria patients are more responsive to some constituent of serum regardless of source, that sera from urticaria patients are more active than healthy sera, and that sera of patients with chronic idiopathic urticaria are no less active in this assay than are sera from patients with chronic autoimmune urticaria (Fig. 4).

Releasability depending upon the presence of the autoantibody

Finally, we wanted to study whether there were differences in releasability with the different stimuli studied between those patients having anti-receptor autoantibody and those that do not. For this purpose, we compared basophil releasability from basophils selected from 10 patients whose sera were able to induce histamine release when incubating with control basophils, and five from those which were not able to do so. We did not find statistically significant differences in any instance. Thus both groups of basophils were equally responsive to bradykinin, C5a, MCP-1, or serum. These data are summarized in Table 2.

Discussion

In this work, we confirm previous reports concerning a diminished histamine release of chronic urticaria basophils when incubated with anti-IgE [6, 9]. These results are not because of a variation in histamine content because we did not find significant differences between healthy control and urticaria basophils. We did, however, observe higher total histamine content and spontaneously released histamine when basophils of atopic subjects were compared with basophils of healthy controls or patients with chronic urticaria. These results are consistent with those published by Wahn [19] and Zuberbier et al. [9].

Although basophils of chronic urticaria patients seem to be less responsive to stimuli such as anti-IgE or C5a, which act through different receptors, the abnormality does not seem to be because of a general impairment of signaling because CU basophils do respond normally to other stimuli that act independently from the IgE receptor such as A23187, FMLP and PAF [9], bradykinin [10], and MCP-1 [20].

In this study in the autoimmune group we routinely do not distinguish between anti-receptor and anti-IgE antibody

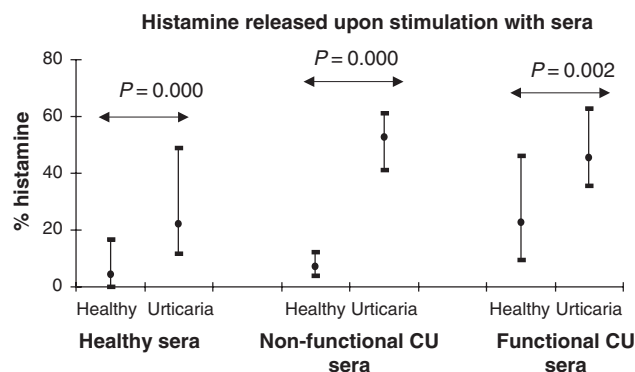


Fig. 4. Basophils from control donors and chronic urticaria patients were incubated with three types of sera: sera from control donors, sera from patients with chronic idiopathic urticaria not having the ability to activate healthy basophils (non-functional CU sera) and sera from patients with chronic urticaria having the autoantibody able to activate normal basophils (functional CU sera) (median interquartile range).

Table 2. Histamine release after incubating with the different stimuli studied, dividing basophils in two groups: one from basophils whose sera has the ability to activate normal basophils, and the other from basophils whose sera does not activate control basophils, so-called functional and non-functional CU, respectively

Stimuli	Functional CU	Non-functional CU	
Anti-IgE	6.71	4.45	NS
Bradykinin 1 µg	1.4	0	NS
Bradykinin 100 ng	0	2.22	NS
Bradykinin 10 ng	0.55	2.22	NS
C5a 1 µg	18.92	25	NS
C5a 100 ng	1.96	2.78	NS
C5a 10 ng	1.60	0.7	NS
MCP-1 100 ng	8.08	6.45	NS
MCP-1 10 ng	2.98	3.64	NS
MCP-1 1 ng	4.08	1.21	NS
CU sera	49.52	35.25	NS

The figures correspond to percentage of histamine release. The results are expressed as median

CU, chronic ordinary urticaria; MCP, monocyte chemoattractant protein-1; NS, not significant.

because anti-IgE is much less common: 5% compared with 40%. It has been hypothesized that hypo-responsiveness is because of *in vivo* desensitization of the basophils [21], but this may not be the case because we also find decreased responsiveness of basophils obtained from patients lacking anti-receptor antibody. In fact, when we compared releasability after incubating (data included in Table 2) basophils from patients with or without identifiable autoantibodies with anti-IgE, we did not find any significant difference. This differs from a prior report of Sabroe et al. [22] but their data were derived from one donor whose basophils were IgE-depleted and a second donor whose basophils were sensitized with IgE, and therefore are not comparable.

Our most surprising finding was that chronic urticaria basophils, in spite of being less responsive to many stimuli, are clearly highly responsive when incubated with sera; even normal sera. One could argue that this effect might be because of variability in donor basophil histamine release; however our study employed the same basophil preparation incubated with different stimuli. When different basophil

preparations were compared, the counts were similar, and the results were strikingly consistent for all CU basophils studied. Moreover, we previously found (as it is observed *in vivo*), that CU basophils release histamine when incubated with their own serum as well as sera of others (data not shown).

In summary, we conclude that chronic urticaria basophils have several specific features that distinguish them from basophils of healthy donors or atopic controls. They are less respondent to anti-IgE and C5a with no difference when stimulated with bradykinin and MCP-1 and have much higher release when incubated with serum. The factor in serum that stimulates these cells has not been identified nor is the abnormal responsiveness of the cells understood. The other known basophil abnormality in chronic urticaria is basopenia, which is present in patients with anti-receptor antibody as well as those with chronic idiopathic urticaria [23, 24].

Although 55% of chronic urticaria patients are still considered idiopathic because the etiology is obscure, including absence of autoantibodies, this is the first demonstration that basophils of this group share abnormal responsiveness with basophils of patients with chronic autoimmune urticaria, just as the histology of the two groups is strikingly similar [25, 26].

Acknowledgements

This work was supported by the Fondo de Investigación Sanitaria of Spain grant FIS #03/0789

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