

Histamine releasability of basophils and skin mast cells in chronic urticaria

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In order to clarify the pathogenetic role of basophils and mast cells in chronic urticaria, histamine and leukotriene (LT)₄ release was examined in washed mixed leukocytes ($n=8$) and skin mast cells ($n=5$) from patients with chronic urticaria and compared with the same cells from normal controls ($n=9$). Anti-IgE-stimulated basophil histamine release was significantly reduced in urticaria patients (median 2.9% vs 15.1% in normal controls), whereas histamine release to A23187, FMLP, and PAF, as well as anti-IgE-induced LTC₄ release, showed no differences in both groups. In contrast, anti-IgE-stimulated skin mast cells from urticaria patients reacted similarly to those of controls (median histamine release 11.4% vs 14.2% in normal controls). Pretreatment of the cells with interleukin (IL)-3 upregulated responsiveness of basophil histamine release to anti-IgE in urticaria patients (median histamine release 14.3%), but pretreatment with the H₂-antagonist cimetidine showed no effect. These data show that reduced basophil histamine releasability in chronic urticaria is not H₂ mediated. It is a stimulus-, mediator-, and cell type-restricted phenomenon that can, at least partially, be reversed in the presence of the cytokine IL-3.

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Urticaria is a common skin disease, approximately 12–15% of the population being affected by acute episodes of whealing at least once in their lifetime (1, 2). In a minority of these patients, the disease becomes chronic, generally defined as urticaria lasting longer than 6 weeks. Histamine, released from cutaneous mast cells, is considered to play the dominant role in the pathogenesis of whealing in chronic urticaria (3, 4). In previous studies on basophil histamine release in chronic urticaria, defects in anti-IgE-induced histamine release have been noted (5–7), but the pathophysiologic significance of this observation has remained unclear. The present study attempts to shed further light on this observation by investigating histamine release from basophils compared to skin mast cells in chronic urticaria.

Material and methods

Materials

The following reagents were purchased: Pipes, collagenase type 1a, hyaluronidase type I-S, N-FMLP,

PAF (Sigma, Deisenhofen, Germany), anti-IgE (polyclonal from goat, Behring, Marburg, Germany), DNase I (Boehringer, Mannheim, Germany), A23187 (Calbiochem, Bad Soden, Germany), cimetidine (SmithKline Beecham, Munich, Germany), MEM/Earle's salts, FCS, IL-3 (Gibco, Berlin, Germany), histamine EIA (highly specific for histamine, sensitivity 0.2 nM; Immunotech, Hamburg, Germany), LTC₄ EIA (cross reaction with leukotriene (LT)₄: 46%, LTE₄: 2% and LTB₄: <0.01%, sensitivity 18 pg/ml, Cayman, Ann Arbor, MI, USA).

Buffers. Pipes A (containing 0.1% HSA), Pipes EDTA (Pipes A containing 2% 0.1 M EDTA), and Pipes ACM (Pipes A containing 2 mM CaCl₂ and 0.5 mM MgCl₂) were used in all experiments at a pH of 7.40.

Medium. In all experiments with mast cells, MEM containing 2% FCS, 1% penicillin and streptomycin, 1% glutamine, 2% MgSO₄, and 10 µg/ml DNase was used during the isolation process.

Patient selection and leukocyte isolation

Venous blood was drawn after informed consent from hospitalized patients with chronic urticaria (four men, four women, age range 24–53 years, daily attacks for at least 3 months) and age-matched nonatopic healthy volunteers (four men, five women). None of the subjects used any medication for at least 5 days prior to venipuncture and biopsy (including antihistamines; none had been treated with astemizole).

Twenty milliliters of blood was drawn and placed into a polypropylene tube containing EDTA (2 ml, 0.1 M), dextrane (5 ml, 6%), and dextrose (0.8 ml, 40%). After sedimentation (90 min, 22°C), the leukocyte-containing upper layer was removed and centrifuged (4°C, 8 min, 130 g). The cells were then washed twice (first Pipes EDTA, then Pipes A) and resuspended in Pipes ACM.

Preincubation with human recombinant interleukin-3 and cimetidine

Leukocytes were preincubated for 15 min at 37°C with recombinant interleukin (IL)-3 (20 ng/ml) or cimetidine (1, 10, 100 µM) and then stimulated as described below. In preliminary experiments, this dose of IL-3 had been found to be optimal for the enhancement of anti-IgE-induced histamine release, but did not induce histamine release itself.

Preparation of human skin mast cells

Mast cells from normal human breast skin (from plastic surgery), or in the case of urticaria patients from 4-mm punch biopsies (uninvolved skin from the upper thorax, were isolated by enzymatic dispersion, with slight modification as described before (8). Briefly, skin was cut into small pieces (1–2 mm²), dispersed in two 1-h cycles with collagenase and hyaluronidase (15 and 7.5 mg/g tissue, respectively, in 2.5 ml medium/g tissue), separated from undissociated tissue by filtration through nylon gauze (150 µm) and then washed twice in Pipes A. Viability was checked by trypan blue staining, and mast cell purity with toluidine blue staining. This procedure yielded $6\text{--}8 \times 10^5$ mast cells/g wet weight tissue, with 3–5% mast cells of total nucleated cells and more than 95% viable cells.

Mediator release

Aliquots of 7×10^5 mixed leukocytes were challenged in a total volume of 0.5 ml in duplicate, with an optimal dosage of the following stimuli dissolved in Pipes ACM, determined in preliminary experiments: anti-IgE (400 IU/ml with 0.417 IU precipit-

ating 1 ng WHO IgE standard), Ca-ionophore (1 µM), N-FMLP (1 µM), or PAF (1 µM).

Aliquots of 10^4 mast cells were challenged in a total volume of 0.1 ml Pipes ACM in duplicate with anti-IgE at an optimal dosage (2000 IU/ml) established in preliminary experiments. Spontaneous histamine release was determined by addition of Pipes ACM instead of stimuli. Total cellular histamine was assayed by lysis of aliquots with 2% perchloric acid. After a 20-min incubation at 37°C (leukocytes) or 30°C for mast cells (optimal release temperature for mast cells), cells were centrifuged, and supernatants were kept for later determination of mediator release. Histamine release is expressed as percent release of total histamine after subtraction of spontaneous release.

Mediator assays

Histamine was either measured by the automatic fluorometric method described by Siraganian (9) (basophils) or determined with a sensitive commercial EIA kit (mast cells). LTC₄ was determined with a commercial EIA kit.

Statistics

As the data were not normally distributed, the nonparametric Wilcoxon test for unpaired data was used throughout (10), and medians, instead of means and SEM, are stated.

Results

In patients with chronic urticaria, anti-IgE-induced basophil histamine release was markedly reduced compared to controls, whereas no differences were observed upon stimulation with the IgE-receptor independent stimuli FMLP, A23187, or PAF (Fig. 1). Fig. 1 shows histamine release with optimal concentrations of stimuli. For both urticaria patients and controls, maximum histamine release was seen with 400 IU anti-IgE (with a range of 50–1600 IU/ml being used for stimulation). The observed reduction of histamine release in urticaria patients was therefore not due to a shift in the dose-response curve to anti-IgE but reflects a decrease of histamine releasability toward stimulation of the IgE-receptor.

Leukocytes of chronic urticaria patients and controls also did not differ in spontaneous histamine release, which was below 5% in all experiments, the number of basophils in the differential blood count and total histamine content (1.1 ± 0.25 pg/basophil in urticaria vs 1.0 ± 0.2 pg/basophil in controls).

Reduction of releasability to anti-IgE was limited to the release of the preformed mediator histamine.

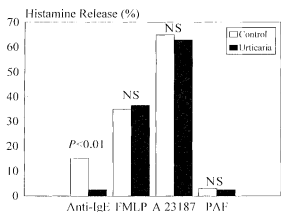


Fig. 1. Median basophil histamine release upon stimulation with anti-IgE (400 IU/ml), FMLP (1 μ M), A23187 (1 μ M), and PAF (1 μ M) in urticaria patients ($n=8$) and controls ($n=9$).

No differences were observed in IgE-receptor dependent release of the newly generated mediator LTC_4 from basophils of urticaria patients compared to controls (median 586 pg/ 10^6 leukocytes, range 253–1564 pg, vs median 594 pg/ 10^6 leukocytes, range 339–1390 pg, in controls, on stimulation with an optimal dose of 400 IU/ml anti-IgE; $n=8$ in both groups). A similar average amount of LTC_4 release and the high interindividual variability have been described previously for healthy donors (11). From these data, it can be concluded that reduced basophil histamine releasability to anti-IgE in urticaria is not due to an impaired responsiveness of the IgE-receptor to cross-linking.

Changes in histamine releasability were also limited to basophils. Skin mast cells of urticaria patients showed a similar histamine releasability to that of controls when stimulated with optimal doses of anti-IgE for mast cells, which are higher than for basophils (median histamine release 11.4% vs 14.2% in normal controls on stimulation with 2000 IU/ml anti-IgE (Fig. 2).

In order to investigate whether the observed decrease of anti-IgE-induced basophil histamine release in chronic urticaria patients was due to a total blockade of IgE-mediated degranulation or might still be modified by cytokines, leukocytes were preincubated with IL-3. This cytokine is known to be a potent enhancer of IgE-induced histamine release in human basophils *in vitro* (12, 13) as well as *in vivo* (14). These experiments showed that pretreatment of leukocytes with IL-3 was able to augment histamine release in urticaria patients (Fig. 3a), although enhancement of median anti-IgE-induced histamine release after IL-3 treatment was less pronounced than that observed in controls (Fig. 3b).

Since the experiments with IL-3 indicated that low basophil histamine release in urticaria patients

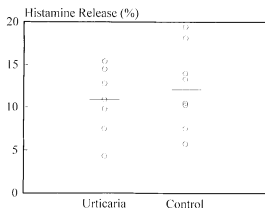


Fig. 2. Skin mast cell histamine release on stimulation with anti-IgE (2000 IU/ml) in urticaria patients ($n=7$) and controls ($n=8$).

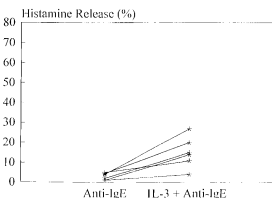


Fig. 3a. Influence of pretreatment with IL-3 (20 ng/ml) on anti-IgE-stimulated (400 IU/ml) basophil histamine release in urticaria patients.

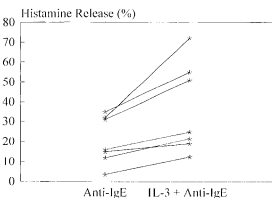


Fig. 3b. Influence of pretreatment with IL-3 (20 ng/ml) on anti-IgE-stimulated (400 IU/ml) basophil histamine release in healthy controls.

was not due to a general defect in secretory activity but could be upregulated in the presence of this cytokine, a possible downregulation in basophil releasability by high serum histamine levels in chronic urticaria due to mast-cell degranulation was

considered. Histamine, at serum concentrations occurring *in vivo* during allergic reactions, is known to inhibit basophil histamine release (15). This inhibitory action is mediated via activation of the H_2 -receptor and can be reversed in the presence of H_2 -antagonists (16, 17), which can even enhance anti-IgE-induced basophil histamine release.

In order to evaluate this possible inhibitory effect of H_2 -receptor activation in urticaria, experiments with the H_2 -blocker cimetidine were performed. In contrast to normal persons in whom cimetidine enhanced histamine release, no effect was observed on anti-IgE-induced basophil histamine release in urticaria patients (median anti-IgE induced histamine release: 8.0% without vs 17.5% with cimetidine in controls, compared to 3.5% vs 3.0%, respectively, in urticaria patients; stimulation with anti-IgE, 400 IU/ml, $n=5$). These data suggest that activation of the H_2 receptor is probably not responsible for low basophil histamine releasability in urticaria.

Discussion

Basophil releasability is defined as the ability to secrete histamine upon a given IgE receptor-dependent stimulus (18). A high inter- and intra-individual variance of releasability is observed in healthy nonatopic persons, but, generally, releasability is higher in atopic subjects (18, 19). In contrast, basophils of patients with chronic urticaria have been shown to release only insignificant quantities of histamine upon stimulation with anti-IgE (5-7), and a defect in the release mechanism has been assumed but has not been further investigated.

Until now, the factors regulating releasability have been only partly understood. Releasability does not correlate with the number of IgE receptors on the cell surface (20) or the number of unoccupied receptors (21), but it can be modified by various cytokines (22).

In the present study, it could be shown that decreased releasability in chronic urticaria was restricted to basophils and to histamine release. Skin mast cells of urticaria patients, which are regarded as the primary effector cells and thus the most relevant sources of mediators in this disease, showed the same releasability as skin mast cells of normal controls. Although the experiments with skin mast cells from urticaria patients could be performed only in the limited number of seven patients and the punch biopsies could not be taken from identical anatomic sites of the thorax compared to normal skin from breast surgery, the results appear relevant since none of the seven mast-cell experiments showed poor releasability, in contrast to patients' basophils where 7/8 experiments showed poor releasability of less than 5% histamine release.

A general defect in the basophil IgE receptor-mediated response can be excluded since anti-IgE-induced LTC_4 release from basophils of urticaria patients was not affected. Furthermore, no differences in histamine release compared to controls were observed when cells were stimulated with other receptor-dependent stimuli (FMLP or PAF) or the unphysiologic stimulus A23187. A total impairment of the ability to secrete histamine can therefore be excluded as well. In addition, the experiments with IL-3 show that low IgE-mediated basophil releasability in urticaria can, at least in part, be reconstituted in the presence of this physiologic cytokine. In summary, these experiments suggest a specific downregulation of IgE-mediated basophil histamine releasability in urticaria, but not a defect in releasability, as previously suspected (5-7).

As skin mast cells are the primary effector cells in urticaria, the observed alterations in basophil histamine release could be a secondary and reversible effect. As histamine inhibits histamine release in a negative feedback (15), elevated serum histamine levels could have been responsible for the inhibition of histamine release in urticaria patients. This inhibition has been shown to be due to an activation of basophil H_2 -receptors by histamine, which, in the presence of H_2 -antagonists, can be reversed, even resulting in enhancement of histamine release (16, 17). As preincubation of the leukocytes with cimetidine did not affect low basophil releasability in urticaria patients, the observed downregulation is probably not histamine mediated; however, since the experiments were performed *in vitro*, an *in vivo* effect of histamine cannot be totally excluded.

In conclusion, reduced basophil histamine releasability to anti-IgE in chronic urticaria appears to be a specific, reversible downregulation, but further studies are necessary to investigate the underlying pathomechanisms and the significance of this finding.

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References

- Sheldon JM, Mathews KP, Lovell RG. The vexing urticaria problem. Present concepts of etiology and management. *J Allergy* 1954;25:525-60.
- Champion RH, Roberts SOB, Carpenter RG, Roger JH. Urticaria and angio-oedema: a review of 554 patients. *Br J Dermatol* 1969;81:588-97.
- Czarnetzki BM. Basic mechanisms. In: Czarnetzki BM, editor. *Urticaria*. Berlin: Springer, 1986:5-24.

4. Schwartz LB. Mast cells and their role in urticaria. *J Am Acad Dermatol* 1991;25:190-204.
5. Greaves MW, Plummer VM, McLaughlan P, Stanworth DR. Serum and cellbound IgE in chronic urticaria. *Clin Allergy* 1974;4:265-71.
6. Kern F, Lichtenstein LM. Defective histamine release in chronic urticaria. *J Clin Invest* 1976;57:1369-77.
7. Czarnetzki BM, Kern F, Lichtenstein LM. Defective release of eosinophil chemotactic factor from peripheral leukocytes in patients with chronic urticaria. *J Invest Dermatol* 1976;67:276-8.
8. Lowman MA, Rees PH, Benyon RC, Church MK. Human mast cell heterogeneity: histamine release from skin, lung, adenoids, tonsils and colon in response to IgE-dependent and non-immunologic stimuli. *J Allergy Clin Immunol* 1988;81:590.
9. Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283-90.
10. Noether GE. Introduction to statistics. The nonparametric way. New York: Springer, 1991.
11. MacGlashan DW Jr, Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J Immunol* 1986;136:2231-9.
12. Hirai K, Morita Y, Misaki Y, et al. Modulation of human basophil histamine release by hemopoietic growth factors. *J Immunol* 1988;141:3958-64.
13. Kurimoto Y, de Weck AL, Dahinden CA. The effect of interleukin 3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation. *Eur J Immunol* 1991;21:361.
14. Merget RD, Maurer AB, Koch U, et al. Histamine release from basophils after *in vivo* application of recombinant human interleukin-3 in man. *Int Arch Allergy Appl Immunol* 1990;92:366-74.
15. Bourne HR, Melmon KL, Lichtenstein LM. Histamine augments leukocyte adenosine 3',5'-monophosphate and blocks antigenic histamine release. *Science* 1971;173:743-5.
16. Tedeschi A, Lorini M, Arquati M, Miadonna A. Regulation of histamine release from human basophil leukocytes: role of H₁, H₂ and H₃ receptors. *Allergy* 1991;46:626-31.
17. Kleine Tebbe J, Buschauer A, Friese A, Schunack W, Kunkel G. Modulation of IgE-mediated histamine release from human leukocytes by a new class of histamine H₂-agonists. *Agents Actions* 1992;35:185-91.
18. Gaddy JN, Busse WW. Enhanced IgE-dependent basophil histamine release and airway reactivity in asthma. *Am Rev Respir Dis* 1986;134:669-74.
19. Casolaro V, Spadaro G, Marone G. Human basophil releasability. VI. Changes in basophil releasability in patients with allergic rhinitis or bronchial asthma. *Am Rev Respir Dis* 1990;142:1108-11.
20. Conroy MC, Adkinson NF, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-21.
21. MacGlashan DW. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993;91:605-15.
22. Dahinden CA, Bischoff SC, Brunner T, Krieger M, Takafuji S, de Weck AL. Regulation of mediator release by human basophils: importance of the sequence and time of addition in the combined action of different agonists. *Int Arch Allergy Appl Immunol* 1991;94:161-4.

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