

Comparison of histamine-releasing factor recovered from skin and peripheral blood mononuclear cells of patients with chronic idiopathic urticaria

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Background: The pathogenesis of chronic idiopathic urticaria is characterized by defective histamine release. Skin mast cells show an increased release of histamine while circulating basophils are less responsive to immunologic stimulus.

Objective: The purpose of the study was to examine and compare the production of the histamine-releasing factor in the skin and within the peripheral blood of patients with chronic idiopathic urticaria and normal control subjects, as a possible factor responsible for the difference observed in the releasability of both skin mast cells and basophils.

Methods: Using the skin chamber technique, histamine-releasing factor production and histamine concentration were assessed in normal-appearing skin of patients with chronic idiopathic urticaria ($n = 12$) and normal controls ($n = 5$) over a 2-hour observation period. In both groups, histamine-releasing factor production by peripheral blood mononuclear cells was also measured.

Results: The weighted average of histamine-releasing factor production during the 2-hour observation period was higher in the non-lesional skin of patients with chronic idiopathic urticaria as compared with normal controls ($5.6 \pm 1.4\%$ versus $0.7 \pm 0.6\%$, $P < .01$). In contrast, less histamine-releasing factor was produced by peripheral blood mononuclear cells in chronic urticaria as opposed to normal controls ($17.2 \pm 2.1\%$ versus $25.7 \pm 2.8\%$, $P < .03$). Spontaneous histamine concentration was not significantly different in patients with chronic urticaria than in normal controls.

Conclusion: Histamine-releasing factor production is increased in the skin, and decreased in the peripheral blood of patients with chronic idiopathic urticaria when compared with nonatopic controls. The lower production of histamine releasing factor in the blood could be explained by the migration of activated T-lymphocytes in the skin.

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INTRODUCTION

During the last decade, the role of cytokines as modulators of cellular responses became an active area of research. Recently, a new family of

molecules called histamine-releasing factors has been investigated. These substances have molecular weights of between 12 and 93 kD and can be produced by a variety of cells including lymphocytes and monocytes. These factors are able to induce basophils and mast cells to secrete histamine,^{1,2} possibly through surface-fixed IgE.² Increased histamine-releasing factor activity has been shown not only in various allergic diseases such as atopic dermatitis associated with food hypersensitivity,³ asthma,⁴⁻⁶ and sea-

sonal allergic rhinitis,⁷ but also in pulmonary fibrosis.⁸ In chronic idiopathic urticaria patients, we have recently demonstrated higher levels of histamine-releasing factor in both lesional and non-lesional skin as compared with normal control subjects.⁹ These data suggested to us that histamine-releasing factor plays a major role in the cutaneous inflammation found in lesional skin of chronic idiopathic urticaria and the increased skin mast cell releasability.

With regard to histamine release in chronic idiopathic urticaria, previous studies on skin and peripheral blood suggest a defective production in both compartments. Spontaneous skin histamine release by mast cells does not differ from that measured in normal control subjects as was previously reported by our group.¹⁰ Skin histamine release, however, was higher after stimulation with compound 48/80,¹¹ a nonspecific mast cell degranulator. This functional abnormality of cutaneous mast cells is not a permanent phenomenon since mast cells return to normal during remission of the disease.¹⁰

When the results from peripheral blood are considered, the opposite situation occurs, mainly because peripheral basophils from patients with chronic urticaria release less histamine than basophils from nonatopic donors.^{12,13} This difference was observed only upon challenge with an immunologic stimulus (anti-IgE) but not following nonimmunologic stimulation with either calcium ionophore or deuterium oxide.^{12,13} These findings led

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the investigators to suggest a state of chronic antigen-induced "desensitization" to explain this paradoxical situation.^{13,14}

In an effort to explain the difference in histamine release observed between skin and peripheral blood, we decided to evaluate histamine-releasing factor activity at those two levels. First, normal controls and patients with active chronic urticaria were examined for the kinetics of histamine-releasing factor activity and histamine secretion, during a period of two hours, in normal appearing skin sites. Second, we measured the in vitro production of histamine-releasing factor by peripheral blood mononuclear cells obtained from the same volunteers.

MATERIAL AND METHODS

Study Groups

Patients were selected for this study if they were nonatopic adults presenting almost daily urticarial lesions for more than 6 weeks. Well-known etiologies of urticaria were ruled out by questionnaire, physical examination, and laboratory evaluation. Personal history of atopic dermatitis, allergic rhinitis, and asthma were negative for all patients. Skin prick tests to common inhalants and food allergens were also negative. Family history of atopy was noted in two patients. Dermographism was absent or minimal on physical examination. All laboratory tests were within normal limits and included complete blood count, sedimentation rate, SMA-12/60, antinuclear antibody, complement profile, thyroid function, and urinalysis. Patients were instructed to discontinue their H₁ and H₂ antihistamine drugs at least 72 hours prior to study. Astemizole and systemic corticosteroids had to be stopped for more than 4 weeks. Skin reactivity was evaluated by histamine prick test (1 mg/mL) at the time of the start of participation.

Healthy nonatopic volunteers were also recruited as controls. They had not been taking any medication at the time of their recruitment. Questionnaires, physical examinations, and screening

laboratory tests were also normal. The study was approved by the Ethics Committee of our Institution and a written informed consent was obtained from all subjects.

Assessment of Clinical Status

Evaluation of the severity of urticaria was performed for each patient enrolled in the study. A "symptom score" between 0 and 9 was calculated on the basis of the evaluation of three variables: intensity of pruritus, number of hives, and degree of activity and/or sleep interference. The following scale was used to score each symptom: 0, no symptoms and no hives evident; 1, mild symptoms and few hives (1 to 6); 2, moderate symptoms and number of hives between 7 to 12; and 3, severe symptoms and number of hives, greater than 12.

Skin Chamber Technique

Skin chamber technique studies were performed as previously reported.¹¹ Briefly, two skin blisters were raised by a combination of heat and suction over normal appearing skin of the forearm. A collection chamber was applied over each denuded blister base and sampling was made at 30 minutes, 1, and 2 hours after the end of blister formation. Each sample was recovered and stored at -70 °C until assayed for histamine concentration and histamine-releasing factor.

Dialysis of Chamber Fluids

After assessment of histamine concentration, skin chamber fluids were dialyzed in order to remove histamine and to equilibrate pH and ion concentration. Dialysis was performed at 4 °C against PBS by ultrafiltration using microconcentrators for small volume concentration (Centricor-3, Amicon, Danvers, MA; 3 KD molecular weight cut-off). The volume of chamber fluids was only slightly affected by dialysis (less than 10%), if indeed at all.

Histamine-releasing Factor Production by Peripheral Blood Mononuclear Cells

Production by peripheral blood mononuclear cells was measured as previ-

ously reported.⁷ In brief, peripheral blood mononuclear cells were isolated from venous blood drawn from all subjects using Ficoll-Hypaque density centrifugation. After washes, the mononuclear cells were resuspended at a concentration of 5×10^6 /mL and then incubated in buffer only for 20 hours at 37 °C in CO₂ 5%. Cell-free supernatant containing histamine-releasing factor was extracted by centrifugation, dialyzed, and stored at -70 °C until the assay was performed.

Assay

Histamine-releasing factor activity was assessed using the in vitro basophil histamine release assay previously adapted by our group.⁷ Briefly, heparinized blood was collected from a single atopic donor and leukocyte-enriched pellets containing basophils were obtained after sedimentation of whole blood with Dextran 6% (Dextran T-500, Pharmacia). After washes, cells were resuspended in 50 µL of cold buffer containing Ca and Mg at a final concentration of 2×10^6 cells/mL, and added to either 100 µL of PBS only or to dialyzed, heated and diluted skin chamber fluids and incubated for 20 minutes at 37 °C. The reaction was stopped by cooling. The cell-free supernatants were recovered for histamine determination. The same procedure was repeated for the cell-free supernatants obtained from peripheral blood mononuclear cells of the study groups to evaluate the peripheral blood production of histamine-releasing factor.

The results were expressed as percent of histamine release (% HR) and calculated as follows:

$$\% \text{ HR} = \frac{(A - B) \times 100}{T}$$

A = histamine-releasing factor-induced HR, B = buffer-induced HR, and T = total histamine of basophils.

Histamine Assay

Histamine concentration in skin chamber fluids or from the basophil histamine release assay was measured by a

single radioenzymatic assay previously described.¹¹

Statistical Analysis

Data have been summarized using means and SEM. Values for histamine concentration and histamine-releasing factor activity from skin chamber fluids as well as supernatants from peripheral blood mononuclear cells for the two study groups, were compared by an unpaired Student's *t* test. All tests were two-sided and differences were considered to be significant at a *P* value of less than .05.

RESULTS

The characteristics of our two study groups are displayed in Table 1. In brief, 12 individuals suffering from chronic idiopathic urticaria and five normal controls were enrolled in the study. An uneven ratio of women to men occurred in our study which is in agreement with a higher prevalence of chronic urticaria in women.^{15,16} All volunteers were Caucasian and were aged from 18 to 66 years old. The mean duration of the disease among the 12 patients with chronic urticaria was 21.5 months and ranged from 3 to 48 months. A symptomatic control of the disease was obtained with only H₁-antihistamine drugs in seven patients but the remaining five required at least one course of systemic corticosteroids in addition to H₁ and H₂ antihistamines. All patients had a skin prick test to histamine of 3 to 6 mm wheal (mean 3.4 mm) at the time of the study.

The profile of spontaneous histamine-releasing factor activity in skin chambers during the 2-hour experiment is shown in Table 2 for both groups. For each time point, histamine-releasing factor levels in chamber flu-

Table 2. Spontaneous Production of Histamine-Releasing Factor in Skin Chamber Fluids in Patients with Chronic Urticaria (n = 12) and Normal Controls (n = 5).

Time, hr	Histamine-releasing factor activity (% histamine release ± SEM)		<i>P</i> <
	Chronic Urticaria	Normal Controls	
0.5	4.1 ± 0.6	0.3 ± 0.4	.01
1	6.1 ± 0.9	0.8 ± 0.2	.01
2	6.2 ± 0.7	0.2 ± 0.4	.01
Weighted average	5.6 ± 1.4	0.7 ± 0.6	.01

ids from patients with active chronic urticaria were significantly higher than the levels of normal controls (*P* < .01). Accordingly, the weighted average of histamine-releasing factor activity for the 2-hour observation was at least 8 times higher in the group with chronic urticaria than among normal controls (5.6 ± 1.4% versus 0.7 ± 0.6%, *P* < .01, Table 2). In other respects, spontaneous histamine secretion in skin chamber fluids (PBS site) was not statistically different between patients with chronic urticaria and normal controls (Fig 1), at any time throughout the experiment.

Finally, histamine-releasing factor production by peripheral blood mononuclear cells that had been isolated from patients with active chronic urticaria the day of the skin chamber study was significantly lower than produc-

tion in normal controls (17.2 ± 2.1% versus 25.7 ± 2.8%, *P* < .03, Fig 2).

DISCUSSION

The purpose of this study was to gain insight into local skin inflammatory response of chronic idiopathic urticaria and to assess its relationship to peripheral blood lymphocytes. Although chronic idiopathic urticaria is probably caused by a heterogeneous group of factors, it is characterized by four major features, namely a dermal infiltration of activated T-cells,^{17,18} an elevated histamine concentration in the tissue fluid of the skin lesion,^{19,20} a hyperreleasability of skin mast cells¹¹ and, finally, an increased cutaneous histamine-releasing factor activity.⁹ In addition to these histopathologic and biochemical features, early reports on chronic idiopathic urticaria have demonstrated that blood leukocyte histamine release was significantly lower than in normal controls.^{12,13} The mechanisms explaining the unresponsiveness noted in the blood cells of urticaria patients did not relate to basophil number, IgE levels, absence of normal IgE molecule, leukocyte cyclic AMP levels, beta adrenergic blockade or defective basophil IgE receptors.

The implications for immunopathogenesis of this paradoxical defect in histamine release in the skin and blood of patients with chronic idiopathic urticaria are not entirely clear. Nonetheless, this discrepancy in the release of histamine is interesting, particularly in light of the fact that similar examina-

Table 1. Characteristics of the Study Groups

Groups	N	Age, yr		Sex
		Mean	Range	M/F
Chronic urticaria	12	35	18-49	1/11
Normal controls	5	41	24-66	2/3

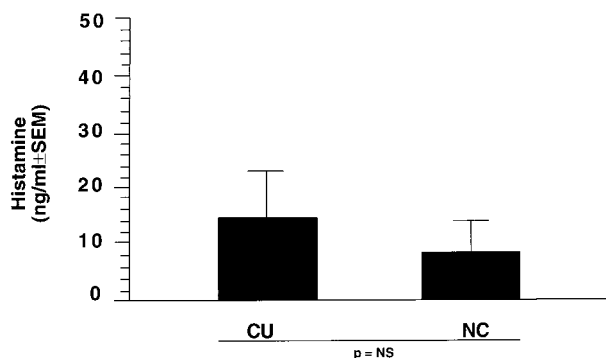


Figure 1. Total histamine release in skin chamber fluids during 2-hour observation period in chronic urticaria and normal control subjects.

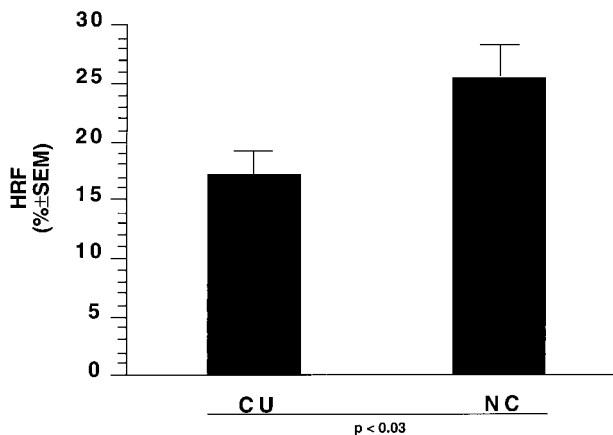


Figure 2. Histamine-releasing factor production from peripheral blood mononuclear cells of patients with chronic urticaria and normal control subjects.

tion of lymphocytes in sarcoidosis and hypersensitivity pneumonitis revealed alterations of CD-4/CD-8 ratios in bronchial lavage fluids that were not seen in peripheral blood of patients during their disease state.^{21,22} We therefore, feel that abnormalities observed in blood do not necessarily contradict what happens in the target organ.

As regards our histamine-releasing factor data, a number of explanations could serve to link the skin and the blood network in chronic idiopathic urticaria. First, we have documented that elevated histamine-releasing factor is not a transient but indeed a persistent finding in the skin of patients with chronic urticaria since it was measured at significantly high levels during at least the 2-hour sampling, and not only at the peak intensity of a skin lesion, as was reported in our previous study.⁹ Further, the activity that we have observed could play an active role in priming skin mast cells to release more histamine.

Interestingly enough, in this study, we demonstrated that blood lymphocytes from patients with chronic urticaria generated weak histamine-releasing factor activity even after one day of cell culture, while the histamine-releasing factor produced from normal control subjects provided a higher response. We hypothesize that one of the reasons for this type of lymphocyte

unresponsiveness could be due to a subset of CD-4 cells that has migrated into the skin, leaving few of these cells in the periphery and leading to lower production of histamine-releasing factor.

In their 1976 study,¹³ the Lichtenstein group suggested that decreased histamine release observed in blood basophils of patients with chronic idiopathic urticaria is due to a defect comparable to the phenomenon of desensitization. It has already been shown in patients undergoing immunotherapy for allergic rhinitis that when their blood leukocytes are either incubated in vitro with antigen in an appropriate "environment" or are given repeated low doses of antigen, these cells do become activated while also releasing a minimum percentage of their total histamine content.^{23,24}

On the basis of our results, which show that histamine-releasing factor production from peripheral blood mononuclear cells of patients with active chronic urticaria is clearly diminished as compared with normal control subjects, we suggest that the desensitization affecting histamine release is not explained by repetitive leukocyte discharge after a series of low doses of stimuli but instead derived from the absence in the media reaction of a co-factor such as histamine-releasing factor, which might prime the threshold of basophil reactivity. The likelihood that

both low doses of antigen and a defective serum factor contribute to the abnormal histamine release process of blood cells is rather remote because in chronic urticaria, antigen-IgE interactions probably play no part.^{12,14}

In summary, our work underlines the importance of histamine release found in patients with urticaria and its modulation by histamine-releasing factor. This study also suggests that the primary source of histamine-releasing factor recovered from the skin of those predisposed to develop hives is derived from mononuclear cells that have migrated from the circulating pool to tissue sites. The nature of the stimuli provoking this chain of events remains as yet unknown.

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INHALANT ALLERGY TO FRESH ASPARAGUS

The authors report two patients who experienced nasal, ocular, and chest symptoms during preparation of fresh asparagus but who could eat cooked asparagus without symptoms. Allergy prick testing disclosed strongly positive reactions to raw asparagus but negative reactions to cooked asparagus. There were also positive reactions to grass pollen and other pollens. Specific IgE to asparagus as well as onion, profilin, birch, and mugwort was found in sera. Inhibition studies disclosed no crossreactivity of asparagus with other allergens.

—RMS

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