Chronic idiopathic urticaria: Possible contribution of histamine-releasing factor to pathogenesis

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Background: *Histamine-releasing factor was recently shown to be clinically relevant in allergic rhinitis and asthma. HRF could also be involved in the pathogenicity of chronic idiopathic urticaria (CU). The purpose of this study was to investigate the role of HRF in the pathophysiology of CU.*
Methods: *Blisters were induced on lesional and nonlesional skin of 12 patients with CU and on normal skin of five control subjects. HRF activity and histamine content were measured in all samples recovered from each skin site.*
Results: *Significantly more HRF was found in blister fluids from lesional skin of patients with CU as compared with nonlesional skin and skin of control subjects. In addition, histamine content in blister fluids from affected skin of patients with CU was significantly higher in comparison with both nonlesional skin of patients with CU and skin of control subjects. HRF activity was also higher in blister fluids from nonlesional skin of patients with CU than that of control subjects, in spite of equivalent histamine content.*
Conclusion: *These data suggest that the inflammatory reaction found in CU disease is associated with the cutaneous release of HRF. (J Allergy Clin Immunol 1993;92:132-7.)*

Key words: *Chronic idiopathic urticaria, histamine-releasing factor, histamine content*

The role of histamine in the pathophysiology of chronic idiopathic urticaria (CU) has been established. Interaction between IgE-bound mast cells and allergen is unlikely to be the mechanism by which histamine release occurs because CU does not represent, in most cases, an IgE-mediated allergic reaction.[1] Therefore a totally different biochemical process could be responsible for localized histamine secretion within separate areas of the skin. There is some evidence to suggest a defective histamine release in CU disease. On the one hand, peripheral blood basophils from patients with CU release significantly less histamine than those from control subjects on challenge with immunologic stimuli.[2] On the other hand, recent data from our laboratory indicate that patients with CU have an increased dermal histamine release, which occurs during the early phase of nonspecific activation of resting cutaneous mast cells.[3, 4] We have also provided evidence that a defective regulation is involved in this phenomenon, rather than an intrinsic skin mast cell abnormality, because this enhanced releasability of histamine disappears when the disease is in remission.[5]

Abbreviations used:
HRF: Histamine-releasing factor
CU: Chronic idiopathic urticaria
NC: Normal control
HR: Histamine release

Recently, a family of cytokines, derived from a large variety of cells that includes lymphocytes, has been shown to potentiate the release of histamine, possibly through surface-fixed IgE.[6, 7] These cytokines, referred to as histamine-releasing factor (HRF), were demonstrated to be clinically relevant in allergic rhinitis and asthma.[8, 9] It is interesting to speculate that HRF could also be involved in the pathogenicity of CU.

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TABLE I. Clinical data of patients with CU

Patient No.	Age (yr)	Sex	Duration of urticaria (mo)	Symptom score (0-9)	Medication to control disease: Antihistamines	Medication to control disease: Systemic steroids	Prick test to histamine* (wheal in mm)
1	33	F	3	5	Anti-H_1 and H_2	Once in 1989	3
2	41	F	48	7	Anti-H_1 and H_2	Once in 1987	3
3	46	F	3	4	Anti-H_1	No	3
4	30	F	30	5	Anti-H_1	No	3
5	49	F	12	7	Anti-H_1	No	3
6	39	F	24	8	Anti-H_1 and H_2	Few courses	5
7	42	M	42	6	Anti-H_1 and H_2	1 dose I.V. in 1990	3
8	18	F	12	5	Anti-H_1	No	3
9	46	F	24	7	Anti-H_1	No	3
10	25	F	30	8	Anti-H_1 and H_2	Once in 1990	3
11	29	F	6	4	Anti-H_1	No	3
12	22	F	24	5	Anti-H_1	No	6
Mean	35		21.5	6	12/12	5/12	3.4

*1 mg/ml.

Earlier reports have confirmed the presence of activated CD_4-positive T lymphocytes in the perivascular infiltrates of skin lesions of individuals with CU.[10] However, there are no indications as to whether this represents a primary or a secondary phenomenon in the pathogenesis of CU and whether these infiltrating cells interact with the surrounding skin mast cells. HRF secretion could explain the relationship between accumulation of T cells and mast cell releasability. Therefore we have undertaken the present study to explore the possible role of HRF in the production of a state of cutaneous mast cell hyperreactivity in CU.

METHODS

Study group

Adults who presented with urticarial lesions almost daily for more than 2 months and who showed no well-identified causative factors were recruited for the study after their written informed consent had been obtained. None were considered to be atopic on the basis of negative personal history of rhinitis, asthma, or atopic dermatitis and on the basis of negative skin prick test results to a panel of common inhalants and food allergens. Familial history of atopy was weakly positive in only two patients. Physical urticarias were excluded by questionnaire and by the absence of significant dermographism on physical examination. Results of all the laboratory tests fell within normal limits and included: complete blood count, sedimentation rate, Sequential Multiple Analysis 12/60, urinalysis, antinuclear antibody, complement profile, and thyroid function. Classic H_1 and H_2 antihistamine drugs were discontinued at least 72 hours before the study. Astemizole and systemic steroids had to be discontinued for more than 4 weeks. A group of normal volunteers was also studied. Each underwent a normal physical examination and screening laboratory tests. None had a personal or family history of atopic diseases, and none were taking any medication.

Assessment of clinical status

At the time of the study, each patient had his or her disease evaluated and scored according to a severity scale. The "symptom score" included three factors: itching, number of hives, and degree of interference with the patient's normal activity or sleep. Each item was scored according to the following scale: 0: absent, no symptom or no hive evident; 1: mild symptoms or few hives (1 to 6); 2: moderate symptoms or number of hives (7 to 12); 3: severe symptoms or number of hives (more than 12). A total symptom score between 0 and 9 was then obtained for each patient with CU (Table I).

Skin blisters

Blisters of a 1 cm diameter base were formed at sites of urticarial lesions and on contralateral normal-appearing skin with a combined heat and suction device.[3] Normal control (NC) subjects had only one skin blister on the volar area of one forearm. Blister fluids were aspirated aseptically from the base of the blister with a 1 ml insulin syringe. Between 100 and 150 μl was recovered from each blister and kept frozen at −70° C until assayed for histamine concentration and HRF.

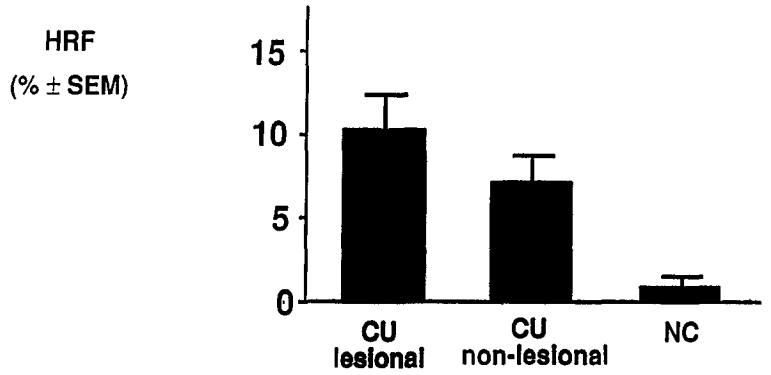

FIG. 1. HRF production (%) in skin blister fluids in lesional and nonlesional skin of patients with CU and in NC subjects.

Dialysis of blister fluids

After measurement of histamine concentration of skin blister fluids, the solutions were dialyzed at 4° C against phosphate-buffered saline by ultrafiltration with microconcentrators for small volume concentration (Centricor-3, Amicon, Danvers, Mass.; 3000 MW cut-off). Dialysis was performed to remove histamine and to equilibrate pH and ion concentration. Dialysis had little or no effect (less than 10%) on the volume of blister fluids. HRF activity of dialyzed blister fluids was identical to that of undialyzed fluids.

In order to eliminate complement products and IgE that could falsely degranulate basophils, skin blister fluids were also inactivated by heating them in a water bath at 56° C for 40 minutes.

HRF assay

HRF activity was assessed with a modified in vitro basophil histamine release assay, as previously reported by our group.[8] Briefly, heparinized blood was collected from a single atopic donor, and leukocyte-enriched pellets containing basophils were obtained by sedimentation of whole blood with Dextran 6% (Dextran T-500, Pharmacia, Uppsala, Sweden). After washing, cells (2×10^6 cells) were resuspended in either 100 μl of buffer alone without calcium (phosphate-buffered saline) or 100 μl of dialyzed, heated, and diluted blister fluids, and incubated for 20 minutes at 37° C. The reaction was stopped by cooling with addition of cold buffer, and cell-free supernatants were recovered for histamine determination. The results were expressed as percentage of histamine release (HR) and calculated as follows:

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

where A = HRF-induced HR, B = buffer-induced HR, and T = total histamine content of basophils.

Histamine assay

Histamine determinations of blister fluid or basophil HR assay were obtained with the use of a single radioenzymatic assay, as described in a previous article.[3]

Statistical analysis

HRF activity levels in skin blister fluids were expressed as means of percentage of HR ± SEM, and histamine content levels were expressed as means ± SEM. Because our study groups are normally distributed, the values were compared by means of one-tailed paired Student's *t* test for matched pairs design or by one-tailed unpaired Student's *t* test for two independent groups design.

RESULTS

Twelve subjects who had CU and five NC subjects were included in the study. The characteristics of our 12 patients with CU are summarized in Table I. The ratio of men to women was uneven, but so far our previous work on CU has not revealed any effect of sex distribution on clinical or biochemical parameters.[3-5] The mean duration of urticaria in the CU group was 21.5 months (range, 3 to 48 months), and the mean symptom score was 6. Regular therapy for urticaria was limited to antihistamines in seven subjects, but five patients with urticaria required at least one corticosteroid treatment in addition to anti-H_1 and H_2 medication. As far as NC subjects are concerned, we studied three women and two men, aged 24 to 66 years (mean age, 41 years).

The HRF measured in skin blister fluids was about ten times higher in lesional skin of patients with CU than that of NC subjects (11.2% ± 1.9% vs 1.1% ± 1.4%, $p < 0.001$), (Fig. 1), whereas normal-appearing skin of patients with CU had about a sevenfold increase of HRF over that of NC subjects (7.4% ± 1.8% vs 1.1% ± 1.4%, $p < 0.03$), (Fig. 1). Moreover, there was a significant difference between levels of HRF when comparisons were made between lesional and nonlesional skin among patients with CU ($p < 0.05$).

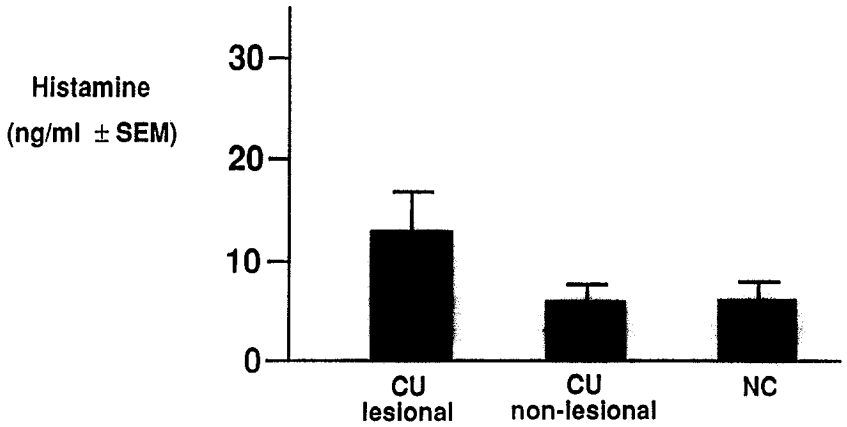

FIG. 2. Histamine content in skin blister fluids in lesional and nonlesional skin of patients with CU and in NC subjects.

The total histamine content of skin blister fluids was 12.6 ± 3.9 ng/ml and 5.7 ± 1.5 ng/ml in lesional skin and nonlesional skin of patients with CU, respectively and, 6.1 ± 1.6 ng/ml for NC subjects (Fig. 2). Therefore it appears that normal skin of both patients and control subjects had tissue fluid histamine in the same range, whereas histamine concentrations at sites of urticarial lesion were two times higher ($p < 0.05$). Also, there was no significant correlation between histamine content of blister fluids and HRF activity found in patients with CU.

DISCUSSION

There is much evidence to support the role of HRF in either acute or chronic allergic skin reactions. Indeed, in 1985 Alam and Roznieckí[11] first reported an immediate wheal and flare skin reaction in atopic subjects after cutaneous injection with HRF derived from mononuclear cells of allergic volunteers. Some of these individuals also exhibited a late skin reaction. Studies by Warner et al.[12] have identified histamine releasing activity in skin blisters during the late-phase reaction induced by pollen antigen challenge. More recently, HRF has been closely linked to atopic dermatitis.[13] Indeed, it was shown that HRF produced by mononuclear cells in children with food allergy and eczema was rather high and could be brought back to normal levels once patients were started on an appropriate specific food avoidance program.

Mast cell activation and degranulation have been reported in a variety of inflammatory diseases in which an IgE-dependent mechanism is not demonstrable; this applies to CU as well. Elias et al.[14] were probably the first to speculate that HRF could be responsible for hyperreactive mast cells in CU, on the basis of their observation of increased numbers of helper T cells and monocytes in the biopsy specimens of skin lesions. In addition, others have shown that urticarial sites are characterized by the involvement of inflammatory cells such as neutrophils and eosinophils, which resemble late-phase cutaneous allergic reactions.[15] This observation also makes the hypothesis of cytokine release in CU attractive.

The blister fluid HRF in our patients with CU was clearly different from that of NC subjects. In both lesional and nonlesional skin sites, high levels of HRF were found. Heat inactivation of specimens ruled out the participation of complement or IgE in the release of histamine. However, because of the small quantity of blister fluid that could be recovered from each skin site, and once HRF and histamine assays were completed, sufficient material was not available for further analysis, such as high performance liquid chromatography. It may be presumed that other histamine-releasing cytokines such as interleukin-3 and granulocyte-macrophage colony-stimulating factor are not involved because they usually induce very low levels of HR (less than 3%), whereas HRF and anti-IgE are more potent.[16] At least two other cytokines are as powerful as HRF, MCAF/MCP-1 and RANTES, and these were found to be potent secretagogues for basophils.[17-19] The HRF activity demonstrated here in CU could be secondary to one of these molecules, and further studies should be conducted in order to clarify this point.

The blister fluid histamine levels from lesional skin of our patients with CU also clearly exceeded

the mean levels of normal volunteers, in accordance with results of a previous study.[20] Parallel in vivo elevation of HRF and histamine secretion provides some support for the hypothesis that HRF increases skin mast cell reactivity. It is true, at least, that the two phenomena occur simultaneously. However, a clear demonstration of a cause-and-effect relationship remains to be made in CU disease.

Interestingly, the elevated blister fluid histamine concentration was not found in the normal-appearing skin of patients with CU. Other studies[20] had previously suggested a higher histamine content of both lesional and nonlesional skin of patients with CU, which has been explained by the possible presence of an increased number of mast cells or an increased histamine content per cell. However, this normal level of histamine in normal-appearing skin of our group of patients with CU is in agreement with our own previous skin biopsy study, which showed a normal number of mast cells and normal histamine content in nonlesional skin of patients with CU.[3] In the present study, it was also interesting to find an elevated HRF activity of intermediate level in nonlesional skin sites of patients with CU without subsequent increase of skin blister histamine content. Hence, we should consider the possibility that this abnormal level of HRF reflects the fact that activated T cells must be present and secrete soluble factors up to a critical concentration before local mast cell activation and mediator release can be induced. Also, T cells can secrete other factors that might prime mast cells. However, we cannot rule out the possibility that HRF persists for long periods subsequent to mast cell degranulation and then to histamine reuptake or catabolism. Furthermore, all of our previous studies on CU with compound 48/80 (Sigma Chemical Co., St. Louis, Mo.) challenge skin windows have confirmed an increased histamine secretory response in normal-appearing skin, which might be attributed to a mast cell priming effect caused by elevated HRF in the skin milieu. Finally, HRF may play a role in the accumulation of mononuclear cells in urticarial sites, since some HRF molecules have been shown to have chemotactic effects.[7, 17, 18]

In conclusion, our results confirm that histamine is a mediator of primary importance in CU and suggests that HRF could be a modulatory factor of skin mast cells in this disease. Further studies are required to better characterize and purify this molecule to strengthen our conclusions. It is also apparent from this study that the pathophysiology of CU is complex and involves important regulatory factors. However, considering the limitations of the human in vivo skin blister model, we believe that we have provided additional data that might link activated T cells[10] and hyperreleasable mast cells in CU.[3]

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