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Basophil Releasability in Patients with Hymenoptera Venom Allergy

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Key Words
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Abstract
Basophils in about 15% of subjects allergic to hymenoptera venom do not release histamine in the presence of antigen. Little is known on the basophil releasability in these patients. We therefore measured maximum percent leukocyte histamine release to antigen (Vespula venom), anti-IgE and formylmethionylphenylalanine (FMP) in 39 patients allergic to wasp venom and compared our results according to basophil responsiveness to antigen. Mean maximum percent histamine release was 39, 34 and 22%, respectively, for venom (100 ng/ml), anti-IgE (0.25 μg/ml) and FMP (10^{-4} *M*). The amount of histamine specifically released by venom correlated significantly with anti-IgE but not with FMP-induced histamine release. Leukocytes were unresponsive to antigen in 10 subjects. The clinical characteristics and anaphylactic symptoms of these patients were not different from those with antigen-responsive cells. Unresponsive leukocytes responded to FMP in all and to anti-IgE in 8 of the 10 subjects. Mean anti-IgE and FMP-induced histamine release were, respectively, lower and higher than those observed with leukocytes responsive to antigen ($p < 0.05$). In unresponsive basophils, there was a negative correlation between maximum percent anti-IgE and FMP-induced histamine release. We confirm that basophils of a minority of the subjects allergic to Vespula venom do not release histamine in the presence of antigen. The negative correlation between anti-IgE and FMP-induced histamine release in unresponsive basophils may suggest individual differences in the ratio of FcεRI and FMP receptors on the surface of basophils.

Introduction

It has recently been reported that basophils from approximately one fifth of the population do not release histamine and leukotrienes in the presence of any IgE cross-linking stimulus in spite of a normal response to nonimmunological secretagogues including formylated peptides (formylmethionylphenylalanine, FMP) [1]. A similar situation may occur in type I allergic patients and is well exemplified in hymenoptera venom allergy. It is indeed recognized that basophils in about 15% of subjects with hymenoptera venom allergy and sting anaphylaxis do not release histamine in the presence of antigen at the optimal concentration [2, 3]. The releasability of these antigen unresponsive basophils to anti-IgE and FMP has not been systematically studied in wasp venom allergy. We therefore measured maximum percent leukocyte histamine release to antigen, anti-IgE and FMP in wasp venom allergic

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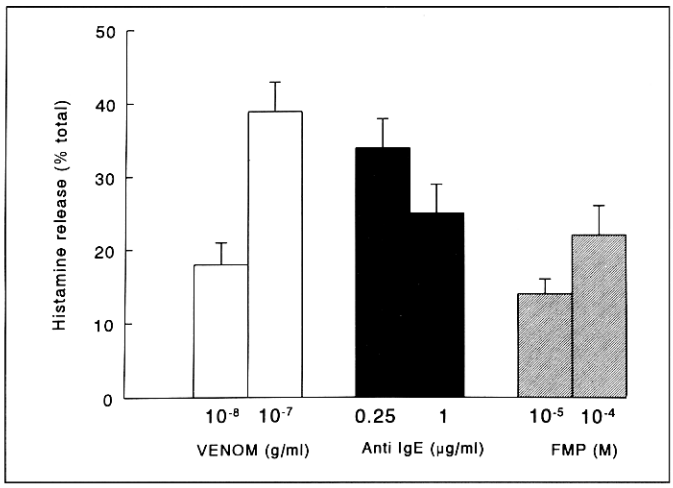

Fig. 1. Mean leukocyte histamine release to antigen, anti-IgE and FMP in 39 Vespula venom-allergic subjects.

patients and analyzed our results according to basophil responsiveness to antigen. Our results show that antigen-unresponsive leukocytes are more sensitive to FMP than responding cells and that this parameter is negatively correlated with anti-IgE-induced histamine release.

Subjects and Methods

Thirty-nine patients (mean age 33 ± 7 years, range 8–71, 22 males, 17 females) with a documented history of anaphylactic sensitivity to wasp venom *(Vespula germanica* or *Vespula vulgaris)* were seen between 1987 and 1990 and evaluated for the first time 6–12 weeks after sting anaphylaxis. No patients with large local reaction were included in this study. The evaluation included intradermal skin tests with venom (100 ng/ml), measurement of serum venom-specific IgE by radioallergosorbent test (RAST) (Pharmacia, Uppsala) and histamine release from leukocytes using wasp venom (Pharmalgen, Pharmacia) [4], polyclonal rabbit antihuman IgE (Behringwerke, Marburg, FRG), and FMP (Sigma). All the patients had a positive cutaneous reaction and venom-specific serum IgE was found in 32 patients.

Leukocyte Releasability

Basophil releasability was determined by measuring leukocyte histamine release with concentrations of antigen, anti-IgE antibodies and FMP that were optimal for histamine release. In our experimental conditions, it was not possible to construct a complete dose-response curve for each subject because 3 different secretagogues were tested on the same blood sample. Previous studies from our laboratory have, however, shown that antihuman IgE diluted to contain 0.25 µg antibodies/ml and FMP 10^{-4} *M* were optimal concentrations for leukocyte histamine release [5]. In preliminary experiments, we first determined the concentration of venom causing maximum specific (i.e. IgE-mediated) histamine release from leukocytes. Only concentrations at 100 ng/ml and below were used because venom exhibited dose-related fluorescence and cytotoxicity both starting at 1 µg/ml. Mean venom specific histamine release was found to be 4, 16 and 37%, respectively, at 1, 10 and 100 ng venom/ml in 10 subjects allergic to wasp venom.

Leukocytic Histamine Release

Peripheral venous blood (100 ml) was collected into heparinized vacutainers (Becton Dickinson), gently mixed and allowed to sediment for 35–45 min. The upper plasma layer containing leukocytes and platelets was carefully decanted and cells were washed 3 times in Hanks' balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}. Cells were counted (Coulter) and resuspended in HBSS containing Ca^{2+} and Mg^{2+} to 2×10^7 cells/ml. To 1 ml of this leukocyte suspension was added the same volume of HBSS containing wasp venom (Pharmacia) at 20 and 200 ng/ml, anti-human IgE diluted to contain 0.5 and 2 µg antibodies/ml or FMP at $2 \cdot 10^{-5}$ and $2 \cdot 10^{-4}$ *M*. The leukocyte suspensions were incubated for 30 min at 37 °C, centrifuged and histamine measured spectrofluorometrically in the supernatant fluid as previously described [6]. Histamine release was expressed as a percent of total histamine content of the leukocyte suspension. At the concentrations used, the secretagogues had no direct fluorescence. Wasp venom (Pharmalgen) at 100 ng/ml caused only negligible cytotoxic leukocytic histamine release (i.e. below 8% of total in 5 of 30 normal subjects). Therefore, basophils were considered antigen-responsive only when the amount of histamine released by venom at 100 ng/ml was > 10%. Histamine release caused by FMP or anti-IgE was considered significant when > 5% of total.

Statistical Analysis

Results are expressed as mean ± SEM. Student's t test was used to compare mean values between the groups. Linear regression and correlation was applied to measure the association between variables. All results were considered to be significant when $p < 0.05$.

Results

Figure 1 shows mean percent leukocyte histamine release to wasp venom (10 and 100 ng/ml), antihuman IgE (0.25 and 1 µg/ml) and to FMP (10^{-5} and 10^{-4} *M*) in 39 patients allergic to wasp venom, tested 1.5–3 months after anaphylaxis. Mean maximum percent histamine release was 39, 34 and 22%, respectively, for venom (100 ng/ml), anti-IgE (0.25 µg antibodies/ml) and FMP (10^{-4} *M*). Venom-induced maximum percent histamine release correlated significantly with anti-IgE (fig. 2) but not with FMP-induced histamine release [data not shown].

Leukocytes of 10 patients did not respond to antigen (i.e. released no histamine in the presence of venom at 10 ng/ml and less than 10% at 100 ng/ml). The clinical characteristics, cutaneous reaction to venom (100 ng/ml) and anaphylactic symptoms of these patients were similar to venom-allergic individuals with antigen-responsive basophils. Three patients, in the nonreleaser group, experienced vascular collapse and loss of consciousness; none

was on β-blockers at the time of the sting anaphylaxis. Serum venom-specific IgE were found in 7 of the 10 nonreleasers and in 25 of the 29 patients whose basophils responded to antigen.

Antigen-unresponsive basophils responded to FMP in all (range 6–83) and to anti-IgE in 8 (range 10–64) of the 10 subjects. Mean histamine release to anti-IgE and FMP of leukocytes responsive and unresponsive to antigen is shown in figure 3. Unresponsive leukocytes released significantly less histamine to anti-IgE and more to FMP than antigen-responding cells ($p < 0.05$). In the 10 nonreleasers, there was a negative correlation between maximum percent anti-IgE and FMP-induced histamine release (fig. 4).

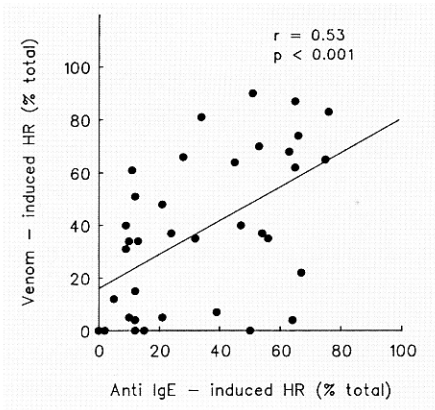

Fig. 2. Relationship between maximum percent leukocyte histamine release (HR) induced by venom (100 ng/ml) and anti-IgE (0.25 μg/ml) in Vespula venom-allergic subjects.

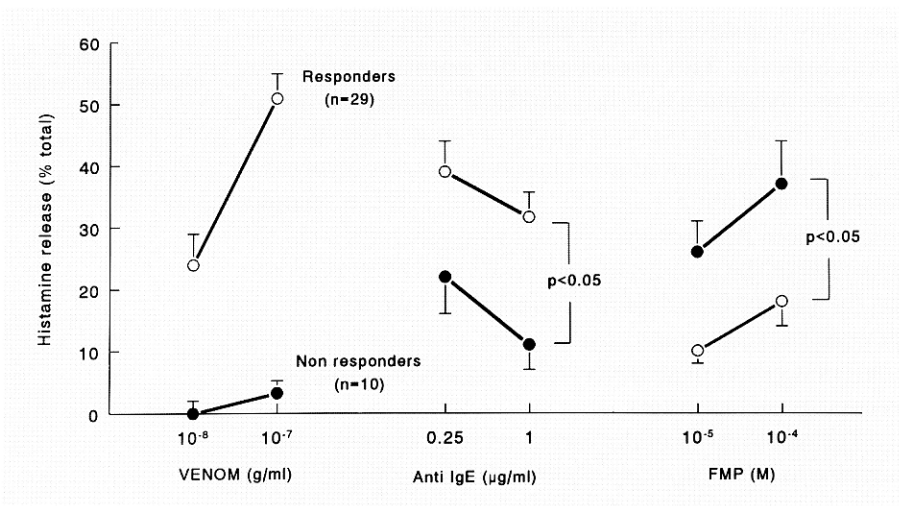

Fig. 3. Leukocyte histamine releasability to anti-IgE and FMP in 39 Vespula venom-allergic subjects according to basophil responsiveness to antigen. Both responder and nonresponder groups were comparable in the severity of sting anaphylactic reactions and in the positiveness of venom-specific skin tests (0.1 μg/ml) and RAST IgE.

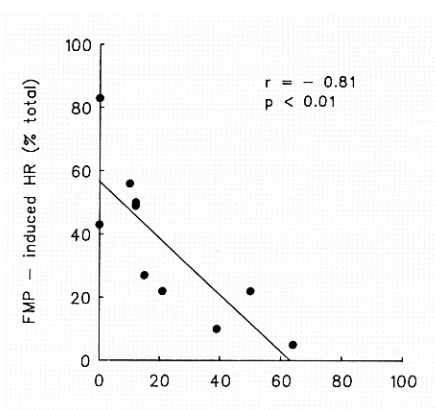

Fig. 4. Relationship between maximum FMP- and anti-IgE-induced histamine release (HR) in antigen unresponsive basophils from 10 subjects allergic to Vespula venom.

Discussion

The main purpose of this study was to compare leukocyte histamine releasability to anti-IgE and FMP in wasp venom-allergic patients according to basophil responsiveness to antigen. We found that leukocytes of 10 out of 39 subjects with wasp sting anaphylaxis did not release histamine in the presence of an optimal concentration of antigen (nonreleasers). The clinical characteristics and anaphylactic symptoms of the nonreleasers were not different from those with antigen-responsive cells. The paradox between the clinical situation and basophil response to antigen may reflect intrinsic differences between mast cells and basophils or day to day variations in the releasability of the basophils. It is indeed appreciated that the release of basophil mediators does not only depend on the amount of cell-bound IgE but also on poorly defined mechanisms controlling their release [1, 7].

Our results show that antigen-unresponsive basophils are more sensitive to FMP and less to anti-IgE than responding cells. This finding and the negative correlation between maximum anti-IgE and FMP-induced histamine release in nonreleasers may suggest some interaction between the two different transduction pathways involved in basophil activation following IgE and peptide receptor stimulation. Alternatively, it could suggest individual differences in the proportions of FcεRI and FMP receptors on the surface of basophils. Conceivably 'anaphylactic' reactions to wasp venom could select in the general population a subset of subjects with a high density of peptide receptors on basophils.

The significance of the increased responsiveness of basophils to FMP in wasp venom-allergic patients whose cells do not respond to antigen cannot, at present, be assessed. Formylated peptides interact with a specific receptor on basophils [8]. This receptor may be of interest as a releasing mechanism because many bacterial peptides terminate with a formylmethionine sequence [9, 10]. Hymenoptera venoms are complex materials containing peptides, vasoactive amines and enzymes (phospholipase, hyaluronidase, etc.). Wasp venom contains significant amounts of several peptides (the mastoparans) which are capable of degranulating mast cells [11, 12]. Mastoparans also enhance phospholipase A_2 activity of both venom and victim [13] and promote exocytosis of 5-hydroxytryptamine from human platelets [14]. Whether these peptides can induce histamine release from human basophils or mast cells remains to be determined. Several clinical observations indicate that life-threatening 'anaphylactic' reactions to insect stings are not always IgE-mediated. Insect sting anaphylaxis may occur in patients without detectable venom-specific IgE or after a first sting [15]. The mechanism of these 'anaphylactoid' reactions is unknown. It is conceivable that basophils in a minority of subjects would be exquisitely sensitive to the direct histamine-releasing properties of hymenoptera venom and would release their mediators by nonimmunological mechanisms.

In conclusion, leukocytes in approximately one fifth of wasp venom allergic patients do not release histamine in the presence of antigen. These unresponsive leukocytes are more sensitive to FMP and less to anti-IgE than basophils responding to antigen. The negative correlation between anti-IgE and FMP-induced histamine release in antigen unresponsive leukocytes suggests individual differences in basophil populations with respect to their FcεRI and peptide receptor ratio.

Acknowledgment

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