

# The human recombinant histamine releasing factor: Functional evidence that it does not bind to the IgE molecule

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**Background:** We have previously shown that the human recombinant histamine releasing factor (HrHRF) caused histamine release from a subset of basophils from donors with allergy, and this release seemed to be dependent on the presence of a certain type of IgE, termed *IgE+*. IgE molecules that did not support HrHRF-induced histamine release were termed *IgE-*. However, subsequently we demonstrated that HrHRF primes anti-IgE-antibody-induced histamine release from all basophils, irrespective of the type of IgE on the cell surface.

**Objective:** Because these data suggested that HrHRF does not exert its biologic effects by binding to IgE, but rather that it interacted with a surface receptor on the basophil, we wanted to obtain functional evidence that HrHRF did or did not bind to the IgE molecule.

**Methods:** The rat basophilic leukemia cell line (RBL-SX38), which has been transfected to express a functional human FcεRI (α-, β-, and γ-chains of the receptor) in addition to the normal rat FcεRI, was used. The presence of the human FcεRI receptor enables these cells to be sensitized with human IgE. Cells were passively sensitized with 1000 ng/mL human IgE+ or 1000 ng/mL human IgE- for 60 minutes at 37°C. Unsensitized cells served as a control. After the cells were washed,  $1 \times 10^5$  cells were stimulated in the presence of 1 mmol/L  $\text{Ca}^{2+}$  with 0.1 μg/mL anti-IgE, 40 μg/mL HrHRF, or 40 μg/mL mouse recombinant HRF (MrHRF), which has 96% homology to HrHRF.

**Results:** Mean anti-IgE-induced histamine release was  $33\% \pm 15\%$ , and there was no difference between IgE+ sensitization ( $32\% \pm 12\%$ ) and IgE- sensitization ( $34\% \pm 18\%$ ). However, in contrast to human basophil experiments, neither HrHRF ( $0\% \pm 0\%$ ) nor MrHRF ( $3\% \pm 5\%$ ) caused histamine release in RBL cells sensitized with IgE+. In addition, priming the transfected RBL-SX38 cells or the parental cell line, RBL-2H3 cells, with HrHRF or MrHRF did not increase anti-IgE-induced histamine release.

**Conclusion:** The results indicate that HrHRF does not bind to IgE, either IgE+ or IgE-. Therefore it appears likely that rHRF signals through its own specific receptor, which is not

expressed or functional on RBL-SX38 or RBL-2H3 cells, but which seems to be expressed on basophils of atopic and nonatopic donors. (*J Allergy Clin Immunol* 1999;103:642-8.)

**Key words:** Rat basophilic leukemia cells (RBL-SX38, RBL-2H3), basophils, IgE heterogeneity, FcεRI

The human recombinant histamine-releasing factor (HrHRF; also called p23<sup>1,2</sup> and translationally controlled tumor protein<sup>3</sup>) was subcloned<sup>4</sup> and was shown to cause histamine release and IL-4 protein secretion from a subset of basophils from donors with allergy.<sup>4,5</sup> The mechanism by which HrHRF induced histamine release was originally thought to be dependent on a certain type of IgE, termed IgE+.<sup>6,7</sup> IgE molecules that did not support HrHRF-induced histamine release were termed IgE-. Because of these findings, it appeared likely that HrHRF did bind to the IgE+ molecule but not to IgE-.<sup>8</sup> However, additional data have shown that HrHRF will augment or prime anti-IgE-antibody-induced histamine release and IL-4 and IL-13 production from all basophils, irrespective of the type of IgE on the cell surface.<sup>9</sup> Furthermore, HrHRF caused chemotaxis and augmented IL-8 production from human eosinophils of donors with mild allergy.<sup>10</sup> Although there have been reports that eosinophils from patients with hypereosinophilia express FcεRI<sup>11</sup> and that mRNA for the FcεRI can be detected in eosinophils from patients with asthma,<sup>12</sup> eosinophils from our donor pool do not express measurable surface levels of FcεRI by flow cytometry.<sup>13</sup> Taken together, these data suggest that HrHRF does not exert its biologic effects by binding to IgE, but rather that it interacts with another cell surface receptor.

The aim of this study was to demonstrate whether IgE (either as IgE+ or IgE-) could mediate the signaling caused by HrHRF. To accomplish this goal, the rat basophilic leukemia (RBL)-SX38 cell line was used. These RBL cells express a functional human high-affinity IgE receptor, FcεRI receptor, with all 3 chains (α, β, and γ). This enables these cells to be passively sensitized with human IgE molecules and subsequently stimulated with agents that crosslink human IgE and lead to mediator secretion. To compare the RBL-SX38 data with previous results obtained in human basophils, the RBL-SX38 cell sensitivity (defined as the number of FcεRI crosslinks required to induce half-maximum histamine release) was assessed. HrHRF priming experiments were performed to examine the HRF reactivity of RBL-SX38 cells. To determine whether an HRF signaling pathway may have been

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#### Abbreviations used

HRF:	Histamine-releasing factor
HrHRF:	Human recombinant histamine-releasing factor
HSA:	Human serum albumin
MrHRF:	Mouse recombinant histamine-releasing factor
PAG:	PIPES/albumin/glucose
PIPES:	Piperazine-N-N'-bis [2-ethane sulphonic acid]
RBL:	Rat basophilic leukemia

lost by transfecting the RBL-SX38 cells with the human FcεRI receptor, additional HrHRF and mouse recombinant histamine-releasing factor priming experiments were done in RBL-2H3 cells, which are the parental RBL cells.

## METHODS

### Materials

HrHRF and mouse recombinant histamine-releasing factor (MrHRF) were subcloned and expressed as described previously.<sup>4</sup> The HrHRF stock solutions used in these experiments were prepared at a concentration of 100 µg/mL (HrHRF) and up to 375 µg/mL (MrHRF). The serum containing IgE+, drawn after informed consent from patient 1, contained 12,000 ng IgE/mL. The serum containing IgE-, drawn from a patient with myeloma, was a gift of Dr H. Gewurz (Rush Medical College, Chicago). After purification by anion exchange chromatography,<sup>14</sup> the IgE content was 10,000 ng IgE/mL. In previous experiments, the biologic activity of IgE+ and IgE- in these sera was determined.<sup>4</sup> By definition, IgE+ did transfer sensitivity to HrHRF, and IgE- did not. Anti-human IgE antibody (anti-IgE) was generated in a goat and provided by Dr N. F. Adkinson of our Division. The hybridoma (H1-DNP/ε26) was obtained previously from Dr D. Katz (La Jolla Institute of Cellular and Developmental Immunology), and ascites were generated and used without purification. DNP-human serum albumin (HSA) was synthesized.<sup>15</sup>

All reagents used for buffers, piperazine-N-N'-bis [2-ethane sulphonic acid] (PIPES), EDTA, heparin, lactic acid, and glucose, were purchased from Sigma (St Louis, Mo). HSA was purchased from Calbiochem-Novabiochem Corp (La Jolla, Calif) and Dextran 70 6% was obtained from McGaw (Irvine, Calif).

### Buffers

All RBL cell histamine release experiments were performed in PIPES/albumin/glucose (PAG) buffer containing 25 mmol/L PIPES, 110 mmol/L NaCl, 5 mmol/L KCl, 0.003% HSA, and 0.1% D-glucose in the presence of 1 mmol/L CaCl<sub>2</sub>. The pH of the buffer was adjusted to 7.40. All basophil histamine release experiments were also performed in PAG buffer but in the presence of 5 mmol/L CaCl<sub>2</sub>.<sup>4</sup> Ca<sup>2+</sup> free PAG buffer was exclusively used for washing RBL cells and basophils.

Lactic acid containing 0.01 mol/L lactic acid, 140 mmol/M NaCl, and 5 mmol/L KCl adjusted to pH 3.90 by 0.1 mol/L NaOH was used to remove surface IgE from basophils.

Acetate elution buffer containing 0.05 mol/L acetate, 0.85% NaCl, 0.01 mol/L EDTA, 0.03% HSA, and pH 3.7 was used for total IgE measurements.

### RBL-SX38 cells and culture

Experiments were performed with rat basophilic leukemia cells (RBL-SX38), a gift of Dr J. P. Kinet (Boston, Mass). These cells

express the rat high-affinity IgE receptor, FcεRI, and have additionally been transfected with a functional human FcεRI, possessing the α-, β-, and γ-chains.<sup>16</sup> Cells were cultured at 37°C in 95% oxygen and 5% carbon dioxide in EMEM (1 × minimal essential medium with Earle's salts and glutamine; Paragon Biotech Inc, Baltimore, Md) containing 1.2 mg/mL geneticin (G418; Sigma, St Louis, Mo), 10% heat-inactivated FCS (Sigma), 100 U/mL penicillin, and 100 µg/mL streptomycin solution (Paragon Biotech Inc). After the cells reached confluence in 75 cm<sup>2</sup> flasks (Sarstedt, Newton, NC), the G418-containing medium was changed to EMEM for secretion experiments. Cells were kept in G418-free EMEM for at least 12 hours to a maximum of 36 hours before the initiation of an experiment. These adherent cells were harvested with 2.0 mL of 0.05% trypsin/0.53 mmol/L EDTA × 4Na (Gibco, Grand Island, NY) for 2 minutes, resuspended in 10 mL EMEM, centrifuged for 5 minutes at 100g, and counted. Treating basophils with 0.05% trypsin/0.53 mmol/L EDTA × 4Na for 2 minutes did not affect HrHRF-induced histamine release. For passive sensitization, cells were divided into 3 pots (control cells, IgE+ sensitization, and IgE- sensitization) to obtain an approximate final number of 1 × 10<sup>5</sup> cells per reaction tube for histamine release experiments. Viability of cells used was 95% or more.

### RBL-2H3 cells and culture

RBL-2H3 cells, a gift from Dr C. Fewtrell, Cornell University, were used for HrHRF and MrHRF priming experiments only. Cells were grown in G418-free EMEM as described earlier. Cells were passively sensitized with mouse anti-DNP-IgE.

### Peripheral blood basophils

After informed consent, 40 mL of peripheral blood was obtained by venepuncture of healthy volunteers and mixed with 12.5 mL of 6% Dextran 70, 5 mL of 0.1 mol/L EDTA, and 375 mg glucose.<sup>4</sup> After 90 minutes of sedimentation at room temperature, the leukocyte-rich plasma was transferred to 50 mL flat-bottomed tubes and spun for 8 minutes at 150g. Basophils were then washed with cold PAG (4°C) followed by 0.9% cold saline solution. Surface IgE was removed by adding 5 mL of cold (4°C) lactic acid pH 3.90 for 3.5 minutes.<sup>17</sup> Immediately afterwards, 40 mL of PAG was added, and basophils were washed twice. For passive sensitization, cells were divided into 3 pots (IgE-stripped control cells, IgE+ sensitization, and IgE- sensitization) to obtain an approximate final number of 2 × 10<sup>4</sup> basophils per reaction tube for histamine release experiments.

### Passive sensitization of RBL-SX38 cells, RBL-2H3 cells, and basophils

Passive sensitization of RBL cells was done according to a modification of the method of Levy and Osler.<sup>18</sup> RBL-SX38 cells were passively sensitized with 1000 ng/mL IgE+ (serum, patient 1) or 1000 ng/mL IgE- (IgE myeloma serum, patient 2) for 60 minutes in an incubator at 37°C with 95% oxygen and 5% carbon dioxide. EMEM medium was added to obtain a total volume of 1000 µL, but no EDTA/heparin was added. Unsensitized RBL cells served as controls.

RBL-2H3 cells were passively sensitized with 1000 ng/mL mouse anti-DNP-IgE for 60 minutes as described earlier. Again unsensitized cells served as controls.

Basophils were passively sensitized with 1000 ng/mL IgE+ (serum, patient 1) or 1000 ng/mL IgE- (IgE myeloma serum, patient 2) for 60 minutes at 37°C in the presence of 200 µL of EDTA (4 mmol/L/mL)/heparin (10 µg/mL).<sup>18</sup> Additional PAG was added to obtain a total volume of 1000 µL. IgE-stripped, but unsensitized, basophils served as controls. Based on previous data<sup>4</sup> and evaluation of FcεRI receptor density on the cells, 1000 ng of IgE

provides an excess amount of IgE (approximately equivalent to  $3.3 \times 10^{15}$  molecules) for sensitization of RBL cells and basophils.<sup>19</sup> After incubation, both the RBL cells and the basophils were washed twice in PAG and resuspended in the appropriate volume of PAG.

### HRF priming of RBL-SX38 and RBL-2H3 cells

IgE+ and IgE- sensitized RBL-SX38 cells were primed with 40  $\mu$ g/mL HrHRF for 15 minutes, then anti-human-IgE antibodies were added and histamine release was performed. To determine whether an HRF signaling pathway may have been lost by transfecting the RBL-SX38 cells with the human Fc $\epsilon$ RI receptor, additional HrHRF priming experiments were done in RBL-2H3 cells, which are the parental RBL cells. Anti-DNP-IgE sensitized RBL-2H3 cells were primed with 0.04  $\mu$ g/mL, 0.4  $\mu$ g/mL, 4  $\mu$ g/mL, and 40  $\mu$ g/mL HrHRF or MrHRF for 15 minutes; histamine release was performed.

As a positive control, although basophil data have been previously published,<sup>9</sup> native human basophils were primed with 0.04  $\mu$ g/mL, 0.4  $\mu$ g/mL, 4  $\mu$ g/mL, and 40  $\mu$ g/mL HrHRF or MrHRF for 15 minutes; histamine release was performed.

### Histamine release assay

The histamine release assay was performed in a final volume of 100  $\mu$ L in the presence of Ca<sup>2+</sup>. Based on previous experience in kinetic experiments, 1 mmol/L CaCl<sub>2</sub> was used for the RBL cell histamine release experiments and 5 mmol/L CaCl<sub>2</sub> for the basophil experiments.<sup>7</sup> An approximate number of  $1 \times 10^5$  RBL cells and  $2 \times 10^4$  basophils were used per reaction tube. Cells were added to prewarmed (37°C) tubes and incubated for 45 minutes at 37°C in a waterbath. In HrHRF/MrHRF-priming experiments, cells were primed for 15 minutes at 37°C, then anti-IgE (0.1  $\mu$ g/mL final) was added to the primed and to the control cells and incubated for 45 minutes. In RBL-2H3 cell experiments, DNP-HSA (0.1  $\mu$ g/mL final) was used as the antigen. At the end of the incubation, 900  $\mu$ L of cold (4°C) PAG was added to stop histamine release, and tubes were centrifuged (basophils, 2 minutes at 1000g; RBL cells, 5 minutes at 100g). Cell-free supernatants were collected and assessed for histamine content by the automated fluorometric assay of Siraganian.<sup>20</sup> Results were based on the mean of duplicate determinations and were expressed as a percentage of histamine release by dividing the total histamine after subtracting the spontaneous release of unstimulated cells (20% to 25% for RBL cells and 25% to 5% for basophils after 45 minutes). Total histamine was obtained by lysing the cells with 2.0% perchloric acid.

### RBL-SX38 cell sensitivity

To interpret the results obtained appropriately, we investigated and compared the sensitivity (defined as the density of IgE required to obtain half-maximum histamine release) of RBL-SX38 cells with known sensitivity data from human basophils.<sup>19</sup> For 30 minutes,  $1 \times 10^6$  cells were sensitized at 37°C in the incubator with several concentrations of human antibody to glycoprotein 120 peptide of the HIV envelope coupled to ovalbumin (anti-gp 120-ovalbumin) IgE (2000, 666, 222, 74, and 25 ng/mL). Unsensitized cells served as the control. After the cells were washed, histamine release was performed as described earlier with 0.2  $\mu$ g/mL gp120-ovalbumin or 0.2  $\mu$ g/mL anti-human IgE antibodies as stimuli. In addition,  $2 \times 10^5$  sensitized or unsensitized cells were used for FACS analysis. Cells were incubated with 1.6  $\mu$ g/mL of the anti-id antibody AB-19-4 (Dr Frances Davis, Tanox Corp, Houston, Texas), which binds only to anti-gp120 IgE, or with a 1/900 dilution of the anti- $\alpha$  chain antibody, 22E7 (Dr Jarema Kochan, Hoffmann LaRoche, Nutley, Pa) for 30 minutes in PAG/HSA/EDTA (PAG + 0.06% HSA and 0.01 mol/L EDTA) on ice. Cells were spun (4 minutes at 150g), the supernatant was removed, and the cells were washed in 500  $\mu$ L of

PAG/HSA/EDTA. After spinning, cells were resuspended in 100  $\mu$ L of phycoerythrin-labeled goat anti-mouse antibody (3.33  $\mu$ g/mL; BioSource, Camarillo, Calif) and incubated for 30 minutes on ice. Afterwards, cells were spun (4 minutes at 150g), and the supernatants were removed and resuspended in 150  $\mu$ L PAG/HSA/EDTA. FACS analysis was performed on an Epics-Profile 2 (Coulter Counter, Hyla, Fla). To calibrate the flow cytometric data (ie, to convert arbitrary flow units to absolute cell surface receptor densities), a portion of the cells labeled with either 2000 or 666 ng/mL of anti-gp 120 IgE were both counted and "stripped" of their IgE molecules with acetate buffer, pH 3.7.<sup>7</sup> The cells were sedimented, and the supernatant fluid was immediately titrated to neutrality with 1 mol/L NaOH. Total IgE in the supernatants of the acetate-treated cells was measured by the IMx System (Abbot, Abbott Park, Ill) according to the manufacturer's specifications. Acetate elution removes all of the IgE molecules from the surface of the cell but, unlike lactic acid treatment, does not yield a viable cell that can be passively sensitized. On the basis of this, the RBL-SX38 cells expressed 70,000 to 150,000 receptors per cell. One flow unit represents  $1400 \pm 700$  IgE molecules per cell.

### Statistical analysis

For statistical analysis, the Wilcoxon signed-rank test and the Kruskal Wallis test were used.

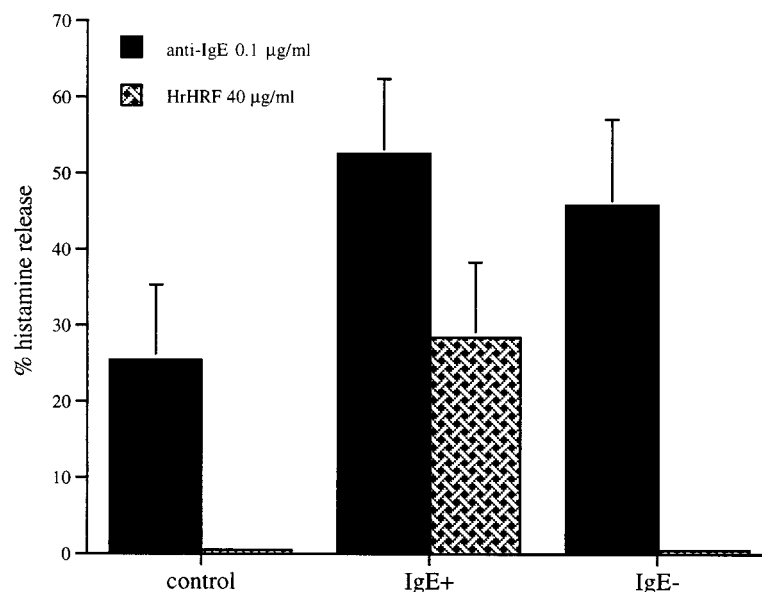
## RESULTS

### Histamine release to HrHRF and anti-IgE in human basophils

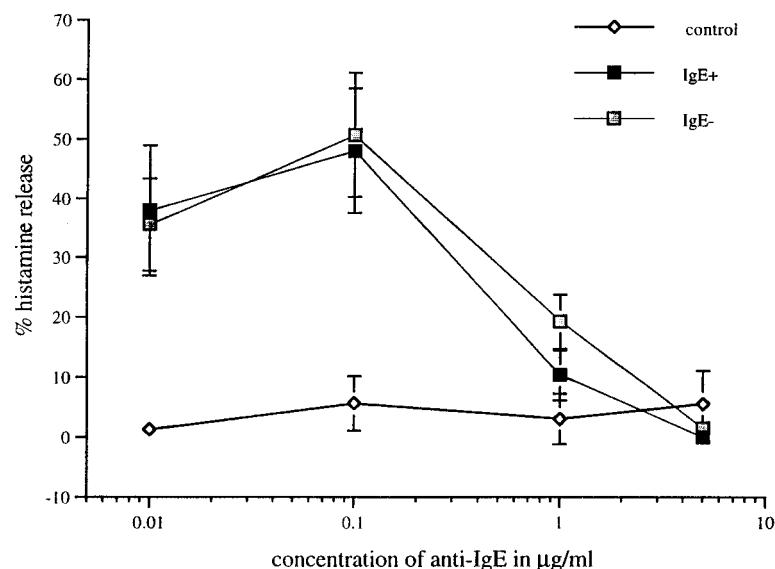
In human basophil experiments ( $n = 5$ ), HrHRF at 40  $\mu$ g/mL induced  $29\% \pm 9\%$  histamine release in IgE+ sensitized cells ( $P < .04$ , Wilcoxon signed rank test). As expected, neither the IgE- ( $0\% \pm 0\%$ ) nor the IgE-stripped basophils ( $0.2\% \pm 0.4\%$ ) responded to HrHRF. On the other hand, anti-IgE-induced histamine release with the optimal dose of 0.1  $\mu$ g/mL<sup>5</sup> resulted in the same amount of histamine release in IgE+ ( $52\% \pm 9\%$ ) and IgE- ( $46\% \pm 10\%$ ;  $P =$  not significant) sensitized cells. Because lactic acid treatment removes approximately 90% of surface IgE from Fc $\epsilon$ RI receptors on the basophil,<sup>7</sup> the IgE-stripped cells released  $25\% \pm 9\%$  histamine to anti-IgE (Fig 1).

### Histamine release to anti-IgE in RBL-SX38 cells

In pilot experiments ( $n = 3$ ), using anti-IgE-antibodies at a final concentration of 0.01  $\mu$ g/mL, 0.1  $\mu$ g/mL, 1.0  $\mu$ g/mL, and 5.0  $\mu$ g/mL, the optimum dose for histamine release by anti-IgE antibodies was determined to be 0.1  $\mu$ g/mL (Fig 2). This concentration therefore was used for anti-IgE-induced histamine release in all further experiments with passively sensitized RBL-SX38 cells. As expected, the results showed no difference in histamine release in IgE+ and IgE- sensitized cells and no histamine release in unsensitized cells. These results gave strong evidence for binding IgE+ and IgE- to the transfected human Fc $\epsilon$ RI and functional signaling through the human Fc $\epsilon$ RI in these RBL-SX38 cells. Human IgE is well known not to bind to the rat Fc $\epsilon$ RI<sup>21</sup> and therefore does not contribute to the results of this figure.



**FIG 1.** Histamine release to HrHRF and anti-IgE in lactic acid stripped (control), IgE+, and IgE- sensitized basophils (mean of  $5 \pm$  SD). HrHRF induced significant ( $P < .04$ ) histamine release in IgE+ sensitized basophils.



**FIG 2.** Anti-IgE dose-response curve in unsensitized (control), IgE+, and IgE- sensitized RBL-SX38 cells (mean of  $3 \pm$  SD).

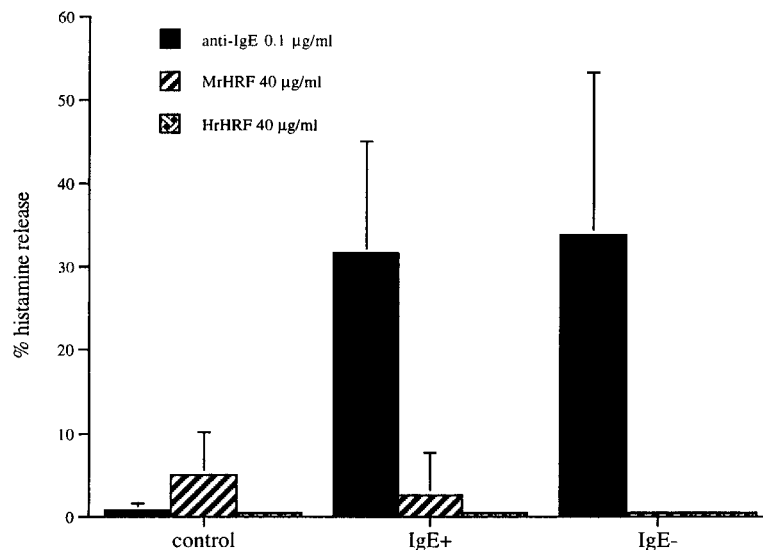
### Histamine release to HrHRF and MrHRF in RBL-SX38 cells

In 6 experiments, RBL-SX38 cells passively sensitized with IgE+ did not release histamine to either HrHRF ( $0.0\% \pm 0.0\%$ ;  $P =$  not significant) or to MrHRF ( $3\% \pm 5\%$ ,  $P =$  not significant) with a final HrHRF concentration of  $40 \mu\text{g/mL}$  (Fig 3). In addition, neither IgE- sensitized nor unsensitized cells (control) released histamine to HrHRF or MrHRF (Fig 3). Although HrHRF and MrHRF have 96% homology, we included the

MrHRF in these experiments because the RBL cells might have been more reactive to the MrHRF than to the HrHRF.

### HRF priming of RBL-SX38, RBL-2H3 cells and human basophils

Priming RBL-SX38 cells with  $40 \mu\text{g/mL}$  HrHRF ( $n = 3$ ) did not increase the anti-IgE-induced histamine release, in either IgE+ or in IgE- sensitized cells (data not shown). In addition, priming RBL-2H3 cells that had been sensitized with mouse DNP specific IgE with  $0.04$



**FIG 3.** Histamine release to HrHRF, MrHRF, and anti-IgE in unsensitized (control), IgE+, and IgE- sensitized RBL-SX38 cells (mean of  $6 \pm \text{SD}$ ). Neither HrHRF nor MrHRF induced histamine release in IgE+ sensitized basophils.

$\mu\text{g/mL}$ , 0.4  $\mu\text{g/mL}$ , 4  $\mu\text{g/mL}$ , or 40  $\mu\text{g/mL}$  of HrHRF or MrHRF ( $n = 3$ ) did not increase histamine release induced by DNP-HSA. In human basophils, HrHRF and MrHRF in a concentration of 4  $\mu\text{g/mL}$  and 40  $\mu\text{g/mL}$ , but not 0.04  $\mu\text{g/mL}$  or 0.4  $\mu\text{g/mL}$ , enhanced anti-IgE-induced histamine release (Fig 4).

### RBL-SX38 cell sensitivity

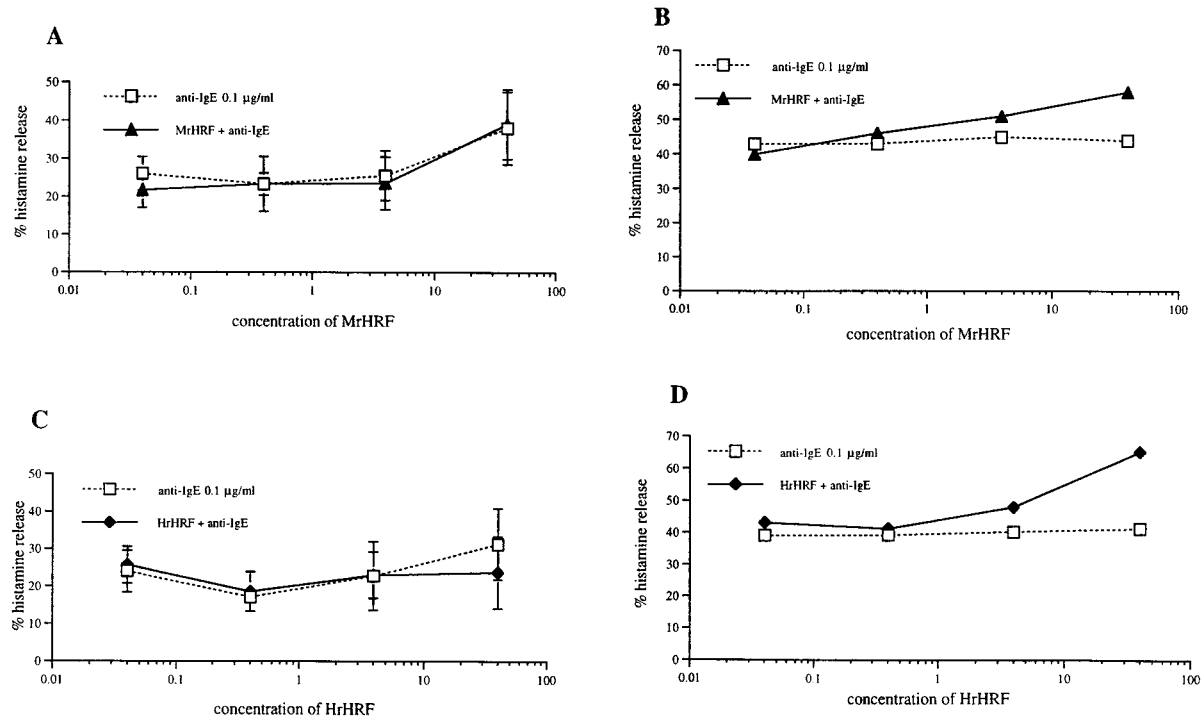
Based on the assessment of IgE density on sensitized RBL cells with acetate stripping, our RBL-SX38 cells express a mean of  $134,400 \pm 16,800$  FcεRI on their cell surface ( $n = 3$ ). RBL-SX38 cells require approximately the same number of IgE crosslinks as human basophils to obtain 50% of maximum histamine release.<sup>19</sup> On the basis of these data, the transfected RBL cells are approximately as sensitive as are human basophils (Fig 5).

### DISCUSSION

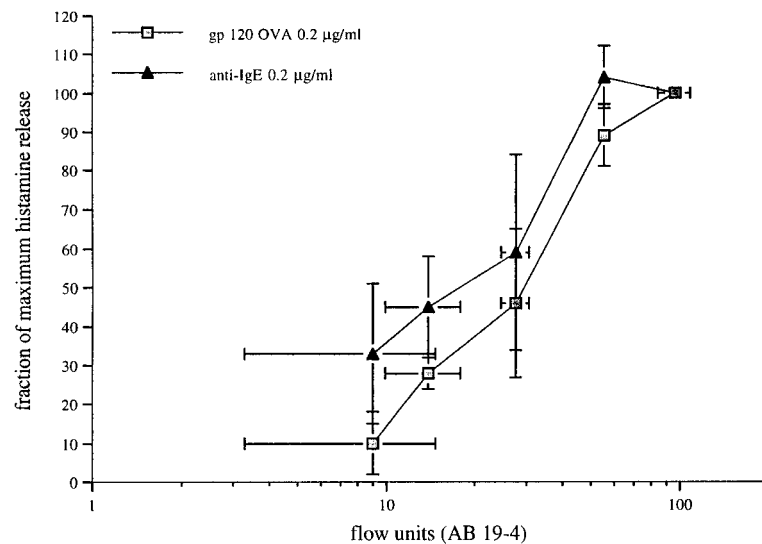
We have demonstrated that HrHRF and MrHRF failed to induce histamine release in the presence of IgE+ in RBL-SX38 cells. This finding is in contrast to our results obtained in human basophils, which release 29% histamine to HrHRF in IgE+ sensitized cells ( $P < .04$ ). There are several possible explanations for these results. First, it appears likely that these RBL cells simply lack an HRF receptor, although the existence of an HRF receptor still remains hypothetical. Previous studies indicated that HrHRF does not bind to solid-phase bound IgE+ (Susan M. MacDonald, unpublished data) and that HrHRF primed anti-IgE-antibody-induced histamine release and IL-4 and IL-13 production from all basophils, irrespective of the type of IgE on the cell surface.<sup>9</sup> Taken together, these data suggested that basophils have a distinct receptor for HRF. More recent studies have shown that rottlerin, a nonstaurosporin-derived kinase inhibitor, significantly

enhanced HrHRF-induced histamine release but not anti-IgE-induced histamine release in basophils from IgE+ donors. These data suggest that the signaling pathway triggered by HrHRF differs from that of IgE-dependent stimuli.<sup>22</sup> Furthermore, HrHRF caused chemotaxis and augmented IL-8 production from human eosinophils.<sup>10</sup> It cannot be absolutely excluded that RBL-SX38/RBL-2H3 cells express an HRF receptor that did not bind to HrHRF or could not signal after binding to HrHRF. The absence of an effect was observed with humans and MrHRF, which was included in the experiments because RBL cells are rat cells, which may be more closely related to the mouse system than to the human system. With the use of the PCR technique, it has been shown that RBL cells neither contain nor secrete HRF (Susan MacDonald, unpublished data). By definition, RBL cells are considered to be a mast cell line, and experiments with human mast cells also indicate that they fail to react to HrHRF (Susan MacDonald, unpublished data).

The fact that HrHRF and MrHRF did not induce histamine release in IgE+ sensitized RBL-SX38 cells is strong evidence that HrHRF does not exclusively bind to the human IgE molecule to induce aggregation and mediator release from human basophils. It is important to consider that the RBL-SX38 cells appeared to be as sensitive to IgE-mediated activation as human basophils. Additionally, it should be noted that the ability to fill 100,000 of the RBL-SX38 human FcεRI should be sufficient to demonstrate secretion from these cells. Even crosslinking 500 receptors on normal human basophils would generate a modest response so that, if HrHRF were inducing a low level of aggregation on the RBL-SX38 cell, it would have to be less than 1% of the cell surface IgE to be undetectable. It is more difficult to factor in how the size of any aggregate formed might influence the ability to induce histamine release with recombinant



**FIG 4.** HrHRF and MrHRF priming of DNP-IgE sensitized RBL-2H3 cells (mean of  $3 \pm$  SD). HrHRF neither augmented nor primed anti-IgE-induced histamine release at 40  $\mu$ g/mL, 4  $\mu$ g/mL, 0.4  $\mu$ g/mL, or 0.04  $\mu$ g/mL (A and C). In contrast, HrHRF and MrHRF augmented or primed anti-IgE-induced histamine release in human basophils at 40  $\mu$ g/mL and 4  $\mu$ g/mL (B and D;  $n = 1$ ). Data are given in percentage of histamine release compared to the anti-IgE-induced histamine release control (0.1  $\mu$ g/mL in all conditions).



**FIG 5.** RBL-SX38 cell sensitivity. RBL-SX38 cells express about  $134,000 \pm 16,800$  human Fc $\epsilon$ RI (mean of  $3 \pm$  SD); 1 flow unit is equal to  $1400 \pm 700$  human IgE molecules. Histamine release is shown as a fraction of maximum histamine release (anti-IgE, 48% release; gp 120-OVA, 54% release).

HRF. RBL cells are known to be relatively insensitive to aggregates in the size of dimers but so too are most human basophils. Overall, the data summarized earlier provide additional functional evidence that HRF does not induce the aggregation of IgE directly.

The passively sensitized RBL-SX38 cells did not release histamine to recombinant HRF, either HrHRF or MrHRF, the latter showing 96% homology to HrHRF at the protein level. Priming the RBL-SX38 cells with HrHRF (40  $\mu$ g/mL) for 15 minutes did not increase the

anti-IgE-induced histamine release, either in IgE+ or in IgE- sensitized cells (data not shown). In addition, priming the parental RBL cells, RBL-2H3, with various concentrations of HrHRF and MrHRF for 15 minutes did not increase the DNP-HSA-induced histamine release. In contrast, priming basophils, which formerly did not release histamine to HrHRF, for 15 minutes with HrHRF significantly increased their anti-IgE-induced histamine release.<sup>9</sup> When these data are compiled, it appears unlikely that RBL-SX38 or RBL-2H3 cells respond to either recombinant HRF. Thus the most probable explanation for these findings is the lack of an HRF-specific receptor on RBL cells.

With the evidence suggesting that HRF does not directly interact with IgE but only acts in a cytokine-like manner, the dependence of HRF-induced histamine release on IgE+ raises additional questions about the mechanism for this reaction. In this context, it should be noted that other priming agents can be shown to have a dependence on the presence or absence of IgE+. For example, IL-3 does not normally directly induce histamine release from human basophils but if the cells are first sensitized with IgE+, but not IgE-, IL-3 induces release.<sup>23</sup> A similar result can be found for the ability of deuterium oxide (partial replacement of the H<sub>2</sub>O in the buffers with D<sub>2</sub>O) to directly induce release.<sup>8</sup> The working hypothesis is that IgE+ has the property of inducing a low level of FcεRI aggregation, not detected by incubating cells in normal buffers. However, any agent that significantly "primes" or enhances basophil function reveals this low level of aggregation and induces histamine release. Furthermore, mouse recombinant IL-3 priming for 15 to 30 minutes did not enhance anti-IgE-induced histamine release in these cells (data not shown), despite the fact the IL-3 is known to prime basophils for histamine release.<sup>24-26</sup> These results also suggest that the RBL cell does not express a receptor capable of binding HrHRF, which would "prime" the cell.

In conclusion, these results provide evidence that HrHRF does not directly induce histamine release through interaction with IgE antibody alone. In conjunction with other data, it seems likely that HRF does not interact with IgE at all. Therefore it appears likely that HrHRF signals through its own specific receptor, which is not expressed or is not functional on RBL-SX38 or RBL-2H3 cells but which seems to be expressed on basophils of atopic and nonatopic donors.

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