

NEUTROPHILS AND MAST CELLS

Comparison Of Neutrophil-Derived Histamine-Releasing Activity With Other Histamine-Releasing Factors

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Human neutrophil-derived histamine-releasing activity (HRA-N) was partially purified and found to contain a heat-stable 1400 to 2300-Da fraction which caused human basophils and rat basophil leukemia cells (RBL) to degranulate. The capacity of HRA-N to activate basophils was not related to the gender or atopic status of the basophil donor, but was related to anti-IgE responsiveness. Several lines of evidence suggest that HRA-N and anti-IgE induce histamine release through distinctly different mechanisms: 1) the time course of HRA-N- and anti-IgE-induced RBL histamine release are different; 2) HRA-N causes histamine release from RBL with and without surface-bound IgE; 3) lactic acid stripping of IgE from human basophils reduces anti-IgE-induced histamine release, but has no consistent effect on HRA-N-induced histamine release; and 4) passive sensitization of lactic acid-stripped basophils with IgE restores anti-IgE-induced histamine release but not HRA-N-induced histamine release.

Several histamine-releasing factors (HRF) were compared with HRA-N. Human nasal HRF (HRF-NW, crude and partially purified fractions of 15 to 30, 3.5 to 9, and <3.5 kDa), like HRA-N, caused equal histamine release from both native and IgE-sensitized RBL. However, only the 15- to 30-kDa fraction caused histamine release from human basophils in the doses tested. Mononuclear cell HRF (HRF-M, crude and a partially purified 25 kDa *M_r* fraction) and platelet HRF (HRF-P, crude preparation) failed to cause histamine release from either native or IgE-sensitized RBL but caused $30 \pm 5.5\%$ and $20 \pm 10\%$ net histamine release from human basophils, respectively. HRA-N and HRF-NW were both stable to boiling.

These data, taken together, suggest that the capacity of HRA-N to induce RBL and human basophil histamine release and of HRF-NW to stimulate RBL histamine release is independent of IgE. The data further suggest that HRA-N and HRF-NW can be distinguished by size, and that they both differ from

mononuclear cell HRF and platelet HRF. Thus, it appears that inflammatory cells generate a family of distinct HRF.

A human neutrophil-derived histamine-releasing activity, termed HRA-N,² has recently been described which causes dose-related, temperature- and calcium-dependent, noncytotoxic histamine release from cultured RBL cells, human basophils, human cutaneous mast cells, cultured mouse bone marrow-derived mast cells *in vitro*, and guinea pig and rat cutaneous mast cells *in vivo* (1).³ Neutrophils from approximately 80% of donors spontaneously release HRA-N into their supernatants. HRA-N release itself is not related to the release of lysosomal enzymes from either primary or secondary granules. In general, donors who release HRA-N, do so consistently over at least a 2-yr span. The ability to release HRA-N is not related to atopic status, gender, or the presence of contaminating mononuclear cells or platelets, but is inversely related to the presence of eosinophils in the PMN preparation.

HRF have also been described from other inflammatory cells, including mononuclear cells and platelets (2-4). Nasal washings from unstimulated human subjects and supernatants of lung macrophages also contain factors which cause histamine release from human basophils (5, 6). The HRF derived from nasal washings (HRF-NW) and lung macrophages (HRF-MP) may cause basophil degranulation through an IgE-dependent pathway as suggested by the following observations: 1) basophils desensitized by exposure to anti-IgE in the absence of calcium no longer respond to either HRF-NW or HRF-MP and conversely, histamine released in response to anti-IgE is reduced after incubation with either HRF-NW or HRF-MP in the absence of calcium; 2) removal of basophil IgE by exposure to lactic acid renders the basophil unresponsive to HRF-NW and HRF-MP, and reduces the response

² Abbreviations used in this paper: HRA-N, neutrophil-derived histamine-releasing activity; RBL, rat basophil leukemia cells; LPR, late phase allergic reaction; PMN, polymorphonuclear neutrophil; HSA, human serum albumin; HRF, histamine-releasing factor; HRF-M, mononuclear cell-derived HRF; HRF-P, platelet-derived HRF; HRF-MP, human lung macrophage-derived HRF; HRF-NW, HRF in nasal washings; NW 1, 2, and 3, partially purified nasal wash fractions 1 (15,000 to 30,000 Da), 2 (3,500 to 9,000 Da), and 3 (<3500 Da); PAG, Pipes buffer without Ca^{2+} or Mg^{2+} , with 0.1% albumin, and 0.1% glucose.

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to anti-IgE; 3) incubation of lactic acid-stripped basophils with IgE myeloma or high titer IgE sera from certain individuals restores the basophil response to HRF-MP and HRF-NW, respectively; and 4) the capacity of IgE-containing sera to restore responsiveness is destroyed by passage over an anti-IgE-Sepharose column (6, 7). Thus, the activity of both HRF-NW and HRF-MP is thought to be dependent upon IgE. It was therefore of interest to compare the HRF derived from nasal washings and other inflammatory cells with HRA-N.

MATERIALS AND METHODS

Materials. Hanks' buffer without phenol red, with and without calcium and magnesium, heat-inactivated FCS, PBS, and RPMI 1640 (GIBCO, Grand Island, NY); O-phenylenediamine hydrochloride, histamine diphosphate, Histopaque, trypan blue dye, EDTA, and Tris (Sigma Chemical Co., St. Louis, MO); DE52 (DEAE-cellulose (Whatman Ltd., Maidstone Kent, England); Ficoll-Hypaque, Sephadex G-25, Sephadex G-50, and Dextran T 500 (Pharmacia Fine Chemicals, Uppsala, Sweden); anti-human IgE (Melo Laboratories, Springfield, VA); streptokinase-streptodornase (Lederle, Pearl River, NY); YM2, YM5, YM30, and YM100 membranes (Amicon, Danvers, MA); 50 × 0.6 cm TSK G-3000 column (Varion, Walnut Creek, CA); heparin sodium injection USP (Organon Inc., Orange, NJ); sodium hydrochloride, hydrochloric acid, absolute ethanol, heptane, butanol, Brij 35, and phosphoric acid (Fisher Scientific, Springfield, NJ); Eagle's MEM, with Earl's balanced salt without L-glutamine, trypsin-Versene mix, and L-glutamine (M.A. Bioproducts, Walkersville, MD); 150 cm² tissue culture flasks and 24-well tissue culture plates (Costar Labs, Cambridge MA); 500-ml filters (Nalgene Labware, Rochester NJ); 0.22 µm filter units (Millipore Corp., Bedford MA); Nunc cryotubes (Intermed Interlab, Thousand Oaks CA); and polypropylene falcon tubes (Becton Dickinson Labware, Oxnard CA) were obtained from the manufacturers. HSA was obtained from the Michigan Department of Public Health, Lansing, MI. RBL cells (Dr. Reuben Siraganian, NIDR), IgE IR-162, rabbit anti-IgE IR-162 (Dr. Howard Boltansky, NIAID), IgE* (Dr. Anne Kagey-Sobotka, Johns Hopkins University), and nasal washings (Dr. Gordon Raphael, NIAID), were each generous gifts.

Isolation of PMN. PMN were isolated from human venous blood by Ficoll-Hypaque (1.077) centrifugation followed by dextran sedimentation as previously described (8). The resulting isolate was resuspended in HBSS with 0.2% HSA at 5×10^7 cells/ml, unless otherwise stated. Purity was greater than 94% neutrophils unless otherwise specified, and viability was greater than 95% as determined by trypan blue dye exclusion and lactate dehydrogenase determination.

Generation of PMN supernatants. PMN, 5×10^7 /ml, were incubated at 37°C for 1 h in HBSS-2% HSA and the supernatants collected. All experiments were completed within 4 h of blood collection in order to maintain PMN viability.

PMN equivalent/ml is defined as the number of PMN, expressed on a per ml basis, from which the supernatant was generated. For example, a supernatant generated from 5×10^7 PMN/ml would contain 5×10^7 PMN equivalents/ml.

Partial purification of HRA-N. Aliquots of HRA-N containing PMN supernatants (7.5 ml) were boiled 10 min, centrifuged at $3000 \times g$ for 5 min, and the supernatants concentrated to 0.3 ml at 4°C under pressure over a YM2 (1000 m.w. cutoff) membrane. The retentate was applied to a Sephadex G-25 column (1 × 37 cm) and eluted in 0.55 ml fractions at 37°C with HBSS with Ca and Mg.

Generation of mononuclear cell and platelet supernatants (HRF-M and HRF-P). After informed consent, normal healthy volunteers were leukapheresed in order to obtain large numbers of cells. Approximately 200 ml of cells were obtained from each procedure. The cells were diluted to 360 ml with Dulbecco's PBS, layered over a Ficoll-Hypaque density gradient (1.077), then centrifuged for 15 min at $1000 \times g$. The cells concentrated at the upper interface were collected, washed twice with PBS, and centrifuged for 10 min at $400 \times g$. The cell pellets were resuspended in PBS and platelets separated from cells as previously described (2) by low speed centrifugation for 7 min. The platelet-rich supernatants were aspirated, suspended in 200 ml PBS, and cultured for 16 to 20 h at 37°C and 5% CO₂. The platelet-depleted mononuclear cell pellets, which were greater than 98% viable by trypan blue dye exclusion, were resuspended in RPMI 1640 at 25 to 40×10^6 cells/ml in 50 ml conical polypropylene tubes. The mononuclear cells were stimulated with 25 U/ml of streptokinase-streptodornase and cultured 16 to 20 h at 37°C and 5% CO₂. Supernatants from the mononuclear cell and platelet suspensions

were removed separately and centrifuged at $38,000 \times g$ for 30 min. The platelet supernatant was concentrated 50-fold by using positive pressure filtration (Amicon YM 5 membrane). The mononuclear cell-conditioned media was passed through a YM 100 membrane followed by 50-fold concentration by positive pressure filtration using a YM5 membrane. Unless otherwise stated, these crude concentrated supernatants were diluted 10-fold before use.

Partial purification of HRF-M. Concentrated mononuclear cell-conditioned media was applied to a 75 × 0.6 cm TSK-3000 column. The operating parameters were controlled by a Waters HPLC system with detection of proteins by absorbance at 280 nm. The isocratic runs, with PBS containing 0.3% polyethylene glycol-8000, pH 7.4, were carried out at a constant flow rate of 0.5 ml/min. One-minute fractions were collected and assayed for the ability to induce histamine release from freshly isolated human basophils. The major HRF component identified and employed throughout these studies corresponded to a m.w. of about 25,000. Unless otherwise stated, these preparations were diluted 10-fold before use in experiments.

RBL cell culture. The 2H3 subline of RBL cells was maintained in 150 cm² tissue culture flasks in Eagle's MEM supplemented with 4 mM glutamine, 200 U/ml penicillin, 200 µg/ml streptomycin, 500 ng/ml fungizone, and 15% heat-inactivated FCS. The cells were treated with trypsin-Versene, resuspended in media, and passaged every 3 to 7 days. Cells were maintained in a 5% CO₂ humidified atmosphere at 37°C.

Nasal washings. Nasal washings were collected over a 1-yr span from both atopic and nonatopic donors as previously described (8). Briefly, nasal washings were obtained by washing human nares with 4 ml of normal saline under constant gentle suction through a size 8 French rubber urethral catheter. Recovery generally was 2 to 3 ml. Washings were immediately placed on ice and frozen at -70°C until use. Atopic donors were defined as persons with symptoms of seasonal asthma, seasonal allergic rhinitis, and/or eczema with positive skin tests to inhalant allergens.

Partial purification of human nasal washings. Nasal washings were purified by using a modification of the method of MacDonald et al. (7). Briefly, 8-ml pools (combining 4 to 8 donors) of crude nasal washings were mixed with 800 mg DE52 preequilibrated with 1.2 ml Tris (0.01 M Tris, 0.15 M NaCl (pH 7.4) at 4°C) and stirred gently overnight at 4°C. The slurry was washed with 1.6 ml Tris, centrifuged at $3000 \times g$ for 5 min, and the supernatant filtered through a 22 µm filter. The filtrate was then passed through a YM30 (30,000 m.w. cutoff) membrane and the filtrate concentrated under pressure to 0.3 ml over a YM2 (1,000 m.w. cutoff) membrane. The retentate was then applied to a Sephadex G-50 column (1 × 37 cm), eluted at 4°C with HBSS with Ca, Mg, and collected in 0.55-ml fractions. Column fractions were stored at -70°C until use.

Histamine release experiments. 1) RBL: HRA-N activity was determined by measuring the percent histamine released from triplicate aliquots of RBL exposed to PMN supernatants as follows: 10^5 RBL/well were plated in a 24-well culture plate and allowed to adhere overnight. The wells were then exposed to IgE, 5 µg in 0.02 ml PBS or to PBS for 45 min, and washed twice with ice cold HBSS without Ca²⁺ or Mg²⁺. Unless otherwise specified, RBL were incubated at 37°C with PMN supernatants, 0.2 ml, 5×10^7 PMN equivalents/ml for 20 min, a time determined experimentally to be optimal for maximum histamine release. In experiments with HRF generated from nasal washings, platelets and mononuclear cells, the incubation time was increased to 45 min. Rabbit anti-IgE, 1/1000 in HBSS with 0.1% HSA, was used to induce histamine release in the IgE-sensitized replicates as the positive control, and buffer alone was used for the negative control. Reactions were stopped by the addition of 0.6 ml ice cold EDTA, 0.1 M. Supernatants were centrifuged at 4°C for 3 min, $400 \times g$, to remove RBL. The adherent cells were removed from the wells with trypsin-Versene and lysed with 6% perchloric acid. Samples were stored at -70°C until assayed for histamine.

2) Human basophils: Venous blood, 40 ml was mixed with 4 ml EDTA (0.1 M), and 10 ml Dextran 75 (6% in 5% dextrose) and allowed to sediment for 60 to 90 min. The basophil containing supernatant was then aspirated, centrifuged, and diluted in HBSS-2% HSA.

Release experiment. Mixed leukocytes, 0.03 ml were added to 0.170 ml of releasing agent and incubated at 37°C for 20 min. In experiments with HRF generated from nasal washings, platelets, and mononuclear cells, the incubation time was increased to 45 min. Cells were adjusted such that total histamine content was approximately 40 ng/tube. Leukocytes were incubated with anti-human IgE (1/1000) or buffer for the positive and negative controls, respectively.

Serotonin release experiments. RBL serotonin release experiments were performed exactly as described for RBL histamine release experiments except that cells were incubated overnight with 1 mCi [³H]serotonin. After incubation, serotonin was assayed in the

supernatants and cells by counting radioactivity. Values were corrected for background radioactivity. Cells were removed from the wells with Triton X. Twelve RBL experiments were assayed for both serotonin and histamine release with comparable results (data not shown).

Lactic acid stripping of IgE from human basophils. Acid stripping was performed as previously described (10). Briefly, human basophils were extensively washed, suspended in 5 ml lactic acid (0.9%, pH 3.9, 25°C) for 3.5 min then flooded with PAG and washed extensively. Control cells were incubated in parallel with PAG. Aliquots of lactic acid-stripped cells were passively sensitized for 30 min at 37°C with IgE⁺ (500 ng/ml) in PAG containing 20% heparin/EDTA solution (9 ml EDTA 0.1 M, 4.5 ml heparin 500 USP, 4.5 ml PAG, 27 ml normal saline). Control cells were incubated in parallel in PAG, 20% heparin/EDTA.

Histamine assay. After precipitation of protein with 6% perchloric acid, histamine was assayed fluorometrically as previously described (10). The assay is accurate to $\pm 3\%$ between 1.0 and 100 ng/ml. The perchloric acid does not affect the measurement of histamine in known standards. Percent histamine release is defined as:

$$\frac{\text{Histamine in supernatant}}{[\text{Histamine in cells}] + [\text{Histamine in supernatant}]} \times 100$$

Net histamine release is defined as:

$$\% \text{ Histamine released} - \% \text{ histamine released from negative controls.}$$

Statistics. Student's one- and two-tailed *t*-test, linear regression analysis, and Fisher exact test were employed to analyze the results.

RESULTS

Partial purification of HRA-N. As previously reported, HRA-N is stable to boiling (see footnote 3). When boiled PMN supernatants known to contain HRA-N were concentrated over a YM2 filter, passed over a Sephadex G-25 column, and fractions tested for RBL serotonin releasing activity, a peak of activity was detected in fractions corresponding to a M_r of 1400 to 2300 Da (Fig. 1). This peak of activity was detected in all preparations tested. Two preparations of partially purified HRA-N₁₄₀₀₋₂₃₀₀ (fractions 35-59, Fig. 1) were tested on human basophils and caused $8 \pm 0.5\%$ histamine release ($N = 4$). Chromatography of several additional HRA-N preparations also revealed a second smaller peak of histamine-releasing activity fractionating at the void volume. Thus, partially purified HRA-N is active on both RBL and human basophils and contains at least two active species, one of which fractionates with a M_r between 1400 and

2300 Da and a second peak of activity seen less frequently and fractionating with a M_r of greater than 5000 Da.

Characterization of HRA-N responsive basophil donors. Eight of 14 donors' basophils released histamine on exposure to PMN supernatants known to contain HRA-N, as assessed by RBL histamine-releasing activity. To determine whether the capacity of basophils to respond to HRA-N was related to their ability to respond to anti-IgE, histamine release from basophils exposed to both HRA-N and anti-IgE was compared (Table I). All eight donors' basophils which released histamine in response to HRA-N also responded to anti-IgE. In contrast, only two of the six basophil preparations which failed to respond to HRA-N released histamine after anti-IgE stimulation ($p = 0.015$ by Fisher's exact test). Thus, a significant correlation between basophil responsiveness to HRA-N and anti-IgE was identified. However, there was no correlation between the magnitude of response to HRA-N and anti-IgE (linear regression analysis). The gender and atopic status of donors of HRA-N responsive and nonresponsive basophils were analyzed (Table II), and no differences were found between the two populations. Thus, the capacity of basophils to respond to HRA-N is unrelated to the donor's atopic status or gender, but is correlated with basophil responsiveness to anti-IgE.

Role of IgE in RBL histamine release induced by HRA-N and nasal washings. Since the capacity of basophils to respond to HRA-N is related to anti-IgE responsiveness, the relationship between IgE and basophil reactivity to HRA-N was examined. To answer this question, several lines of investigation were undertaken. The time course of HRA-N and anti-IgE-induced histamine release from RBL were compared (Fig. 2). In four separate experiments performed in triplicate, RBL were exposed to PMN supernatants (5×10^7 PMN equivalents/ml), or after passive sensitization with IgE, exposed to three concentrations of anti-IgE. Histamine release was stopped by addition of 0.1 M EDTA at various intervals up to 30 min. HRA-N caused 15% net histamine release. The HRA-N induced reaction was 50% complete within 75 s and complete by 2 min. Basophils were examined on three separate occasions employing anti-IgE concentrations adjusted to cause 14, 23, and 42% net histamine

Figure 1. Partial purification of HRA-N. Boiled HRA-N containing PMN supernatants (7.5 ml) were concentrated over a YM2 membrane, passed over a Sephadex G-25 column, and fractions of 0.55 ml collected and assayed for their capacity to stimulate serotonin release from RBL (●—●). The data are the mean of duplicate samples. This fractionation was repeated on five separate purifications and the results presented are representative, except that several of the purifications also revealed a fraction with less activity eluting with the void volume (◇—◇ = OD₂₈₀).

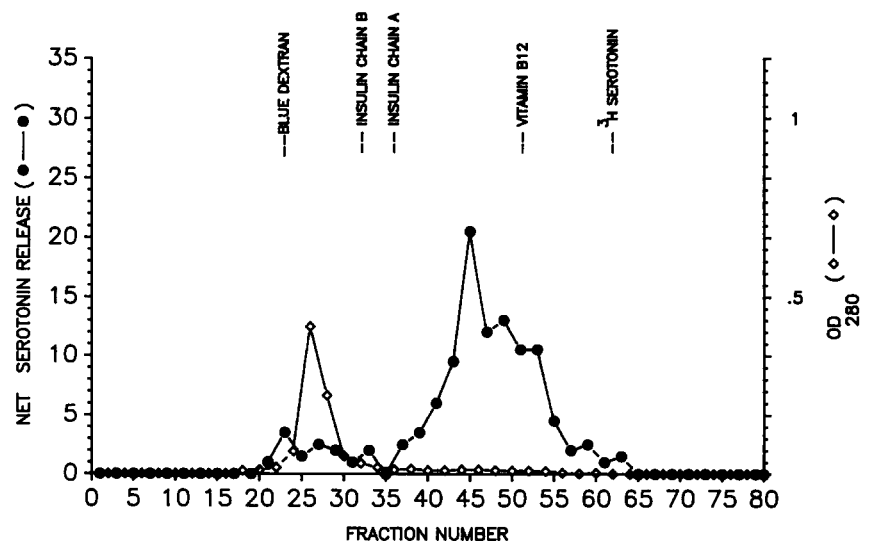


TABLE I

Comparison of human basophil response to HRA-N and anti-IgE^a

Histamine Release in Response to HRA-N	Histamine Release in Response to Anti-IgE	
	Yes	No
Yes	8	0
No	2	4

^a Basophils from 14 donors were incubated with either anti-IgE (1/1000) or HRA-N and histamine release measured. Basophils which released less than 5% of total histamine after correcting for background release (3%) were classified as nonresponsive. $p = 0.015$ (Fisher's exact test).

TABLE II

Characteristics of HRA-N responsive and nonresponsive donors^a

Histamine Release in Response to HRA-N	Gender	Atopic	
	Male	Female	No
Yes	5	3	4
No	3	3	2

^a Basophils which released less than 5% of total histamine after correcting for background release (3%) were classified as nonresponsive. $p = NS$.

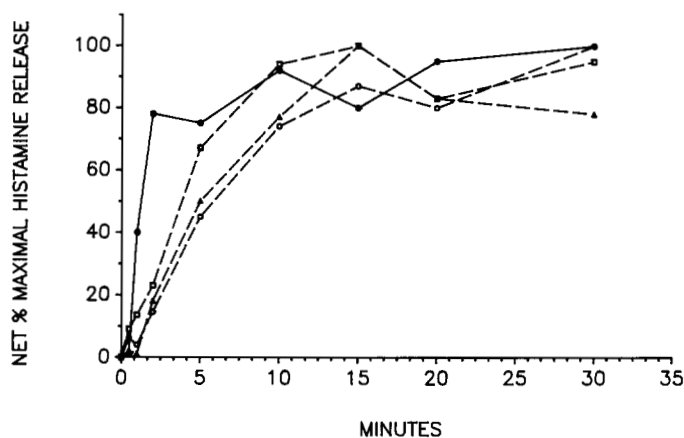


Figure 2. Time course of HRA-N and IgE-induced RBL histamine release. RBL (10^5) were incubated at 37°C with HRA-N (5×10^7 PMN equivalents/ml) or various dilutions of anti-IgE. Reactions were terminated at various intervals by addition of ice cold EDTA 0.1 M and histamine release measured. Anti-IgE, 1:1000 (O---O), 1/25,000 (Δ --- Δ), and 1/50,000 ($\square$ --- $\square$) caused 42, 23, and 14% net histamine release, respectively, whereas HRA-N ($\bullet$ — $\bullet$) caused 15% net histamine release. The results are expressed as the mean of three experiments.

release. Anti-IgE-induced histamine release was 50% complete by 5 to 6 min and complete by 15 min when maximal release was 23 and 42%. When maximal anti-IgE-induced release was equivalent to maximal HRA-N-induced release (14%), the release reaction remained significantly slower than HRA-N-induced release: half-maximal release was achieved by 4 min, whereas maximal release occurred at 10 min of incubation. Thus HRA-N causes rapid histamine release from RBL, and the time course of histamine release is more rapid than that of anti-IgE-induced release, even when the magnitude of induced release is equivalent.

HRA-N induces histamine release from RBL which are grown in the absence of rat IgE and are therefore free of surface-bound IgE. Thus, HRA-N-induced RBL histamine release is independent of IgE. In order to determine the effect of adding surface-bound IgE on HRA-N-induced histamine release from RBL, aliquots of RBL were passively sensitized with IgE before exposure to five crude and three partially purified preparations of HRA-N (Table

III). RBL exposed to IgE alone or to buffer released 4.6 ± 0.2 and $2.8 \pm 0.2\%$ histamine, respectively. The eight HRA-N preparations induced $15 \pm 5\%$ (range 5 to 50%) and $13 \pm 3.5\%$ (range 5 to 37%) net histamine release from native and IgE-sensitized RBL, respectively ($p = NS$). In no case was an effect of IgE on HRA-N-induced histamine release observed.

When the same experiment was performed on RBL by using the HRF-NW, the results were less clear (Table IV). Twelve individual nasal washings were obtained from nine normal, and three allergic donors; four subjects had two to five separate nasal washings examined. Initial examination of seven separate nasal washings obtained from five subjects suggested that histamine release from RBL exposed to HRF-NW might indeed be IgE dependent. Several of these subjects were not available for repeated study (subjects 3, 5, and 6), however, two of the initial subjects were reexamined (subjects 1 and 2). In both instances, the initial results were not reproducible. For instance, nasal washings from subject 1 in two experiments initially caused 2 and 6% net histamine release from native RBL and 13 and 18% from IgE sensitized RBL, respectively. On repeat study, this subject's nasal washings caused 9 and 6% net histamine release from native and IgE-sensitized RBL, respectively. Nasal washings from subject 2 initially caused 0 and 6% net histamine release from native and IgE-sensitized RBL, respectively, and in four subsequent studies, caused 10, 5, 16, and 9% histamine release from native RBL and 9, 4, 14,

TABLE III

Effect of IgE on HRA-N induced RBL histamine release^a

Experiment	Net % Histamine Release	
	Native RBL	IgE sensitized
1	20 ± 2	22 ± 2
2	6 ± 0.5	6 ± 0
3	5 ± 3	8 ± 0
4	6 ± 0	7 ± 0
5	10 ± 0.5	5 ± 1
6	50 ± 2.5	37 ± 3
7	11 ± 1.5	10 ± 1.5
8	12 ± 0.5	11 ± 0.5
Mean $\pm$ SEM	$15 \pm 5^*$	13 ± 3.5

^a Eight different HRA-N preparations were added to RBL, half of which had been sensitized with IgE ($10 \mu\text{g/ml}$ for 45 min at 37°C), and histamine release was measured. Histamine release in response to anti-IgE was $43 \pm 2\%$. Experiments 1 to 5 were performed with crude HRA-N, whereas 6 to 8 were performed with partially purified HRA-N preparations. Results are expressed as mean $\pm$ SEM of duplicate determinations. $^*P = NS$.

TABLE IV

Effect of IgE on RBL histamine release induced by nasal washings^a

Experiment	Net % Histamine Release	
	Native RBL	IgE sensitized
1	6 ± 1.5	12 ± 3
2	8 ± 2	8 ± 1.5
3	2 ± 1	8.5 ± 2.5
4	18.5 ± 7.5	15 ± 6
5	2 ± 1	7 ± 1
6	0 ± 0	8 ± 1.5
7	6 ± 1	4 ± 0
8	24 ± 6	24 ± 10.5
9	20 ± 1	20 ± 0
10	13 ± 0.5	10 ± 1.5
11	7 ± 0	5.5 ± 1.5
12	8 ± 0	10.5 ± 0.5
Mean $\pm$ SEM	9.5 ± 2	11 ± 1.5

^a Nasal washings from 12 subjects were added to RBL, half of which had been incubated with IgE ($10 \mu\text{g/ml}$ for 45 min at 37°C). Net % histamine release was measured after 45 min of incubation at 37°C. Anti-IgE induced $70.5 \pm 0.5\%$ net histamine release. Results represent the mean $\pm$ SEM of one to five separate experiments performed in duplicate.

and 7.5% net histamine release from IgE-sensitized RBL, respectively. Eight additional washings from seven subjects were examined, and none caused more histamine release from IgE-sensitized RBL than from native RBL. Thus, even including the initial studies, there was no difference in the magnitude of HRF-NW-induced histamine release from native and IgE-exposed RBL ($9.5 \pm 2\%$ and $11 \pm 1.5\%$, respectively, $p = \text{NS}$). Therefore, both HRA-N and HRF-NW induce histamine release from RBL which is neither dependent upon, nor enhanced by surface-bound IgE.

Partial purification of human nasal washings. To determine whether human nasal washings might contain individual HRF capable of causing IgE-dependent activation of RBL, pooled human nasal washings were analyzed after column chromatography. The fraction of pooled nasal washings which filtered with a molecular size less than 30,000 Da and which was nonadherent to DE52 was passed over a Sephadex G-50 column and the fractions assayed for their capacity to cause serotonin release from native RBL. Two active fractions were identified (Fig. 3). The major activity fractionated with a M_r between 3,500 and 30,000 Da. This area of activity was divided into two parts: a 15,000- to 30,000-Da fraction (fractions 21–32, HRF-NW 1) to correspond to the activity described by MacDonald et al. (6), and a 3,500- to 9,000-Da fraction (fractions 33–46, HRF-NW 2). A third low M_r fraction with histamine-releasing activity (fractions 60–73, HRF-NW 3) was also identified, however the corresponding M_r was below the linear range of the column's calibration curve.

When the three active HRF-NW fractions were incubated with five preparations of human basophils, $8 \pm 0.5\%$, $3 \pm 0.5\%$, and $3 \pm 1\%$ net histamine release was induced by fractions 1, 2, and 3, respectively (Table V, $n = 5$). Thus, in the doses tested, HRF-NW 1 causes degranulation of both human basophils and RBL, whereas HRF-NW 2 and HRF-NW 3 are active on RBL but not on human basophils.

Role of IgE in partially purified HRF-NW induced histamine release from RBL. The effect of surface bound

IgE on partially purified HRF-NW induced histamine release from RBL was next examined. Pooled HRF-NW 1, 2, and 3 were incubated for 45 min with native or IgE exposed RBL and net histamine release measured (Table VI). In two experiments performed in duplicate, all three fractions caused equivalent amounts of histamine release from native and IgE exposed RBL. Thus, the three active fractions identified in human nasal washings cause RBL histamine release which is neither dependent upon nor enhanced by surface bound IgE.

Role of IgE in HRA-N-induced histamine release from human basophils. The effect of surface bound IgE on HRA-N-induced histamine release from human basophils was examined. Basophils from four donors were treated with lactic acid to remove approximately 80% of surface bound IgE (6). An aliquot of lactic acid-stripped cells was then passively sensitized by exposure to IgE from a donor responsive to HRF-NW. The effects of lactic acid stripping and passive sensitization on spontaneous and anti-IgE-induced histamine release is shown in Figure 4. Acid stripping increased spontaneous histamine release from $3.5 \pm 1.5\%$ to $13 \pm 4\%$ ($n = 12$) and passive sensitization had no further effect ($10 \pm 2.5\%$) on spontaneous histamine release. Base line anti-IgE-induced histamine release ($26 \pm 2.5\%$) was decreased 42% by lactic acid stripping, and restored to 125% of base line by passive resensitization with IgE ($15 \pm 5.5\%$ and $32.5 \pm 5\%$, respectively $p = \text{NS}$). Although the differences are not statistically significant, they are similar to results obtained by other investigators (9).

The effect of lactic acid stripping on HRA-N-induced basophil histamine release is summarized in Table VII. These experiments were performed in parallel with the experiments summarized in Figure 4. Four different donors' basophils were incubated with one to three different HRA-N preparations before and after lactic acid treatment $\pm$ passive sensitization with IgE. Lactic acid treatment caused enhancement of HRA-N-induced histamine release from two donors' basophils, inhibition of release from a third donor's basophils and caused enhancement or had no effect on a fourth donor's basophils in two

Figure 3. Column chromatography of the histamine releasing activity in nasal washings. Pooled human nasal washings (8 ml) were mixed overnight with DE52, and the supernatant passed through a YM30 membrane. The filtrate was concentrated over a YM2 membrane, the retentate passed over a Sephadex G-50 column and the fractions (0.55 ml) assayed in duplicate for serotonin release from RBL. The data shown are the results of a single purification and are representative of two additional purifications ($\bullet$ = serotonin release, $\diamond$ = OD_{280}).

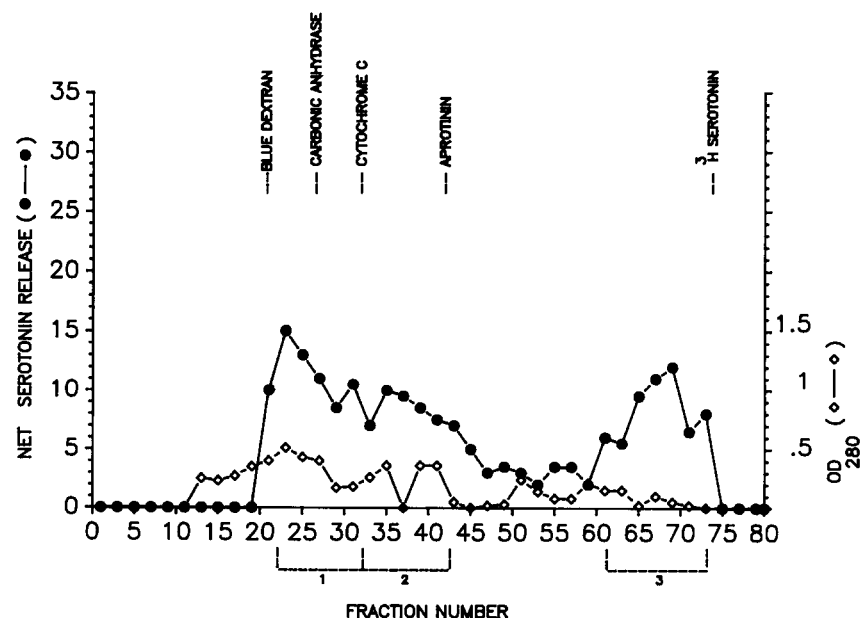


TABLE V

Human basophil histamine release induced by partially purified HRF-NW and anti-IgE^a

Donor	HRF-NW Fraction			
	Anti-IgE			
1	9	3	1	37
2	8	4	3	66
3	10	0	3	39
4	5	5	7	17
5	8	3	3	39
Mean ± SEM	8 ± 0.5	3 ± 0.5	3 ± 1	40 ± 7

^a Partially purified preparations of human nasal washings (Fig. 3) and anti-IgE were incubated for 45 min at 37°C with human basophils and histamine release measured. Individual results are expressed as the mean of duplicate experiments.

TABLE VI

Effect of IgE on RBL histamine release induced by partially purified nasal washings^a

HRF-NW Fraction	Native RBL	IgE Sensitized
HRF-NW 1	7 ± 1.5	6 ± 1.5
	10 ± 1.5	14 ± 2
Mean ± SEM	8.5 ± 1.5	10 ± 4
HRF-NW 2	14 ± 0.5	15 ± 5
	12 ± 2	12 ± 1.5
Mean ± SEM	13 ± 1	13.5 ± 1.5
HRF-NW3	6 ± 0.5	7 ± 2.5
	15 ± 1.5	14 ± 2
Mean ± SEM	10.5 ± 1.5	10.5 ± 3.5

^a Native and IgE-exposed (10 µg/ml for 45 min at 37°C) RBL were incubated in duplicate for 45 min at 37°C with two preparations each of three partially purified fractions of human nasal washings (Fig. 3) and histamine release measured. Results are expressed as mean ± SEM. *p* = NS for all three fractions.

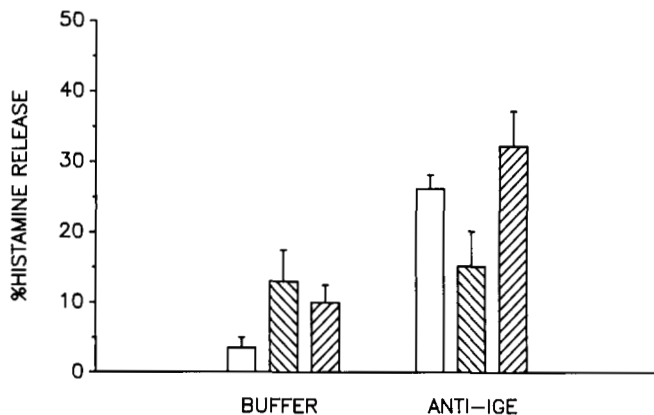


Figure 4. Effect of lactic acid stripping and passive sensitization with IgE on spontaneous and anti-IgE-induced net histamine release from human basophils. Aliquots of mixed leukocytes from the donors in Table V were exposed to lactic acid (pH 3.9, 3.5 min, 25°C) and aliquots of these cells were subsequently incubated in human IgE containing serum. The cells were then incubated in duplicate with buffer or with anti-IgE (1:1000) and histamine release measured. (□) basophil containing leukocyte preparation, (▨) acid stripped leukocytes, (■) passive sensitization after acid stripping. The results are expressed as the mean ± SEM.

separate experiments. In no case did passive sensitization with IgE reverse the effect of lactic acid treatment. When the data were statistically evaluated, there were no significant differences between the control, acid treated or passively sensitized groups (12.6 ± 1.9 , 16.6 ± 3.5 , and $16.8 \pm 3.3\%$ histamine release, respectively, *p* = NS).

Taking the data as a whole, lactic acid treatment inhibited anti-IgE-induced basophil histamine release but had no consistent effect on HRA-N-induced histamine release. Passive sensitization of lactic acid-treated basophils with IgE restored the ability to respond to anti-IgE

TABLE VII

Effect of lactic acid treatment on HRA-N induced histamine release from human basophils^a

Donor	HRA-N Preparation	% Histamine Release with Human Basophil Preparation:		
		Base line	Lactic acid	IgE-sensitized
1	A	16.5	20.3	29
	B	15	14	14
2	A	16	5	15.5
	B	21.5	2.5	2
3	A	8	16	19.5
4	A	7.5	30	21
	B	7.5	16	19
	C	9	29.5	24.5
Mean ± SEM		13 ± 2	17 ± 4*	17 ± 3*

^a Basophils from four donors were exposed to lactic acid in order to remove IgE. Aliquots of the acid-treated cells were resensitized by exposure to IgE*. The various cell preparations were exposed in duplicate to one to three different HRA-N preparations (denoted as A, B, or C) and histamine release measured. Control values obtained during these experiments are shown in Figure 2. Results are expressed as the mean of duplicate experiments. **p* = NS compared with base line.

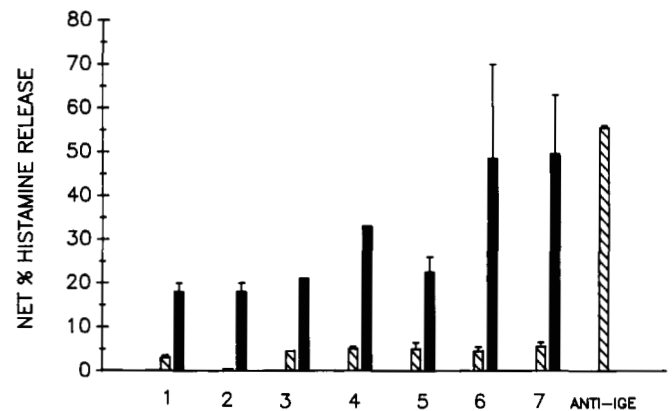


Figure 5. Effect of HRF-M on human basophils and RBL. IgE exposed RBL (□) or human basophils (■) were incubated for 20 minutes with HRF-M preparations diluted 10-fold, and histamine release measured. Preparations 1 and 2 are partially purified preparations, whereas preparations 3 to 7 are crude mononuclear cell supernatants. For comparison, IgE-exposed RBL were also incubated with anti-IgE (1/1000). All results are corrected for histamine release induced by buffer alone. *N* = 2 for RBL and 3 for human basophil experiments. The results are expressed as mean ± SEM.

but had no effect on the basophils' response to HRA-N, suggesting that HRA-N does not require the presence of IgE on the surface of basophils in order to cause histamine release.

Effect of HRF-M on human basophils and RBL. The capacity of HRF-M to cause histamine release from human basophils and RBL was examined. Shown in Figure 5 is the net percent histamine released from human basophils and IgE-exposed RBL incubated with seven different HRF-M preparations. In every instance, HRF-M failed to induce histamine release from native RBL (which are not shown on the figure), and induced minimal histamine release from IgE-sensitized RBL ($4 \pm 0.7\%$ net, range 0.5 to 5.5), compared with 56% net histamine release induced by anti-IgE. In contrast, all seven preparations induced significant histamine release from human basophils ($30 \pm 5.5\%$ net release, range 21 to 50%) obtained from both atopic and nonatopic donors. Thus, HRF-M causes histamine release from human basophils, but not from RBL, and the inability to cause histamine release from RBL is unaffected by the presence of surface

bound IgE.

It seemed possible that the lack of RBL reactivity to HRF-M might reflect a difference in RBL and human basophil sensitivity. Therefore, the ability of more concentrated (10-fold) preparations of crude and partially purified (the 25,000 m.w. fraction of HRF-M after HPLC) HRF-M to trigger RBL histamine release was examined. Shown in Figure 6 is the net percent histamine released from native and IgE sensitized RBL exposed to various dilutions (1/10 undiluted) of two preparations of concentrated HRF-M. For comparison, the net percent histamine released by human basophils exposed to the unconcentrated preparations, and by IgE sensitized RBL exposed to anti-IgE is shown. No appreciable amount of histamine was released by either native or IgE exposed RBL in response to any concentration of either crude or partially purified HRF-M. By contrast, human basophils released 70 and 30% of available histamine in response to the crude and partially purified HRF-M, respectively, and RBL exposed to anti-IgE released 55% net histamine. Thus, both native and IgE exposed RBL fail to release histamine on exposure to HRF-M concentrated 10-fold more than required to cause histamine release from human basophils. Further, it is unlikely that this lack of response is due to an inhibitor present in the crude preparation, since there was no RBL response to the partially purified HRF-M. The data also indicate differences between HRF-M and both HRA-N and HRF-NW.

Heat stability of HRA-N and HRF-NW. RBL were incubated 45 min with native and boiled (10 min) preparations of crude HRA-N and HRF-NW 1, 2, and 3, and histamine release measured (Table VIII). The capacity of all four preparations to cause RBL histamine release proved to be resistant to boiling. Thus, HRA-N and HRF-NW are stable to boiling.

Effect of HRF-P on human basophils and RBL. The effect of HRF-P on RBL and human basophils was then examined. In two experiments performed in duplicate, HRF-P caused $20 \pm 10\%$ net histamine release from

human basophils compared with $5.5 \pm 4\%$ and $4 \pm 1.5\%$ from native and IgE exposed RBL, respectively (Fig. 7). Thus, HRF-P causes histamine release from human basophils, but only minimal histamine release from RBL, and this activity is not affected by the presence of surface bound IgE.

DISCUSSION

Human neutrophils spontaneously release a heat-stable, 1400- to 2300-Da factor which causes histamine release from both RBL and human basophils. Supernatants from the PMN of some donors also contain a second histamine-releasing activity which has a molecular size greater than 5000 Da. In addition to HRA-N, human monocytes and platelets generate and nasal washings contain factors which also cause basophil histamine release. Only the HRA-N- and HRF-NW-derived factors activate native RBL, however.

The capacity of human basophils to respond to HRA-N appeared to reflect inherent "releasability" rather than the donor's gender or atopic status. Basophils of both atopic and nonatopic donors were responsive to HRA-N and similar results have been obtained with HRF-M (11). All donors' basophils that responded to HRA-N also responded to anti-IgE, whereas only two of the six basophil preparations which failed to respond to HRA-N responded to anti-IgE (Fisher exact test, $p = 0.015$). Thus, the ability of human basophils to respond to HRA-N is related to the ability to respond to anti-IgE. In contrast, HRF-NW has been reported to cause histamine release only from atopic individuals' basophils (12). However, in these experiments, HRF-NW induced histamine release from a single nonatopic donor's basophils as well as from the basophils of atopic donors.

HRF-NW or HRF-MP have been reported to require the presence of IgE in order to cause histamine release (6, 7). There are several lines of evidence to suggest that HRA-N is not IgE dependent. First, the time course of HRA-N-induced histamine release is different from that of IgE-

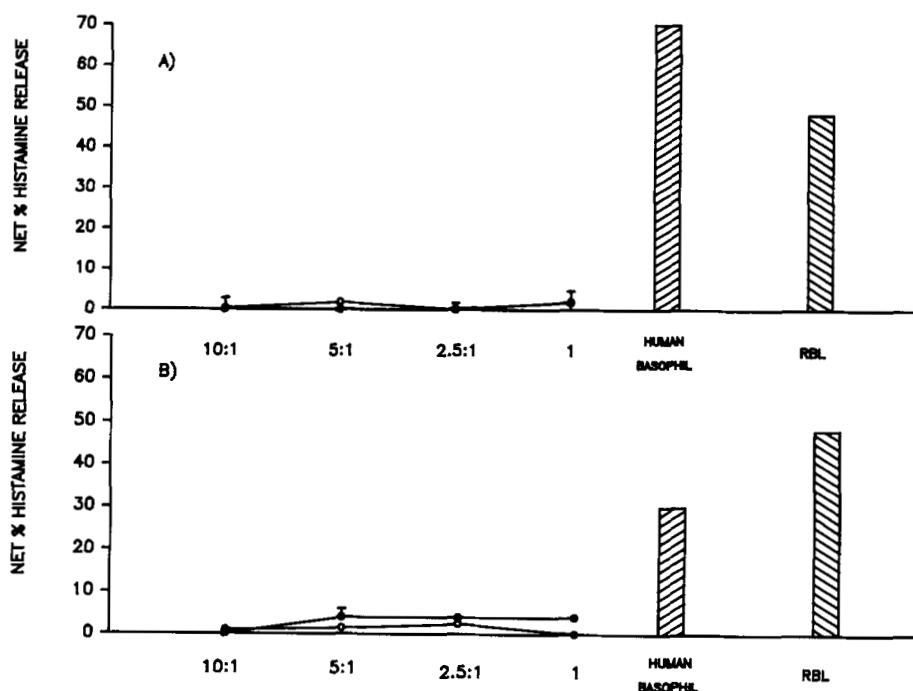


Figure 6. Capacity of crude (A) and partially purified (B) HRF-M to induce histamine release from RBL. Native (○—○) and IgE-exposed RBL (●—●) were incubated for 20 min at 37°C with various dilutions of concentrated crude and partially purified HRF-M, and histamine release measured. For comparison, human basophil (▨) and RBL (▨) histamine release induced by 10-fold dilutions of the HRF-M preparations and anti-IgE (1/1000), respectively, is shown. All results are corrected for background release. $N = 2$ for RBL and 3 for human basophil experiments. Results are expressed as mean $\pm$ SEM.

TABLE VIII
Comparison of heat stability of HRA-N and HRF-NW^a

Fraction	Treatment			N
	Native	56°C 30 min	Boll 10 min	
HRA-N	11 ± 2	14 ± 2.5	21 ± 1	3
HRF-NW 1	8.5 ± 2	ND	10.5 ± 3	2
HRF-NW 2	8.5 ± 1	ND	9.5 ± 2.5	2
HRF-NW 3	10 ± 2	ND	10 ± 3	2

^a HRA-N and partially purified HRF-NW (Fig. 3) were subjected to 56°C for 30 min or boiled for 10 min and assayed on RBL for histamine-releasing activity. Results are expressed as mean ± SEM of two to three separate experiments performed in duplicate.

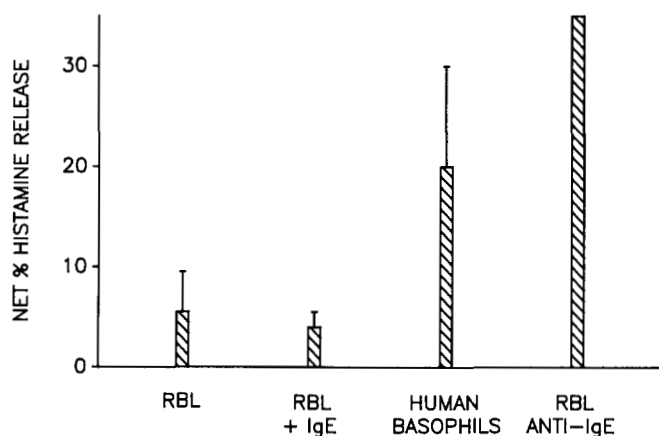


Figure 7. Effect of HRF-P on RBL and human basophils. Native RBL and IgE-exposed RBL and human basophils were incubated 20 min, 37°C, with a single crude HRF-P preparation and histamine release measured. For comparison, RBL histamine release induced by anti-IgE (1/1000) is shown. Results are corrected for background histamine release. *N* = 2 for RBL and 3 for human basophil experiments. Results are expressed as mean ± SEM.

induced histamine release from RBL. HRA-N-induced histamine release was complete by 2 min. Maximal anti-IgE-induced release (42%) was not complete until 15 min. When anti-IgE was diluted to induce histamine release equivalent to that induced by HRA-N (14 and 15%, respectively), release was complete by 10 min. Thus, the kinetics of HRA-N and anti-IgE induced histamine release are distinct, suggesting that HRA-N and anti-IgE may act through different mechanisms.

The effect of IgE on HRA-N-induced RBL histamine release was examined carefully. The advantage of using RBL is that they are grown in the absence of IgE, thus obviating the need for IgE stripping protocols which may cause subtle cell damage. In eight separate experiments with both crude and partially purified HRA-N, RBL preincubated with rat IgE were no more sensitive to HRA-N than were RBL lacking surface-bound IgE. Thus HRA-N-induced RBL histamine release is neither dependent upon nor enhanced by the presence of surface-bound IgE, suggesting that IgE does not play a role in HRA-N-induced histamine release.

The results were extended to human basophils, employing lactic acid stripping to remove IgE from basophil surface receptors. Acid treatment enhanced spontaneous histamine release and inhibited anti-IgE-induced basophil histamine release. The reduction of anti-IgE-induced histamine release after acid stripping could be reversed by replenishing IgE on the basophil surface by exposure to IgE containing serum.

Acid treatment, however, had no consistent effect on HRA-N-induced histamine release. In eight lactic acid-

stripping experiments, histamine released in response to HRA-N was increased in five, decreased in two, and unchanged in one. In all cases the response of acid-stripped basophils to HRA-N was the same before and after passive sensitization with IgE. These data suggest that lactic acid affects the basophil's capacity to respond to HRA-N, but that the mechanism involved in unrelated to IgE, and further suggests that the human basophil's response to HRA-N is not dependent upon IgE. The HRF-NW reacts quite differently from HRA-N with regard to these same experiments. MacDonald et al. reported that HRF-NW is totally inactive on acid-stripped cells, whereas the response is restored by IgE repletion (6).

The effect of IgE on HRF-NW induced RBL histamine release was also examined. Native and IgE exposed RBL released histamine equally in response to crude nasal washings obtained from both atopic and nonatopic donors. Although initial experiments suggested that a few donor's nasal washings caused histamine release only from IgE exposed, and not native RBL, these observations were not substantiated upon more extensive study. Thus, the differences between the effect of HRF-NW on native and IgE-sensitized RBL was not statistically different. Therefore, the results indicate that the suggestion by MacDonald et al. (6), that HRF-NW induced human basophil histamine release is IgE dependent, does not extend to RBL. MacDonald, however, was working with a 15,000- to 30,000-Da fraction of partially purified HRF-NW obtained from pooled nasal washings. When pooled nasal washings were subjected to a similar purification procedure in our laboratory, three fractions capable of causing RBL histamine release were identified (HRF-NW 1, 2, and 3, corresponding to 15,000- to 30,000-Da, 3,500- to 9,000-Da, and <3,500 Da, respectively). All three fractions caused equivalent histamine release from native and IgE-exposed RBL, but only the 15,000- to 30,000-Da species caused histamine release from human basophils. Thus, HRF-NW contains at least one species capable of causing histamine release from both RBL and human basophils, and in contrast to the results obtained by MacDonald et al. (6) with human basophils, the RBL histamine release induced by HRF-NW 1 is neither dependent upon nor enhanced by the presence of surface bound IgE.

The magnitude of histamine release obtained with HRF-NW 1 in this paper is lower than that reported by MacDonald et al. (6). One probable explanation is that MacDonald was purifying batches of 400- to 600-ml pooled nasal washes while 8-ml pools were used herein. Thus the partially purified nasal wash preparation used by MacDonald had a final concentration at least two to four times that of the preparations used in this paper.

HRF-NW also contains two additional fractions capable of causing IgE independent RBL, but not human basophil, histamine release. The effect of these two HRF on human mast cells is unknown. Inasmuch as HRA-N causes human basophil histamine release and HRF-NW at the concentrations tested was inactive on human basophils, the data suggest that HRF-NW 3 is not HRA-N, despite the similar size.

The effect of HRF-M and HRF-P on RBL and human basophils was also examined. Seven different HRF-M preparations caused 21 to 50% net histamine release from human basophils, but failed to induce significant

TABLE IX
Comparison of HRF

Comparisons	Source of HRF			
	HRA-N	HRF-M	HRF-P	HRF-NW
Histamine release				
Human basophil:				
Native	+	+	+	+
Lactic acid exposed	+	ND	ND	-(7)
RBL:				
Native	+	-	-	+
With IgE	+	-	-	-
Molecular weight (Sephadex):	1400-2300	25,000	ND	15,000-30,000
Heat stability:				
56°C	+	ND	ND	ND
Boil 10 min	+	ND	ND	+

histamine release from either native or IgE-sensitized RBL. Human basophils desensitized to IgE, however, continued to respond to HRF-M (11). Tenfold concentrates of crude or partially purified HRF-M failed to cause significant RBL histamine release. Thus, the data suggest that the lack of response of RBL to HRF-M is not related to the presence of IgE and probably cannot be related to the presence of inhibitors in the crude preparation, because partially purified HRF-M caused the same results. Similarly, HRF-P caused 10 to 30% histamine release from human basophils, but caused only minimal ($5.5 \pm 4\%$, net) histamine release from native RBL and this response was not augmented by IgE sensitization ($4 \pm 1.5\%$, net). Thus, the data suggest that the activity profiles of HRF-P and HRF-M on RBL and human basophils are similar, and that the two factors are different from both HRA-N and HRF-NW. HRA-N can be distinguished by its low m.w., while HRF-NW and HRF-M are similar in size. Both HRA-N and HRF-NW are stable to boiling (Table IX).

The biologic role of these HRF is undefined. Histamine release has been found during the initial PMN-rich inflammatory phase of LPR. It has been suggested that neutrophils and eosinophils attracted to sites of LPR might release HRA-N and major basic protein or eosinophil peroxidase, respectively (13, 14), causing mast cell or basophil activation. Likewise, mononuclear cells, which infiltrate LPR as the reaction matures, might release HRF-M, further propagating the cycle. On the other hand, the capacity of such a diverse group of inflammatory cells to cause histamine release suggests that recruitment of mast cells or basophils into participation in inflammatory processes may be a fundamental event. Although mast cells and basophils have classically been considered to be involved only in allergic reactions, their distribution and potent inflammatory properties suggest

that they could contribute to other inflammatory processes. Thus, the uncovering of a family of histamine-releasing activities may lead to a better comprehension of inflammation and its modulation.

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