

STUDIES OF IgE-DEPENDENT HISTAMINE RELEASING FACTORS: HETEROGENEITY OF IgE¹

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Nasal lavage fluids from unstimulated individuals contain a histamine-releasing factor (HRF) similar to those which we have previously described from macrophages, platelets, and from blister fluids obtained during the late cutaneous reaction. The nasal HRF was partially purified by ion-exchange chromatography and gel filtration. Although some m.w. heterogeneity was observed, the majority of the HRF eluted at an apparent m.w. range of 15,000 to 30,000. This partially purified HRF induced histamine release from basophils of certain individuals. Histamine release occurred via a mechanism which is IgE-dependent in that: 1) basophils desensitized by exposure to anti-IgE in the absence of calcium no longer respond to HRF, and desensitization with HRF reduces responsiveness to anti-IgE; and 2) removal of IgE from the basophil surface by using lactic acid renders cells unresponsive to HRF. We have further defined this IgE dependence and have shown that the reason that only selected basophil donors respond to HRF is due to a previously unrecognized, functional heterogeneity of IgE. Thus, passive sensitization using sera from responders restored the responsiveness of acid-stripped basophils and conferred responsiveness to basophils of a nonresponder with naturally unoccupied IgE receptors. Sera from nonresponders failed to do this even though similar numbers of IgE molecules were put onto the basophil surface in each case. This property of responder sera was due to IgE because both heating sera at 56°C for 2 hr and passage of sera over anti-IgE-Sepharose (which removes >90% of the IgE) markedly reduced the ability of sera to induce responsiveness, and because an excess of either purified IgE myeloma or purified penicillin-specific IgE antibody from a nonresponder competitively inhibited the ability of IgE from responder

sera to induce responsiveness to HRF. We conclude that nasal lavage fluids contain an HRF which induces basophil histamine release in a specific, IgE-dependent fashion but only from individuals with the appropriate type of IgE. Because we have shown that basophils are recruited into the nose during the late-phase reaction, we suggest that nasal HRF may induce these cells to release histamine and other mediators which could contribute to the symptomatology of the late-phase reaction.

The exposure of an allergic individual to an appropriate antigen results in an immediate pathophysiologic response. After the initial reaction subsides, most individuals, if the dose of antigen used is sufficiently high, will develop a recrudescence of symptoms within 3 to 11 hr after antigen challenge (1). This late-phase reaction (LPR)⁶ is characterized by edema and erythema in the skin (2) and by increased resistance to airflow in the nose and lungs (3). It has recently been appreciated that this LPR is a more appropriate model of allergic inflammation than is the acute response to antigen. The pathogenesis of the LPR, however, is complex and poorly understood. It seems clear that basophils and/or mast cells are activated in vivo during the LPR, because antigen bronchoprovocation leads to elevated plasma levels of histamine and neutrophil chemotactic factor (4, 5). By using an in vivo model of intranasal antigen challenge, mast cell and basophil mediators, such as histamine and leukotrienes, have been found to be elevated in nasal washes coincident with symptoms in individuals experiencing an LPR (6-8). In both the skin and the nose, it appears that this delayed mediator release is not due to continued antigenic stimulation, leaving the mechanism of cellular activation unknown.

Preliminary studies indicate that lavage fluids obtained during the LPR in the nose contain histamine-releasing (HR) factors (HRF) which have characteristics similar to those from macrophages (9) and those found in blister fluids during the LPR in the skin (10). Other investigators have reported the in vitro production of lymphokines that release histamine from human basophils. Thuesen et al. (11, 12) were the first to report such a factor, called HR activity (HRA), from cultured human mononuclear cells. HRA was further characterized by Lett-Brown and col-

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⁶ Abbreviations used in this paper: LPR, late-phase reaction; HRF, histamine-releasing factor(s); anti-IgE, goat anti-human IgE; HRA, histamine-releasing activity; f-met-leu-phe, formyl-methionine-leucine-phenylalanine; HSA, human serum albumin; DE52, diethylaminoethylcellulose; PAG, PIPES buffer containing HSA and glucose; PAGCM, PAG containing Ca⁺⁺ and Mg⁺⁺; LTC₄, leukotriene C₄; HR, histamine release.

leagues (13, 14) and was found to be heterogeneous with two m.w. species of 15,000 and 50,000. Subsequently, Bamzai and Kretschmer (15) described a histamine release-enhancing factor from phytohemagglutinin-stimulated mononuclear cells from asthmatic patients, and Alam et al. (16) have also reported HRF from cultured mononuclear cells from asthmatic patients. Ida et al. (17) found a histamine release-enhancing factor produced by lymphocytes from BCG-positive donors after incubation with purified protein derivative. That HRF is produced by cultured lymphocytes has been confirmed subsequently by Sedgwick et al. (18), Ezeamuzie and Assem (19), and Kaplan et al. (20). Recently, Goetzl et al. (21) have reported a basophil-activating factor derived from human lymphocytes that is an acidic protein with an estimated m.w. of 70,000 to 90,000. All of these factors mentioned above induce noncytotoxic histamine release from human leukocytes, which resembles IgE-mediated release in that it is temperature-dependent and requires metabolic energy and calcium. However, evidence for the IgE dependence of release by these mononuclear cell products has either not been sought or is circumstantial.

Here we describe an HRF obtained from nasal lavage fluids which is similar to those previously described by us in molecular characteristics and mode of releasing mediators from basophils. The release obtained is shown to be due to an interaction of HRF with basophil cell surface IgE. Not all basophil donors respond to this nasal HRF, and we demonstrate that the response is a function of different IgE molecules. Our results indicate a hitherto undescribed, functional heterogeneity of the human IgE molecule.

MATERIALS AND METHODS

Materials. The serum from a patient with IgE myeloma (H.L.) was obtained from Dr. H. Gewurz, Rush Medical College, Chicago, IL, and the purified IgE myeloma (P.S.) and goat anti-human IgE were kindly provided by Dr. K. Ishizaka, The Johns Hopkins University School of Medicine. Ragweed antigen E was provided by Dr. T. P. King, Rockefeller Institute, New York, NY. Other supplies were purchased as indicated: PIPES, EDTA, lactic acid, Tris (hydroxymethyl) aminomethane, glucose, dextran, formyl-methionine-leucine-phenylalanine (f-met-leu-phe), human serum albumin (HSA) (Sigma Chemical Co., St. Louis, MO); DE-52 (diethyl amino ethyl cellulose) (Whatman, Clifton, NJ); Sephadex G-75 (Pharmacia, Uppsala, Sweden); Amicon YM2 membranes (Amicon, Danvers, MA).

Buffers. PIPES (P) contains 25 mM PIPES, 110 mM NaCl, and 5 mM KCl adjusted to pH 7.4; PA also has 0.003% HSA; PAG additionally contains 0.1% D-glucose; and PAGCM also contains 1 mM CaCl_2 and 1 mM MgCl_2 . Lactic acid is 90.1 mg % lactic acid, 0.82 g % NaCl, 37.3 mg % KCl, adjusted to pH 3.9 with 10 N NaOH. Acetate buffer has 0.05 M acetate, 0.85% NaCl, 0.01 M EDTA, 0.03% HSA, adjusted to pH 3.7 at 0°C.

Preparation of nasal wash samples. After informed consent, healthy human volunteers had 5 ml normal saline instilled into each nostril. Samples were collected after a 10-sec delay and were frozen at -20°C until pooled for chromatography.

Preparation of peripheral blood basophils. After informed consent, peripheral blood was obtained by venipuncture. For most experiments, mixed leukocytes containing basophils were prepared by dextran sedimentation as described (22). In brief, 40 ml of venous blood were mixed with 12.5 ml of 6% clinical dextran and 5 ml of 0.1 M EDTA and were allowed to sediment for 90 min at room temperature. The leukocyte-containing plasma layer was then removed and cells were sedimented by centrifugation ($150 \times G$, 8 min), were washed twice with PAG, and were resuspended in PAGCM for use in the histamine release assay.

It should be noted that of 80 subjects tested (approximately two-thirds atopic, one-third normals), only certain donor's basophils responded to nasal HRF. In general, only donors whose basophils responded to nasal HRF with $\geq 10\%$ histamine release were employed in these experiments.

In some experiments, basophils were purified by sensitization with human IgE anti-penicillin antibody followed by affinity chromatography on benzyl-penicillin-Sepharose as described (23).

Mediator assays: a) *HR.* Reaction volumes for the HR assay were 0.1 ml. Nasal HRF was incubated in tubes containing PAGCM and mixed leukocytes. Cell concentrations were adjusted so that there were approximately 2×10^4 basophils per tube (i.e., a total of 20 ng histamine per tube). Based on kinetic experiments, an incubation period of 45 min at 37°C was chosen. At the end of the incubation, 0.9 ml of cold PAG was added to each tube. The tubes were then centrifuged ($1000 \times G$, 2.5 min, 23°C), and aliquots of the cellfree supernatant were assessed for histamine content by the automated fluorometric assay of Siraganian (24). The results were based on the mean of duplicate determinations and were expressed as percent histamine release, by dividing by total cellular histamine after subtracting for spontaneous release of unstimulated basophils (typically, 2 to 5% during the 45-min assay). Total cellular histamine was obtained by lysing cells in 2% perchloric acid.

b) *Leukotriene assay.* The radioimmunoassay for sulfidopeptide leukotrienes employed dextran-coated charcoal as a separation technique, and a rabbit anti-leukotriene C_4 (LTC_4) antiserum which has been described (25). The limit of detection of LTC_4 in this assay is 15.6 pg/0.1 ml.

Desensitization experiments. Basophils were desensitized by preincubating leukocytes with stimuli in the absence of calcium (26). Leukocytes and stimuli were preincubated for 90 min at 37°C in PAG containing 4 mM EDTA, were washed twice in PAG, and were resuspended in PAGCM. No histamine release occurred during the preincubation. These washed cells were then challenged with relevant stimuli in the presence of 1 mM Ca^{++} in a standard 45-min HR assay.

Lactic acid elution of basophil IgE. The method of Pruzansky et al. (27) was used to dissociate IgE from basophils. Leukocytes were washed once in PAG, once in unbuffered normal saline, and the cell pellet was resuspended in 5 ml of 0.01 M lactic acid, pH 3.9 (containing 140 mM NaCl, 5 mM KCl), was incubated (23°C, 3.5 min), was then diluted in 30 ml of PAG, and was centrifuged twice in PAG. The cells were resuspended in the appropriate volume of PAGCM for the HR assay.

Passive sensitization of lactic acid-treated basophils. Passive sensitization was accomplished by the method of Levy and Osler (28). Acid-treated leukocytes were incubated (37°C, 90 min) with serum or various sources of purified IgE in PAG containing 4 mM EDTA and 10 $\mu\text{g}/\text{ml}$ heparin. In most experiments, the serum was diluted to contain 300 ng/ml IgE. The purified IgE myeloma (P.S.) was used at a concentration of 25 $\mu\text{g}/\text{ml}$, whereas serum containing the IgE myeloma, H.L., was diluted to match the IgE concentration in the responder sera used. The highly purified penicillin-specific IgE antibody, from a single donor, was used at a concentration of 750 or 2500 ng/ml.

Acetate elution of basophil IgE. For assessment of basophil IgE content, aliquots of cells were assessed before and after sensitization with saturating concentrations of IgE-containing sera. Control and sensitized leukocytes were washed twice with PAG buffer. After centrifugation, the cells were resuspended in 1 ml of acetate buffer, pH 3.7, and were allowed to incubate for 10 min at 0°C. The cells were sedimented, and the supernatant fluid was immediately titrated to neutrality with 1 N NaOH. This technique has been described in detail by Ishizaka and Ishizaka (29) and by our laboratory (30). All supernatant fluids were analyzed for total IgE content. Acetate elution removes all of the IgE molecules from the surface of the basophil but, unlike lactic acid treatment, does not yield a viable cell that can be passively sensitized.

Total IgE measurements. The amount of IgE eluted from acetate-treated cells and total serum IgE were measured by a direct (noncompetitive) radioimmunosorbent test (31) which has been described in detail (32). This assay is sensitive to ≤ 1 ng/ml IgE with a mean coefficient of variation for low-range samples of 10%.

Chromatography of nasal HRF. After pre-equilibration with 0.01 M Tris/0.15 M NaCl, pH 7.4, 50 g of DE-52 cellulose (Whatman) were added to a 400- to 600-ml sample of pooled nasal lavages and were incubated overnight at 4°C with gentle stirring. This slurry was then added to a $5 \times 15\text{-cm}$ column. The column was washed with 100 ml of 0.01 M Tris/0.15 M NaCl, pH 7.4, and the flow-through was collected. This pool, containing the HRF activity, was concentrated 400-fold by ultrafiltration using a YM 2 membrane (Amicon). This concentrate was applied to a calibrated column ($1.6 \times 90.5\text{-cm}$) of Sephadex G-75 equilibrated in 0.01 M Tris/0.15 M NaCl, pH 7.4. The column was eluted at a flow rate of 12 ml/hr. Fractions of 2-ml vol were collected and were monitored for their absorbance at 280 nm. The fractions were also assessed, at a final dilution of 1/3, for their capacity to induce HR from the basophils of a sensitive donor. The fractions containing HRF were pooled and were concentrated

20-fold by ultrafiltration using a YM 2 membrane. This partially purified HRF was stored in aliquots at -80°C and was used at a final concentration of 1/15 or 1/21 (depending on the donor) to give approximately 15% HR.

Statistical analysis. The results are expressed as percent HR of duplicate samples. Error bars represent standard error of the mean (SEM), with N denoting the number of experiments performed.

RESULTS

As noted above, HRF has been described in nasal lavages obtained from individuals during the LPR (41). We found that we could obtain similar HRF activity from nasal lavage fluids of unstimulated individuals. In contrast to other sources of releasing activity, this nasal lavage HRF is available in large quantities. All results presented in this report were obtained by using the nasal HRF obtained following the chromatographic procedures described below.

Purification of HRF from nasal washes. Nasal secretions were obtained from approximately equal numbers of allergic and nonallergic volunteers; the donors utilized (less than one-half of subjects tested) had previously been screened and were found to have secretions which caused $\geq 10\%$ basophil HR when used at a final dilution of $\frac{1}{2}$ in the standard histamine assay. Seven pools of ~ 500 ml each were obtained over a 12-mo period and were subjected to ion-exchange chromatography. Greater than 90% of the HRF did not bind to DE-52 at pH 7.4 under isotonic conditions. After concentration of the flow-through material from the DE-52 chromatography, it was applied to a Sephadex G-75 column as described above. The majority of the protein (presumably representing immunoglobulins) eluted at the void volume. Although a small amount (usually $\leq 10\%$, depending on the donor used) of the HRF activity was detected at the void volume, the majority of the activity eluted as a broad peak with an apparent m.w. range of 15,000 to 30,000 (Fig. 1). Similar results were obtained with each of the seven pools used. For the current studies, the concentrated 15,000 to 30,000 m.w. fractions from three different pools were used as sources of nasal HRF.

Characteristics of release by HRF. Nasal wash-derived HRF induced histamine release that was calcium-dependent. At 1 mM calcium, the optimal concentration for anti-IgE-induced HR, the release was only 60% of that occurring with 5 mM calcium. The experiments to be described were, however, carried out at physiologic cal-

cium levels (1 mM). The temperature dependence of the release reaction was similar to that observed for anti-IgE, in that no release occurred at 0°C or 22°C , and maximal release occurred at 37°C (data not shown).

As shown in Figure 2, the kinetics of the HR were slow, in that 50% of maximal release occurred after 5 to 10 min. This rate is similar to that of antigen (not shown) or anti-IgE, and considerably slower than that of C5a- or f-met-leu-phe-induced release (33, 34).

The nasal lavage HRF was tested on basophil-enriched preparations to determine that the factor acted directly on basophils. When basophils from a known responder individual were purified ~ 100 -fold (68% purity), HR was 12.3% when the cells were stimulated with $0.1 \mu\text{g/ml}$ anti-IgE, whereas HRF led to 11.1% HR. It should be noted that with purification some donors have decreased basophil responsiveness. A similar result was obtained in a second experiment. HRF (unlike C5a) also causes LTC_4 production. In the experiments with purified basophils, LTC_4 production was 26.2 and 15.5 $\text{ng}/10^6$ basophils on challenge with anti-IgE or HRF, respectively (data not shown). The background LTC_4 production was less than the limit of detection of the assay, 15.6 $\text{pg}/0.1$ ml.

HR by nasal HRF is IgE-dependent. The characteristics of release by HRF suggested that it might interact with cellbound IgE. To test this possibility, we pursued several lines of experimentation. First, we utilized anti-IgE-induced desensitization, which has been shown to be specific for IgE-mediated stimuli; secretagogues which act in a fashion other than by the cross-linking of cell surface IgE are unaffected, or their ability to release is increased (26, 34). When basophils were treated with anti-IgE in the absence of Ca^{++} , they were rendered unresponsive not only to anti-IgE but also to nasal HRF. Figure 3 shows the results of five experiments in which cells were preincubated with buffer, anti-IgE, or nasal HRF at 37°C for 90 min without Ca^{++} . The cells were then washed, each set was rechallenged with the three stimuli (anti-IgE, nasal HRF, or f-met-leu-phe) in the presence of 1 mM Ca^{++} , and the subsequent HR was measured. Desensitization with anti-IgE decreased

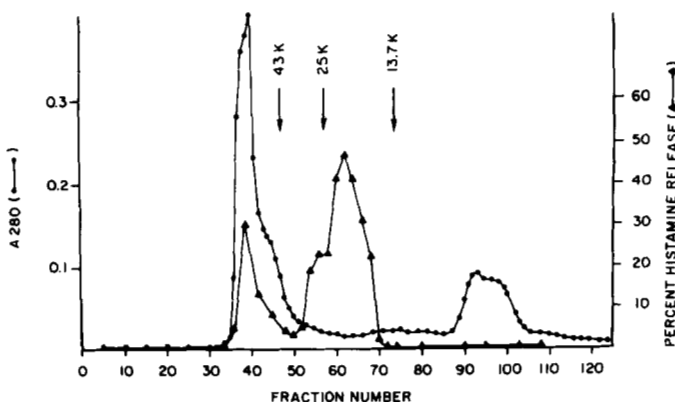


Figure 1. Sephadex G-75 chromatography of concentrated nasal HRF obtained after passage over DE-52. Fractions of 2 ml were assayed for absorbance at 280 nm (●—●) and HR (▲—▲). Fractions 52 to 70 were pooled, were concentrated 20-fold, and were used for subsequent experiments.

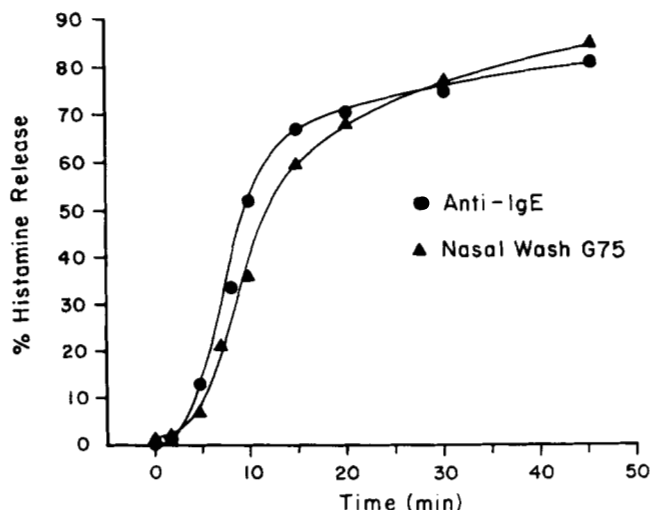


Figure 2. Kinetics of HR by partially purified nasal HRF (▲, nasal HRF pool from Sephadex G-75) at a dilution of 1/21 and anti-IgE (●) at a final concentration of $0.1 \mu\text{g/ml}$ are shown. With both stimuli, release is slow, with 50% of maximal release occurring at 5 to 10 min.

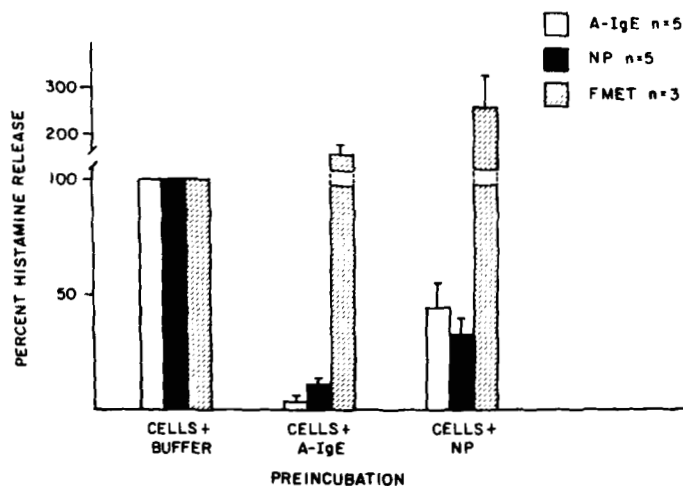


Figure 3. Effect of desensitization of basophils with three stimuli: A-IgE (anti-IgE, 0.1 μ g/ml), NP (pooled nasal HRF, 1/21 dilution), and F MET (f-met-peptide, 2×10^{-6} M). The results are presented as the mean $\pm$ SEM HR, expressed as a percent of the release induced from control cells. Control release for anti-IgE ranged from 38 to 64%, for nasal HRF from 15 to 62%, and for F MET from 9 to 43% (n = number of experiments).

release caused by subsequent challenge with anti-IgE to $3.9\% \pm 2$ and decreased nasal HRF release to $11.3\% \pm 2.4$. Similarly, preincubation with nasal HRF decreased release on challenge with the same stimulus to $44\% \pm 10.9$ ($p \leq 0.0005$) and decreased anti-IgE-induced release to $32.9\% \pm 6.9$ ($p \leq 0.005$). In contrast, there was augmentation of release ($149\% \pm 8$ and $246\% \pm 44$) caused by f-met-leu-phe after desensitization with both HRF and anti-IgE, respectively.

Another type of experiment was utilized to further establish that release induced by nasal HRF was dependent on cell surface IgE. Cells were treated with lactic acid (0.01 M, pH 3.9, 3.5 min, 23°C), which causes 80 to 98% of the IgE to be dissociated from the basophil IgE receptors but preserves basophil function (27). The lactic acid-treated basophils can be resensitized by incubation with IgE antibody-containing serum or IgE myeloma protein. Figure 4 presents a composite of nine experiments in which lactic acid treatment of basophils resulted in virtually complete loss of responsiveness to nasal HRF, and in a reduction of responsiveness to anti-IgE. It is not surprising that acid treatment did not abolish responsiveness to anti-IgE, because only 1000 to 3000 molecules of IgE/basophil are required to cause near-maximal release by this stimulus (35). Lactic acid treatment did not decrease the HR induced by f-met-leu-phe, which acts on basophil receptors distinct from those for IgE (34). Attempts to resensitize the cells with IgE myeloma (P.S. or H.L.) at a concentration of 25 μ g/ml or with a preparation of highly purified penicillin-specific IgE (L.L., from a single donor) at 750 ng/ml, however, failed to restore responsiveness to nasal HRF ($6.7\% \pm 1.7$ HR, $p > 0.1$) for reasons to be considered below.

Further studies of IgE dependence: heterogeneity of IgE molecules. While pursuing these studies, we had found that only certain donors' basophils responded to HRF by releasing histamine. This variable response could be due to "releasability" parameters intrinsic to basophils (36), to properties of IgE receptors, or to properties of IgE molecules themselves. Because we had evidence that our HRF was IgE-dependent, we first investigated the role of serum IgE molecules from specific donors. We compared

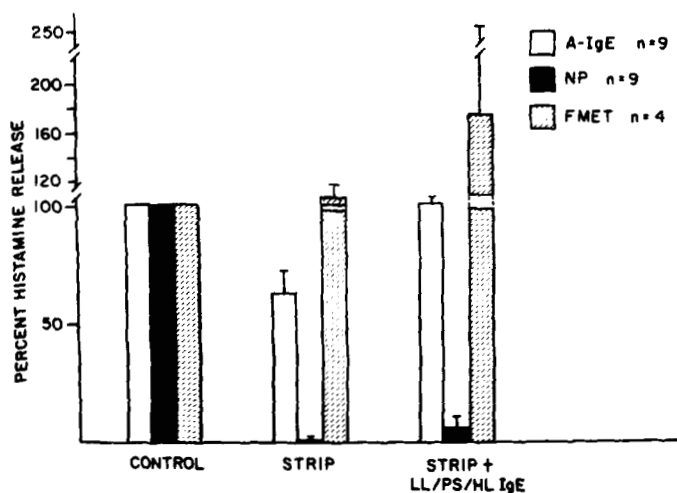


Figure 4. Effect of lactic acid treatment of basophils on subsequent release by three stimuli. The results are expressed as the mean $\pm$ SEM HR, presented as a percent of the release induced from control cells. In nine separate experiments, cells were treated with buffer (CONTROL), lactic acid (STRIP), or lactic acid followed by passive sensitization with polyclonal, penicillin-specific IgE (L.L., $n = 5$), P.S. myeloma ($n = 3$), or H.L. myeloma ($n = 1$). Cells from each condition were then stimulated with anti-IgE (A-IgE, 0.1 μ g/ml), nasal HRF (NP, 1/21 dilution), or f-met-peptide (FMET, 2×10^{-6} M). Control release for anti-IgE ranged from 20 to 66%, for nasal HRF from 2 to 26%, and for FMET from 36 to 40% (n = number of experiments with each stimulus).

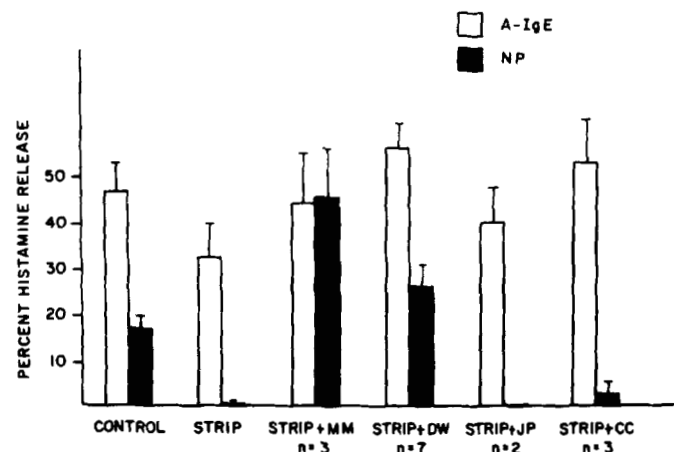


Figure 5. Effect of passive sensitization of lactic acid-treated basophils with responder or nonresponder serum. In eight experiments, cells were incubated with buffer (CONTROL) or lactic acid (STRIP). Lactic acid-treated cells were then incubated with serum from responders (M.M. and D.W.) or nonresponders (J.P. and C.C.) all diluted to contain 300 ng/ml of IgE. The results are expressed as percent HR by anti-IgE (A-IgE, 0.1 μ g/ml) or nasal HRF (NP, 1/21 dilution). The values are mean $\pm$ SEM of duplicate determinations from the designated number of experiments using each serum.

sera from "good responders" (i.e., donors whose basophils respond to the nasal HRF) to sera from "nonresponders" in terms of their ability to passively sensitize (90 min, 37°C) acid-treated basophils (28). Sera were diluted so that the total IgE concentrations in the incubation mixtures were equal.

As shown in Figure 5, the sera of basophil responders (M.M. and D.W.), but not the sera of nonresponders (J.P. and C.C.), restored the responsiveness of acid-treated basophils to nasal HRF. In contrast, the sera from all subjects were effective in restoring responsiveness to anti-IgE. Similar results have been obtained with five responder and five nonresponder sera (data not shown). These results demonstrated that only certain sera conferred sensitivity to HRF, and that there was, therefore,

a functional heterogeneity of the IgE molecules.

The transfer of responsiveness was not an artifact of acid treatment because sensitization to HRF also occurred when recipient nonresponder basophils, which were not acid-treated but had unoccupied IgE receptors in their basal state, were incubated with the serum of an HRF responder. Specifically, control cells released $2\% \pm 0.7$ histamine when exposed to nasal HRF, and this increased to $17\% \pm 2$ after incubation of cells with responder serum ($n = 3$, data not shown).

It was possible that the reason that certain sera failed to make basophils sensitive to HRF was that these IgE molecules did not bind to the receptors on the lactic acid-treated basophils. This possibility was tested by measuring the number of IgE molecules placed on the basophils during passive sensitization (29, 30). Representative data are shown in Figure 6. Cells were treated first with lactic acid and then were passively sensitized with responder serum (M.M.) at 350 ng/ml or with penicillin-specific IgE (L.L.) at 2,000 ng/ml. Control cells were not acid-treated. A sample from each condition was saved for challenge with HRF and histamine measurement, while the remainder of the cells were treated with acetate buffer, and the total number of molecules of IgE per cell was measured. As shown, the control cells had 335,500 IgE molecules per cell (66% HR on challenge with HRF); treatment with lactic acid reduced this number to 18,850 (3% HR); after sensitization with M.M. (42% HR), there were 125,000 molecules per cell. When the cells were sensitized with the higher concentration of L.L. (5% HR), there were 315,800 IgE molecules per cell. A second experiment confirmed these results. Thus, the IgE molecules which failed to induce sensitization to HRF were indeed present on the basophils.

To demonstrate that this passive sensitization was indeed due to IgE, we carried out a series of experiments in which we "blocked" the open receptors on acid-treated basophils with IgE myeloma (25 μ g/ml) or a highly purified penicillin-specific IgE preparation (2500 ng/ml) from a nonresponder. Figure 7 shows the results of five exper-

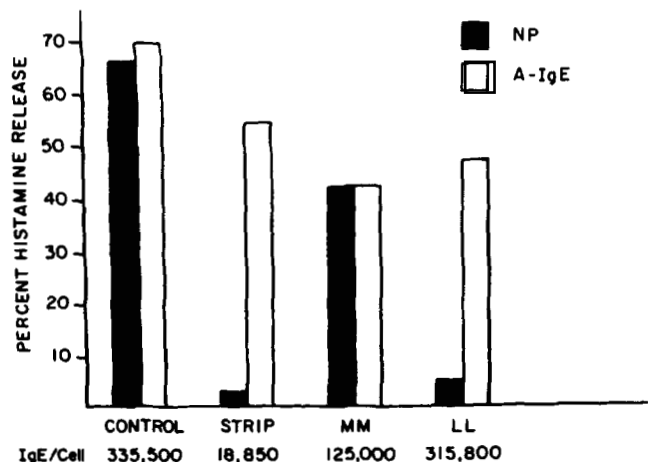


Figure 6. Determination of the number of IgE molecules per cell before and after passive sensitization. Cells were treated with buffer (CONTROL) or lactic acid (STRIP). Lactic acid-treated cells were then sensitized either with responder serum (M.M., 350 ng/ml) or nonresponder serum (L.L., 2000 ng/ml). A portion from each condition was saved for HR by the nasal HRF (NP, $1/21$ dilution) or anti-IgE (A-IgE, 0.1 μ g/ml). The remaining cells from each condition were treated with acetate buffer, pH 3.7, and from the total IgE content of the resulting supernatants, the number of molecules per cell was calculated.

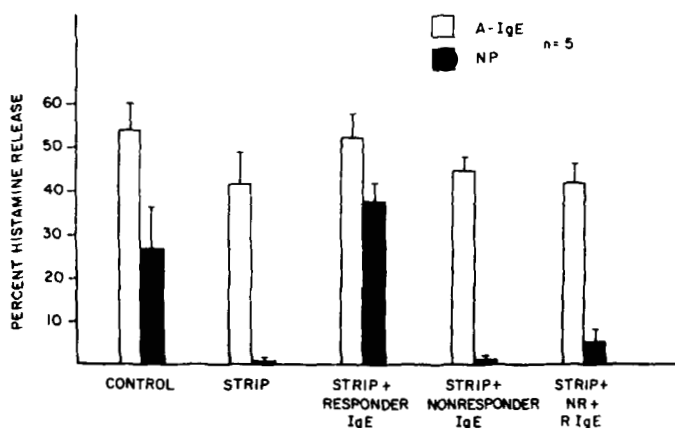


Figure 7. Effect of saturating IgE receptors with nonresponder IgE on the ability of responder IgE to interact with nasal HRF. The results are expressed as the mean $\pm$ SEM of duplicate determinations from the designated number of experiments. Cells were incubated with buffer (CONTROL) or lactic acid (STRIP). Lactic acid-treated cells were sensitized with responder IgE (300 ng/ml) or nonresponder IgE (25 μ g/ml when myeloma was used or 2500 ng/ml when highly purified penicillin-specific IgE was employed). Lactic acid-treated cells were also sensitized with a mixture of nonresponder and responder IgE (STRIP + NR + R IgE). The cells were washed and were challenged with either anti-IgE (A-IgE, 0.1 μ g/ml) or nasal HRF (NP, $1/21$ dilution).

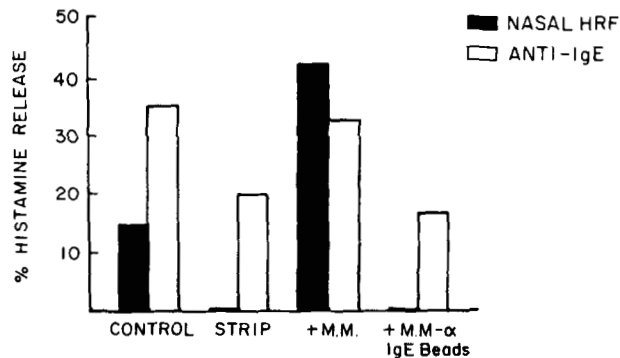


Figure 8. Effect of absorbing serum with anti-IgE Sepharose. Cells were incubated with buffer (CONTROL) or lactic acid (STRIP). Lactic acid-treated cells were then sensitized with responder serum (M.M., 300 ng/ml) or responder serum incubated for 24 hr with anti-IgE covalently linked to Sepharose (MM- α IgE Beads). The results from a single experiment are expressed as percent HR. Similar results were obtained in an additional experiment.

iments in which passive sensitization of acid-treated cells with serum from HRF responders (300 ng/ml) restored the ability of the HRF to induce HR ($37\% \pm 2$ HR) while IgE myeloma and purified IgE from a nonresponder did not ($1.4\% \pm 0.4$ HR). When the acid-treated basophils were first incubated with an eight- to 80-fold molar excess of either highly purified nonresponder IgE or IgE myeloma and were then exposed to the responder serum, the latter was no longer effective in sensitizing the cells: release was only $5.2\% \pm 1.4$ and was not different from the response of the acid-treated cells ($p > 0.1$). These results indicate that when noneffective IgE molecules occupy all of the available IgE receptors, the HRF-binding IgE molecules were unable to bind the basophil cell surface to participate in release. This documents both the IgE dependence of HRF-induced release and the existence of IgE heterogeneity.

To rule out a role for a serum component other than IgE in the response to HRF, two further types of experiments were performed. Responder serum was absorbed with anti-IgE-Sepharose, which removed $\geq 90\%$ of the IgE (37) (Fig. 8), or was heated at 56°C for 2 hr, which markedly

reduces the capacity of IgE to bind to basophil receptors (38) (data not shown). Similar results were obtained in an additional experiment using both IgE Sepharose-absorbed and heated serum. These treatments abolished the capacity of the serum to transfer responsiveness to nasal HRF, and markedly reduced the response to anti-IgE. These experiments, coupled with the data from experiments using an excess of nonresponders' IgE to inhibit the binding of responders' IgE to IgE receptors on the basophil (see Fig. 7), established that responder IgE molecules are required to confer sensitivity to nasal HRF.

DISCUSSION

We have been conducting a series of experiments, of which the studies presented in this paper are a part, in an attempt to understand the mechanism of mediator release during the LPR in man. Before initiating the present study, we had found an HRF in blister fluids obtained during the late phase (but not in the early phase), after antigen challenge in the skin (10). There was insufficient material to characterize the nature of this factor, but based on the kinetics of HR and on the facts that desensitization with anti-IgE or removal of basophil IgE abolished its effect, we suspected that this factor caused release via an IgE-mediated mechanism (10). This efficacy on human basophils was of interest because we had previously postulated (6), and have recently demonstrated, that basophils accumulate at the site of the LPR (39). In continuing this line of experimentation, we attempted to discover which cells produced HRF, and described materials from macrophages (9) and platelets (40) which seemed similar to the several lymphokines which had previously been described to be HRF (11-20). With the additional material obtained from macrophages and platelets, we developed further evidence that these HRF cause HR by an IgE-dependent mechanism. The experiments involving removal of cell surface IgE antibody described in this manuscript have also been carried out by using these cell-derived HRF. However, in our experimentation with these cellular HRF, we failed to obtain definitive proof for the involvement of IgE because we could not resensitize the cells to HRF with myeloma.

In seeking additional HRF from relevant biological fluids, we performed a series of studies with our model of nasal challenge with antigen, doing lavages during the LPR. These fluids contained materials which released histamine from human basophils (41). In the controls for these experiments, we found that many individuals had similar quantities of HRF in their upper respiratory passages without antigen challenge. Parenthetically, we have no idea why this activity should be present, but it should be noted that some individuals also have high concentrations of histamine and other mediators in their nasal fluids before challenge; perhaps these mediators occur because of the presence of HRF. More to the point of this manuscript, however, the ready availability of HRF from nasal lavage fluids of untreated, normal, and atopic individuals provided us with the materials to carry out these studies.

With the large quantities of nasal lavage fluids available, we have started to characterize this HRF and have defined its molecular size and mechanism of release of histamine from basophils. Despite the data using desensitization and lactic acid treatment of basophils which

supported the concept that an HRF-IgE interaction caused the release observed with cell-derived HRF, the following two observations were initially confusing. First, when IgE was restored to the surface of acid-treated basophils by using IgE myeloma, HR by anti-IgE was restored, but release by the platelet HRF (40) or the blister fluid HRF (10) was not. The activity of the macrophage HRF was inconsistently restored by (IgE) myeloma (42), and the release to nasal HRF was not restored. Secondly, only certain donors' basophils responded to HRF (9), a restriction also true for the HRA described by Kaplan et al. (20) and Thuesen et al. (12).

The implications of these observations were clarified when we recognized that there were differences between IgE molecules. By using passive sensitization of basophils from various subjects, we found that sera from HRF-responsive subjects rendered lactic acid-treated cells responsive to HRF as assessed by HR. In contrast, sera from nonresponders, at the same IgE concentrations, did not restore the ability of HRF to induce HR. In both instances, the molecules of IgE were shown to bind effectively to the surface of the basophil. The transfer of sensitivity did not appear to be an artifact of the acid treatment of the cells, because non-acid-treated basophils with naturally unoccupied IgE receptors were rendered responsive when sensitized with a responder's serum. Furthermore, an excess of nonresponder IgE molecules effectively blocked responder IgE from binding to the basophil surface, ablating HRF-induced HR. Finally, depletion of a serum's capacity to sensitize basophils, by passage over anti-IgE affinity columns or by heating at 56°C, documented the IgE dependence. These results show clearly that the release by this HRF depends on cell surface IgE molecules, and furthermore establish the functional heterogeneity of human IgE molecules. Although an IgE-dependent mechanism of HR was expected from our previous work, the fact that there is IgE heterogeneity was far more surprising. Again, the differential ability of responder and nonresponder sera and the inability of IgE myeloma to confer HRF responsiveness make this point clear.

Inasmuch as there are no subclasses of IgE molecules described to date, we have no basis for suggesting the nature of this heterogeneity. We suspect, however, that because the IgE molecule is heavily glycosylated, variations in carbohydrate groups may account for our observations.

Our work and that of other investigators suggest that there is a family of HRF derived from several cell types. Based on the work of Spiegelberg (43), these cells are similar in that they all contain low affinity IgE receptors. Whether the HRF from these cell types known to produce HRF are related to this receptor in some way remains to be seen, but thus far, in our laboratory, all seem to release by an IgE-related mechanism. The production of these factors by cells known to be involved in inflammatory reactions and their appearance in fluids during the late inflammatory response suggest a fundamental, pro-inflammatory cycle whereby an original antigenic insult leads to the generation of HRF which continue the process by interacting with IgE in a non-antigen-specific fashion. Because there are probably many ways to generate HRF aside from antigenic stimulation, our results suggest a novel way to trigger basophils in inflammatory

reaction sites by utilizing IgE-related mechanisms by nonspecific means. The increased bronchial sensitivity to nonspecific stimuli, which is associated with the LPR, may well be the result of such an activation process. We propose that the interaction of various HRF with subsets of IgE molecules from responder individuals represents an important element in the pathogenesis of a variety of inflammatory disease processes. Therefore, we are now attempting to purify the nasal HRF to homogeneity by conventional chromatographic techniques followed by affinity chromatography, using columns containing purified responder IgE.

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