

IL-8 INHIBITS HISTAMINE RELEASE FROM HUMAN BASOPHILS INDUCED BY HISTAMINE-RELEASING FACTORS, CONNECTIVE TISSUE ACTIVATING PEPTIDE III, AND IL-3¹

PIOTR KUNA, SSHA R. REDDIGARI, DOREEN KORNFELD, AND ALLEN P. KAPLAN²

From the Division of Allergy, Rheumatology, and Clinical Immunology, Department of Medicine, SUNY-Stony Brook, Health Sciences Center, Stony Brook, NY 11794-8161

We have previously purified and partially characterized histamine releasing factors (HRF), which were derived from a mixture of human mononuclear cells and platelets. We now report the effect of IL-8 upon HRF-, connective tissue activating peptide III (CTAP III)-, and IL-3-induced histamine release from human basophils. We determined that IL-8 itself, at concentrations between 10^{-7} to 10^{-11} M, does not release histamine from basophils, although positive results are observed in two of 26 subjects at 10^{-7} M. Unfractionated (crude) HRF released histamine in 25 of 26 donors, in the range of 6.7% to 100% of total basophil histamine stores. When basophils were preincubated with IL-8 (10^{-7} to 10^{-11} M) for 5 min, followed by a 40-min incubation with HRF, histamine release was significantly inhibited in 20 of 25 donors. Inhibition was observed at as little as 10^{-11} M IL-8, with maximal inhibition being attained at 10^{-9} M. HRF-containing supernatants contain a mixture of different histamine-releasing moieties. To better define which factor(s) may be inhibited by IL-8, fractionated supernatants, purified CTAP III, and IL-3 were studied. Histamine release produced by two different HRF-containing chromatographic fractions (HRF_{void} and HRF_{peak 2}) and purified CTAP-III (5 μ g/ml) was inhibited by IL-8 in 10 of 12 donors, three of three donors, and seven of 10 donors, respectively. IL-3 (5000 U/ml)-dependent histamine release was inhibited by IL-8 in all subjects tested. In contrast, histamine release by anti-IgE and FMLP was not affected by IL-8. Thus, IL-8 appears to be an inhibitor of cytokine-like molecules that induce histamine release and may represent the previously described 8-kDa histamine release inhibitory factor present in mononuclear cell supernatants.

HRF³ are a family of molecules that interact with basophils and/or mast cells to release histamine. They have been identified as products of mononuclear cells (1, 2), T

lymphocytes (3, 4), B lymphocytes (3), platelets (5), and human lung macrophages (6). Our laboratory has previously reported purification of three forms of HRF from a mixture of streptokinase/streptodornase-stimulated human mononuclear cells and platelets, with apparent molecular masses of 10 to 12, 15 to 17, and 40 to 41 kDa (7). The first form of HRF (10-12 kDa) can be separated from the other two forms by anion exchange chromatography and corresponds to CTAP III and a proteolytic derivative of CTAP III that lacks the amino-terminal 15 amino acids (8). This latter moiety stimulates neutrophils and is called neutrophil-activating peptide 2. Both CTAP III and neutrophil-activating peptide 2 cause activation and proliferation of fibroblasts (9). It has also been reported that IL-3 and, to a lesser degree, granulocyte/macrophage CSF release histamine from basophils in a subpopulation of subjects (10, 11).

A specific antagonist of HRF that inhibits histamine release from basophils has been found in the supernatant of stimulated mononuclear cell cultures and BAL fluids (12, 13). This HRIF was shown to be a heat-stable protein resistant to freezing and thawing, with a molecular mass in the range of 8 to 10 kDa (12). IL-8, which is chemotactic for neutrophils and T lymphocytes and causes neutrophil activation and secretion, possesses similar physical and chemical properties (14, 15). IL-8 has also been reported to induce histamine release from human basophils, but the concentration required was 1000 times higher than the quantity required for neutrophil activation (16). We, therefore, reexamined the direct effect of IL-8 on basophil histamine release, as well as its effect upon HRF-induced histamine release. Human endothelial cell-derived rIL-8, at concentrations sufficient to activate neutrophils, inhibited histamine release induced by HRF, purified CTAP III, and human rIL-3. Thus, IL-8 may represent one form of HRIF.

MATERIALS AND METHODS

Materials. Chemicals used in the production of HRF and the histamine release assay were purchased as previously described (7). Human endothelial rIL-8, with maximal chemotactic activity on human neutrophils at 5 ng/ml, was from Pepro Tech Inc. (Rocky Hill, NJ). Human rIL-3 (specific activity, 10^{-8} U/mg) was obtained from Amgen Biologicals (Thousand Oaks, CA); Percoll from Pharmacia (Uppsala, Sweden); FMLP from Sigma (St. Louis, MO); Spectrapor dialysis tubing from Spectrum Medical Industries (Los Angeles, CA); and tissue culture flasks from Corning Glass Works (Corning, NY). mAb 18-1 was prepared as described (17).

Preparation of HRF-containing supernatants. PBMC were isolated from buffy coats obtained from normal blood bank donors. Leukopheresis packs of approximately 200 ml were diluted to 360 ml in Dulbecco's PBS without calcium and magnesium, containing 4 mM EDTA (PBS-EDTA). Thirty milliliters of cellular suspension were layered over 15 ml of Ficoll-Paque in 50-ml polypropylene

Received for publication February 28, 1991

Accepted for publication June 19, 1991

The cost of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was presented, in part, at the 47th Annual Meeting of the American Academy of Allergy and Immunology, San Francisco, March 1991.

² Address correspondence and reprint requests to Allen P. Kaplan, M.D., Department of Medicine, SUNY-Stony Brook, Health Sciences Center, Stony Brook, NY 11794-8160.

³ Abbreviations used in this paper: HRF, histamine-releasing factors; CTAP III, connective tissue activating peptide III; HRIF, histamine release inhibitory factor; BAL, bronchoalveolar lavage; HSA, human serum albumin; HBS, HEPES-buffered saline.

tubes. The suspension was centrifuged for 35 min at $400 \times g$. The interface was collected, washed with PBS-EDTA, and centrifuged at $400 \times g$ for 10 min. After a third wash, the cells were suspended in 5 ml of PBS-EDTA, layered over discontinuous Percoll gradients (30.0 ml of 7% Percoll layered over 15.0 ml of 30% Percoll), and centrifuged for 25 min at $150 \times g$. The cell pellet was collected and washed three times with PBS. This protocol achieved 99.9% platelet depletion; the platelet/mononuclear cell ratio varied from 1:10 to 1:20. The cells were resuspended at a concentration of 6×10^6 cells/ml in RPMI 1640 with L-glutamine, 0.03% BSA (RIA grade), 100 μ g/ml penicillin, 100 μ g/ml streptomycin, and streptokinase/streptodornase at 13 U/ 10^6 cells. The cells were then cultured for 24 h at 37°C with 5% CO_2 , in 75- cm^2 tissue culture flasks. At the end of the culture period, the conditioned media were pooled, centrifuged at $400 \times g$ for 15 min, and clarified at $38,000 \times g$ for 20 min. The supernatant was concentrated 50-fold by using YM-3 membranes, dialyzed (m.w. cut-off, 3,500) against PBS, and stored at -70°C . Cell viability at the end of the culture was $>95\%$, as determined by trypan blue exclusion.

Partial purification of HRF. The 10- to 12-kDa (HRF_{void}) and 15- to 17-kDa ($\text{HRF}_{\text{peak 2}}$) forms of HRF were partially purified from supernatant containing HRF by using anion exchange HPLC (quaternary methyl ammonium), as previously described (7).

Purification of CTAP III. CTAP III was purified according to the procedure described by Reddigari et al. (18). Briefly, platelets were isolated from normal human donors, suspended in PBS containing 1 mM EDTA, 5 mM glucose, and 0.1 mM PMSF, and stimulated with 20 μ M calcium ionophore A-23187 at 37°C for 30 min, according to the method of Collier et al. (19). mAb 18-1 to CTAP III was coupled to tressyl-activated agarose (10 mg of IgG/ml of Affinica gel; Schleicher and Schuell, Inc.) in 0.2 M NaHCO_3 , 0.5 M NaCl, pH 8.5, according to the manufacturer's recommendations. The gel was equilibrated with 0.01 M sodium phosphate buffer, pH 7.5, containing 0.5 M NaCl, 0.1 mM PMSF, 0.1 mM EDTA, and 0.02% azide (equilibration buffer). Platelet releasate was applied to the column and washed with the equilibration buffer until the $\text{OD}_{280\text{ nm}}$ of the wash fractions returned to the baseline. Fractions of 0.5 ml were then eluted with 3.0 M potassium thiocyanate, and peak protein fractions were pooled, dialyzed against PBS (pH 7.4) containing 0.1 mM PMSF, and stored at -70°C . Purity of CTAP III was $>95\%$, as determined by SDS-PAGE.

Basophil histamine release. The procedures for basophil isolation and histamine release have been described in detail (1). Briefly, the cells were prepared by dextran sedimentation (0.6% dextran, 0.6% glucose, 0.02% EDTA), the basophil-containing layer was washed twice with HBS containing 0.3% HSA, and the cells were resuspended in HBS-HSA containing 2 mM CaCl_2 and 2 mM MgCl_2 . Thirty microliters of each sample or control medium were incubated with 3×10^6 cells, in a total volume of 300 μ l of HBS-HSA, for 40 min in a shaking water bath at 37°C . The supernatant were separated from the cells by centrifugation at $700 \times g$ for 5 min at 4°C . The total basophil histamine content was determined by boiling two of the aliquots. Results are expressed as a percentage of histamine release. Spontaneous histamine release from the cells was usually $<5\%$ and was subtracted from total before histamine release was calculated.

Inhibition of histamine release by IL-8. The experiments were performed in a shaking water bath at 37°C . After warming the cells to 37°C , IL-8 was added and cells were preincubated for 5 min. Leukocytes were then challenged with 30 μ l of $\text{HRF}_{\text{crude}}$, HRF_{void} , $\text{HRF}_{\text{peak 2}}$, CTAP III, or IL-3. The percentage of inhibition of histamine release was calculated according to the formula of Alam et al. (12), as follows: percent inhibition = $(b - a) - (d - c) \times 100 / (b - a)$, where a is leukocytes plus buffer preincubation and buffer challenge; b is leukocytes plus buffer preincubation and HRF, IL-3, or CTAP III challenge; c is leukocytes plus IL-8 preincubation and buffer challenge; and d is leukocytes plus IL-8 preincubation and HRF, IL-3, or CTAP III challenge.

Histamine assay. The radioenzyme assay for histamine was performed as described by Beaven et al. (20).

Statistical analysis. Results are presented as mean $\pm$ SEM, and the statistical analysis was performed with Wilcoxon's signed rank test or Student t -test (paired), by using the computer program StatWorks (Cricket Software, Philadelphia, PA).

RESULTS

Inhibition of HRF-induced histamine release by IL-8. We first determined the ability of IL-8 to induce histamine release in our subject population. Twenty-six persons were tested at a dose range of 10^{-11} to 10^{-7} M IL-8. There was no histamine release in 24 subjects at any of

the concentrations tested, whereas two subjects responded to the 10^{-7} M dose and released 11% and 14.6% histamine. Next, we tested the ability of IL-8 to inhibit HRF-induced histamine release by preincubating the basophils with IL-8 at concentrations ranging from 10^{-11} to 10^{-7} M. When 30 μ l of a crude HRF were tested, 25 of 26 subjects released histamine and, of those 25, 20 were inhibited by preincubation with 10^{-9} M IL-8 (Fig. 1A). Five subjects showed no inhibition (Fig. 1B). Combined, these data indicated an inhibition of 37% (Fig. 1C). An identical experiment was then performed by using the HRF_{void} fraction from quaternary methyl ammonium anion exchange chromatography. Thirteen subjects were tested and 12 responded, with a mean of 26% histamine release. IL-8 at 10^{-9} M inhibited release in 10 of these 12 donors; the inhibition ranged from 25% to 92%. The pooled data (including the two subjects not inhibited) are shown as HRF_{void} in Figure 1. A mean of 57% inhibition is seen. In a similar experiment with $\text{HRF}_{\text{peak 2}}$ in 15 subjects, three responded, with an average 33% histamine release, and all were inhibited by IL-8 (Fig. 1). The percentage of inhibition for $\text{HRF}_{\text{peak 2}}$ was in the same range as that obtained with $\text{HRF}_{\text{crude}}$ as well as HRF_{void} . There was no inhibition of anti-IgE- and FMLP-induced histamine release and, in fact, release due to FMLP was slightly enhanced.

Effect of IL-8 upon CTAP III-induced histamine release. CTAP III was used at a concentration of 5 μ g/ml. The identity of CTAP III used by us was established by amino-terminal sequence analysis (18). Ten of the 18 subjects tested released histamine upon CTAP III stimulation. Of these 10 subjects, seven demonstrated significant inhibition when their basophils were preincubated with 10^{-9} M IL-8 (Fig. 2). The inhibition was significant (Wilcoxon signed rank test) when all 10 subjects were considered ($p < 0.02$) or when only the seven demonstrating inhibition were examined ($p < 0.01$).

Effect of IL-8 on IL-3-induced histamine release. In these experiments, we utilized 5000 U/ml human rIL-3, to which five of the 15 subjects tested responded posi-

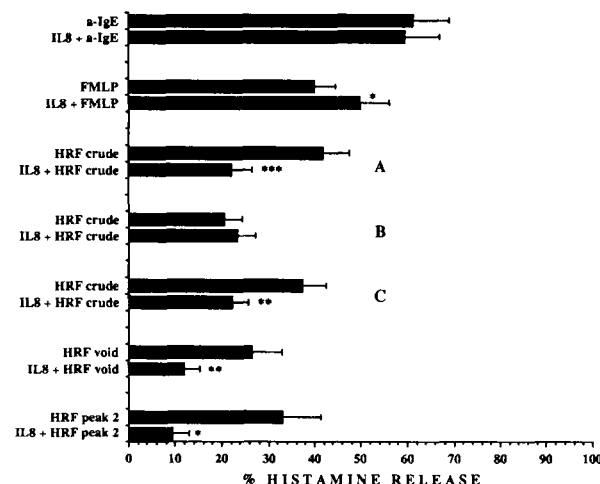


Figure 1. Effect of IL-8 on histamine release produced by various secretagogues. Basophils were preincubated with or without 10^{-9} M IL-8 for 5 min and then challenged with anti-IgE (1 μ g/ml; $n = 17$), FMLP (10^{-5} M; $n = 17$), $\text{HRF}_{\text{crude}}$ (300 μ g/ml; $n = 25$) [A, all 20 subjects tested with $\text{HRF}_{\text{crude}}$ in whom inhibition was seen; B, five subjects with no evident inhibition; C, all 25 subjects], HRF_{void} (30 μ g/ml; $n = 12$), or $\text{HRF}_{\text{peak 2}}$ (30 μ g/ml; $n = 3$), for 40 min. Data are expressed as mean $\pm$ SEM. Statistical significance is shown. *, $p < 0.03$; **, $p < 0.01$; ***, $p < 0.001$ (Student t -test).

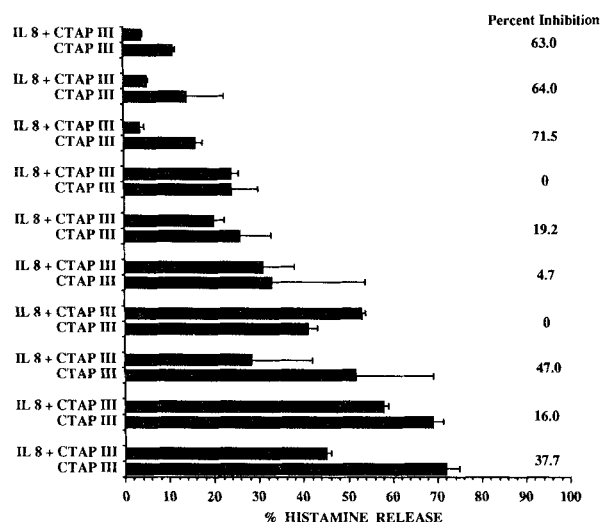


Figure 2. Effect of IL-8 on histamine release produced by CTAP III. Basophils were preincubated with or without IL-8, at a concentration of 10^{-9} M, for 5 min and then challenged with CTAP III ($5 \mu\text{g/ml}$) for 40 min. The percentage of inhibition for each donor test is shown. Seven of 10 donors gave $>15\%$ inhibition. The results are expressed as the mean $\pm$ SEM from duplicate determinations.

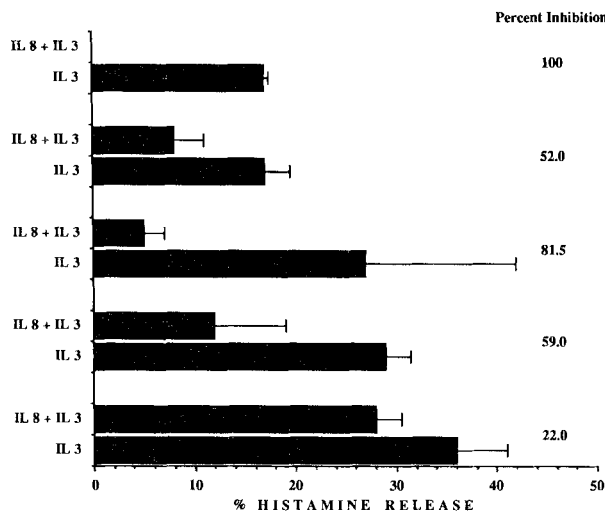


Figure 3. Inhibition of IL-3-induced histamine release by IL-8. Basophils were preincubated without or with IL-8, at a concentration of 10^{-9} M, for 5 min. The cells were then exposed to IL-3 (5000 U/ml) for 40 min. The results are expressed as the mean $\pm$ SEM from two separate experiments.

tively. As shown in Figure 3, inhibition ranged from 22% to 100%. When we combined individual results, IL-8 decreased IL-3-induced histamine release from $25.2 \pm 3.7\%$ to $9.2 \pm 4.9\%$. The difference is statistically significant ($p < 0.01$, Student *t*-test).

Characteristics of IL-8 inhibition. In a dose-response experiment, the effect of 10^{-7} to 10^{-11} M IL-8 was studied in four subjects by using HRF_{crude}. As shown in Figure 4A, maximal inhibition was observed at 10^{-9} M. Interestingly, the inhibition was found to decrease at higher concentrations. We then determined the effect of IL-8 upon histamine release due to IL-3, CTAP III, and various HRF preparations, as shown in Figure 4B. In each instance, inhibition was evident, and a maximal effect was observed at 10^{-9} M. We also sought synergism between IL-8 and various agonists, by preincubation of basophils with 10^{-6} M IL-8 for 10 min before addition of HRF_{crude}, IL-3, or CTAP-III, in five donors. The results were additive but not synergistic (data not shown).

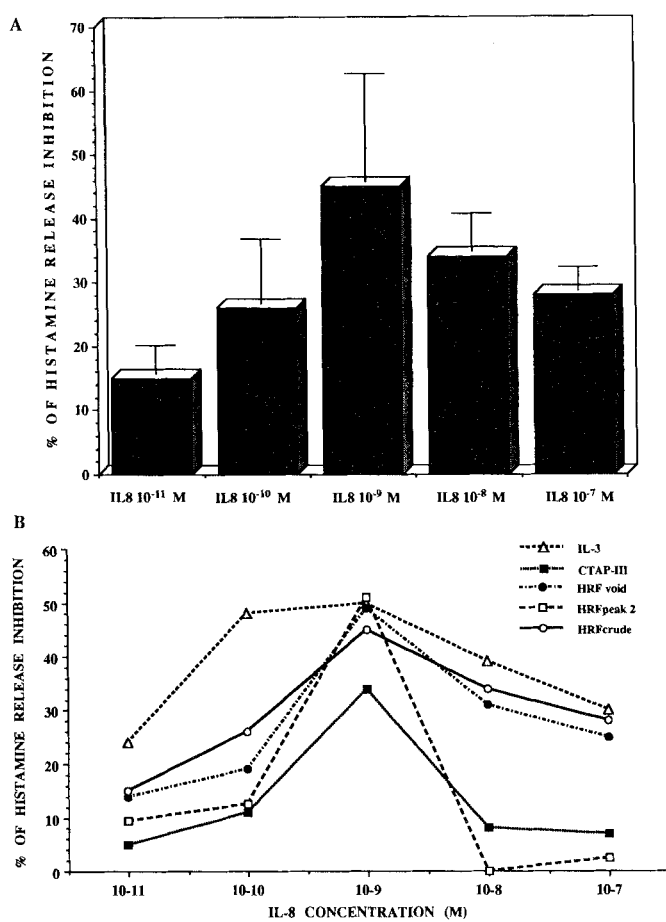


Figure 4. A, Dose-dependent inhibition of histamine release by IL-8. Basophils were preincubated with or without 10^{-11} to 10^{-7} M IL-8. The cells were then exposed to HRF_{crude} for 40 min. Results are mean $\pm$ SEM of two experiments in four different subjects. B, basophils were preincubated with or without 10^{-11} to 10^{-7} M IL-8. The cells were then exposed to various forms of HRF, CTAP III, or IL-3. Results are mean of two experiments in three donors for HRF_{peak 2} and four donors for the other agonists. The SE bars are omitted so the data could be shown more clearly.

IL-8 was present in the aforementioned experiments concomitant with CTAP III, IL-3, or different HRF preparations. We next determined whether removal of IL-8 after preincubation, by extensive washing (three times at 50 times the pellet volume), affected the response to various HRF preparations, IL-3, and CTAP III. The results are shown in Figure 5. Comparisons indicated that percentage of inhibition in the presence of IL-8 (unwashed) and after its removal was similar for all the agonists tested. Washing did not abrogate the effect of IL-8.

DISCUSSION

Our data suggest that IL-8 has a role in cytokine-dependent stimulation of histamine release from human basophils. It does not, however, appear to cause significant basophil histamine release directly, although some release can be demonstrated at very high and, therefore, unphysiologic ($\geq 10^{-7}$ M) concentrations (16). It may also act as an agonist if cells are first primed with IL-3, IL-5, or granulocyte/macrophage CSF (21, 22). We have demonstrated, however, that preincubation of basophils with IL-8 causes inhibition of histamine release by human HRF (either unfractionated concentrate, HRF_{void}, or HRF_{peak 2}) and it also inhibits histamine release due to purified CTAP III or rIL-3. There is no inhibition of anti-

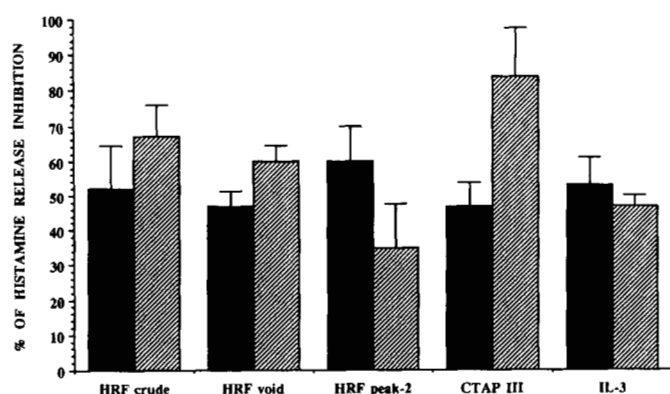


Figure 5. Effect of removal of IL-8 after preincubation on HRF-induced histamine release from basophils. Replicate samples of basophils were preincubated with or without 10^{-9} M IL-8 for 5 min. The cells were then either washed extensively or left unwashed and challenged with agonist. ■, percentage of inhibition seen in the presence of IL-8 with no wash step; ▨, inhibition when the pellets were washed before the addition of agonist. The results are expressed as the mean $\pm$ SEM of two experiments in four different subjects (HRF_{crude}, HRF_{void}, CTAP III, and IL-3) or three different subjects (HRF_{peak-2}).

IgE- or FMLP-dependent histamine release. Thus, IL-8 appears to specifically inhibit histamine release produced by cytokine-like molecules in most but not all of our basophil donors. Heterogeneity of basophil response is a function of the responder rather than the IL-8, and the variability is well known, e.g., anti-IgE response may vary from 0 to 80%, depending on whether the donor is or is not allergic. Recently, Alam et al. (23) reported that IL-8 inhibited HRF-stimulated histamine release in 17 of 20 donors tested and, similar to our observation, they did not find inhibition of histamine release due to anti-IgE or FMLP.

We do not know why IL-8 has specificity as an antagonist for cytokine-like stimulation, rather than that due to anti-IgE or FMLP. Data on IL-8R and signal transduction are just beginning to be accumulated, and they suggest that intracellular signaling may involve GTP-binding protein subunits and activation of protein kinase C (24). The mechanism would presumably differ in some fundamental way from that of anti-IgE (Ag) or FMLP. IL-8 need not be in the reaction mixture after the basophils have been exposed to it, although a fraction may remain bound to the cells. Regardless, we interpret the data to suggest a down-regulation of basophil responsiveness, perhaps via a specific receptor, rather than interaction with the agonist. Recent evidence of basophil chemotaxis to IL-8 also suggests the presence of a specific receptor (25). Some effects of IL-8 are inhibiting ones, such as reported herein. For example, endothelial cell-derived IL-8 has been shown to inhibit neutrophil adhesion to IL-1-activated endothelial cells and protects activated endothelial cell monolayers from neutrophil-mediated damage (26), while the supernatant harvested from granulocytes exposed to IL-8 blocks adhesion of the myeloid cell line HL-60 to endothelial leukocyte adhesion molecule (27). Thus, although IL-8 is a potent neutrophil stimulant (chemotaxis and cell secretion), it also has inhibitory effects upon some inflammatory reactions, and additional studies will be needed to discern which effects are important in differing circumstances.

The histamine release experiments we performed did not utilize D₂O to enhance secretion, and they confirm the previously reported basophil responsiveness to CTAP III and IL-3, as well as that to HRF (7, 10, 17). However,

it should be noted that the concentration of IL-3 utilized herein (5000 U/ml) corresponds to 50 ng/ml and exceeds the amounts of IL-3 present in our HRF supernatants. On the other hand, CTAP III is a plentiful protein, and the concentrations of CTAP III found in human serum after platelet activation during blood clotting is 5.0 to 35.0 μ g/ml (28). Although CTAP III is related to IL-8 and the platelet factor 4 family of proteins thought to participate in the inflammatory response, it is not chemotactic for neutrophils and has minimal capacity to inhibit IL-8 binding to neutrophils (29). Thus, IL-8 and CTAP III may utilize different receptors. The antagonistic effect of IL-8 on HRF- and CTAP III-induced histamine release suggests that IL-8 may provide a local control mechanism for basophil histamine release.

HRF may have an important role in allergic disorders. HRF-like activity has been found in blister fluids induced over late phase cutaneous reactions (30); there is evidence that PBMC spontaneously produced HRF in subjects with atopic asthma and that immunotherapy diminished the allergen-dependent synthesis of HRF (31). HRF have also been shown to be produced spontaneously upon culture of PBMC derived from atopic dermatitis patients who were hypersensitive to certain foods (32). Both HRF and HRIF have been found in BAL fluid (13). Because IL-8 is produced by a variety of cells, including monocytes (15), pulmonary epithelial cells (33), lung macrophages (34), and endothelial cells (35), it is possible that the HRIF present in BAL fluids might be IL-8. Although characterized in only a preliminary fashion, the HRIF described previously was resistant to heating at 56°C and to freezing, had a molecular mass of 8 to 10 kDa, was protease sensitive, and inhibited histamine release due to HRF but not to anti-IgE, Con A, PMA, or FMLP (12, 13). HRIF required a preincubation with the cells for 5 to 10 min for maximal inhibition, and this inhibitory effect was not abolished by subsequent washing (12). All these observations are consistent with the known properties of IL-8 and our observations regarding its modulation of basophil histamine release. We, therefore, suggest that IL-8 is one form of human HRIF.

REFERENCES

- Kaplan, A. P., M. Haak-Frendscho, A. Fauci, C. Dinarello, and E. Halbert. 1985. A histamine-releasing factor from activated human mononuclear cells. *J. Immunol.* 135:2027.
- Thuesen, D. O., L. S. Speck, M. A. Lett-Brown, and J. A. Grant. 1979. Histamine-releasing activity (HRA). I. Production by mitogen or antigen stimulated human mononuclear cells. *J. Immunol.* 123:626.
- Alam, R., P. A. Forsythe, M. A. Lett-Brown, and J. Andrew Grant. 1989. Cellular origin of histamine-releasing factor produced by peripheral blood mononuclear cells. *J. Immunol.* 142:3951.
- Sedgwick, B. D., P. G. Holt, and B. Turner. 1981. Production of a histamine releasing lymphokine by antigen or mitogen-stimulated human peripheral T cells. *Clin. Exp. Immunol.* 45:409.
- Orchard, M. A., A. Kagey-Sobotka, D. Proud, and L. M. Lichtenstein. 1986. Basophil histamine release induced by a substance from stimulated platelets. *J. Immunol.* 136:2240.
- Schulman, E. S., P. C. Liu, D. Proud, D. W. MacGlashan, Jr., L. M. Lichtenstein, and M. Plaut. 1985. Human lung macrophages induce histamine release from basophils and mast cells. *Am. Rev. Respir. Dis.* 131:230.
- Baeza, M. L., S. R. Reddigari, M. Haak-Frendscho, and A. P. Kaplan. 1988. Purification and further characterization of human mononuclear cell histamine releasing factor. *J. Clin. Invest.* 83:1204.
- Reddigari, S. R., M. L. Baeza, D. Kornfeld, N. Ramani, E. M. Smith, P. A. Hossler, C. W. Castor, P. G. Gorevic, and A. P. Kaplan. 1989. Relationship of one form of human histamine releasing factor (HRF) to connective tissue activating peptide-III (CTAP-III). *Cytokine* 1:152 (Abstr.).
- Castor, C. W., J. W. Miller, and D. A. Walz. 1983. Structural and biological characteristics of connective tissue activating peptides

- (CTAP-III), a major human platelet derived growth factor. *Proc. Natl. Acad. Sci. USA* 80:765.
10. Haak-Frendscho, M., N. Arai, K.-I. Arai, M. L. Baeza, A. Finn, and A. P. Kaplan. 1988. Human recombinant granulocyte-macrophage colony-stimulating factor and interleukin 3 cause basophil histamine release. *J. Clin. Invest.* 82:17.
 11. MacDonald, S. M., R. P. Schleimer, A. Kagey-Sobotka, S. Gillis, and L. M. Lichtenstein. 1989. Recombinant IL-3 induces histamine release from human basophils. *J. Immunol.* 142:3527.
 12. Alam, R., J. A. Grant, and M. A. Lett-Brown. 1988. Identification of a histamine releasing inhibitory factor produced by human mononuclear cells in vitro. *J. Clin. Invest.* 82:2056.
 13. Alam, R., J. Welter, P. A. Forsythe, M. A. Lett-Brown, J. A. Rankin, M. Boyars, and A. Grant. 1990. Detection of histamine release inhibitory factor- and histamine releasing factor-like activities in bronchoalveolar lavage fluids. *Am. Rev. Respir. Dis.* 141:666.
 14. Larsen, C. G., A. O. Anderson, E. Appella, J. J. Oppenheim, and K. Matsushima. 1989. The neutrophil activating protein (NAP-1) is also chemotactic for lymphocytes. *Science* 243:1464.
 15. Schröder, J. M., U. Mrowietz, E. Morita, and E. Christophers. 1987. Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. *J. Immunol.* 139:3474.
 16. White, M. V., T. Yoshimura, W. Hook, M. A. Kaliner, and E. J. Leonard. 1989. Neutrophil attractant/activation protein-1 (NAP-1) causes human basophil histamine release. *Immunol. Lett.* 22:151.
 17. Baeza, M. L., S. R. Reddigari, D. Kornfeld, N. Ramani, E. M. Smith, P. A. Hossler, T. Fischer, C. W. Castor, P. G. Gorevic, and A. P. Kaplan. 1990. Relationship of one form of human histamine releasing factor to connective tissue activating peptide-III. *J. Clin. Invest.* 85:1516.
 18. Reddigari, S. R., P. Kuna, G. Miragliotta, D. Kornfeld, C. W. Castor, and A. P. Kaplan. 1991. Connective tissue activating peptide-III and neutrophil activating peptide-2 both release histamine from human basophils. *J. Allergy Clin. Immunol.* 87:241.
 19. Collier, B. S., E. I. Peerschke, L. E. Scudder, and C. A. Sullivan. 1983. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombosthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. *J. Clin. Invest.* 72:325.
 20. Beaven, M. A., A. Jacobsen, and Z. Horakova. 1972. Modification of an enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum, and urine. *Clin. Chem. Acta* 37:91.
 21. Bischoff, S. C., T. Brunner, A. L. DeWeck, and C. A. Dahinden. 1990. Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. *J. Exp. Med.* 172:1577.
 22. Dahinden, C. A., Y. Kurimoto, A. L. De Weck, I. Lindley, B. Dewald, and M. Baggiolini. 1989. The neutrophil-activating peptide NAF/NAP-1 induces histamine release and leukotriene release by interleukin 3-primed basophils. *J. Exp. Med.* 170:1787.
 23. Alam, R., Y. Bodenbun, P. A. Forsythe, M. A. Lett-Brown, and J. A. Grant. 1991. Agonistic-antagonistic property of interleukin 8 on basophils: identification of IL-8 as a potent inhibitor of cytokine induced histamine release. *J. Allergy Clin. Immunol.* 87:241 (Abst).
 24. Bacon, K. B., and R. D. R. Camp. 1990. Interleukin IL-8 induced in vitro human lymphocyte migration is inhibited by cholera and pertussis toxins and inhibitors of protein kinase C. *Biochem. Biophys. Res. Commun.* 169:1099.
 25. Leonard, E. D., A. Skeel, T. Yoshimura, K. Noer, S. Kutvirt, and D. V. Epps. 1990. Leukocyte specificity and binding of human neutrophil attractant/activation protein-1. *J. Immunol.* 144:1323.
 26. Gimbrone, M. A., M. S. Obin, A. F. Brock, E. A. Luis, P. E. Hass, C. A. Hebert, Y. K. Yip, D. W. Leung, D. G. Lowe, W. J. Kohr, W. C. Darbonne, K. B. Bechtol, and J. B. Baker. 1989. Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. *Science* 246:1601.
 27. Walz, G., A. Aruffo, W. Kolanus, M. Bevilacqua, and B. Seed. 1990. Recognition by ELAM-1 of the sialyl-Le determinant on myeloid and tumor cells. *Science* 250:1132.
 28. Castor, C. W., D. A. Walz, C. G. Ragsdale, P. A. Hossler, E. M. Smith, M. C. Bignall, B. P. Aaron, and K. Mountjoy. 1989. Connective tissue activation. XXXIII. Biologically active cleavage products of CTAP-III from platelets. *Biochem. Biophys. Res. Commun.* 163:1071.
 29. Leonard, E. J., T. Yoshimura, A. Rot, K. Noer, A. Walz, M. Baggiolini, D. A. Walz, E. J. Goetzl, and C. W. Castor. 1991. Chemotactic activity and receptor binding of neutrophil attractant/activation protein-1 (NAP-1) and structurally related host defense cytokines: interactions of NAP-2 with the NAP-1 receptor. *J. Leuk. Biol.* 49:258.
 30. Warner, J. A., M. M. Pienkowski, M. Plaut, P. S. Norman, and L. M. Lichtenstein. 1986. Identification of histamine releasing factor(s) in the late phase of cutaneous IgE-mediated reactions. *J. Immunol.* 136:2583.
 31. Kuna, P., R. Alam, B. Kuzminska, and J. Rozniecki. 1989. The effect of preseasonal immunotherapy on the production of histamine releasing factor (HRF) by mononuclear cells from patients with seasonal asthma: results of a double blind placebo-controlled, randomized study. *J. Allergy Clin. Immunol.* 83:816.
 32. Sampson, H. A., K. R. Broadbent, and J. Bernhisel-Broadbent. 1989. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N. Engl. J. Med.* 321:228.
 33. Standiford, T. J., S. L. Kunkel, M. A. Basha, S. W. Chensue, J. P. Lynch, G. B. Toews, J. Westwick, and R. M. Strieter. 1990. Interleukin-8 gene expression by a pulmonary epithelial cell line: a model for cytokine networks in the lung. *J. Clin. Invest.* 86:1945.
 34. Rankin, J. A., I. Sylvester, S. Smith, T. Yoshimura, and E. J. Leonard. 1990. Macrophages cultured in vitro release leukotriene B₄ and neutrophil attractant/activation protein (interleukin 8) sequentially in response to stimulation with lipopolysaccharide and zymosan. *J. Clin. Invest.* 86:1556.
 35. Schröder, J. M., and E. Christophers. 1989. Secretion of novel and homologous neutrophil-activating peptides by LPS-stimulated human endothelial cells. *J. Immunol.* 142:244.