

Monocyte Chemotactic and Activating Factor Is a Potent Histamine-releasing Factor for Human Basophils

By Piotr Kuna,* Sessa R. Reddigari,* Doreen Rucinski,*
Joost J. Oppenheim,† and Allen P. Kaplan*

From the *Division of Allergy, Rheumatology and Clinical Immunology, Department of Medicine, SUNY-Stony Brook, Health Sciences Center, Stony Brook, New York 11794; and the †Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, National Cancer Institute, Frederick, Maryland 21702

Summary

Recombinant monocyte chemotactic-activating factor (MCAF) has been shown to induce histamine release from human basophils with a dose response between 10^{-9} and 10^{-6} M. The peak of activity was reached at 10^{-7} M. Histamine release by MCAF was rapid with an initial rate comparable with histamine release by an optimal dose of anti-IgE. MCAF led to peak histamine release within 1 min. 80% of the subjects tested were responsive to MCAF or anti-IgE, while all were responsive to FMLP. The percentage histamine release by MCAF was, however, less than that seen with anti-IgE or FMLP, but this was attributable to a lesser percent release in nonatopic subjects; atopic subjects responded similarly to all three agonists. MCAF was also shown to activate highly purified human basophils more readily than mixed leukocytes, and its activity was inhibited by a polyclonal rabbit antibody. At a suboptimal concentration (2.5×10^{-9} M), MCAF was unable to prime the basophil to histamine release by other secretagogues. However, interleukin 3 (IL-3) and IL-5 could each prime basophils for MCAF-induced secretion. Therefore, our results suggest that MCAF may be a major contributor to the histamine-releasing activity seen in peripheral blood mononuclear cell supernatants that has been designated histamine releasing factor(s).

We have characterized histamine-releasing factors (HRF)¹ from PBMC and platelets, and a number of cytokine-like molecules have been shown to influence histamine release from human basophils (1). Low concentrations of IL-1, IL-3, IL-5, and GM-CSF can prime human basophils to cause augmented release of histamine and/or leukotriene C₄ upon incubation with anti-IgE, C5a, or C3a (2, 3). IL-3 and GM-CSF, at higher concentrations, can directly release histamine from some donors, most of whom are atopic (4, 5). However, previous studies have demonstrated that the factor responsible for the major activity in PBMC supernatant is different from any of these cytokines (4, 6).

Purification of HRF from stimulated PBMC and platelet supernatants reveals three separate peaks of histamine-releasing activity that do not correspond to aforementioned cytokines and have apparent molecular masses of 8–10, 15–17, and 35–41 kD (7). Further characterization of the 8–10-kD material re-

vealed one active component to be connective tissue-activating peptide III (CTAP III) and its derivative neutrophil-activating peptide 2 (NAP-2) (8), which are derived from platelet basic protein. Cytokines resembling these proteins in physicochemical properties (size and sequence homology) include monocyte-derived neutrophil chemotactic factor, or IL-8 (9), and monocyte chemotactic-activating factor (MCAF) (10, 11). IL-8 has been demonstrated to inhibit histamine release from basophils by partially purified HRF, IL-3, and CTAP III/NAP-2 (12), and as such, is likely the histamine release inhibitory factor (HRIF) described previously (13). Here we report that the human recombinant MCAF causes histamine release from basophils, and is the most potent cytokine-like agonist thus far described.

Materials and Methods

Materials. Chemicals used in the histamine and leukotriene release experiments and mediators assays were purchased as previously described (7). Human rIL-3 (sp act, 10^8 U/mg) and rIL-5 (sp act, 10^6 U/mg) (Amgen Biologicals, Thousand Oaks, CA), anti-human Leu-M3 (CD14), anti-Leu-5b (CD2), anti-Leu-16

¹ Abbreviations used in this paper: CTAP III, connective tissue-activating peptide III; HRF, histamine-releasing factor; MCAF, monocyte chemotactic-activating factor; NAP-2, neutrophil-activating peptide 2.

(CD20), mouse mAb (Becton Dickinson & Co., San Jose, CA), goat anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), affinity-purified goat anti-human IgE antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD), recombinant human MCAF, with maximal chemotactic activity on human monocytes at 20 ng/ml (Peppo Tech Inc., Rocky Hill, NJ), Percoll (Pharmacia, Uppsala, Sweden), human recombinant C5a, FMLP, and bacitracin (Sigma Chemical Co., St. Louis, MO) were used. The C5a had activity similar to serum-derived C5a.

Basophil Donors. Donors were recruited from laboratory and hospital personnel. Allergic subjects had a history of rhinitis, rhinoconjunctivitis, or asthma, and positive skin tests to common aeroallergens. Normal subjects were skin test-negative healthy persons with negative personal or family history of any allergic disease. All allergic subjects were off medications for at least 3 d before blood was drawn. The protocol for blood donation was approved by the human subjects committee (SUNY-Stony Brook).

Cell Preparation. The procedure for leukocyte isolation has been described in detail (14). In some experiments basophils were purified from peripheral blood on Percoll gradients according to Leonard et al. (15), followed by negative panning selection as described (16) using anti-Leu-5b, anti-Leu-16, and anti-Leu-M3 mAbs. The panning yielded basophils of >85% purity. The average recovery of basophils in six donors studied was 20%.

Histamine Release from Basophils. Aliquots of 15 μ l of MCAF, anti-IgE, or FMLP were incubated with 135 μ l of prewarmed leukocytes or basophil suspension at 37°C for 45 min. Total histamine in basophils was released by heating in a boiling water bath. Histamine release experiments were done in duplicate and results expressed as the percentage released. Spontaneous release was <5% and was subtracted from the calculated histamine release. The radioenzymatic assay for histamine was performed in duplicate for each sample according to Beaven et al. (17).

Anti-MCAF Antibody Preparation. Rabbit antiserum to MCAF (kindly provided by Dr. Christine Burns, Advanced Magnetics, Boston, MA) and normal rabbit serum were made 45% in ammonium sulfate, centrifuged at 10,000 g for 5 min, the supernatant was discarded, and the precipitate was dissolved in PBS and dialyzed against PBS. A 1:800 dilution of the rabbit antiserum inhibited monocyte chemotaxis induced by 2.5×10^{-9} M MCAF by 50%.

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

Results

Characteristics of MCAF-induced Histamine Release. We initially determined the release of histamine from the basophils of six subjects with 10^{-11} to 10^{-6} M MCAF. As shown in Fig. 1, MCAF released a significant percent of histamine at concentrations of 10^{-9} M and above, with a maximal effect at $\sim 10^{-7}$ M. The curve appeared to plateau between 10^{-7} and 10^{-6} M. The kinetics of histamine release by MCAF was extremely rapid with the peak release observed within the first minute with no further increment between 1 min and 1 h. Thus, histamine release by MCAF is among the most rapidly acting basophil secretagogues thus far described and resembles C5a and FMLP (18, 19). We also determined that MCAF requires divalent cations for its effect. When the

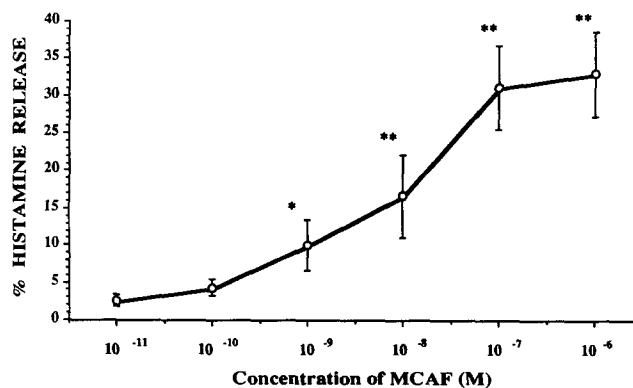


Figure 1. Dose-dependent histamine release by MCAF. Basophils of six individuals were challenged with MCAF from 10^{-11} to 10^{-6} M, and the percentage histamine release was determined. Results are expressed as a mean percentage of histamine release \pm SEM. * $p < 0.022$; ** $p < 0.014$ vs. histamine release induced by 10^{-11} M MCAF (Wilcoxon's rank sum test).

dose-response experiment (Fig. 1) was performed in the absence of calcium and magnesium, there was no histamine release. It appears that MCAF-mediated histamine release from basophils is a secretory, noncytolytic process.

We next enlarged the population studied to better appreciate donor responsiveness to MCAF and included atopic and nonatopic subjects. Leukocytes from 31 subjects were tested for histamine release due to MCAF (10^{-7} M), anti-IgE (1 μ g/ml), and FMLP (10^{-5} M). There were 13 atopic subjects and 18 nonatopic subjects. MCAF caused histamine release in 25 of 31 donors, anti-IgE released histamine in 26 of 31 donors, and FMLP released histamine in all subjects tested. The nonresponders to MCAF were not the same subjects who did not respond to anti-IgE. The histamine release by MCAF ($29 \pm 3.3\%$) was significantly less than that by anti-IgE or FMLP, which were $44.9 \pm 4.6\%$ and $44.5 \pm 2.8\%$, respectively. When the subjects were sorted into atopics and nonatopics, nonresponders to MCAF included two atopics and four nonatopics, while nonresponders to anti-IgE included two atopics and three nonatopics. The percentage of histamine release upon stimulation with MCAF in atopics vs. nonatopics was 35 ± 5.7 and 27 ± 4.3 , respectively, and this difference was not statistically significant ($p > 0.05$ student's t test). When the responses to MCAF, anti-IgE, and FMLP were compared in atopic subjects only, there was no significant difference among the three. However, MCAF caused less histamine release in the nonatopic group ($27 \pm 4.3\%$ vs. anti-IgE at $41.8 \pm 5.9\%$; $p < 0.042$, Wilcoxon's rank sum test) or FMLP ($43.6 \pm 3.3\%$; $p < 0.007$, Wilcoxon's rank sum test). Thus, the difference seen in all 31 subjects was reflected by the lesser response to MCAF in the nonatopic group.

Comparison of Histamine Release in Mixed Leukocytes vs. Highly Purified Basophils. Basophils from six separate subjects were isolated as described in Materials and Methods to 85–90% purity, and histamine release to MCAF (10^{-7} M) and anti-IgE (50 ng/ml) was compared with the release seen with mixed leukocytes (1–2% basophils). As seen in Fig. 2, MCAF and

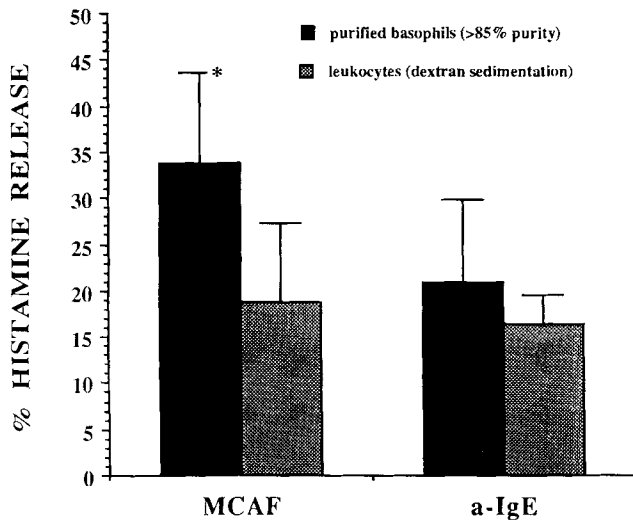


Figure 2. Comparison of the histamine release from dextran-sedimented leukocytes with highly purified basophils by MCAF (10^{-7} M) and anti-IgE (0.05 μ g/ml). The cells were challenged with each agonist for 45 min at 37°C. Each bar represents the mean \pm SEM of six experiments in different donors. *Release with MCAF was significantly greater when the purified basophils were compared with mixed leukocytes ($p \leq 0.023$ Wilcoxon's rank sum test).

anti-IgE released histamine from purified basophils as well as mixed leukocytes. Given the rapidity of histamine release from mixed leukocytes (<1 min) and substantial release from purified basophils, the likelihood of another cell-derived intermediate is extremely low. The augmented histamine release from purified basophils seen with MCAF is unexplained, but may relate to its known interactions with monocytes, which would lessen its effective concentration in mixed leukocyte preparations.

Inhibition of MCAF Histamine-releasing Activity by Anti-MCAF Antibody. MCAF at 10^{-7} M was incubated with either control or anti-MCAF rabbit antibody for 1 h at 37°C. The mixture was microfuged at 10,000 g for 3 min, and the

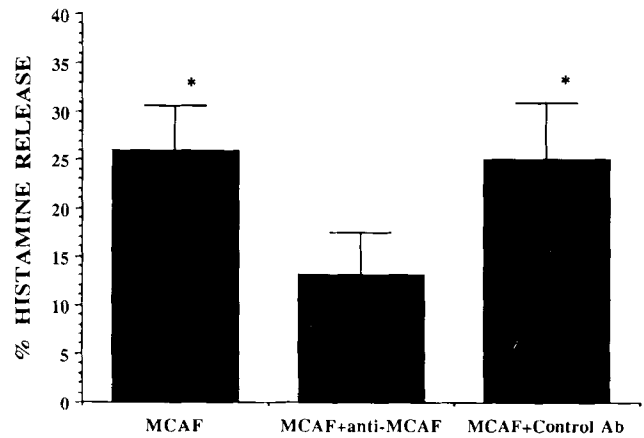


Figure 3. Effect of anti-MCAF rabbit antibody on MCAF-induced histamine release. MCAF (10^{-7} M) was preincubated with rabbit anti-human MCAF antibody or with rabbit control antibody at dilution 1:20 for 60 min at 37°C. The mixture was spun for 3 min at 10,000 g, and the supernatant was assayed for histamine release. The results of six experiments in different donors are shown. Each bar represents the mean \pm SEM. * $p \leq 0.014$ vs. MCAF + anti-MCAF (Wilcoxon's rank sum test).

supernatant added to the leukocytes of six donors, and histamine release determined. As seen in Fig. 3, the anti-MCAF inhibited histamine release by 50% while the control antibody had no effect.

IL-3 and IL-5 Prime Basophils to MCAF-induced Histamine Release. Prior studies have indicated that IL-3, IL-5, and GM-CSF are capable of priming basophils for histamine release due to anti-IgE, C5a, and IL-8 (3). In this study, we wished to corroborate these data and test whether MCAF can act as a primer at low concentrations or whether secretion by MCAF can be primed by the other cytokines. Basophils were preincubated with either buffer, IL-5, IL-3, or MCAF as shown in Table 1 for 10 min at 37°C and then challenged with either buffer, anti-IgE, FMLP, C5a, IL-5, IL-3, or MCAF as potential secretagogues. The low percent release of 8.8% with anti-IgE alone reflects the low concentration (50 ng/ml) used,

Table 1. Effect of Priming Basophils with IL-5, IL-3, or MCAF upon Histamine Release Induced by a Variety of Secretagogues

	Percent of histamine release						
	Buffer	anti-IgE	FMLP	C5a	IL-5	IL-3	MCAF
Buffer	2.2 \pm 0.7	8.8 \pm 3.5	53 \pm 7.3	39 \pm 6.4	6.6 \pm 1.2	6.1 \pm 0.8	16.3 \pm 6.8
IL-5	6.2 \pm 0.9	30.1 \pm 7.4*	72.7 \pm 8.8*	68 \pm 8.4*	-	12 \pm 6.2	35 \pm 6*
IL-3	7.3 \pm 1.4	32.2 \pm 7.5*	74 \pm 8.7*	66.3 \pm 8.2*	11.1 \pm 1.8	-	36.5 \pm 6.3*
MCAF	17.1 \pm 6.2	26.9 \pm 8.8	66.3 \pm 11.3	55.2 \pm 9.5	24.9 \pm 9	24.5 \pm 7.2	-

Leukocytes of six separate donors were preincubated in buffer or with cytokines IL-5 (100 ng/ml), IL-3 (5,000 U/ml), MCAF (20 ng/ml) for 10 min, and further challenged for 35 min with anti-IgE (0.05 μ g/ml), FMLP (10^{-5} M), C5a (10^{-8} M), IL-5 (100 ng/ml), IL-3 (5,000 U/ml), and MCAF (20 ng/ml). The mean \pm SEM of histamine release is shown.

* Statistically significant augmentation of release ($p \leq 0.05$) as a result of preincubation with either IL-5 or IL-3.

so that any enhancing effect is easily seen. The percent release of 6.6 and 6.1% for IL-5 and IL-3 is considered borderline. Substantial release is seen with FMLP, C5a, and MCAF when tested alone with values of 53 ± 7.3 , 39 ± 6.4 , and $16.3 \pm 6.8\%$, respectively. Preincubation with either IL-5 or IL-3 significantly enhanced the percent histamine release with all four secretagogues, but IL-5 and IL-3 did not prime each other. When MCAF was used as the primer (2.5×10^{-9} M), the sum of its release (17.1%) plus that seen with anti-IgE (8.8%), FMLP (53.7%), and C5a (37.6%) alone are equal to that seen in the sequential additional experiment, thus, no priming effect was evident. MCAF therefore appears to be a releasing agent and not a primer. Its activity as a secretagogue, however, can be primed by either IL-3 or IL-5.

Discussion

MCAF belongs to the proinflammatory "intercrine" cytokine family. It has been purified as a 15-kD factor from LPS-stimulated myelomonocyte cell line THP-1 and from cultured supernatants of PHA-stimulated human PBMC (10, 11). The amino acid sequence of MCAF has 20–55% homology with other members of intercrine family, including RANTES, IL-8, GRO/MGSA, PF-4, CTAP III, and its derivatives β -thromboglobulin and NAP-2 (20). MCAF is a potent chemoattractant for monocytes but not for neutrophils or lymphocytes, and it activates monocyte superoxide anion production and release of lysosomal enzymes in the presence of cytochalasin B (21).

We have demonstrated that some members of this group of inflammatory mediators release histamine from basophils and contribute to the histamine-releasing activity present in supernatants of PBMC and platelets (8, 22). IL-8, for example,

is a weak agonist and can stimulate histamine release at concentrations $>10^{-6}$ M (23). However, at lower concentrations, IL-8 functions as a histamine release inhibitory factor (12). CTAP III and NAP-2 are also weak agonists for histamine release on a molar basis (peak activity, 10^{-6} to 10^{-5} M); however, these proteins are so abundant in platelet releasate that they can contribute significantly to the histamine-releasing activity found in the 8-kD fraction of stimulated supernatants (22). We have now investigated the histamine-releasing capability of MCAF and demonstrate that it is the most potent cytokine-like secretagogue hitherto described. It acts as an agonist for basophils of most subjects irrespective of allergic status, and is active at concentrations as low as 10^{-9} M with peak activity at 10^{-7} M. The magnitude of histamine release by MCAF can be augmented if the cells are primed by IL-3 or IL-5. However, MCAF does not act as a primer for other agonists. Histamine release from human basophils is most likely due to direct interaction of MCAF with the surface of basophils rather than via another cell-derived intermediate, but we have not performed binding experiments in the present studies. We infer the presence of a receptor because the release is extremely rapid, and is augmented when highly purified basophils are compared with mixed leukocytes.

Histamine releasing factors (HRF) are thought to contribute significantly to the protracted histamine release and basophil and/or mast cell degranulation seen in a wide variety of immunologic disorders, including late phase allergic reactions (1), chronic urticaria (24), atopic dermatitis (25), asthma (26, 27), and rheumatoid arthritis (28). The specific contribution of MCAF to histamine release in such disorders is the subject of ongoing investigations, and its relationship to the purified components derived from mononuclear cell supernatants requires further definition.

Address correspondence to Allen P. Kaplan, Department of Medicine, SUNY-Stony Brook, HSC T-16, Stony Brook, NY 11794.

Received for publication 8 October 1991 and in revised form 31 October 1991.

References

1. Kaplan, A.P., S. Reddigari, M. Baeza, and P. Kuna. 1991. Histamine releasing factors and cytokine-dependent activation of basophils and mast cells. *Adv. Immunol.* 50:237.
2. Bischoff, S.C., A.L. De Weck, and C.A. Dahinden. 1990. Interleukin 3 and granulocyte/macrophage-colony-stimulating factor render human basophil responsive to low concentration of complement component C3a. *Proc. Natl. Acad. Sci. USA.* 87:6813.
3. Bischoff, S.C., T. Brunner, A.L. De Weck, and C.A. Dahinden. 1990. Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonist. *J. Exp. Med.* 172:1577.
4. 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.
5. MacDonald, S.M., R.P. Schleimer, A. Kagey-Soboka, S. Gillis, and L.M. Lichtenstein. 1989. Recombinant IL-3 induces histamine release from human basophils. *J. Immunol.* 142:3527.
6. Alam, R., J.B. Welter, P.A. Forsythe, M.A. Lett-Brown, and J.A. Grant. 1989. Comparative effect of recombinant IL-1, -2, -3, -4, and -6, IFN-gamma, granulocyte macrophage-colony-stimulating factor, tumor necrosis factor- α , and histamine releasing factors on the secretion of histamine from basophils. *J. Immunol.* 142:3431.
7. Baeza, M.L., S.R. Reddigari, M. Haak-Frendscho, and A.P.

- Kaplan. 1989. Purification and further characterization of human mononuclear cell histamine releasing factor. *J. Clin. Invest.* 83:1204.
8. 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.
 9. Matsushima, K., K. Morishita, T. Yoshimura, S. Lavu, Y. Kobayashi, W. Lew, E. Appella, H.F. Kung, E.J. Leonard, and J.J. Oppenheim. 1988. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J. Exp. Med.* 167:1883.
 10. Yoshimura, T., E.A. Robinson, S. Tanaka, E. Appella, and E.J. Leonard. 1989. Purification and amino acid analysis of two monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes. *J. Immunol.* 142:1956.
 11. Matsushima, K., C.G. Larsen, G.C. DuBois, and J.J. Oppenheim. 1989. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J. Exp. Med.* 169:1485.
 12. Kuna, P., S.R. Reddigari, D. Kornfeld, and A.P. Kaplan. 1991. IL-8 inhibits histamine release from human basophils induced by histamine releasing factors, connective tissue activating peptide III, and IL-3. *J. Immunol.* 147:1920.
 13. 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.
 14. 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.
 15. Leonard, E.J., R.L. Roberts, and A. Skeel. 1984. Purification of human blood basophils by single step isopycnic banding on percoll. *J. Leukocyte Biol.* 35:169.
 16. Wysocki, L.J., and V.L. Sato. 1978. "Panning" for lymphocytes: A method for cell selection. *Proc. Natl. Acad. Sci. USA.* 75:2844.
 17. 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.
 18. Grant, J.A., E. Dupree, A.S. Goldman, D.R. Schultz, and A.L. Jackson. 1975. Complement-mediated release of histamine from human leukocytes. *J. Immunol.* 114:1101.
 19. Siraganian, R.P., and W.A. Hook. 1977. Mechanism of histamine release by formyl methionine containing peptide. *J. Immunol.* 119:2078.
 20. Oppenheim, J.J., C.O.C. Zacharie, N. Mukaida, and K. Matsushima. 1991. Properties of the novel proinflammatory supergene "Intercrine" cytokine family. *Annu. Rev. Immunol.* 9:617.
 21. Zachariae, C.O.C., A.O. Anderson, H.L. Thompson, E. Appella, A. Mantovani, J.J. Oppenheim, and K. Matsushima. 1990. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. *J. Exp. Med.* 171:2177.
 22. 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. (Abstr.)
 23. 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.
 24. Elias, J., E. Boss, and A.P. Kaplan. 1986. Studies of the cellular infiltrate of chronic idiopathic urticaria: Prominence of T-lymphocytes, monocytes, and mast cells. *J. Allergy Clin. Immunol.* 78:914.
 25. 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.
 26. Alam, R., P. Kuna, J. Rozniecki, and B. Kuzminska. 1987. The magnitude of spontaneous production of histamine releasing factor (HRF) by lymphocytes in vitro correlates with the state of bronchial hyperreactivity in patients with asthma. *J. Allergy Clin. Immunol.* 79:103.
 27. 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.
 28. Gruber, B., M. Poznansky, E. Boss, J. Partin, P. Gorevic, and A.P. Kaplan. 1986. Characterization and functional studies of rheumatoid synovial mast cells: Activation by secretagogues, anti-IgE, and histamine-releasing lymphokine. *Arthritis Rheum.* 29:944.