

Histamine-releasing factors

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There is increasing evidence that human basophils accumulate at sites of chronic inflammation, and, in particular, in allergic asthma. Investigators have, therefore, become very interested in identifying proteins that activate these cells. Recently, the gene encoding a candidate for this function, a novel molecule, the IgE-dependent histamine-releasing factor, was cloned.

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Abbreviations

BAL	bronchoalveolar lavage
GM-CSF	granulocyte macrophage-colony stimulating factor
HRF	histamine-releasing factor
IL	interleukin
LPR	late-phase reaction
MCP	monocyte chemotactic protein
MIP	macrophage inflammatory protein
PAF	platelet activating factor
RANTES	regulated on activation, normal T cell expressed and secreted
rHRF	recombinant IgE-dependent HRF

Introduction

It has long been appreciated that exposure of an allergic individual to an antigen produces an immediate response, often followed by a late reaction. This late reaction has been studied in multiple species by many groups. In humans, it occurs within 6-24 hours of challenge and is characterized by edema and erythema of the skin and by increased resistance to air flow in the nose and lungs. There are several reasons why the late-phase reaction (LPR) may be a more appropriate model of allergic inflammation than is the acute response to antigen: its duration; its susceptibility to oral steroids; and its cellular influx, characterized by eosinophils, basophils and monocytes, resembling that of chronic allergic inflammation. The pathogenesis of this late reaction is complex, however, and not well understood. An initial event in the human response to antigen might well be mast cell degranulation and release of mediators that, in turn, attract inflammatory cells and upregulate adhesion molecules. The basophils and eosinophils that infiltrate during the LPR are known to be activated or primed to be hyper-responsive to stimuli. There is growing evidence to suggest that the basophil, and not the mast cell, plays the more significant role in the LPR. First, the mast cell mediators that are found associated with the symptoms of the acute reaction, such as, histamine, prostaglandin D₂, tryptase

and leukotriene C₄ are clearly different than those found in the late reaction, that is, histamine and leukotriene C₄, but no prostaglandin D₂ or tryptase. Basophils do not contain prostaglandin D₂ and have very little, if any, tryptase. Second, by morphological and flow cytometrical evidence, basophils clearly appear during antigen-induced LPRs in skin chambers and in the nose. Furthermore, the IgE-bearing cells in late-phase nasal lavages express CD18 leukocyte adhesion molecules. This adhesion molecule is found primarily on basophils and other leukocytes, not on lung mast cells.

Basophils also have been identified as the histamine-containing cells in bronchoalveolar lavage fluids (BALs) obtained during the late response. Of the cells positive for Alcian blue that increased 15-fold in the antigen site versus the saline site in late-phase BAL fluids, 96% were morphologically and functionally defined as basophils (reviewed in [1]). Additionally, Maruyama *et al.* [2] have shown that basophils accumulate during airway narrowing in asthmatic patients. Finally, Koshino *et al.* [3] have shown an increase in basophils in biopsies from patients dying with asthma compared to biopsies from patients with other diseases and that basophil numbers significantly correlate with bronchial hyper-responsiveness in those patients experiencing an asthmatic episode. These data suggest that the infiltration of proinflammatory cells, including basophils, is of critical importance in the LPR of allergic, asthmatic diseases. Since basophils are present *in vivo* in states of chronic inflammation, the stimuli for basophil activation, the so-called histamine-releasing factors (HRFs), have become an area of intense investigation. This review will describe the biological properties and clinical relevance of the recombinant IgE-dependent HRFs (rHRFs) and compare it to with those of the other known HRFs.

History

The history of HRFs dates back to 1979 when Thueson *et al.* [4] first described a histamine-releasing activity produced by cultured peripheral blood mononuclear cells that had been stimulated with mitogens or antigens. This HRF was further characterized and found to be very heterogeneous, containing molecular weight species ranging from 15 000 to 50 000 kDa. A number of other groups confirmed this finding (reviewed in [1]). HRFs are produced *in vitro* by a variety of cell types such as T and B lymphocytes, mononuclear cells, alveolar macrophages, platelets, vascular endothelial cells and various cell lines, including the U937 monocyte/macrophage-like cell line and the RPMI 8866 B-cell line. Not only is HRF found *in vitro*, but it is also found *in vivo* in resting and late-phase nasal lavages, late-phase skin blister fluids and BALs [1].

In the context of purifying HRFs, it became apparent that there were two distinct mechanisms of HRF-induced histamine release, one that is dependent on cell surface IgE and another that is IgE independent. The Johns Hopkins Asthma and Allergy Center laboratory aggressively pursued the purification of the rHRF (see below). Two large groups of cytokines, the chemokines and the interleukins, comprise the HRFs that cause basophilic histamine release that is independent of IgE. These will be discussed first.

Chemokines

The chemokines and their actions on lymphocytes have been recently reviewed by Hedrick and Zlotnik [5]. Briefly, they consist of a superfamily of proteins with a molecular weight range of 6000 to 14000 kDa that are classified according to the position of cysteine residues. Currently, there are three subgroups: the C-X-C, (two cysteines with an intervening amino acid); C-C (two adjacent cysteines); and recently discovered, the C subfamily (of which lymphotactin is the only member to date [6]). The number of known chemokines presently exceeds 30 and the list is increasing. Table 1 describes the ability of chemokines to act as HRFs and cause histamine release from human basophils.

Table 1

Chemokines that cause basophil histamine release.

Chemokine	Histamine release	Reference
C-C		
MCP-4	+	[9]
MCP-3	+++	[8]
MCP-2	++	[10]
MCP-1	+++	[8]
RANTES	+/-	[1]
MIP-1 α	+/-	[1]
C-X-C		
IL-8	+/-	[1]
CTAP-III(a)	+/-	[1]
NAP-2(b)	+/-	[1]

(a)CTAP-III, connective tissue activating peptide III; (b)NAP-2, neutrophil-activating peptide 2.

The C-C chemokines

As is evident from Table 1, the C-C chemokines are more active as stimuli for histamine release from basophils, but their degree of potency varies. It has been known since the early part of this decade that monocyte chemoattractant protein (MCP)-1 is a potent agonist for basophil histamine release. Regulated on activation, normal T cell expressed and secreted protein (RANTES), on the other hand, causes chemotaxis of basophils and eosinophils but is a weak stimulus for histamine release. MCP-3, which shares a high degree of homology with MCP-1, is both a chemotactic agent and a histamine-releasing agent (reviewed in [1,7]). The gene encoding the most recent member of the C-C chemokine family, MCP-4, originally

termed CK β 10, was cloned and expressed and some of its product's biological activities determined [8]. MCP-4 shares 56-61% sequence identity with MCP-1, -2 and -3 as well as 60% identity to eotaxin, another C-C chemokine which causes chemotaxis of eosinophils [9]. MCP-4 has similar biological properties to those of MCP-3 and eotaxin. Since MCP-1 and MCP-3 have previously been shown to be potent HRFs for human basophils, one might predict that MCP-4 would also stimulate histamine release from basophils. Indeed, Stellato *et al.* [10] have recently demonstrated that MCP-4 was a potent stimulus of chemotaxis of human eosinophils and induced histamine release in IL-3-primed human basophils with a potency equal to that of MCP-3. Another member of this family, MCP-2, has also been shown to induce histamine release from IL-3-primed basophils, as well as to induce a calcium signal in eosinophils and basophils, but these effects were less potent than for MCP-1 [11]. Thus, all members of the MCP family affect human basophils but with varying degrees of efficacy. The reason for this remains an enigma. Several possible explanations include receptor occupancy by the various MCP homologues; clearly there are multiple chemokine receptors (see below) and they differ in their affinity for the MCP members. Another possibility is structural differences in the amino terminus of the molecule. Weber *et al.* [12 $\bullet$] have recently demonstrated that amino-terminally truncated analogs of MCP-1 dramatically altered the histamine-releasing capacity of this molecule. They showed that removing the amino-terminal amino acids 2-76 led to a 50-fold reduction in both histamine and sulfidopeptide leukotriene release from human basophils. Truncation of amino acids 6-76 resulted in total loss of activity. Interestingly, while removal of amino acids 2-76 reduced the potency of MCP-1 activity on basophils, this analog maintained activity on eosinophils as shown by chemotaxis and cytosolic free calcium changes [12 $\bullet$].

The C-C chemokine receptors

As mentioned above, in addition to the identification of new members of the chemokine family, the number of chemokine receptors known is also growing. A review of the various receptors is beyond the scope of this manuscript. It is important to note, however, that the gene coding for a novel C-C receptor was recently cloned using the human immature basophilic cell line, KU812 [13]. The ligands for this receptor identified thus far are macrophage inflammatory protein (MIP)-1 α , RANTES and MCP-1, but not MCP-2. To date, multiple laboratories have demonstrated the existence of several different chemokine receptors with overlapping specificities in varying cell types. At this point it is not clear whether activation of different receptors determines the diverse cell biology of the chemokines or whether different ligands binding to the same receptor alter signal transduction events. Indeed, the field is even further complicated by the fact that Bacon *et al.* [14 $\bullet\bullet$] have described two different signaling pathways when the same chemokine,

RANTES, was used to activate T cells. One pathway involved a guanine-nucleotide-binding G protein and another was dependent on protein tyrosine kinases. The pathways were responsible for different cellular responses; the G protein pathway was associated with chemotaxis, while the protein tyrosine kinase pathway was associated with responses dealing with T cell activation, such as calcium flux, cytokine production and T cell proliferation. The complexity of the biological functions of the chemokines and their multiple receptors with varying specificity may well explain the data of Kuna *et al.* [15]. These investigators examined multiple C-C and C-X-C chemokines and showed that RANTES, MIP-1 α , MIP-1 β , connective tissue activating peptide III, platelet factor 4, IL-8 and inflammatory protein 10 could inhibit MCP-1-induced histamine release. As additional chemokines and receptors are discovered, their actions on human basophils will undoubtedly be explored, perhaps adding to the stimuli for these very responsive cells.

As a final comment with respect to the HRF activity of chemokines, it is worthwhile to note that three different groups have confirmed the actions of chemokines on basophils and have demonstrated no effect on human mast cells. Hartmann *et al.* [16] looked specifically at skin mast cells, Okayama *et al.* [17] investigated skin and lung mast cells, and Fureder *et al.* [18•] showed no effect of chemokines on mast cells obtained from lung, uterus, skin or tonsils, either directly or when these mast cells were preincubated with stem cell factor, a known potentiator of mast cell histamine release. Thus, the chemokines' ability to release histamine and act as IgE-independent HRFs is due solely to their actions on human basophils.

Interleukins

Another group of IgE-independent HRFs includes several ILs. It is well accepted that IL-3 at high concentrations causes direct histamine release from basophils of a subpopulation of allergic donors and that at lower concentrations it primes or augments histamine release when cells are stimulated with other agonists. Likewise, other members of the IL-3 gene cluster, namely IL-5 and GM-CSF, prime basophils to release histamine but to a lesser extent than IL-3. Recent studies by Sarmiento *et al.* [19•] have shown that IL-3, IL-5 and GM-CSF, in a dose-dependent manner, enhance basophilic histamine and leukotriene release induced by the eosinophil product major basic protein. Furthermore, this work demonstrates that basophils and eosinophils stain brightly for the IL-3 receptor using an antibody to the α chain of the receptor. These data suggest a link between the basophil and eosinophil in perpetuation of the inflammation seen in chronic allergic diseases.

Tedeschi *et al.* [20] have extended the observations of the effects of IL-3 on human basophils. They have shown that extracellular sodium acts as an inhibitor of IL-3-induced basophil histamine release and that gramicidin D, the

sodium ionophore, markedly inhibits release induced by IL-3 and by anti-IgE. In another *in vitro* study, Columbo *et al.* [21] demonstrated the importance of the kinetics of basophil exposure to extracellular calcium and the secretion of histamine induced by platelet activating factor (PAF). Their data indicate that exposure of human basophils to extracellular calcium for 2–5 minutes leads to secretion induced by PAF and that longer exposure actually decreases release by this secretagogue. These data suggest that redistribution of extracellular calcium affects signal transduction in basophils in a complex manner. These authors also showed, as have others, that IL-3 augmented PAF-induced histamine release.

The importance of IL-3 regulation of the ability of basophils to release histamine was definitively shown by Yamaguchi *et al.* [22••]. Using basophils of enriched purity, they demonstrated that basophils from donors that did not release histamine in response to anti-IgE antibody were converted to basophils that did release histamine after three days in culture with 300 pmol l⁻¹ of IL-3. They further showed that the defect in these basophils was not at the level of the high-affinity IgE receptor, Fc ϵ RI. The mechanism of this conversion to releasing-basophils is presently unknown, but their data suggest that early signaling effectors, such as the tyrosine kinases Lyn and Syk, may be altered.

Finally, the importance of IL-3 on basophil function was shown *in vivo* by Van Gils *et al.* [23]. They studied IL-3 receptor regulation and cellular characterization of bone marrow and peripheral blood cells in rhesus monkeys undergoing IL-3 treatment. Their data demonstrated that *in vivo* therapy with IL-3 upregulated the number of high- and low-affinity receptors for IL-3 on basophils but not on mast cells or monocytes. This could provide a possible explanation for the increased incidence of allergic reactions documented with IL-3 administration.

The IgE-dependent HRF

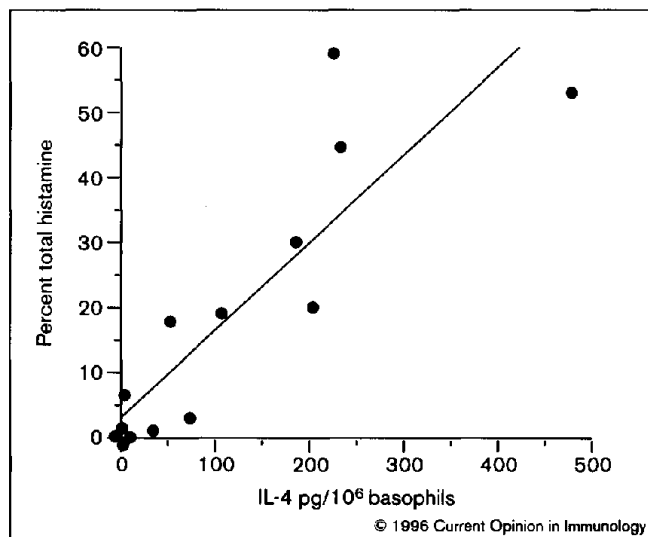
As mentioned above and as reviewed in [1], the rHRF is found *in vivo* in LPR fluids from nasal lavages, skin blister fluids and BAL. This HRF causes histamine release from basophils of a subpopulation of allergic donors. Originally, we thought that this HRF interacted with certain IgE molecules which we termed IgE⁺. By definition, those basophils with IgE⁻ were not responsive to this HRF. While HRF is a complete secretagogue for basophils possessing IgE⁺, we now have additional data that shows HRF can act on all basophils and eosinophils (see below).

For purification of the rHRF, 50 L of the human monocyte/macrophage cell line, U937, were sequentially chromatographed and the protein purified. Amino-terminal sequencing revealed 94% homology to p21, a protein from mouse tumor cells, as well as identity to p23, the human homologue. Both p21 and p23 genes were previously cloned on the basis of their abundant expression in

tumor cells, but no function had been ascribed to either molecule. Both p21 and p23 were subcloned from the U937 cell line. Both recombinant proteins caused histamine release from human basophils of a subpopulation of donors, those who possessed IgE⁺ [24**].

As previously discussed, basophils infiltrate tissues and play an important role in the pathogenesis of chronic allergic diseases. This concept is supported by studies showing that basophils generate and secrete high levels of IL-4 and IL-13 [25,26,27*]. Both of these cytokines have immunoregulatory properties that affect many different cell types that are present during allergic inflammation. As shown in Figure 1, there is a significant correlation between IL-4 protein production and histamine release in basophils stimulated with the rHRF [28*]. Our data also demonstrate rHRF is as potent a stimulus for IL-4 production as is anti-IgE antibody, and much superior to other stimuli such as IL-3 and MCP-1. rHRF also causes IL-13 production by human basophils (J Schroeder, SM MacDonald, unpublished data).

Figure 1



IL-4 protein and histamine induced by rHRF. The secretion of IL-4 protein from basophils challenged with rHRF significantly correlates with histamine release. Basophils from fourteen donors were challenged with recombinant p21 (8 µg ml⁻¹) or recombinant p23 (7.5 µg ml⁻¹) for 4 h before harvesting cell-free supernatants for histamine and IL-4 protein measurements. The secretion of IL-4 significantly correlated ($r=0.90$, $p=0.001$) with histamine release, as determined by Spearman's rank correlation coefficient.

Since the rHRF causes histamine release only from basophils possessing IgE⁺, a heterogeneity of the IgE molecule was uncovered. We have failed to show direct binding of HRF to IgE using enzyme-linked immunosorbent assay (ELISA) technology and affinity chromatography, however, and have asked whether HRF might have

its own receptor on the cell surface. Since IL-3 is known to augment basophil histamine release, we assessed the ability of HRF to do the same. In fact, basophils from IgE⁻ donors secreted histamine and IL-4 protein when the cells were pre-incubated with HRF and stimulated with subthreshold doses of anti-IgE antibody (J Schroeder, SM MacDonald, unpublished data). Thus, HRF acts as a complete secretagogue in those donors who possess IgE⁺ and enhances histamine release in donors with IgE⁻ in a manner similar to IL-3.

There is a large body of literature that supports the role of the eosinophil in allergic asthma (reviewed in [29]). Eosinophils as well as basophils are important, however, in allergic inflammation. In this context, my colleagues and I have data showing the ability of HRF to cause chemotaxis, calcium flux and IL-8 production from human eosinophils purified to >99% (SM MacDonald, B Bochner, unpublished data). We are currently pursuing HRF-eosinophil interactions *in vitro* and *in vivo*.

The rHRF is a unique molecule with no homology to any known interleukin, chemokine or antigen. This molecule uncovered a functional heterogeneity of the IgE molecule, but the molecular basis of this heterogeneity is not understood at present. By virtue of the ability of the rHRF to activate basophils and eosinophils and release mediators and cytokines, we suggest that this HRF plays a key role in the pathogenesis of human allergic disease.

Clinical relevance of HRF

Chemokines with IgE-independent-histamine releasing activity have been found *in vivo*. Alam *et al.* [30] have shown that there are increased levels of MCP-1, RANTES and MIP-1α in BAL fluid of asthmatic patients when compared to normal subjects. Their work also shows that mRNA for MCP-1, MCP-3, RANTES, MIP-1α and IL-8 was detectable by the polymerase chain reaction, in cells from BAL from both asthmatic and normal subjects. Sim *et al.* [31] have shown that IL-8, MIP-1α, RANTES, IL-1β and GM-CSF were elevated in nasal secretions during the LPR in ten allergic patients. The elevation of these chemokines correlated with symptom scores during the LPR and topical steroid treatment reduced both the symptoms and levels of these cytokines. Finally, MIP-1α and IL-16 were detected in BAL fluids from asthmatic subjects six hours after antigen challenge [32].

As noted, the rHRF has been detected in human respiratory secretions, late skin blister fluids and BAL fluids [1]. Sampson *et al.* [33] have shown that the production of the rHRF is associated with the clinical status of food allergy and atopic dermatitis. Using blood from food-allergic children with atopic dermatitis, they found that the patients' basophils showed a high spontaneous release of histamine and their mononuclear cells spontaneously produced HRF in culture. When these children are placed on an avoidance diet that eliminates

the offending food allergen, they improve clinically, and their basophils no longer spontaneously secrete histamine and their mononuclear cells no longer spontaneously produce HRF.

With the availability of recombinant material, my colleagues and I examined the lymphocytes of allergic and nonallergic patients for the generation of HRF mRNA and protein. Twelve patients (four allergic IgE⁺, four allergic IgE⁻ and four nonallergic) were recruited. Blood was drawn for serum IgE measurements and for basophil histamine release in response to rHRF and anti-IgE. In addition, peripheral blood mononuclear cells were cultured for HRF protein production and processed for mRNA extraction and subsequent reversed transcribed polymerase chain reaction for HRF mRNA. The geometric mean serum IgE levels were 356 ng ml⁻¹ in the IgE⁺ group versus 52 µg ml⁻¹ and 4.2 µg ml⁻¹ in the IgE⁻ and nonallergic subjects, respectively. Histamine release in response to the rHRF paralleled that of our native HRF preparation in that only the four IgE⁺ patients released histamine to this stimulus. The quantity of mRNA for HRF, when compared to that for beta actin, the housekeeping gene, did not appear different among the groups. The bioactivity of the rHRF on lactic acid treated cells passively sensitized with an IgE⁺-containing serum, however, was greater than in the allergic IgE⁺ patients and in the nonallergic subjects [34]. Thus, it appears that all individuals make mRNA for HRF but atopic subjects more effectively translate it to protein. My colleagues and I are now developing an HRF-specific ELISA to quantitate levels of HRF in fluids from experimental allergen challenge reactions in humans.

Conclusions

The stimulus for basophils and eosinophils during the LPR has thus far been an enigma. ILs and chemokines act as HRFs and also stimulate eosinophils, but there are few reports linking them to the LPR. As additional chemokines are discovered, elucidation of their biological actions will be pursued. We believe that expression of the recently cloned gene encoding rHRF, which activates all basophils and eosinophils from allergic donors and which is found in LPR fluids, plays an important role in perpetuating the LPR and the chronic inflammation that is the hallmark of allergic disease, in particular of allergic asthma. Identification of a receptor for this HRF could lead to strategies to develop therapeutic approaches for the management of a broad spectrum of allergic inflammation.

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