

Histamine releasing factors

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Histamine Releasing Factors (HRF) are defined as products of cells that are capable of interacting with basophils and/or mast cells to cause release of histamine [1,2]. It is also likely that they activate such cells to release other products such as leucotrienes, but the array of basophil/mast cell products released has not been studied in detail. Histamine Releasing Factors have been identified as products of human mononuclear cells [1,2], neutrophils [3] platelets [4], and alveolar macrophages [5] and there is evidence that many types of mononuclear cells may be capable of HRF synthesis including T lymphocytes [6], B lymphocytes [7], and monocytes [8]. B lymphocytes, in particular, appear to be a major source of HRF production when mixtures of mononuclear cells are assessed [8].

Two approaches to the identification of HRF have been taken. In the first, one focuses on known cytokines and tests each to determine which, if any, are capable of functioning as a HRF. In this fashion, interleukin 3 [9], granulocyte-macrophage colony stimulating factor [9], interleukin 8 [10], and interleukin 1 [11,12] have each been reported to possess such activity. The second approach is to directly purify HRF(s) from activated supernatants of the cells in question, and identify or characterize the purified protein(s). The former approach can identify new functions of purified cytokines which may or may not be sufficient in quantity or potency to account for the histamine releasing activity of an activated cell supernatant. Nevertheless, antisera can be used to deplete supernatants of suspected cytokines to determine their contribution to the total activity seen. The latter approach identifies those factors responsible for the activity in question by purifying and characterizing them from cell supernatants. In this manuscript, I will review the data accumulated thus far on the characterization of HRF(s) and focus particular attention on one we have purified from supernatants of mononuclear cells and platelets. We demonstrated that it is closely related to connective tissue activating peptide III (CTAP III) and neutrophil activating peptide-2 (NAP-2), which were originally described as derivatives of the α granules of platelets.

Cytokines and Histamine Release

Interleukin 1 was reported to cause a dose response release of histamine from basophils in about one-third of subjects tested. The responsive individuals appeared to be those with particularly high percentage of histamine release when tested with anti-IgE. The dose response curve, however, required an amount of interleukin 1 that was many hundreds-fold greater than its dose response range in causing fever, activating endothelial cells, or as a participant in activating T lymphocytes. Thus, histamine release is not likely to be a physiologic function. Another study found that preincubation of basophils with low doses of interleukin 1 did not cause

histamine release, but potentiated histamine release when the cells were subsequently stimulated with other secretagogues [13]. Thus, interleukin 1 (and a variety of other factors) may contribute to priming of basophils. Since IL-1 is a monokine (monocyte derived) which shares many functions with other monokines such as tumor necrosis factor α (TNF α) and interleukin 6, we also tested these two cytokines for histamine releasing activity. These were consistently negative when assayed over a wide dose range.

We have reported that interleukin 3 can cause histamine release from human leucocytes (basophils) (Tab 1) and the dose response range was between 0.4 and 400 pg/ml which is within its physiologic range [9]. The kinetics of release was very slow relative to anti-IgE or C5a stimulation of basophils and required eight minutes of incubation to detect any release, and thirty minutes to reach a plateau. Two other studies have confirmed the ability of IL-3 to cause histamine release although the three studies differ in the reported dose response range and the percentage of subjects showing a response [14,15]. We reported that about two-thirds of subjects tested had significant (>5 percent) histamine release within the dose range tested and these included atopic and non-atopic individuals. The atopic individuals were more responsive in terms of number of positive assays and magnitude of histamine released [9,14]. The study of Alam et al [15] reported only a small number of responders, each of whom was an atopic individual. Of particular interest is the evidence that low concentrations of IL-3, insufficient to cause basophil histamine release, can prime cells and markedly augment their responsiveness to other secretagogues including anti-IgE, C5a, and interleukin 8 [16]. Concentrations of IL-3, as little as 100 pg/ml, are active, therefore, this cytokine may be an important basophil primer in immunologic reactions. Considering these results, it is reasonable to question whether IL-3 could correspond to one of the forms of HRF found when human mononuclear cell supernatants are fractionated. We concluded that IL-3 is not HRF [9] because subjects' responsiveness to HRF did not parallel responsiveness to IL-3, and immunoblotting of IL-3 in mononuclear cell supernatants revealed bands in positions that differed from HRF. Alam et al also concluded that HRF is distinct from IL-3 because the kinetics of histamine release are so different as was subject responsiveness [15].

Granulocyte-macrophage colony stimulating factor (GM-CSF) has many physicochemical properties which resemble those of interleukin 3 including the possibility of shared receptors, although receptors specific for each seem to exist also. It therefore seemed reasonable to check GM-CSF for histamine releasing activity. We found that it was active in some subjects with a slow time course that was indistinguishable from that of IL-3 and a dose response range of 8 to 800 pg/ml. Since IL-3 and GM-CSF are each colony stimulating factors that act on bone marrow stem cells or other poorly differentiated marrow precursor cells, and IL-1 is identical to a marrow stimulating factor known as hematopoietin, it appeared that activation/priming of basophils might be a function of colony stimulating factors in general. We then assayed factors that act on more differentiated cells, have histamine releasing capability, including granulocyte colony stimulating factor (G-CSF) and macrophage colony stimulating factor (M-CSF or CSF-1) but these studies were consistently negative [9].

We have assessed a variety of interleukins, colony stimulating factors, or growth factors that are products of human mononuclear cells and the ones lacking HRF-like activity include IL-2, 4, 5, and 6, (IL-7, 9, and 10 have not been tested), G-CSF, M-CSF, γ interferon, and transforming growth factor β (Tab 1).

Table I.**Cytokines and Histamine Release**

<i>Positive</i>	<i>Negative</i>	<i>Not Studied</i>
Interleukin 3	Interleukin 1 (?)	Interleukin 7
GM-CSF	TNF α	Various Growth Factors
Interleukin 8 (MDNCF or NAP-1)	Interleukin 2	
	Interleukin 4	
	Interleukin 5	
	Interleukin 6	
	G-CSF	
	M-CSF	
	γ Interferon	
	TGF β	

Interleukin 8 is identical to monocyte derived neutrophil activating peptide-1 (NAP-1) and is chemotactic for neutrophils and causes neutrophil activation and secretion [16,17]. Two studies have assessed its ability to cause histamine release from basophils. One study was positive [10] with a dose response ranging from 3×10^{-7} M to 4×10^{-6} M, while the other was negative and found no direct histamine releasing activity [18]. Dahinden et al reported that IL-8 was a potent releaser if the cells were first primed with IL-3 but did not observe release in the absence of IL-3. It was suggested that HRF might represent factors such as IL-8 (or IL-8 itself) which act on cells that are primed (pre-activated) in vitro or in vivo. Thus, the phenomenon would require more than one molecular interaction with the cell to ultimately cause secretion.

Table 1 summarises the histamine releasing capabilities of those factors studied thus far. Certain factors are capable of priming basophils using concentrations insufficient to cause direct secretion. Gamma interferon is the only substance thus far described which primes basophils but has not been reported to cause secretion.

Purification and Characterization of HRF(s)

Lymphophoresis packs (250 ml) were obtained from normal volunteers or patients with multiple sclerosis who were receiving apheresis as a therapeutical trial. The cells were fractionated by Hypaque-Ficoll density gradient sedimentation, low speed centrifugation to deplete platelets, and the cells cultured overnight in streptokinase/streptodornase. The supernatants were then concentrated 15-fold and assayed for HRF activity [2]. HRF was purified by sequential chromatography by HPLC gel filtration, Accell QMA exchange chromatography,

and preparative SDS gel electrophoresis [19]. Gel filtration yields a broad peak between molecular weight 15-40 kd (peak at 20-25 kd) and Accell QMA anion exchange chromatography yields three peaks of HRF activity. The first peak does not adhere to the column, and the second and third peaks are eluted at 0.5 and 0.8 molar acetate buffer respectively. When each of these three peaks are subjected to preparative SDS gel electrophoresis, HRF is purified at molecular weights of 8-12 kd, 15-17 kd, and 35-41 kd respectively (Fig. 1). Each of these eluates were radiolabelled with ¹²⁵iodine, subjected to SDS gel electrophoresis, and radioautograms obtained. Each revealed a single band with no evident contamination even after prolonged exposure time. The 8-12 kd material is typically seen at the bottom of a 12% SDS gel, thus, to improve the resolution we used 15% gels. A single band was again obtained but it appeared quite broad. A replicate gel was not stained but was blotted onto nitrocellulose paper and then stained with amido schwarz. The band was divided into an upper and lower half and each was subjected to amino acid sequence analysis. As shown in Table I, the upper half of the band (designated HRF-1) was virtually identical to CTAP III, an α granule consistent of platelets which stimulates collagen and proteoglycan synthesis by fibroblasts [20]. The lower half of the band (HRF-2) was homologous to the same protein if the sequence alignment begins with amino acid 16. This protein is called NAP-2 since it is a neutrophil activating peptide derived from CTAP III by proteolytic cleavage to remove the N-terminal 15 residues. We found both proteins to be active in causing basophil histamine release in the same dose range as they each activate fibroblasts. (CTAP III does not, however, activate neutrophils and must first be converted to NAP-2.) It is of interest that these proteins are part of a family of factors that are homologous in structure and this includes NAP-1/interleukin 8. We prepared monoclonal antisera to this 8-12 kd form of HRF and exchanged antisera with Dr. William Castor of the University of Michigan who supplied polyclonal antibody to CTAP III. They were completely cross reactive. We then used our monoclonal antibody to isolate the 8-12 kd form of HRF directly from stimulated supernatants in a single step. We isolated pure CTAP III when platelet supernatants were applied to the column and eluted, and we isolated a mixture of CTAP III and NAP-2 when mononuclear cell supernatants were processed in the same fashion.

HRF PURIFICATION PROTOCOL

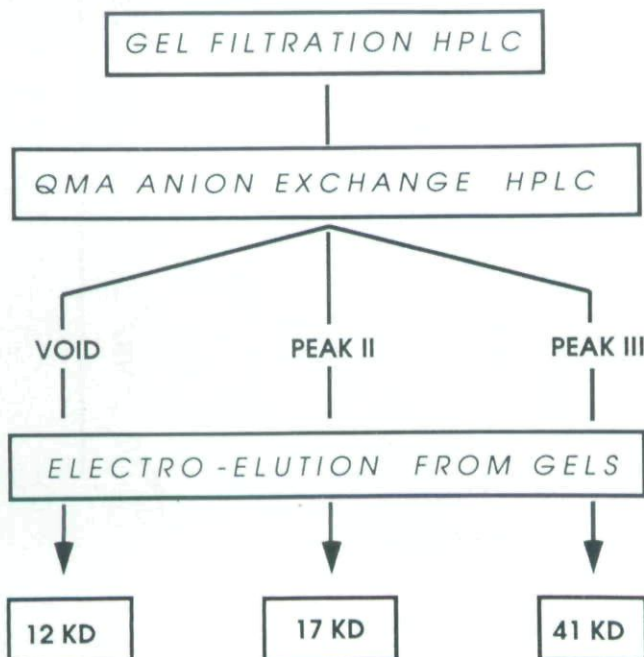


Table II, N-terminal sequence comparison of CTAP III and HRF

Residue No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CTAP III:	ASN-LEU-ALA-LYS-GLY-LYS-GLU-SER-LEU-ASP-SER-ASP-LEU-TYR-ALA-															
HRF 1:	ASN-LEU-ALA-LYS-GLY-LYS-GLU-SER-LEU-ASP-SER-ASP-LEU-TYR-ALA-															
HRF 2:	ALA-															

Residue No.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
CTAP III:	-GLU-LEU-ARG-CYS-MET-CYS-ILE-LYS-THR-THR-SER-GLY-ILE-HIS-PRO-															
HRF I	-GLU-LEU-----MET															
HRF 2:	-GLU-LEU-ARG-----MET-CYS-ILE-LYS-THR-THR-SER-GLY-ILE-HIS-PRO-															

Residue No.	32	33	34	35	36	37	38	39	40
CTAP III:	LYS-ASN-ILE-GLN-SER-LEU-GLU-VAL-ILE								
HRF 1:									
HRF 2:	LYS-ASN-ILE-GLN-SER								

Platelet contamination of mononuclear cell preparations becomes a major issue in interpreting all HRF data from these cells. Most prior publications have not carefully assessed platelet contamination and have assumed that SK/SD or Concanavalin-A stimulation of cells will be selective for the mononuclear cells. We found that removal of 99% of platelets from a mononuclear cell preparation yields a platelet/mononuclear cell ratio of 1:1. Thus, significant platelets remain. Using Percoll gradients we have been able to reduce platelet contamination by a hundred-fold which will assist in distinguishing platelet-derived HRF from mononuclear cell derived HRF.

The 15-17 kd and 35-41 kd forms of HRF have not been characterized further, however, they are not reactive with the monoclonal antibody to the 8-12 kd HRF and likely represent different gene products. We have tried to obtain an amino acid sequence on each of these and find no free N-terminus suggesting presence of a blocked protein. Studies are in progress to digest the proteins, isolate and sequence an internal fragment, and use the sequence to prepare an oligonucleotide probe. Our goal is to obtain a complete sequence by cloning the gene.

Interaction of HRF with basophils

An intriguing series of articles have suggested that HRF (or one of the forms of HRF) acts on basophils via surface bound IgE and include HRF derived from alveolar macrophages [21], platelets [4], and nasal washings [22]. These data are based on the following observations: 1) Desensitization of basophils with anti-IgE markedly diminishes responsiveness to HRF while HRF desensitization reduces responsiveness to anti-IgE. 2) Acid stripping basophils of IgE so as to eliminate secretion by anti-IgE (but not by other secretagogues such as Con A), reduces responsiveness to HRF. 3) There is great variability in response to IgE and about half of atopic subjects respond to it, while non-atopics are generally unresponsive [23]. 4) Elution of IgE from the cells of responders and incubation of that eluate with acid-stripped cells of non-responders, converts the latter to a responsive cell. The responder IgE is called IgE (+) and the non-responder IgE is called IgE (-). Thus, a form of IgE heterogeneity is thought to be defined by responsiveness to HRF and preliminary evidence suggests that this may be accounted for by variability in carbohydrate content.

It is also clear that there are forms of HRF that do not work via IgE and this would include CTAP III/NAP-2 as well as any of the known cytokines. It is possible, however, that the 15-17 kd and/or 35-41 kd forms of HRF might correspond to the form that fulfills the aforementioned criteria suggesting a requirement for surface bound IgE. Because of these observations, we considered the possibility that the low affinity IgE receptor (CD 23) derived from B-lymphocytes or platelets might represent one form of HRF and would obviously bind to IgE. However, antisera to CD 23 did not react with any form of HRF and purified low affinity receptor did not have HRF-like activity [7]. In fact, studies demonstrating a need for basophil IgE for HRF responsiveness have yet to demonstrate any direct interaction of any form of HRF with IgE. We have attempted to fractionate HRF by affinity chromatography using sepharose-bound IgE myeloma proteins, and presented evidence to suggest that one of the myeloma tested would bind a portion of HRF present in an unfractionated supernatant but the material eluted from the columns has not been further characterized [24].

Concluding Comments

There are three situations relevant to allergic diseases in which HRF(s) may have an important role. The first is the late phase reaction in which cellular infiltration and mediator release occurs at the site of an IgE-mediated reaction to cause a subacute or chronic inflammatory reaction. With a single antigen challenge, the late phase can last from 3-12 hours and with continual or continuous challenge, it can theoretically last months as may occur in seasonal allergic rhinitis or extrinsic asthma. There is infiltration with neutrophils within the first few hours, followed by eosinophils, basophils, and mononuclear cells, and there is evidence that these cells are activated and secretory. Further, there is release of histamine during the late phase as assessed in nasal washings of antigen challenged patients with allergic rhinitis, bronchoalveolar lavage fluids, and plasma histamine levels during late phase asthmatic reactions, and protracted histamine release in cutaneous reactions as assessed by antigen challenge of induced blister sites. The source of histamine is likely basophils and/or mucosal mast cells in the respiratory tract and cutaneous mast cells and perhaps basophils in skin. At this stage, the reaction appears to no longer be IgE-dependent, thus a mechanism by which histamine (and other basophil/mast cell products) is released is uncertain. HRF represents a likely candidate and such activity has been observed in cutaneous late phase reactions [25].

A similar argument can be made for the cell-cell interactions that are presumed to occur in atopic dermatitis in which there is diffuse infiltration with CD4(+) T lymphocytes, some monocytes, and there is mast cell degranulation. Although associated with elevated IgE levels, the nature of any IgE-dependent reaction is unclear in terms of an identifiable antigen, although foods seem to have an exacerbating role in about 15% of children. HRF has been shown to be produced spontaneously upon culture of mononuclear cells derived from patients with atopic dermatitis who are hypersensitive to foods, and the HRF appeared to be IgE dependent [26]. Our own studies of chronic urticaria leads to a similar speculation and represents the original reason my laboratory sought factors such as HRF. The lesion is characterized as a non-necrotizing perivascular infiltrate with mononuclear cells (50% T lymphocytes, 20% monocytes, 20% identity unclear, but not B cells) and a 9-fold increase in mast cells relative to normal skin. This reaction is not IgE-mediated and represents some variation of a cellular response in which there is mast cell degranulation that seems dependent on the surrounding mononuclear cells. In this instance, we proposed that one or more factors derived from T lymphocytes or monocytes activate cutaneous mast cells [27].

The disorders in which HRF might be relevant should not be confined to "allergic" diseases. For example, mast cells have been shown to proliferate about erosive lesions seen in sera (+) rheumatoid arthritis and we have shown that synovial mast cells respond to HRF [28]. In addition, early lesions of scleroderma are characterized by a lymphocytic infiltration and degranulated mast cells, thus, there may be an important role for mast cells in the regulation of connective tissue (collagen) deposition [29]. In each instance, HRF may be among the molecules causing mast cell degranulation.

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