

IgE-Dependent Histamine-Releasing Factors

A Brief Review¹

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Abstract. A cytokine, termed histamine-releasing factor (HRF) and produced by many cell types, has become the focus of research by many investigators due to its potential importance as a stimulus in chronic inflammation. We are producing and characterizing an HRF which causes IgE-mediated histamine release from human basophils. Following extensive purification procedures, the molecule will be sequenced and synthesized. A functional heterogeneity of IgE molecules was revealed by these studies. We are currently producing IgE antibody in vitro and testing the hypothesis that differential glycosylation is the basis for the heterogeneity. Knowledge of the structures and interactions of these molecules should advance our understanding of allergic and more chronic diseases.

In 1979, Thuesen et al. [1] were the first to report a histamine-releasing activity, subsequently termed histamine releasing factor (HRF), from cultured human mononuclear cells. Since that time, HRF has been reported from a variety of cell sources including alveolar macrophages, platelets, vascular endothelial cells, B and T lymphocytes, the U937 monocyte/macrophage-like cell line, and the RPMI 8866 B cell line [2-5]. Furthermore, HRF has been found in in vivo fluids from nasal lavages, late-phase blister fluids, late-phase nasal lavages and bronchoalveolar lavage fluids [6-9]. All of these HRF induce noncytotoxic calcium-dependent histamine release from human basophils.

The exact mechanism of HRF-induced histamine release has been proposed by our laboratory to be IgE-dependent [6, 10]. This has recently been confirmed by Alam et al. [9]. That HRF interacts with cell-bound IgE was approached by two types of experiments: desensitization and removal of surface

IgE from basophils [11, 12]. First, basophils were desensitized by incubating them with anti-IgE in the absence of calcium. Such treatment renders the cell unresponsive to subsequent stimulation by any other IgE-mediated stimulus in the presence of calcium [13, 14]. Desensitization with anti-IgE ablated subsequent release with each of our HRF and desensitization with HRF blocked the response to anti-IgE, strongly suggesting that HRF acts via cell surface IgE. The second type of experiment that supports the IgE dependence of our HRF involves removal of IgE from the basophil surface with lactic acid [12]. HRF does not cause histamine release from cells treated with lactic acid. A response can be restored with only IgE molecules from individuals who respond to HRF. Thus, we uncovered a heretofore unappreciated functional heterogeneity of IgE. We have designated the IgE which binds HRF as 'IgE⁺'.

In an effort to define the responding population, 64 atopic patients were compared to 17 skin-test-negative, normal subjects with respect to the ability of their basophils to release histamine to HRF. Fifty percent of atopics responded, whereas none of our normal donors responded [15]. Since HRF is found in

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vivo in late-phase nasal lavages and late-phase skin blister fluids, we asked the question: Is there a link between basophil responsiveness to HRF and the late-phase reaction? In a nasal challenge study of patients with known late-phase reactions, there was a significant correlation between the basophil histamine release to HRF and the severity of late symptoms in those patients experiencing a late-phase reaction [16]. Since, as mentioned, only certain donors respond to HRF, the link between HRF and the late-phase reaction may explain (1) why only a subset of individuals experience a late-phase reaction (i.e. only those with IgE⁺ which can bind HRF), and (2) why certain patients experience more severe allergic respiratory disease. Some of the best clinical data supporting the relevance of HRF come from the work of Sampson et al. [17]. They showed that spontaneous production of an IgE-dependent HRF in food-allergic children with atopic dermatitis was abolished with a strict food elimination diet.

Ongoing experiments in our laboratory involve purifying HRF in order to understand the function of this cytokine and decipher the molecular basis and importance of this apparent IgE heterogeneity. It is well recognized that HRF from mononuclear cell supernatants, nasal lavages and from the U937 cell line are very heterogeneous. Even after a Sephadex G75 sizing column, the nasal-lavage-derived HRF has a large peak of histamine-releasing activity with a distinct shoulder. Because a large constant source of HRF is necessary in order to purify this cytokine, we are currently using the U937 monocyte/macrophage-like cell line which produces HRF. We have developed a purification scheme for the IgE-dependent U937 HRF. The cytokine is generated by growing the cells in RPMI without phenol red for 24 h. The resultant supernatant is concentrated and placed on a Sephadex G75 column in physiological Tris buffer. The HRF activity elutes in a broad peak between 15 and 30 kilodaltons. The fractions containing HRF activity are concentrated and placed on an anion-exchange MONO-Q column in Tris buffer and eluted with an increasing salt gradient. HRF activity is found in 3 distinct regions – from 0 to 0.35 M sodium chloride; between 0.4 and 0.6 M sodium chloride, and finally from 0.65 to 1 M sodium chloride. The IgE-dependent HRF is found in the second peak, 0.4–0.6 M sodium chloride. This fraction is next concentrated and placed on a reverse-phase high-performance liquid chromatographic C4 column in the presence of 0.1%

trifluoroacetic acid (TFA) and eluted with an increasing acetonitrile gradient.

The HRF activity is reproducibly found in the 65% acetonitrile fraction. After the reverse-phase column, this IgE-dependent HRF retains biological activity. It should be noted that this IgE-dependent HRF has a chromatographic pattern distinct from an HRF homologous to connective tissue activating peptide III, purified by Baeza et al. [18]. Our future plans include refining this purification scheme using a large amount of starting material as well performing Western blots to document IgE⁺ binding. Once this is accomplished, we will transfer the material to Immobilon for direct amino acid sequencing.

An obvious question arises. Is HRF a known cytokine? To date, despite the fact that some cytokines, notably human recombinant interleukin-3, do cause basophil histamine release, their mechanism of release does not resemble that of HRF-induced histamine release [19–22]. Thus, HRF does indeed appear to be a unique cytokine.

The other major interest of our laboratory is to define the nature of IgE heterogeneity at the molecular level. Several known observations have allowed us to focus on a particular mechanism. There is only one gene for the Fc region of human IgE [23]. This would suggest that heterogeneity might occur posttranslationally. Furthermore, IgE from ND myeloma has 30% more carbohydrate than does PS myeloma [24]; therefore, there is known differential glycosylation of the IgE molecule. Lectins are known to cause histamine release by cross-linking carbohydrates on IgE [25]. Also, in data we have published in abstract form, lentil lectin, at a concentration of 5 µg/ml, differentiates IgE⁺ and IgE[−] [26]. Therefore, we hypothesize that the difference between IgE⁺ and IgE[−] is most probably at a posttranslational level, in particular, differential glycosylation.

Based on the method of Lohman and Halonen [27], we have purified polyclonal IgE from human serum. This was not only laborious but also yielded small amounts of purified IgE. With the availability of human recombinant interleukin-4, the production of IgE in vitro became feasible. In addition, based on the work of Sarfati et al. [28], many investigators have been able to produce large quantities of IgE in the test tube. We asked the question: if IgE can be produced in large quantities in vitro, can IgE⁺ and IgE[−] be produced in vitro? Several definitions are important to this question. (1) IgE⁺ donors are those who have

greater than 10% histamine release when stimulated with HRF. (2) IgE⁺ is that IgE which passively sensitizes lactic-acid treated basophils and renders them responsive to HRF. We are currently producing IgE⁺ and IgE⁻ in vitro using human recombinant interleukin-4 and manipulating the culture conditions with carbohydrate-processing inhibitors in order to test our hypothesis that differential glycosylation is the basis for the heterogeneity.

Thus, the discovery of HRF and the observation of a functional heterogeneity of the IgE molecule have added additional complexity to allergic disease. However, understanding these complex relationships should lead to a better ability to control the allergic response and the severity of chronic inflammation.

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