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Histamine-releasing Factors and Heterogeneity of IgE

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Abstract. The duration and severity of the allergic response are variable. Even though antigens are rapidly cleared from the individual, an acute allergic response is frequently followed by a recrudescence of symptoms hours or even days after the initial exposure. Experimentally, the cellular infiltrates and mediators released during this late response resemble those associated with chronic inflammatory disease. Although basophils are present in this late reaction, the stimuli for their activation remain unknown. A heterogeneous group of unique cytokines called histamine-releasing factors (HRF), discovered over a decade ago, may well play a role in stimulating basophils during this late-phase reaction. These factors have been reported from a variety of cell sources including alveolar macrophages, platelets, vascular endothelial cells, B and T lymphocytes, mononuclear cell cultures, the U937 monocyte/macrophage-like cell line and the RPMI 8866 B cell line. These ubiquitous factors cause non-cytotoxic, calcium-dependent mediator release from human basophils *in vitro* and are also present and active *in vivo*. Purification attempts have revealed that HRF exists in at least three forms, based on molecular weight. In our hands, the mechanism of mediator release by one of the forms of HRF is IgE dependent. Since only about 50% of allergic donors' basophils respond to HRF, a heretofore unappreciated heterogeneity of IgE was revealed. The presence of HRF has been shown to correlate with severity of allergic disease in children with food allergies, with symptoms in the late-phase response in adults and with severity of the allergic response to an inhaled antigen. Thus, the study of HRF has evolved over the last decade and may lead to better understanding of the complex allergic response.

Introduction

A century ago Blackley [15] noted that exposure of an allergic individual to an antigen produced an immediate response followed by a late reaction. Interest in

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this late reaction resurfaced when Pepys et al. [51] noted dual skin reactions in patients with allergic bronchopulmonary aspergillosis. The deposition of IgG, IgM and complement components in the skin during the late reaction as well as the time course of development suggested an Arthus type reaction, i. e., IgG-mediated inflammation. It was not until Dolovich et al. [19] demonstrated late cutaneous reactions after injections of anti-IgE that IgE antibody was recognized as important in the pathogenesis of the late-phase reaction (LPR). Soon thereafter, definitive evidence that IgE antibody was involved in the LPR was provided by Solley et al. [62] in passive-transfer experiments. More recently, Larsen and co-workers have shown that this late response occurs in rabbits who make only IgE antibodies in response to antigens [14, 60].

The characteristics of the human response to antigen administration involves first an immediate reaction, occurring within 5–15 min. Subsequently, if the dose of antigen used is sufficiently high, most individuals will develop a recrudescence of symptoms within 3–11 h after antigen challenge [19]. This late response is characterized by edema and erythema in the skin [62] and by increased resistance to airflow in the nose and lungs [45]. It has recently been appreciated that the late reaction is a more appropriate model of allergic inflammation than is the acute response to antigen. Microscopic evaluation of this response in the skin reveals cellular infiltrates similar to those found in chronic inflammatory reactions [21, 23]: eosinophils, neutrophils, and variable numbers of basophils at the site of the lesion [21]. Our laboratory has reported that basophils also appear during the antigen-induced LPR in skin chambers [17] and in the nose [12]. More recently, the histamine-containing cells in bronchoalveolar lavage have been identified as basophils and not mast cells [27]. Alcian blue-positive cells were increased 15-fold in the antigen verses the saline sites in bronchoalveolar lavage fluids 19–22 h after ragweed-antigen challenge via a wedged bronchoscope in allergic, asthmatic subjects. Of these alcian blue cells, 96% were basophils as defined morphologically and functionally. These data would suggest that the basophil plays a crucial role in this response.

The pathogenesis of this late reaction, however, is quite complex and poorly understood. Perhaps an initial event in the human response to antigen is mast cell and/or basophils degranulation and release of mediators which in turn attract inflammatory cells. The basophils and eosinophils which infiltrate are activated or primed to be hyperresponsive to stimuli [46]. Hours after antigen bronchoprovocation, elevated levels of histamine and neutrophil chemotactic factor have been found in the blood [20, 48], and using an *in vivo* model of intranasal antigen challenge, our laboratory has demonstrated that mediators such as histamine, kinins, leukotrienes, and N- α -P-tosyl-L-arginine-methyl ester (TAME)-esterase activity are elevated in nasal washes coincident with symptoms in individuals experiencing a LPR [18, 46, 47]. It has been shown, both in the skin and the nose, that this late mediator release is not due to continued antigenic stimulation. Additionally, Bochner et al. [16] have shown that interleukin 1 (IL1), a known promotor of cellular recruitment and a cytokine which potentiates mediator release, is released locally during the cutaneous allergic LPR. Thus, although mediator release clearly occurs *in vivo* during the late response to antigen, the stimulus for this activation remains unknown.

In studies in our laboratory which were designed to answer this question, we found that nasal lavages obtained during the LPR contained histamine-releasing factors (HRF) which had characteristics similar to those from cultured human alveolar macrophages [58] and those in blister fluids during the LPR in the skin [69]. In addition, the macrophage-like cell line, U937, also produces a similar factor in large quantities [53]. Our hypothesis is that the macrophage-derived and the nasal HRF are related molecules, and that this family of molecules is an important stimulus for basophils during the late phase of IgE-mediated reactions. Studies of these HRF will allow us to understand better the pathophysiology of late IgE reactions and the nature of cell-cell interactions.

History

Several investigators have reported the *in vitro* production of human-derived lymphokines that release histamine from human basophils. Thueson et al. [65, 66] were the first to report such a factor, which they called histamine-releasing activity (HRA), from cultured human mononuclear cells. HRA, subsequently termed HRF, was further characterized by Lett-Brown et al. [35, 36] and was found to be heterogeneous with two molecular weight species of 15,000 and 50,000. Subsequently Bamzai and Kretschmer [11] described a histamine-release-enhancing factor from phytohemagglutinin (PHA)-stimulated mononuclear cells from asthmatic patients, and Ida et al. [29] found a histamine-enhancing factor produced by lymphocytes from BCG-positive donors following incubation with purified protein derivative. Furthermore, these latter authors showed that this enhancing factor was not interferon. Alam et al. [2] have also reported HRF production by cultured mononuclear cells from asthmatic patients. Their factor was released spontaneously, but increased amounts were obtained following specific antigenic stimulation. The release of histamine by this factor *in vitro* was not dependent on the sensitivity of basophils to the stimulating antigens, since they originally reported that histamine release was also observed with basophils from non-allergic subjects. In their studies, lymphocytes from normal controls also produced an HRF, but only after stimulation with PHA. That HRF is produced by cultured human lymphocytes has subsequently been confirmed by Sedgwick et al. [59], Ezeamuzie and Assem [22] and Kaplan et al. [33]. Strickland et al. [63] reported that an HRF was produced by human T lymphocytes if peripheral blood lymphocytes were stimulated with streptokinase/streptodornase, whereas, if the cells were stimulated with an antigen, such as house-dust mite, the HRF was produced by B lymphocytes and monocytes.

Sources

Over the last 5 years there has been increased interest in the sources of HRF and vigorous efforts have been aimed at the purification of these ubiquitous molecules. In addition to cultured mononuclear cells, 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. Furthermore, HRF has been found in *in vivo* nasal lavages, late-phase blister fluids, late-phase nasal lavages and bronchial alveolar lavages. In addition to being present in *in vivo* fluids, several investigators have shown that some HRF are active *in vivo*. Thueson et al. [67] reported that his HRA caused wheal and flare responses in monkeys. Alam and Rozniecki reported that their lymphocyte supernatant induced bronchoconstriction and a wheal and flare in asthmatic patients which was not due to histamine in the supernatants [1]. Platelets stimulated with thrombin produce a factor, distinct from platelet factor 4, that induces histamine release from human basophils [49]. Weiss et al. showed that 9 of 12 extrinsic asthmatic subjects had immediate wheal and flare reactions to intradermal administration of autologous platelet-derived HRF, while none of 5 normals, 5 intrinsic asthmatics or 5 allergic rhinitics responded [70].

Mechanism of HRF-induced Mediator Release

All of the factors mentioned above induce non-cytotoxic histamine release from human leukocytes that is temperature dependent, and requires metabolic energy and calcium. Our laboratory has proposed an IgE-dependent mechanism of histamine release by the subset of HRF that we study [37, 39]. This proposal that the ability of HRF to induce mediator release by an interaction with cell-bound IgE was based on the following data. The kinetics of HRF-induced histamine release resemble those of IgE-mediated stimuli in that they are slow, and the temperature optimum is 37 °C. In contrast, C5a or f-met peptide cause very rapid release [26, 61]. Stronger evidence that the several HRF we study appear to induce release by cross-linking IgE on the surface of the cell has been obtained using two types of experiments. First, basophils were desensitized by incubating them at 37 °C with optimal concentrations of anti-IgE in the absence of calcium [52]. Such treatment renders basophils unresponsive to the subsequent addition of anti-IgE or any other IgE-mediated stimulus, in the presence of calcium [32, 42]. In contrast, release caused by a wide variety of other signals such as anaphylatoxins, f-met peptide, hyperosmolarity, calcium ionophore and the phorbol ester, TPA, is not affected or is increased [24, 57]. Desensitization with anti-IgE ablated subsequent release with each of our HRF, strongly suggesting that they are interacting with cell surface IgE (Fig. 1). The second type of experiment which supports the IgE dependence of our HRF involves removal of IgE from the basophil surface with lactic acid [54]. This procedure leads to removal of 80%–90% of the IgE from the surface of the cell. HRF from various sources including macrophages, platelets, blister fluids, nasal lavages and the U937 cell line did not cause histamine release from cells treated with lactic acid.

In this context, it is important to note that only certain donors' basophils respond to HRF [39, 58], a restriction also true for the histamine-releasing activity described by Kaplan et al. [33]. In fact, as will be noted later, most of the individuals who respond to our HRF are atopic or atopic asthmatic subjects. We hypothesized, therefore, that there might be a difference among IgE molecules

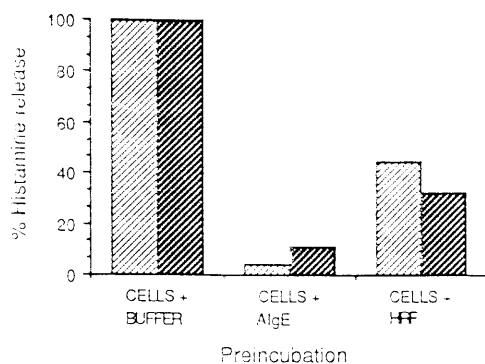


Fig. 1. Effect of desensitization of basophils with two stimuli: anti-IgE (AlGE, 0.1 μ g/ml) and histamine-releasing factor (HRF, from a pool of nasal lavages, 1/21 dilution). The results are presented as the mean histamine release, expressed as a percent of the release induced from control cells of five experiments. Control release for anti-IgE ranged from 38% to 64%, and for HRF from 16% to 62%. $\square$ AlGE; $\blacksquare$ HRF

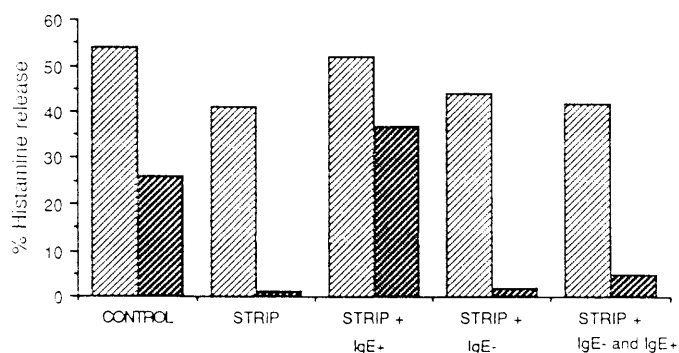


Fig. 2. Effect of saturating IgE receptors with non-responder IgR (IgE⁻) on the ability of responder IgE (IgE⁺) to interact with HRF. The results are expressed as the mean of five experiments. Cells were incubated with buffer (CONTROL) or lactic acid (0.1 M, pH 3.9 for 5 min, designated STRIP). Lactic acid-treated cells were passively sensitized with IgE⁺ (300 ng/ml) or IgE⁻ (25 μ g/ml when IgE myeloma was used or 2,500 ng/ml when polyclonal penicillin-specific IgE was employed). Lactic acid-treated cells were also sensitized with a mixture of IgE⁻ and IgE⁺. All cells were washed with buffer and challenged with anti-IgE (AlGE, 0.1 μ g/ml) or HRF (1/21 dilution). $\square$ AlGE; $\blacksquare$ HRF

from atopics and controls. Passive sensitization of lactic acid-treated basophils with serum from HRF-responsive subjects rendered these acid-treated cells responsive to HRF as assessed by histamine release. In contrast, serum from non-responders, at the same total IgE concentration, did not restore the ability of HRF to induce histamine release. Furthermore, preincubation of acid-treated cells with serum from non-responders inhibited the ability of responder serum to bind to the basophil and interact with HRF (Fig. 2). Moreover, serum from responders converted acid treated, non-responder basophils to responders: i.e., non-responders' basophils following incubation with a responders' serum now released histamine when stimulated with HRF. These data indicate that it was the serum

source, not intrinsic properties of the basophil, which conferred sensitivity to HRF. Also, this transfer of sensitivity did not appear to be an artifact of the acid treatment of the cells, since basophils with naturally occurring unoccupied IgE receptors were rendered responsive when sensitized with a responders' serum. In all instances, using responders' or non-responders' serum, the release of histamine with anti-IgE as the stimulus was unaffected. These results demonstrated that these HRF interact with IgE molecules of some but not all donors. Thus, there is a functional heterogeneity of IgE. We have tentatively designated the IgE which binds HRF as "IgE⁺" (therefore, by definition the remaining IgE molecules are IgE⁻).

To document that IgE⁻ bound effectively to the high-affinity Fc ϵ R1 on the surface of the basophil, we measured the total number of IgE molecules per basophil (Fig. 3). Cells were treated with lactic acid and then passively sensitized with IgE⁺, IgE⁻, or a mixture of IgE⁺ and IgE⁻. As expected, IgE⁺ allowed significant release by HRF, while IgE⁻ inhibited the ability of IgE⁺ to support release. Aliquots of cells from each reaction condition were stripped with acetate buffer pH 3.7. This treatment removes virtually all of the IgE molecules, but leaves the cells unable to be passively sensitized [31]. When the total IgE molecules per cell were measured by Radio Immuno Sorbent Test (RIST), IgE⁻ as well as IgE⁺ were equally effective in binding to the high-affinity IgE receptor. Therefore, IgE⁻ binds to cells, but does not interact with HRF.

To prove definitively that it was the IgE in the serum and not an additional serum factor that conferred responsiveness to HRF, we affinity-purified polyclonal IgE from an IgE⁺ donor using a modification of the method of Lohman and Halonen [38]. Serum was subjected to two sequential ammonium sulfate precipitations (25% and 55%). The 55% precipitate was resuspended, dialyzed and placed on a concanavalin A column. IgE was eluted with 0.2 M α -methyl mannoside,

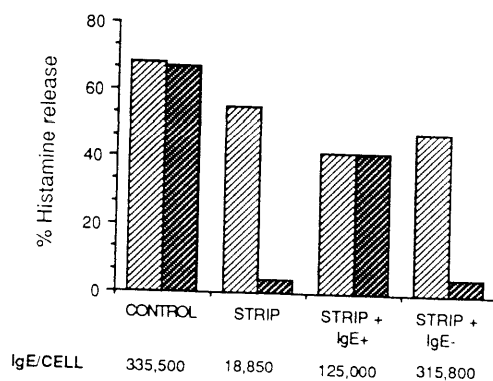


Fig. 3. Determination of the number of IgE molecules per cell before and after passive sensitization with responder IgE (IgE⁺) or non-responders IgE (IgE⁻). Cells were treated with buffer (CONTROL) or lactic acid (STRIP). Lactic acid-treated cells were then sensitized with responder serum (IgE⁺, 350 ng/ml) or non-responder serum (IgE⁻, 2000 ng/ml). A portion from each condition was saved for histamine release using two stimuli: AlGE (anti-IgE, 0.1 μ g/ml) or HRF (dilution 1/21). The remaining cells from each condition were treated with acetate buffer, pH 3.7, and the total IgE content of the supernatant was determined. The total number of IgE molecules per cell was calculated.

▨ AlGE; ■ HRF

the sugar which binds to concanavalin A. The fraction containing IgE was placed on a Protein A sepharose column to remove IgG. Finally, the IgE was eluted from an anti-IgE column with glycine buffer. SDS-PAGE (10% acrylamide gel, non-reducing conditions) showed approximately a 95% purification of this polyclonal IgE. There was minimal contamination with IgG and albumin. The calculated yield was 25%. This affinity-purified IgE was subjected to high-pressure liquid chromatography (HPLC) using two columns in series (TSK 4000 and 3000) in phosphate-buffered saline and had a molecular weight less than 300 000, but greater than 160 000. This affinity-purified IgE⁺ was next used to passively sensitize lactic acid-treated cells and restored release by HRF in a dose-dependent fashion from 255 to 32 ng/ml of IgE.

Just as HRF are heterogeneous, the mechanisms by which they cause release are probably varied. While it is somewhat unsettled at the moment, it seems likely that there are two major mechanisms of release, one of which is dependent upon IgE⁺ [39], while the other does not seem to involve IgE. The question has been difficult to resolve because the original description of the cytokine, HRF from human mononuclear cells, stated that it caused a non-IgE-dependent histamine release from human basophils [65]; their more recent data clearly demonstrate that this cytokine causes IgE-dependent release (personal communication, paper submitted). They showed that the response to mononuclear cell-derived HRF is reduced following lactic acid treatment of basophils, and that the response to HRF is restored with IgE from allergic donors, but not with PS myeloma IgE. This is the first confirmation of our data indicating an IgE-dependent mechanism for HRF-induced histamine release. Another group actively purifying HRF from mononuclear cell culture supernatants showed that a portion of their HRF bound to an ADZ myeloma IgE affinity column, but not to a PS myeloma IgE affinity column [7]. This group has not specifically addressed the mechanism of release. These differences will no doubt be definitively settled when purified HRF is available.

Purification

If progress is to be made in this area, the nature of HRF must be determined. Active efforts toward purification of HRF are currently being pursued by each of the laboratories interested in this novel cytokine. In everyone's hands, the HRF from mononuclear cell supernatants, nasal lavages or the U937 cell line are very heterogeneous. In our hands, U937-derived HRF has a broad peak of activity which elutes from a Sephadex G75 column with an apparent molecular mass between 15–30 kDa. We have further purified this activity on an anion exchange column with an increasing salt gradient and found three distinct areas of activity. Currently, we are focusing on the one particular area that has an IgE-dependent mechanism of histamine release utilizing reverse-phase HPLC. The greatest progress in purification, thus far, has been accomplished by Baeza and Kaplan [8]. They reported that streptokinase/streptodornase-stimulated mononuclear cell supernatants containing HRF activity contain multiple forms with molecular masses ranging from 12–19 kDa. They further characterized a 30 kDa HRF activity

from HPLC gel filtration on a Accell-QMA anion exchange HPLC column with an increasing salt gradient. HRF activity was recovered in three distinct areas: the effluent, at 0.5 M NaCl and a peak preceding the 1.0 M NaCl wash. These three distinct peaks were subjected to SDS gel electrophoresis and revealed heterogeneous areas of HRF activity with predominant activity in three molecular mass ranges: 11–12 kDa, 17 kDa, and 41 kDa. In two recent abstracts, this group reported that they had successfully prepared a monoclonal antibody that recognizes the lowest molecular weight HRF activity [9]. Utilizing this monoclonal antibody to affinity purify this HRF activity, they found significant sequence homology of this low molecular mass form of HRF with a platelet-derived factor, CTAP [10]. Thus, progress in purification is underway, but, by no means complete at this moment in time.

The Natural Inhibitor

Since immune regulation is often balanced by stimulatory and inhibitory processes, the question arises: is there a naturally occurring inhibitor of HRF activity? Indeed, when human mononuclear cells, known to produce HRF in culture, were cultured by one group in the presence of physiological concentrations of histamine (10^{-10} to 10^{-6} M) an 8–10 kDa factor was produced that inhibited the ability of HRF to cause histamine release. This cytokine, appropriately named histamine-release inhibitory factor (HRIF), was reported to be specific for inhibiting HRF-induced histamine release from human basophils, and did not affect other secretagogues such as allergen, anti-IgE, C5a, concanavalin A or phorbol myristate acetate [4]. The biological activity of HRIF was protease-sensitive, neuraminidase-resistant and relatively heat-stable. To date, further efforts to purify this factor have not been reported. This work, if it is confirmed by other laboratories, could be quite important since this group finds that HRIF production is influenced by immunotherapy, one of the major modalities used to treat allergic disease.

Relationship to Known Cytokines

Since HRF can be derived from mononuclear cell supernatants in culture and established cell lines, such as the U937 line, and has a molecular weight similar to many known cytokines, a question was whether HRF was a known cytokine. With purification and recombinant techniques several cytokines such as human recombinant IL1 α and β , IL2, IL3, IL4, IL5, IL6, interferon-gamma, granulocyte-colony-stimulating factor (G-CSF), and granulocyte/macrophage-CSF (GM-CSF) are available for testing to see if these cytokines stimulate human basophils to secrete histamine. In one report, IL1 was found to be a histamine-releasing agent in the absence of D₂O, but in our hands it did not have this activity, rather it enhanced IgE-mediated release [44, 64]. It is clear that IL3 causes histamine release from basophils, either in the absence or in the presence of D₂O [28, 41]. This is not surprising, since receptors for IL3 have been found on human basophils using chronic basophilic leukemia cells [68]. It is also clear, from kinetic studies

and the lack of even self-desensitization, that IL3 stimulates basophils in a very different manner from HRF. GM-CSF, in the presence of D₂O, also causes basophil histamine release, but it was not equal to HRF [28]. IL2, IL4, IL5, IL6 and G-CSF do not stimulate basophils to release histamine [5, 28]. Thus, though several known cytokines act as histamine-releasing agents, they appear to be different from HRF.

Clinical Correlates

Since, as previously mentioned, only certain donors' basophils respond to HRF, our laboratory made an effort to define the characteristics of the responding population. Sixty-four atopic patients with a history of seasonal allergic rhinitis and a positive intradermal skin test to the appropriate allergen at < 10 PNU allergen/ml were studied. These patients were compared to 17 non-atopic controls who were skin test negative to a panel of 15 allergens. Sensitivity to our HRF was restricted to atopic individuals: histamine release (defined as greater than or equal to 10% above spontaneous release) occurred with the basophils of 31/64 skin test-positive subjects and 0/17 skin test-negative controls ($P < 0.01$) [40]. There was no correlation between the response to HRF and to anti-IgE. Furthermore, the response to HRF did not correlate with the total serum IgE level.

Knowing that HRF caused basophil histamine release in a subset of allergic donors and that it is found in late phase blister fluids and nasal lavages, we queried whether responsiveness to HRF was an indicator of disease severity. Indeed, in a separate study of 55 ragweed hayfever patients, there was a significant correlation between intensity of symptoms in the LPR and basophil histamine release to HRF ($P < 0.02$) [30]. Furthermore, only basophils from allergic asthmatics, but not non-allergic asthmatics, responded to an IgE-dependent platelet-derived HRF [25]. Additionally, for those allergic asthmatics who responded to this HRF in vitro ($n = 8$), methacholine sensitivity and asthma symptoms were highly correlated ($r = 0.87$; $P < 0.005$) [25].

Not only is responsiveness to HRF associated with disease status, this also is true in some instances for the production of HRF. The best evidence for the latter is found in a study by Sampson et al. [55]. Basophils of food allergic children with atopic dermatitis have a high spontaneous release of histamine and their mononuclear cells in culture spontaneously secrete an IgE-dependent HRF. With strict adherence to a diet that eliminated the offending food allergen for an extended period of time, both spontaneous basophil histamine release and spontaneous production of HRF were ablated. Thus, we believe that the interaction of various HRF with the IgE⁺ molecules on the basophils of responder individuals may represent an important element in the pathogenesis of a variety of inflammatory, allergic disease processes.

Other groups have made observations on the clinical importance of HRF. Alam et al. [3] studied 20 patients with mild to severe asthma. They cultured peripheral blood mononuclear cells for 24 h and correlated the spontaneous production of HRF by these cells with the patients' state of bronchial hyperreactivity as assessed by inhaled histamine. They observed that the magnitude of the spontaneous HRF

production was related to the clinical status of the patient. A highly significant inverse correlation was noted between the spontaneous HRF production by mononuclear cells and the sensitivity of the patient to histamine ($r = -0.86$; $P < 0.001$). A group in Poland reported an even more striking correlation between the change in spontaneous HRF production by mononuclear cells and the change in PC_{20} for histamine ($r = -0.93$; $P < 0.0001$) after 2 years of preseasonal immunotherapy in a group of grass pollen allergic subjects [34]. Thus, if this cytokine, HRF, is as important in the pathogenesis of allergic disease as we have hypothesized, antagonists, whether they be other cytokines or chemotherapeutic agents, may well be of clinical importance.

Heterogeneity of IgE

The nature of IgE heterogeneity, as defined by response to HRF, is presently unknown, and we are working to define the difference at the molecular level. Since it is known that the human heavy chain constant region of IgE is coded for by one gene, a pseudogene, and a processed gene [13], this heterogeneity most likely represents a post-translational event. A logical post-translational modification that could explain this heterogeneity would be differential glycosylation. IgE is heavily glycosylated and is known to contain *N*-linked oligosaccharides. Furthermore, there is a precedent for differential glycosylation of IgE since ND myeloma contains 30% more carbohydrate than PS myeloma [6]. Moreover, differential glycosylation of acute-phase proteins is known to occur within 48 h of culturing a human hepatocyte cell line and can be detected by changes in relative affinity to lectins as assessed by agarose affinity electrophoresis [43].

Efforts to obtain large quantities of IgE⁺ have been hampered for two reasons: first, it is extremely difficult to purify large quantities of IgE⁺ from non-myeloma, allergic patient sera; second, to date, all 15 IgE myelomas tested have been IgE⁻. Recently, it has become apparent that IgE can be cultured in vitro using human recombinant IL4 [50]. This technique became applicable to our work with the report of Sarfati and Delespesse, who showed that manipulation of culture techniques could produce significant quantities (ng/ml range) of IgE [56].

Since IgE can be produced in significant quantities in vitro, it was important to establish whether both IgE⁺ and IgE⁻ can be produced in vitro. If this were so, the biochemical basis of the difference would be easier to define. Two definitions are important to this question. First: IgE⁺ donors are those donors who have >10% basophil histamine release when stimulated with HRF. Second: in vitro IgE⁺ is that IgE (produced from cultured IgE⁺ donor mononuclear cells) which passively sensitizes lactic acid-treated basophils and renders them responsive to HRF.

We have begun to address this question. Peripheral blood mononuclear cells were harvested from the Ficoll Paque interface, washed and cultured in HB 101 media containing 5% fetal calf serum, 10^{-5} M hydrocortisone, penicillin, streptomycin, and L-glutamine; 2×10^6 cells/ml were cultured in the presence of 20 ng/ml of human recombinant IL4 (a gift from Sandoz) for 14 days. At the end of culture, the supernatants are harvested and the total IgE measured by RIST.

The total IgE levels obtained in culture ranged from 100–700 ng/ml. In preliminary experiments, it appears that both IgE⁺ and IgE⁻ can be produced in vitro from the mononuclear cell cultures of the respective donors types.

Additionally, to determine if glycosylation explains the difference in IgE⁺ and IgE⁻, experiments are ongoing using carbohydrate processing inhibitors. Through recent advances in carbohydrate biochemistry, specific inhibitors of normal processing of asparagine-linked oligosaccharides are available for use in cell culture systems without introducing the toxic side-effects common with tunicamycin. These experiments may provide the answer to the basis of IgE heterogeneity. It is likely that exposure to food allergens in the case of Sampson's studies, or to inhalent allergens in our studies, alters the glycosylation of IgE.

Conclusion

Over a decade has past since the initial discovery of HRF. The field has expanded tremendously since then, with exponential growth in the last few years. With purification efforts and recombinant technology, investigators in this field are closing in on this ubiquitous cytokine. Since IgE-dependent HRF are present in vivo, particularly in the LPR, they are likely to participate in the in vivo inflammatory response. Additionally, as has been discussed, the pathophysiology of the LPR, which includes mediator release and cellular influx, more accurately reflects the clinical syndromes that involve chronic inflammation (i. e., asthma and allergic rhinitis) than does the acute reaction. Based on the data presented in this review, as well as the lack of antigenic involvement in the LPR, it is possible to speculate that the cytokine, HRF, is one of the contributing causes to mediator release and thereby stimulates the cellular infiltrate found in the late response, a response which would occur only in those individuals with the IgE, IgE⁺, capable of interaction with HRF. This speculation is strengthened by the fact that late IgE-dependent reactions occur only in a subset of subjects. Further understanding of these HRF may help explain the varying severity of allergic disease.

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