

Original article

Reactivity to IgE-dependent histamine-releasing factor is due to monomeric IgE

Background: IgE-dependent histamine-releasing factor (HRF) can distinguish between IgE⁺ and IgE[−]. In contrast to IgE[−], IgE⁺ sensitizes basophils to release histamine in response to HRF. But we do not know what particular feature distinguishes IgE⁺ from IgE[−]. The objective was to investigate the hypothesis that IgE⁺ is polymeric IgE.

Methods: IgE⁺ plasma was separated by size-exclusion chromatography. The basophil-sensitizing capacity of the fractions was analyzed in response to HRF produced by mononuclear cells.

Results: We showed that monomeric IgE sensitized basophils to release histamine in response to HRF and to house-dust mite, whereas no enhanced reactivity was found in the fractions containing polymeric IgE.

Conclusions: HRF reacts with monomeric IgE, and not (exclusively) with polymeric IgE.

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IgE-dependent histamine-releasing factor (HRF) releases histamine from basophils only in the presence of a special type of IgE called IgE⁺. This is in contrast to IgE[−] sera, which do not sensitize basophils to release histamine in response to HRF. Basophils from 50% of patients with allergic asthma, 29% of patients with nonallergic asthma, and 29% of patients with allergic rhinitis reacted to IgE-dependent HRF produced by mononuclear cells (HRF-mn) (1).

The IgE-dependent HRF cloned by MacDonald et al. (2) is rHRF p23. Although this factor needs IgE⁺ to release histamine from basophils, binding of p23 to IgE has not been detected (3), suggesting that p23 has its own receptor on basophils. This is supported by the observation that the rat basophilic

leukemia cell line expressing functional human Fc ϵ R1 did not release histamine in response to rHRF p23 after sensitization with IgE⁺ (4). These facts indicate that the rHRF activity is not due autoreactivity. Recently, it has also been shown that the signaling pathway of rHRF is distinct from the Fc ϵ R1-mediated pathway used by allergens (5). rHRF p23 turned out to be a protein with multiple functions: besides inducing IgE-dependent histamine release, this protein also enhances anti-IgE-induced histamine release from basophils independently of the type of IgE. Moreover, it induces chemotaxis, calcium flux, and IL-8 production by human eosinophils (3).

Recently, it was shown that thapsigargin and ionomycin can distinguish between IgE⁺ and IgE[−] donors: both agents induced significantly higher histamine release from IgE⁺ donors. No explanation for this enhanced responsiveness has as yet been provided (6). The structural basis of the difference

Abbreviations. HRF: IgE-dependent histamine-releasing factor; HRF-mn: IgE-dependent histamine-releasing factor produced by mononuclear cells; HRA: histamine-releasing activity.

between IgE⁺ and IgE⁻ is unknown. Saxon et al. suggested that IgE⁺ reactivity to recombinant HRF p23 was conferred on basophils by polymeric IgE (American Academy of Allergy, Asthma, and Immunology, Annual Meeting, 1997). One of the IgE isoforms, "tailpiece", which is six amino acids longer than the classic secreted IgE, occurs as a polymer due to a terminal cysteine residue (7, 8).

It is well established that basophils can be stimulated by a combination of two partial agonists. For example, it has been shown that the combination of a cytokine (IL-3, IL-5, or GM-CSF) with thapsigargin (a calcium ATPase inhibitor), which are both incomplete stimuli, is very potent in inducing histamine release from basophils (9). Similarly, HRF might exert a priming effect on basophil histamine release: besides HRF, a special form of IgE (for example, polymeric IgE) would be necessary to achieve full basophil activation.

In this paper, we investigated whether HRF exerts its histamine-releasing activity via monomeric or polymeric IgE.

Material and methods

Materials

The required materials included RPMI (Gibco/BRL, Paisley, UK), fetal calf serum (FCS) (Bodinco, Alkmaar, The Netherlands), streptokinase-streptodornase (SK/SD) (Lederle Laboratories, Pearl River, NY, USA), Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden), HEPES (Sigma Chemicals Co, St Louis, MO, USA), human serum albumin (HSA) (CLB, Amsterdam, the Netherlands), heparin Sepharose CL-6B and Protein A-Sepharose CL-4B (Pharmacia Biotech, Uppsala, Sweden), and AcA34 (Ultragel LKB, Villeneuve-la-Garenne, France). Amicon YM3 (3000 MW) membranes (Amicon, Inc., Beverly, MA, USA) were used to concentrate HRF preparations. *Dermatophagoides pteronyssinus* extract was obtained from ALK (Copenhagen, Denmark). Protein concentrations were determined by BCA protein assay (Pierce, IL, USA).

Plasma samples were obtained, after informed consent, from volunteers from the Department of Plasmapheresis of the CLB. Plasma was defibrinated by recalcification and dialyzed. IgE⁺ plasma no. 151 (1600 IU/ml) and IgE⁻ plasma no. 147 (1400 IU/ml) were positive for IgE to common inhalant allergens. Buffy coats were derived from 1000 ml blood (Haemonetics Plasma Collection System, Haemonetics Corporation, Braintree, MA, USA) from donors from the Department of Plasmapheresis of the CLB.

Methods

Production of HRF-mn. HRF was prepared as described

by Pasmans et al. (1). In short, peripheral blood mononuclear cells obtained by leucapheresis were isolated by Percoll centrifugation and elutriation. The cells (5×10^6 /ml) were cultured for 18 h at 37°C in RPMI containing 5% (v/v) heat-inactivated FCS, 50 µM 2-mercaptoethanol, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 300 µg/ml glutamine, and stimulated by 5.3 µl SK/SD per ml culture supernatant. The conditioned culture medium was concentrated three times, and IgE-independent HRFs were removed by heparin-Sepharose, after which the medium was again three times concentrated. The HRF preparation is referred to as HRF-mn. The concentration of protein of the HRF preparation used in this study was 14 mg/ml.

Histamine-release bioassay. The histamine-release assay was performed as described previously (10) with minor modifications. Basophils from nonatopic donors were enriched (2–5% purity) by Percoll centrifugation. IgE was removed from the surface of the basophils by incubating the cells for 3.5 min with lactic acid buffer (13.4 mM lactic acid, 140 mM NaCl, 5 mM KCl, pH 3.9). A volume of 10 ml of this buffer was added to 1.5×10^8 cells. The cells were sensitized (11) by incubation (37°C, 90 min) with the "sensitization mixture" containing human plasma, 4 mM EDTA, and 10 µg/ml heparin in a total volume of 1 ml. After sensitization, 2 ml HEPES buffer (20 mM HEPES, 132 mM NaCl, 6 mM KCl, 1 mM MgSO₄, 1.2 mM K₂HPO₄, 5.5 mM glucose, and 0.5% (w/v) human serum albumin, pH 7.4) was added to the cells, after which they were washed twice. Before incubation with stimuli, the cells were allowed to recover in HEPES buffer containing 1 mM CaCl₂ for 30 min at 37°C. Incubations (37°C, 60 min) were performed with 250 µl of cells (2.5×10^6 cells) and 50 µl allergen or 100 µl HRF-mn supernatant. HRF-mn and allergen samples were diluted in HEPES buffer containing 1 mM CaCl₂. The reactions were stopped by addition of 750 µl ice-cold 0.9% NaCl, after which the cells were pelleted (3 min at 12 000 g), and 1000 µl supernatant was mixed with 200 µl of 12% (v/v) perchloric acid.

The release of histamine was determined by fluorometric analysis, as described by Siraganian (12). Histamine release was calculated as percentage of the total amount of histamine in the cells, as determined by lysis of the cells by perchloric acid. The results were not corrected for spontaneous release.

Size fractionation of IgE⁺. A volume of 1 ml of IgE⁺ plasma no. 151 was fractionated by size-exclusion chromatography (AcA34, 2 × 28 cm) to separate monomeric from polymeric IgE. The column was equilibrated by HEPES buffer. Fractions of 1 ml were

collected in which the IgM, IgE, and IgG4 concentrations were determined. The concentrations were expressed as percentage of the corresponding Ig present in plasma no. 151.

Immunoglobulin assays: IgG4 RIA. Diluted plasma samples or antibody dilutions (20 μ l per incubation, in duplicate) were incubated overnight with 0.1 mg Protein A-Sepharose (250 μ l). Binding was detected with ^{125} I-labeled anti-IgG4 (MH 164-1M, CLB, Amsterdam, The Netherlands). The amount of IgG4 was calculated with chimeric 2B12-IgG4 (13) as reference reagent.

Total IgE RIA. The amount of IgE was measured as described previously (14). The incubations were performed in duplicate.

Total IgM assay. IgM concentrations were determined by the Behring Nephelometer analyzer II using anti-IgM antibodies (CLB, Amsterdam, The Netherlands).

Results

Histamine release by HRF-mn

The histamine-releasing capacity of HRF-mn was studied with basophils sensitized by plasma no. 151 or no. 147. Fig. 1 shows that no. 151 is IgE⁺ plasma, and no. 147 IgE⁻ plasma. Histamine release of basophils sensitized by plasma no. 151 in response to HRF-mn is very reproducible: with two different basophil donors, the releases were, respectively, $55.1 \pm 5.3\%$ ($n=3$, mean $\pm$ SD) and $58 \pm 2.1\%$ ($n=4$, mean $\pm$ SD), while spontaneous releases were, respectively, $3.1 \pm 0.8\%$ ($n=3$, mean $\pm$ SD) and $1.6 \pm 0.7\%$ ($n=4$, mean $\pm$ SD).

Size fractionation of IgE⁺ plasma

IgE⁺ plasma no. 151 was fractionated by size-exclusion chromatography to separate monomeric and polymeric IgE. IgM (1000 kDa), IgE (180 kDa), and IgG4 (150 kDa) were separated from each other (Fig. 2). The peak of monomeric IgE was present in fraction 40–44; polymeric IgE is expected to elute before fraction 40.

Plasma no. 151 was used as reference to determine the sensitizing activity of fractionated IgE. In Fig. 3, the dose-response curve of basophils sensitized by different amounts of plasma no. 151 is shown in two independent experiments with two different donors.

Four fractions were pooled to analyze the sensitizing capacity of fractionated plasma no. 151. Sensitization of basophils was performed within 15 min after size-exclusion chromatography to minimize the possibility of aggregation. Fig. 4 shows histamine release of basophils in response to HRF-

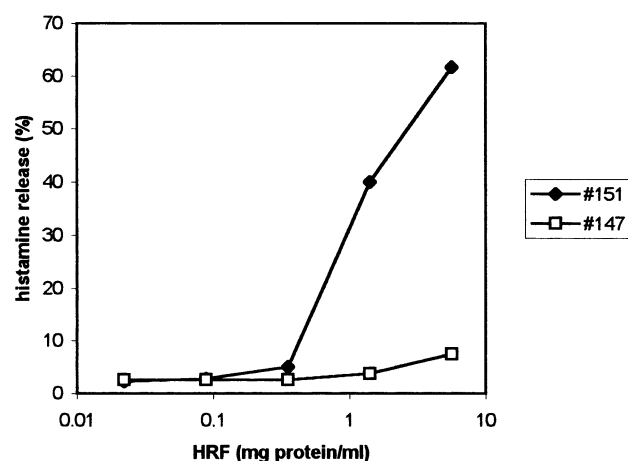


Figure 1. Histamine release of basophils sensitized by IgE⁺ (no. 151) or IgE⁻ (no. 147) in response to HRF-mn.

mn. The sensitizing activity and the concentration of IgE of the fractions are expressed as percentage of plasma no. 151 (Fig. 4). The sensitizing activity of the fractions was also tested with *D. pteronyssinus* extract. The sensitizing activity to both stimuli was coeluted with total IgE, and no enhanced reactivity was found in the fractions preceding monomeric IgE. Fractions eluted before or with IgM did not sensitize. The recovery of sensitizing activity in the fractions was lower than expected on the basis of the IgE yield (Fig. 4).

Size-exclusion chromatography of plasma no. 151 and sensitization of basophils with the fractions were repeated with another basophil donor. The sensitizing activity was again found in the monomeric IgE fraction (data not shown).

Discussion

Our results obtained by size fractionation of IgE⁺ plasma no. 151 indicated that HRF-mn reacts with monomeric IgE. Fractions eluted before monomeric IgE did not sensitize basophils to release histamine to

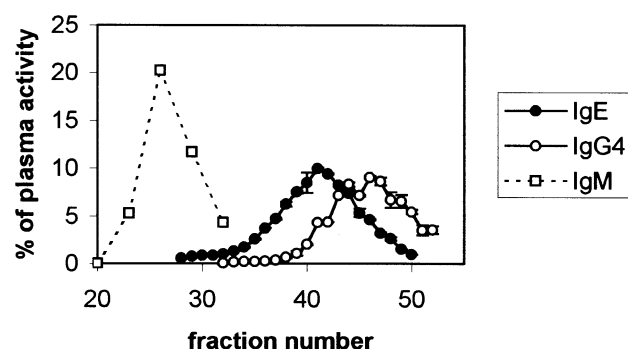


Figure 2. Size-exclusion chromatography on AcA34 of IgE⁺ plasma no. 151. Elution profile of IgM (1000 kDa), IgE (180 kDa), and IgG4 (150 kDa) from this plasma is depicted. Error bars indicate range of duplicates.

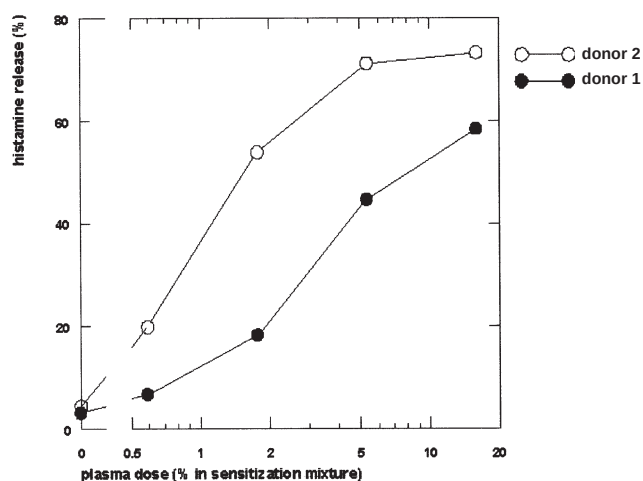


Figure 3. Histamine release of basophils sensitized by increasing amounts of IgE⁺ plasma no. 151 to HRF-mn. In two independent experiments, different basophil donors were used. Sensitization of 20×10^6 cells was performed in volume of 1 ml. Volume of serum is expressed as percentage of total volume. Spontaneous release was 1.5%.

HRF, whereas monomeric IgE did. Therefore, it can be concluded that HRF triggers basophils sensitized by monomeric IgE, and that the histamine-releasing reactivity of IgE⁺ is not an *exclusive* property of polymeric IgE.

After size fractionation of plasma no. 151, the recovery of IgE⁺ was less than that of IgE. This might be caused by specific binding of IgE⁺ to the column material, resulting in a loss of IgE⁺. A structural heterogeneity of IgE⁺ could be responsible for this.

Our results show that IgE⁺ is not likely to be the IgE isoform "tailpiece", which is supposed to form

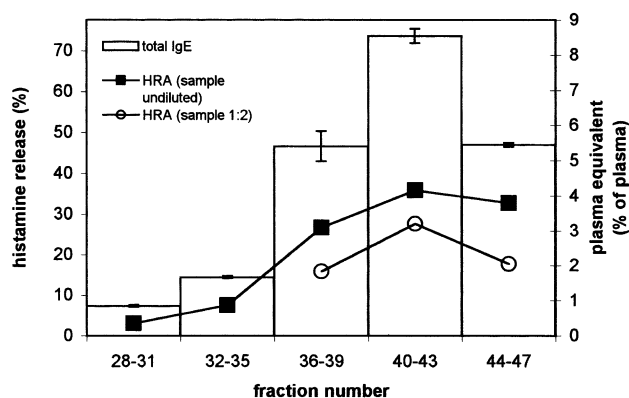


Figure 4. Size-exclusion chromatography profile of IgE⁺ plasma no. 151. Release of histamine of basophils sensitized by plasma fractions, undiluted and 1:2, is depicted in response to HRF-mn. Histamine release is shown as percentage of total amount of histamine in cells. Spontaneous release was 1.5%. With IgE⁺ plasma no. 151 as reference (Fig. 3, donor 1), histamine release is expressed as percentage of plasma activity. Activity of 2.9% corresponds to 25% release of histamine in response to HRF-mn preparation. Total IgE expressed as percentage of IgE⁺ plasma no. 151 is presented as bars. HRA: histamine-releasing activity.

multimers via its additional C-terminal cysteine residues. IgE⁺ might be one of the other splice variants (7, 8, 15, 16). IgE isoform "chimeric" is 10 amino acids shorter than the classic secreted IgE due to replacement of the last 37 amino acids by 27 amino acids of the CH5 site, whereas the IgE isoform "grandé" contains 134 additional C-terminal amino acids. It was demonstrated that B cells from atopic individuals produced relatively higher levels of the isoform "grandé", lower amounts of the IgE isoform "tailpiece", and very low levels of the IgE isoform "chimeric" compared to those from nonatopic individuals (17). IgE isoforms might be differentially expressed under certain disease conditions.

There might be other features that distinguish IgE⁺ from IgE⁻. MacDonald et al. (2) speculated that IgE⁺ reactivity is dependent on the mode of glycosylation of IgE (18). However, no carbohydrate differences were found; Kleine-Tebbe et al. (19) showed that oligosaccharide-specific lectins could not distinguish between basophils sensitized with IgE⁺ or with IgE⁻.

Polyclonal human IgE has a variable binding to staphylococcal protein A (20, 21). This binding is via the Fab fragment, and has been localized to the V region of the Ig H chain. The heterogeneity of IgE that distinguishes IgE⁺ from IgE⁻ might also be caused by VH subgroups. Correlation between these subgroups and IgE⁺ has not yet been reported. IgE⁺ reactivity might also be caused by the presence of IgG antibodies directed against IgE, which have been detected in some allergic conditions (22, 23). These IgG anti-IgE antibodies modulate basophil histamine release in an additive as well as in a blocking manner. However, our study indicated that IgE⁺ reactivity is not caused by the presence of these anti-IgE antibodies. The histamine-releasing activity of fractionated serum was coeluted with total IgE, whereas in the case of IgG-IgE complexes this activity would be present in the fractions eluted before the total IgE peak. Alternatively, if anti-IgE and IgE do not form complexes in serum, anti-IgE could bind IgE when it is present on the surface of the basophil. Then the sensitizing activity would be present between the IgE and IgG elution peaks.

In conclusion, we did not find evidence to support the hypothesis that IgE⁺ is polymeric IgE.

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