

Original article

Human serum IgE-mediated mast cell degranulation shows poor correlation to allergen-specific IgE content

Background: Although allergen-specific IgE content in serum can be determined immunochemically, little is known about the relationship between this parameter and the strength of the degranulation response upon allergen triggering.

Objectives: Analyse the degranulation capacity of immunochemically defined purified and serum IgE after challenge with anti-IgE or allergen using a rat mast cell line (RBL) transfected with the α -chain of the human high-affinity IgE receptor (Fc ϵ RI).

Methods: Purified IgE specific for 4-hydroxy-3-nitrophenylacetyl, purified IgE of unknown specificity, and sera from allergic patients sensitive to *Dermatophagoides pteronyssinus* and *Dactylis glomerata* were assessed. Degranulation was measured by a β -hexosaminidase release assay after anti-IgE or allergen-specific challenge.

Results: For purified monoclonal IgE a significant correlation ($r = 0.97$) was found between the proportion of bound allergen-specific IgE and the strength of the degranulation response. In contrast, no correlation ($r = 0.27$) was detected after sensitization with serum IgE.

Conclusion: Our studies demonstrate that mast cell activation mediated through IgE from allergic patients is a result of complex relationships that are not only dependent on allergen-specific IgE content but also relate to the capacity to efficiently sensitize and trigger the signalling responses that lead to degranulation.

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Allergenicity can be measured by the occurrence of specific IgE in human serum following natural exposure to environmental molecules or therapeutic injection with synthetic or natural substances. The presence of the allergen-specific IgE is commonly evaluated by immunochemical methods for serum IgE and blood basophil mediator release assays for cell-bound IgE (1). While the former techniques require patient serum, which can be stored for prolonged periods of time, the latter requires immediate processing of freshly drawn blood. Owing to the chemical nature of some allergenic molecules, these assays are limited to well-characterized allergens. Indeed, for small molecules no solid-phase preparation can be made without haptenic transformation in order to obtain recognition by the IgE molecules (2). A new means to measure the allergenicity and presence of specific IgE has been proposed in several studies, taking advantage of a rat mast cell line, RBL-2H3, transfected with the α -chain of the human Fc ϵ RI (3–5). Cross-linking of human IgE bound to these cells with anti-human IgE or specific allergen has been shown to trigger the degranulation of the cells.

The relationship between immunochemical parameters of IgE and functional degranulation responses in these transfectants has not been addressed in the past. In this study, we have, therefore, taken advantage of a new humanized Fc ϵ RI α -chain RBL-2H3 transfectant to investigate its capacity to trigger functional degranulation responses using both purified monoclonal and serum-derived polyclonal IgE. A number of experimental parameters were compared to examine the relationship between the total and allergen-specific IgE content and the ability of the cells to induce a degranulation response.

Material and methods

Cell cultures and transfections

A secreting RBL clone was provided by Dr M. Daéron (Institut Curie, Paris France). Cells were cultured at 37°C in DMEM-Glutamax (GIBCO BRL, Eragny, France) containing 10% FCS, 100 U/ml Penicillin and 100 U/ml Streptomycin (all GIBCO BRL). For transfections, cells were trypsinized, washed and resuspended at 1×10^7 cells/ml in complete medium. RBL-2H3 cells (4×10^6) were

transfected with 12 μ g of human Fc ϵ RI α cDNA by electroporation (250 V, 1500 μ F) as described (6). Transfectants were selected after growth in complete medium containing 2 mg/ml G-418 (GIBCO) and 20 mM HEPES, pH 7.3. Resistant clones were selected for expression of human Fc ϵ RI by flow cytometry and maintained in G-418 at 1 mg/ml.

Antibodies and sera

Human IgE of unknown specificity, mouse IgE specific for DNP, anti-human IgE and anti-human IgE-FITC have been described (4). A chimeric human(Fc)/mouse IgE specific for 4-hydroxy-3 nitrophenylacetyl (NP) was purchased from Serotec (Oxford, England). DNP-HSA was purchased from Sigma (St Louis, MO, USA) and NP-BSA from Bioscience Technologies (Novato, CA, USA). Sera were obtained from allergic individuals shown to have IgE specific for *Dactylis glomerata* and *Dermatophagoides pteronyssinus* allergens. Evaluation of total serum IgE was performed by UNICAP TM Pharmacia and specific IgE by Pharmacia CAP System RAST RIA according to the manufacturer's instructions.

Flow cytometry analysis

Cells were cultured for 48 h in medium without IgE or with IgE added for either 1 or 48 h. Cells were harvested by flushing the wells and washed twice with PBS, 1% FCS. An equivalent of 5×10^5 cells were incubated for 30 min at 4°C with 50 μ l of anti-IgE-FITC (1/200). Tubes were then washed twice and cells were analysed by cytofluorometry using cellquest software (Beckton Dickinson, USA).

β -Hexosaminidase release assays

G418 antibiotic was removed from cultures 48 h before stimulation. Transfected or untransfected RBL-2H3 cells were plated at 1×10^4 cells/well in a 96-well plate. After adherence (1 h at 37°C), the supernatant was discarded and replaced by the medium containing IgE or serum samples (1/50 dilution) and cells were further incubated for 48 h. IgE-sensitized untransfected or Fc ϵ RI α -chain transfected RBL-2H3 cells, when stimulated through endogenous receptors were washed once with medium and once with Tyrode buffer (4) and were then challenged with the indicated concentrations of DNP-HSA for 45 min at 37°C in 100 μ l Tyrode buffer. In the case of stimulation through human Fc ϵ RI α , IgE-sensitized cells were stimulated in 100 μ l Tyrode buffer containing 50% deuterium oxide (D₂O) (Sigma) in the presence of anti-human IgE, NP-BSA or *Dactylis glomerata* and *Dermatophagoides pteronyssinus* allergen extracts (Stallergènes, Antony, France). After centrifugation (5 min at 100 g) an aliquot was taken for measurement of β -hexosaminidase activity as previously described (4). All results are expressed as a percentage of total β -hexosaminidase in the cells after correction for spontaneous release in unstimulated cultures (net release).

Results

The cDNA encoding human Fc ϵ RI α -chain was transfected into the RBL cell line. After selection of antibiotic-resistant colonies and further subcloning, the RBL-2H3.E5.D12.8 clone displaying a slight but consistent human IgE binding capacity after a 1-h sensitization period was chosen for analysis (Fig. 1). Incubation with

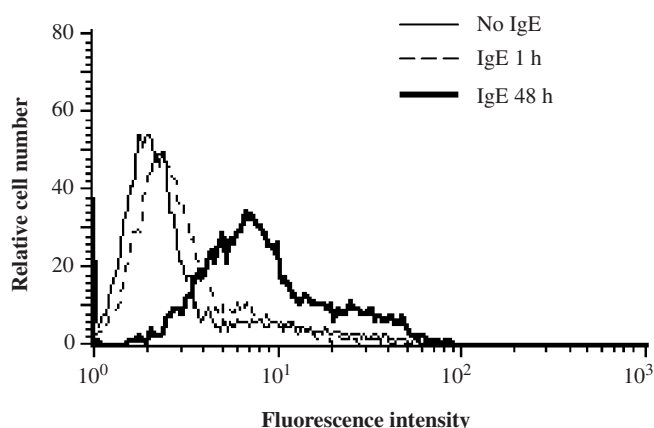


Figure 1. Expression of human IgE binding in RBL-2H3.E5.D12.8 cells. Cells were incubated either in the absence or presence of human IgE (1 μ g/ml) for indicated times. Human IgE binding was measured by cytofluorometry using anti-human IgE-FITC.

human IgE for prolonged periods (48 h) strongly induced Fc ϵ RI α -chain surface expression (Fig. 1).

The ability of this clone to secrete granule-associated mediators was measured by determining β -hexosaminidase release. Untransfected and human Fc ϵ RI α -chain-transfected cells were sensitized with either mouse IgE anti-DNP or human IgE and were then challenged with specific antigen or with anti-human IgE. For the latter, cells were incubated in the presence of 50% D₂O during triggering (7). The results in Fig. 2A demonstrate that normal and Fc ϵ RI α -chain-transfected cells showed equivalent responses upon stimulation through endogenous rat Fc ϵ RI. Only transfected cells responded to stimulation with anti-human IgE. To characterize the inherent releasing capacities of the transfectant we tested the limiting sensitizing concentration of purified human IgE. Figure 2B shows a dose-response experiment using anti-human IgE under three different sensitization conditions (10, 20 and 40 ng/ml). It can be concluded that sensitization with 10 ng/ml of human IgE corresponds approximately to the limiting concentration that enables detection of a degranulation response.

In order to mimic the IgE composition in human sera or on the basophils and mast cell surface, where allergen-specific IgE is present at various proportions, a mixing experiment was performed to achieve defined ratios of receptor occupancy with allergen-specific IgE (8, 9). For this purpose NP-specific IgE was added to human IgE of unknown specificity at different ratios while keeping the total IgE concentration constant. After a 48-h sensitization period to induce human Fc ϵ RI expression, β -hexosaminidase release was determined after challenge with NP-BSA, which aggregates only the fraction of bound NP-specific IgE. Figure 3 shows that β -hexosaminidase release augments with increasing proportions of

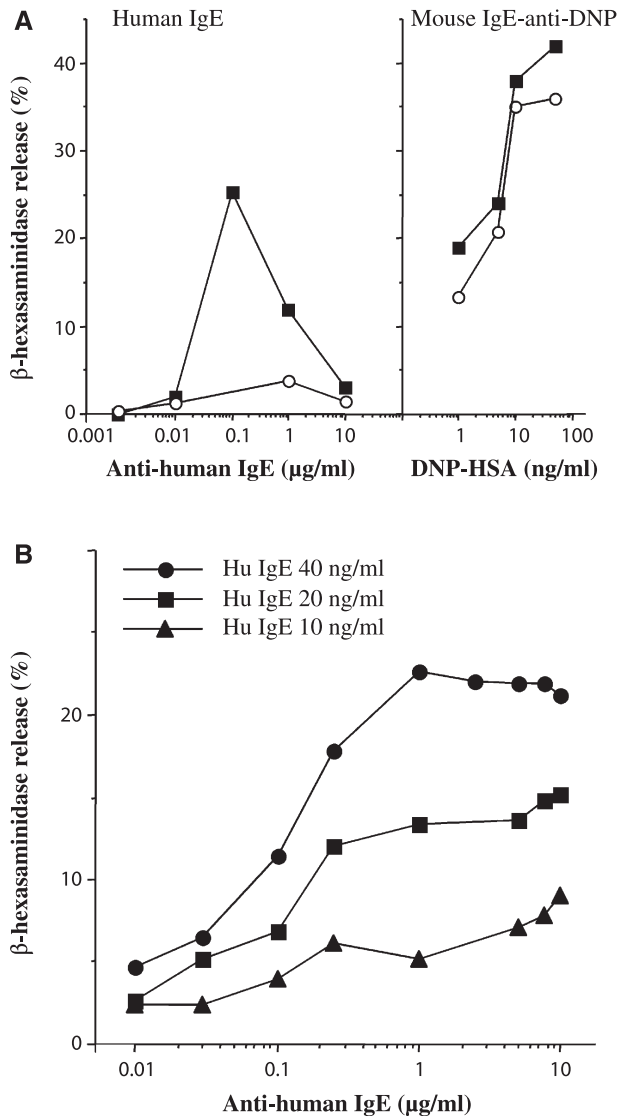


Figure 2. (A) Degranulation response of untransfected and FcεRI α-transfected RBL cells after sensitization with mouse or human IgE. RBL (empty circles) or RBL-2H3.D5.D12.8 cells (filled squares) were sensitized with 1 μg/ml of human IgE or mouse IgE anti-DNP for 48 h and were stimulated with anti-human IgE or DNP-HSA and the amount of secreted β-hexosaminidase (net release) was determined. Anti-human IgE stimulations were performed in the presence of 50% D₂O. Results are representative of at least three experiments. (B) Minimal sensitizing dose of human IgE in RBL-2H3.D5.D12.8 cells. Cells were incubated with the indicated concentrations of purified human IgE and were then stimulated with increasing doses of anti-human IgE in the presence of 50% D₂O. One hour after addition of stimulus the amount of secreted β-hexosaminidase (net release) was determined. Results are representative of two experiments.

NP-specific IgE establishing a quantitative relationship between bound antigen-specific IgE and the degranulation response.

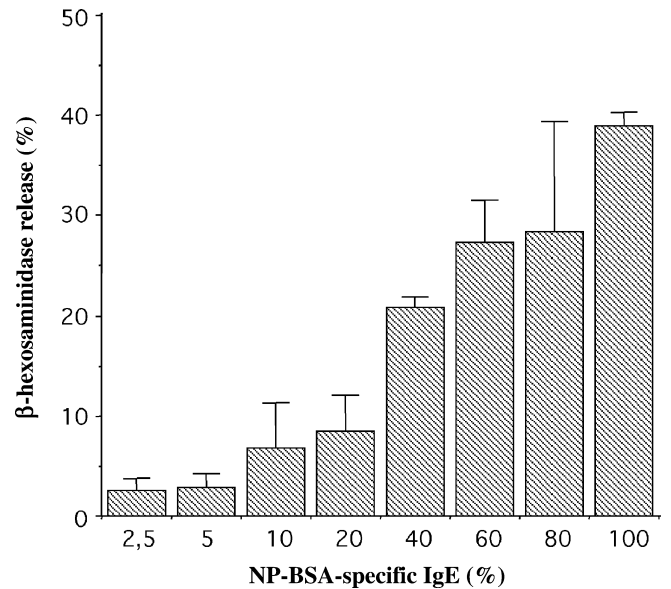


Figure 3. Minimal proportion of antigen-specific human IgE to detect a degranulation response. Human IgE of unknown specificity and NP-specific IgE were adjusted separately to 1 μg/ml before mixing to yield increasing proportions of NP-specific IgE ranging from 2.5 to 100%. RBL-2H3E5.D12 transfectants were sensitized for 48 h with the mixtures and stimulated with 40 ng/ml of NP-BSA in the presence of 50% D₂O and the amount of secreted β-hexosaminidase (net release) was determined. Results show the mean ± SEM and are representative of three experiments.

We then investigated whether the transfectant could also be used as a tool for the detection of human serum IgE. A panel of sera from pollen- and mite-specific allergic individuals was selected. Their total and allergen-specific IgE concentrations are depicted in Table 1. In preliminary experiments we determined the optimal serum concentration for sensitization as 1/50. At lower dilutions cytotoxic effects were observed for some samples (not shown). Figure 4 represents a plot of degranulation capacities under these conditions as a function of IgE concentrations after anti-human IgE stimulation. The results demonstrate a saturable curve that plateaus off between 40 and 60 ng/ml of IgE. Minimal detectable concentrations were around 5–10 ng/ml.

We also tested the capacity of serum-sensitized cells to respond to stimulation with increasing doses of pollen allergen extract. Figure 5A shows the β-hexosaminidase release of the pollen-specific sera after stimulation with anti-human IgE. The response can be compared to the one obtained after triggering with increasing doses of allergen extract. The results demonstrate that seven of the eight sera tested showed a detectable response after anti-IgE stimulation. Four of the eight sera also demonstrated that they can be released after stimulation with allergen as judged from

Table 1. Total and allergen-specific IgE concentrations in *Dactylis glomerata*- and *Dermatophagoides pteronyssinus*-sensitive individuals

Lot (serum) number	Total IgE (kU/l)	Ag-specific IgE (kU/l)	% specific IgE
Sera from <i>Dactylis glomerata</i> -sensitive individuals			
4325 17 03 (1)	360	60	16.7
4409 17 03 (2)	170	44	25.8
4531 17 03 (3)	560	124	22.1
2008 22 03 (4)	1200	35	2.9
2008 22 03 (5)	1020	165	32.4
6307 21 03 (6)	1390	143	10.3
73 23 03 (7)	210	82	39.1
4292 23 03 (8)	810	200	24.7
Sera from <i>Dermatophagoides pteronyssinus</i> -sensitive individuals			
1790 24 03 (9)	1170	223	19.1
1544 23 03 (10)	920	677	73.6
1673 22 03 (11)	1670	219	13.1
2727 18 03 (12)	600	74	12.3
1179 17 03 (13)	325	40	12.4
1317 17 03 (14)	620	83	13.4
1688 17 03 (15)	250	42	16.8
2688 17 03 (16)	1060	158	14.9
604 16 03 (17)	260	84	32.3

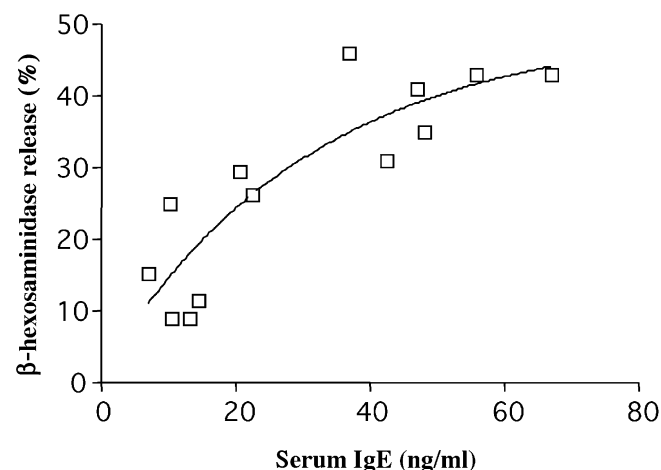


Figure 4. Dose-response evaluation of the triggering capacity of human serum IgE. Transfectants were incubated with serum samples (1/50 dilution). After 48 h cells were stimulated with anti-human IgE (1 µg/ml) and the amount of secreted β-hexosaminidase (net release) was determined. Results are representative of four experiments.

the shown dose-response experiments. Similar data were also obtained for mite-specific sera (Fig. 5B). Seven of the nine sera showed strong stimulation with anti-IgE while two sera showed somewhat smaller, albeit detectable responses ($\approx 10\%$). Stimulation with specific allergen extract also gave positive degranulation responses with four of the sera.

As the antigen-specific response was proportional to the percentage of antigen-specific IgE in purified IgE

preparations, we evaluated whether the allergen-specific IgE content in the sera could also be correlated to the strength of the degranulation response. To this end we had to express the responsiveness as a ratio to the maximal response (defined as the response after anti-human IgE stimulation) to normalize serum samples as they could not be adjusted to the same total IgE concentration as in the case of purified IgE where the maximal response is identical to the response after sensitization with 100% IgE-anti-DNP. The ratio of allergen-specific IgE vs total IgE was then plotted against the ratio of the allergen-specific IgE vs the maximal IgE degranulation response and results were compared for purified and serum IgE samples. For the latter, samples with a limited response to stimulation with anti-IgE (sera 7, 13 and 17) or less than 10% of allergen-specific IgE (serum 4) were excluded from the analysis. The analysis in Fig. 6 reveals that purified monoclonal human IgE shows a very good correlation coefficient ($r = 0.971$) (Fig. 6A). In contrast, no correlation ($r = 0.271$) is seen in the case of serum-sensitized transfectants (Fig. 6B).

Discussion

IgE-mediated allergic manifestations are observed to an increasing number of proteins and compounds (10). For many classical allergic substances immunochemical assays and/or basophil release assays are routinely employed to assess the presence of specific IgE antibodies. However, a number of allergic manifestations exist with no evidence for an IgE-dependent aetiology (11–14). This may be because of the fact that mast cells can be stimulated via other surface receptors or the adequate allergen has not been detected. In the latter case, it would be useful to have a practical experimental device that allows testing the degranulation capacity of serum IgE with an array of specific compounds. Similarly, in situations where only serum can be made available (i.e. during large-scale clinical studies), or in cases where a large panel of mutated recombinant allergen need to be tested, such an assay will allow the functional assessment of an IgE antibody response.

As existing rodent mast cell lines cannot be used because of the species specificity of human IgE (15), several groups have attempted to establish a rat mast cell line (RBL) transfected with the α -chain of the human FcεRI (3–5). These transfectants could be sensitized with IgE from allergic individuals and exhibited a functional degranulation response after allergen-specific triggering. The transfectant have been used for the evaluation of the *in vitro* capacity of some monoclonal anti-human IgE antibodies to interfere with the fixation of human IgE (7, 16). The FcεRI α -chain transfectants could thus serve as a bioassay for IgE-mediated responses. However, so far these transfectants have not been assessed for the

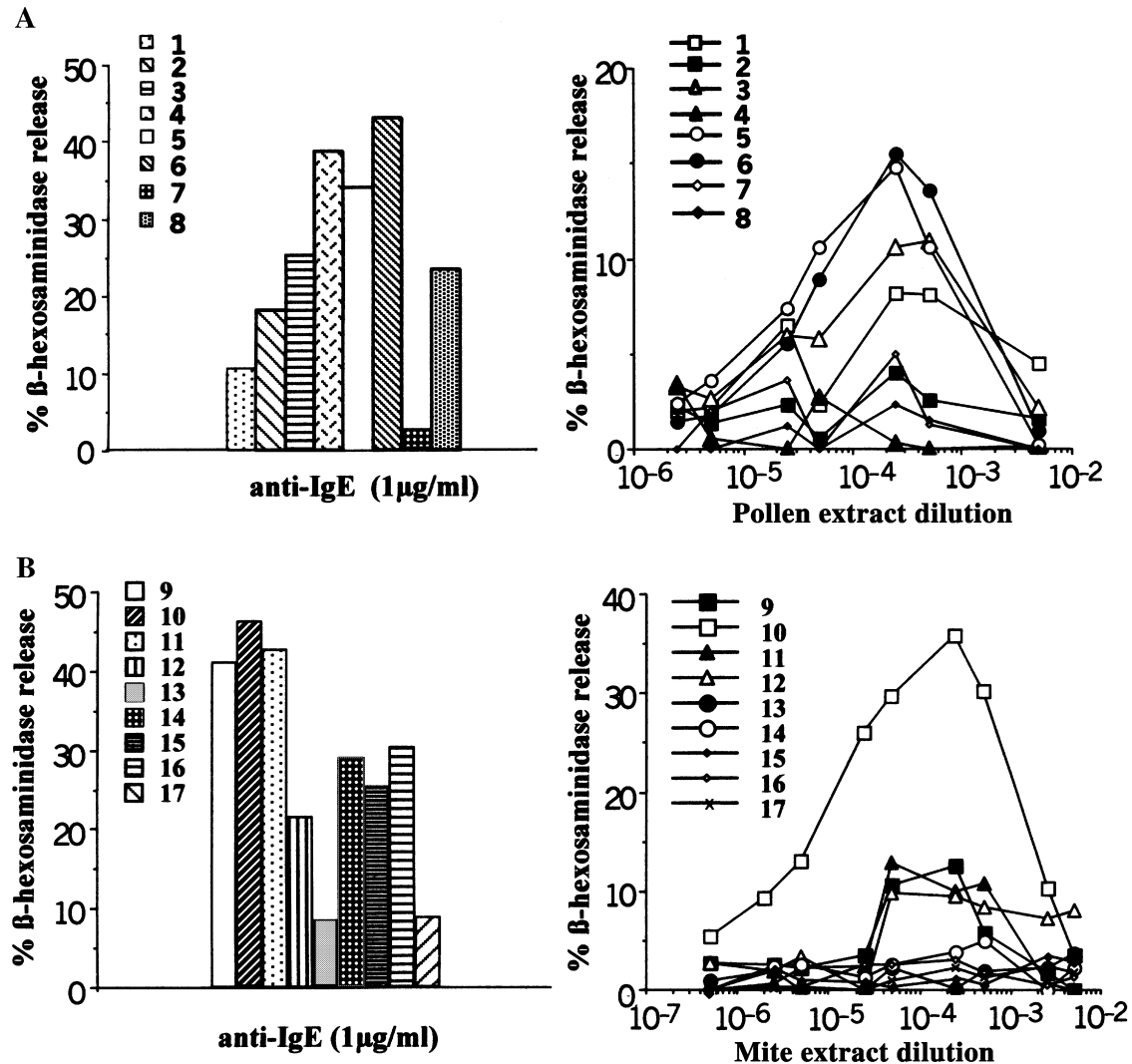


Figure 5. Total and allergen-specific IgE-mediated degranulation in cells sensitized with *Dactylis glomerata* (A) and *Dermatophagoides pteronyssinus* (B) specific sera. RBL-2H3.D5.D12.8 cells were sensitized with serum samples (numbering corresponds to Table 1) at 1/50 dilution. Cells were stimulated with either anti-human IgE (1 μg/ml) to measure maximal responses or with increasing doses of allergen in the presence of 50% D₂O and the amount of secreted β-hexosaminidase (net release) was determined. Results represent the mean ± SEM and are representative of two experiments.

relationship between the strength of the degranulation response and the immunochemically determined allergen-specific IgE content.

Here we have studied a new FcεRI α-chain RBL transfectant for its capacity to degranulate upon exposure to both purified monoclonal and serum-derived polyclonal IgE and have compared the antigen-specific IgE with the total IgE-mediated response. Initial tests showed that the transfectant expressed human IgE binding capacity that was strongly upregulated after prolonged incubation with IgE in agreement with previous data (17–19). Human IgE-sensitized cells responded to stimulation, although this required incubation in the

presence of D₂O most likely by enhancing triggering through chimeric human/rat receptors composed in majority of the less effectively signalling trimeric receptor composed of human α and endogenous rat γ but not β chains (4, 15, 20).

We tested first several experimental parameters using purified human IgE preparations. Dose-response experiments revealed typical profiles reaching plateau levels within about two log scales. On some occasion a bell-shaped dose-response was also obtained (Fig. 2). Minimal IgE sensitizing doses were as low as 10 ng/ml after stimulation with anti-human IgE. In order to mimic *in vivo* conditions where only a fraction of IgE is directed

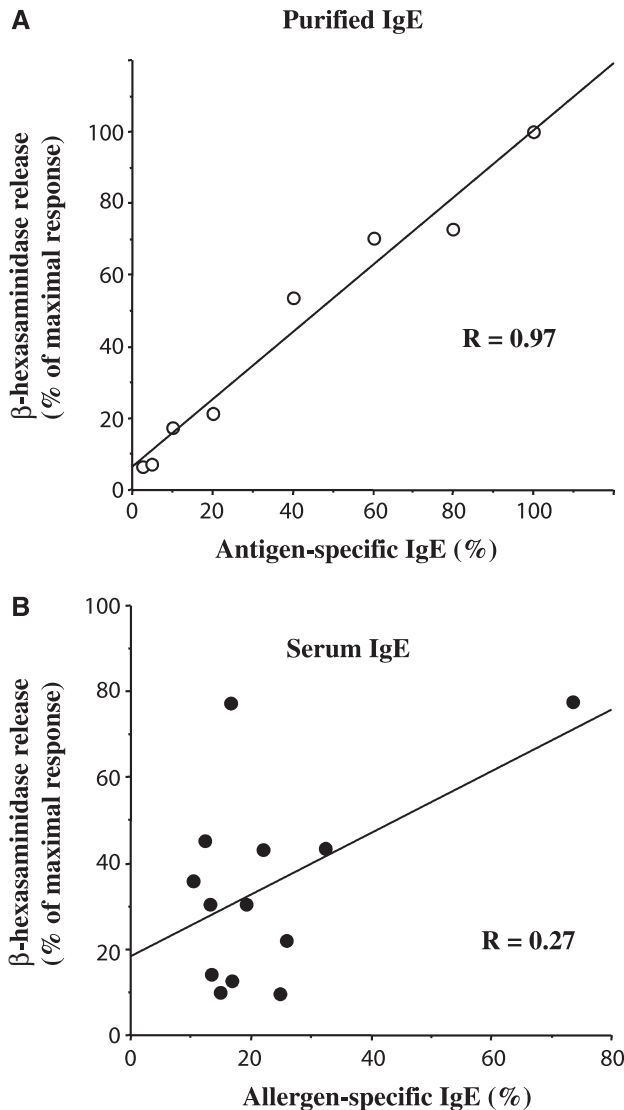


Figure 6. Comparison between the proportion of specific purified (A) or serum (B) IgE and the relative degranulation response. The ratio of allergen-specific vs total IgE was taken from Fig. 3 (purified IgE) or calculated from Table 1 (serum IgE). Data were plotted against the ratio of the allergen-specific vs maximal responses (determined from Figs 3 and 5, respectively). Serum samples with a limited response to stimulation with anti-IgE (sera 7, 13 and 17, see Fig. 5) or less than 10% of allergen-specific IgE (serum 4, see Table 1) were excluded from the analysis. The correlation coefficient is indicated.

to allergens, we evaluated the minimal proportion of antigen-specific IgE necessary to obtain a degranulation response. The results show that about 10% of receptor occupancy with allergen-specific IgE are required to produce a response. The response linearly increased up to 100% occupancy while previous data performed in human basophils (8) or in RBL-2H3 cells stimulated through endogenous rat Fc ϵ RI (9) had revealed plateau

levels at lower occupancy ($\approx$ 30 and 5%, respectively). This may be explained by the low number of expressed hu Fc ϵ RI in the RBL transfectants (4). Indeed, in human basophils the responsiveness was also linear before it had reached the plateau level at about 30% occupancy (8).

After having tested purified human IgE preparations we examined releasability of serum samples with immunochemically defined concentrations of total and allergen-specific IgE. We could measure degranulation upon stimulation with anti-human IgE for almost all sera. Minimal IgE concentrations required for degranulation were close to the values of purified IgE and were in the range of about 5–10 ng/ml. The results were less concordant when allergen-specific responses were studied. Although more than half of the sera triggered a response after allergen challenge, we did not detect a correlation between the allergen-specific IgE content and releasability. This was strikingly different to the experiments using purified IgE samples and suggests that parameters other than the allergen-specific IgE content as determined by immunochemical evaluation methods are important for functional responses with serum samples. Differences in the affinity for allergens or the steric site of allergen recognition that are not taken into account in the immunochemical RAST assay may have an important impact on the response. Indeed, evidence has accumulated that show the dependency on the affinity of the IgE molecule for its allergen to induce functional responses (21, 22). Chemical modification of a specific allergen to obtain different affinities has led to agonist, partial agonist or antagonistic responses (21). Furthermore, experiments using anti-IgE antibodies that recognize the IgE ligand at different sites suggested differences in their capacity to induce degranulation (23, 24). Another factor may also be that the immunological quantification of total- and allergen-specific IgE does not distinguish between free and complexed forms of IgE, which have potentially different biological activities with respect to their bindability to Fc ϵ RI and their capacity to trigger signalling (25–27).

In conclusion, we have tested a new human Fc ϵ RI α -chain transfectant for its ability to degranulate upon aggregation of IgE when sensitized with purified IgE or serum IgE from allergic individuals. The study demonstrates that different serum IgEs trigger mast cell mediator release with variable efficacy and poor correlation to their immunochemically determined allergen-specific IgE content. The degranulation assay with the humanized RBL transfectant may thus provide additional information for the functional evaluation of IgE antibodies in human serum.

Acknowledgments

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