

RBL cells expressing human FcεRI are a sensitive tool for exploring functional IgE–allergen interactions: studies with sera from peanut-sensitive patients

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Abstract

Rat basophilic leukemia cells (RBL SX-38) express the α , β , and γ chains of human FcεRI. Following sensitization with IgE from a subset of allergic human donors, these cells can be triggered by exposure to anti-IgE or to very low concentrations of specific allergens. We examined 18 sera from patients who were highly sensitive to peanuts by history and had anti-peanut IgE by in vitro testing. The ability of these sera to sensitize the RBL SX-38 cells for degranulation with peanut allergens correlates very well with the absolute amount of anti-peanut IgE ($r=0.95$; $p<0.001$). The most effective sera contained at least 50 kU/l of total IgE and at least 15 kU/l of peanut-specific IgE. RBL SX-38 cells sensitized with these sera degranulated optimally upon exposure to anti-IgE (net degranulation of $40 \pm 8\%$, means $\pm$ S.D.; $n=8$) and to a 10^5 – 10^6 dilution of crude peanut extract (CPE) ($37 \pm 7\%$ net degranulation; $93 \pm 13\%$ of that seen with anti-IgE). This assay is quite sensitive. Cells sensitized with selected sera are activated by exposure to a $1:10^7$ dilution of the CPE containing picogram amounts of peanut allergens. This assay is also quite specific. Cells sensitized with sera from patients with anti-peanut IgE and no detectable IgE against soybean, walnut or grass pollen did not degranulate following exposure to these latter antigens. The converse was also true; cells sensitized with sera from patients without anti-peanut IgE did not react to peanut. These data demonstrate that RBL cells expressing human FcεRI form the basis of a useful model system for the detection of allergens and for the study of IgE–allergen interactions.

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Abbreviations: RBL-2H3 cells, rat basophilic leukemia; RBL SX-38 cells, RBL-2H3 cells expressing human FcεRI; FcεRI, Fc-epsilon receptor I; IgE, immunoglobulin E; kU, kilounits; CPE, crude peanut extract.

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1. Introduction

Type I immediate hypersensitivity reactions are mediated through the effects of antigen-specific IgE. IgE binds to high-affinity receptors for IgE (FcεRI) on human mast cells and, when cross-linked by antigen,

causes release of preformed mediators, release of newly formed lipid-derived mediators, and later production of proinflammatory cytokines (Schwartz and Huff, 1998). Allergic individuals express a very wide range of concentrations of total and antigen-specific IgE and this range correlates with the broad spectrum of severity of allergic reactions (Homburger, 1998; Yunginger et al., 2000).

There are an estimated 4 million Americans with well-substantiated food allergies (Sampson, 1997). Reactions to foods are thought to be the most common cause of anaphylaxis when it occurs outside of the hospital and are estimated to cause 125 deaths per year in the U.S. (Burks et al., 1999). A recent analysis of 32 fatalities thought to be due to food-induced anaphylaxis revealed that peanut was the likely responsible food in 62% of the cases (Bock et al., 2001). In placebo-controlled food challenges, peanut-sensitive patients can react to as little as 100 µg of peanut protein (Hourihane et al., 1997). Thus, anaphylactic reactions and other allergic reactions to peanuts are significant medical problems (Hourihane, 1998; Burks et al., 1999; Warner, 1999).

The peanut, *Arachis hypogaea*, is a member of the legume family and is related to beans and peas, but not to tree nuts (Sampson, 1998). Immediate hypersensitivity reactions to peanuts are unusual in that they are much more prevalent and severe than for other legumes and tend to persist into adulthood (Sampson, 1997). For this reason, substantial effort has been made to identify the major peanut allergens in hopes of desensitization of patients or production of peanuts without allergenic activity (Burks et al., 1998; Moneret-Vautrin, 1998; Nelson et al., 1997). Seven potentially important allergens of peanut have been identified (Ara h 1–Ara h 7) based on binding of IgE from allergic sera on Western blots (Burks et al., 1995; Stanley et al., 1997; Kleber-Janke et al., 1999; Rabjohn et al., 1999).

RBL SX-38 cells are rat basophilic leukemia cells that stably express the α , β , and γ chains of the human high-affinity receptor for IgE, Fc ϵ RI (Wiegand et al., 1996). This receptor gives these cells the important property that they can bind IgE from the sera of allergic individuals and can be activated in an allergen-specific manner (Wiegand et al., 1996). We have studied the ability of sera from 18 subjects with documented sensitivity to peanuts to sensitize these

cells for subsequent degranulation following exposure to crude peanut extract (CPE). In this report, we characterize the parameters of those sera that are successful in sensitizing the RBL SX-38 cells to subsequent degranulation upon exposure to the appropriate antigen. An unexpected finding is that, with selected sera, these cells can be activated by dilute extracts containing less than 1 ng/ml of major allergen. RBL cells expressing human Fc ϵ RI should be a useful model for the detection of very small amounts of functional allergen and for the analysis of functional interactions between allergen-specific IgE and allergens.

2. Materials and methods

2.1. Patients and preparation of sera

This protocol was approved by the Colorado Multiple Institutional Review Board. All subjects gave informed consent. We studied sera from a total of 18 patients with a history of systemic reactions to peanuts. Most of these had documented positive skin tests to peanuts. Nine patients were identified by colleagues and referred to SCD. These sera are identified by the letter “D” followed by a number. For these nine patients, blood was drawn into plain (“red top”) tubes (Becton-Dickinson), allowed to clot at room temperature and promptly centrifuged at 2000 rpm at room temperature for 10 min to collect the serum. This serum was then aliquoted and stored at –20 °C. Each of these patients was skin tested to peanuts and at least two of the following: walnuts, soybean, timothy grass, ragweed and cat. Allergen-specific IgE was assayed using the Pharmacia ImmunoCAP method by the clinical laboratory of the National Jewish Medical and Research Center. We also reviewed a database (from SAB’s clinical practice) of approximately 200 patients with a history of peanut allergy and found nine patients who had detectable anti-peanut IgE (Pharmacia ImmunoCAP) and for whom there was >3 ml of available serum as this would allow repeat testing. These sera are identified by the letter “B” followed by a number. These patients had previously given informed consent for the use of their sera to study peanut allergy. Permission to use these previously collected serum was obtained

from the National Jewish Medical and Research Center Institutional Review Board.

2.2. Maintenance of RBL SX-38 cells in tissue culture

RBL SX-38 cells (kind gift of J.P. Kinet) were grown in Eagle's Minimal Essential Medium (EMEM) with 10% defined fetal calf serum (FCS) (HyClone, Logan, UT), 1% penicillin/streptomycin and 1.2 mg/ml G418 (Invitrogen, Carlsbad, CA) as previously described (Wiegand et al., 1996). Every other passage, the G418 was increased to 2.4 mg/ml to maintain a high level of expression of human FcεRI.

2.3. Analysis of binding of IgE to the RBL SX-38 cells

RBL SX-38 cells were handled as above, except that serum was diluted in CM (culture medium) and then allowed to bind to the cells for 24 h. Cells were detached, washed and stained with biotinylated goat anti-IgE (Dreskin et al., 1987). Following a second set of washes, cells were counterstained with phycoerythrin streptavidin (Jackson ImmunoResearch, West Grove, PA), fixed, and analyzed by flow cytometry. The data are expressed as fold-increase in fluorescence compared to cells not sensitized with human IgE but carried through the staining procedure.

2.4. Preparation of RBL SX-38 cells for assay

Two days prior to a planned experiment, cells from a confluent flask were detached, washed and replated at 2×10^5 cells/ml (200 μl per well) in 48-well plates, with 1 μCi/ml titrated 5-hydroxytryptamine (5-HT, serotonin) (New England Nuclear Division of Perkin-Elmer Life Sciences, Boston, MA). After an overnight incubation, human serum (25 μl, from an allergic donor) was added to sensitize the cells and the cells were cultured for 24 additional hours. Background degranulation for replicates not containing serum ranged from 5–10%. Background degranulation for replicates containing serum but not triggered ranged from 10–25%. In later experiments, background degranulation due to serum was minimized (usually to 5–15%) by cross-absorbing the human serum (10% in EMEM without FCS) against the parent RBL-2H3 cells (not expressing FcεRI) for 1

h at 37 °C and then adding this supernatant to the RBL SX-38 cells.

2.5. RBL SX-38 cell triggering by specific antigens

The labeled and sensitized RBL SX-38 cells were washed twice with a sodium PIPES buffer containing 0.1% bovine serum albumin, 25 mM disodium PIPES, 100 mM NaCl, 5 mM KCl, 0.4 mM MgCl₂, 1 mM CaCl₂ and 5.6 mM glucose (pH 7.0). After washing, 100 μl of this PIPES buffer, to which 0.1 mM 5'-N-ethylcarboxamide (NECA) (final DMSO dilution 1:1000) had been added, was placed in each well and the plates were placed in a 37 °C shaking water bath for 5 min (Wiegand et al., 1996).

Degranulation was initiated by adding 5 μl of allergen extracts (diluted to the appropriate concentration in the PIPES buffer) or goat anti-human IgE (final concentration, 3 μg/ml). Negative controls included cells not sensitized with serum and cells sensitized with serum and treated with 5 μl PIPES buffer instead of extract or anti-IgE. After 30–45 min at 37 °C, the plates were removed to ice and the tritium content of the supernatant and of the residual cells was determined. Preliminary experiments demonstrated that degranulation was complete by 20 min (data not shown). In selected experiments, the supernatants were centrifuged to demonstrate that the release of ³H-5-HT is not due to detachment of cells. To determine the residual ³H, the adherent cells were either solubilized with 250 μl of 1% Triton X-100 detergent (Pierce Chemicals, Rockford, IL) at room temperature for 30 min followed by scraping or detached with 100 μl of 0.25% Trypsin/1 mM EDTA (Gibco, Grand Island, NY) at 37 °C for 30 min. All samples were counted in 4 ml of Universol (ICN, Costa Mesa, CA).

2.6. Calculation of percentage release and presentation of the data

The percentage of radiolabeled serotonin release is calculated as previously described (Hohman and Dreskin, 1994): disintegrations per minute (dpm) released (supernatant), divided by dpm released (supernatant) plus dpm in the residual cells. Net release was calculated as the percentage release following incubation with anti-IgE or allergen extracts minus the background seen following incubation with buffer

(see Section 2.4). For each experiment, replicate samples were stimulated with anti-IgE. In each experiment, the net release with anti-IgE varied from 8–67% (see Table 1). In order to compare data from separate experiments, release following exposure to the allergen extracts is expressed as a percentage of that seen with anti-IgE in the same assay.

2.7. Preparation of extract dilutions

Commercially available allergenic extracts (1:10 w/v for peanut, walnut and soybean; 100,000 BAU/ml for timothy pollen) were obtained from ALK Abello (Round Rock, TX) and were diluted into the PIPES buffer.

2.8. Assay of protein and major allergen content of extracts

The content of a major allergenic protein (Phl p 5) in the timothy grass pollen extract was reported by the manufacturer (ALK Abello) to be 750 µg/ml. The crude extract of peanuts (ALK Abello) used in these studies contained 7.5 mg of protein/ml (BCA protein assay, Pierce Chemicals). Dr. Gary Bannon kindly assayed the major allergen content of the peanut extract and found that it contained 8% Ara h 1 and 2% Ara h 2. This translates into an Ara h 1 content of approximately 600 µg/ml and an Ara h 2 content of approximately 150 µg/ml (Gary Bannon, personal communication).

Table 1
Characteristics of sera used in this study

Serum	Age (years)	Symptoms	IgE (IU/ml)	Peanut-specific IgE (IU/ml)	Percentage of IgE specific for peanut	No. of assays	Net release with anti-IgE	Net release with CPE	Percent of release compared with anti-IgE
B20	10	U/AE	689	96	14.1	3	27 ± 2	29 ± 2	107 ± 10
B119	7	U/AE/R	324	73	22.8	1	46	47	102
B73	1	No peanut ever ^a	416	34	8.2	2	33 ± 14	34 ± 17	99 ± 10
B50	1	R/GI	296	28	9.6	3	38 ± 3	36 ± 3	96 ± 10
B8	1	U	298	>100	>33	4	48 ± 4	46 ± 9	93 ± 13
D10	49	Systemic anaphylaxis	52	20	39	6	43 ± 6	38 ± 6	85 ± 3
D17	43	AE/R	180	44	24	4	44 ± 2	38 ± 3	85 ± 4
D13	19	AE	1465	17	1.2	6	47 ± 3	31 ± 4	65 ± 7
B10	8	U/R/GI	639	13	2.0	2	35 ± 7	15 ± 7	39 ± 11
D15	39	U/R	255	9.7	3.8	3	39 ± 2	9 ± 2	24 ± 3
D9	24	U/R	226	5.1	2.2	1	66	14	21
D7	37	U/GI	168	6.3	3.8	3	58 ± 7	10 ± 2	17 ± 2
B139	3	U/AE	62	2.2	3.5	1	51	1	2
D12	38	AE	192	7.6	4.0	2	56 ± 6	1 ± 1	0
D6	45	U/GI	361	0.7	0.2	1	67	0	0
D16	32	AE/R	365	3.1	0.9	2	41 ± 6	0	0
B37	1	U/R/GI	33	4.4	13.4	1	31	0	0
B32	3	R/GI	19	0.7	3.5	1	8	0	0

Sera are listed in the order of their ability to sensitize the RBL SX-38 cells for degranulation when exposed to 1:10⁵–10⁶ dilutions of the CPE and compared to the degranulation with anti-IgE in the same assay (last column). Symptoms of the most severe reaction for each patient are listed. U, urticaria; AE, angioedema; R, wheezing and/or shortness of breath; GI, diarrhea, nausea and/or vomiting.

^a Subject B73 was evaluated for a history of anaphylaxis to milk and was found to have a high in vitro test for IgE to peanut. This patient had no history of exposure to peanuts. Total IgE and anti-peanut IgE were determined by the Clinical Laboratory of the National Jewish Medical and Research Center. Where no error bars are shown, the peanut-induced degranulation of the RBL SX-38 cells compared to the release with anti-IgE was determined in duplicate in one experiment. The duplicate determinations were generally ± 10%. Otherwise, values are means ± S.E.M. of multiple determinations.

2.9. Statistics

Curve fitting and calculation of correlation coefficients were performed with GraphPad Prism version 3.0a for Macintosh, GraphPad Software (San Diego, CA). Correlation coefficients were determined using the Spearman rank method. Spearman r -values and the associated p -values are shown in the relevant figures.

3. Results

3.1. Binding of human IgE to RBL SX-38 cells

Binding of IgE to Fc ϵ RI on these cells was assessed by adding dilutions of serum D15 containing IgE. As shown in Fig. 1, with sera containing greater than 500 ng/ml of IgE/ml (210 IU/ml), when added at a 1:10 dilution (50 ng/ml), binding of IgE to Fc ϵ RI reaches a plateau.

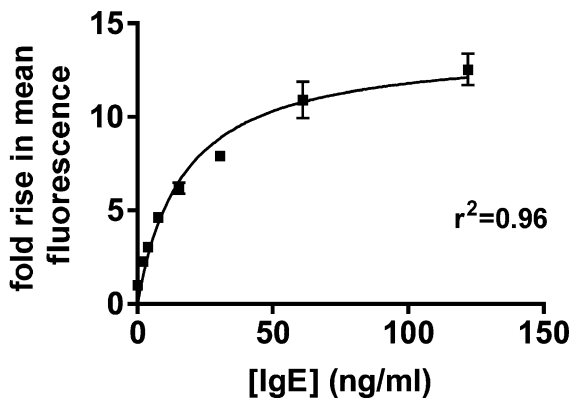


Fig. 1. Binding of human IgE to RBL SX-38 cells. Serum from an allergic donor was diluted in culture medium to contain IgE at the concentrations shown. After an overnight incubation, cells were harvested, washed and stained with biotinylated anti-IgE and then PE–streptavidin. Stain intensity was determined by fluorescent-activated cell analysis and expressed as fold increase over cells that were not incubated with IgE. Error bars represent the range of duplicate determinations. If not visible, this range was smaller than the size of the symbol. These data are representative of three similar experiments. The curve was fitted with a one site-binding model. The r^2 -value for the curve fitting is shown.

3.2. Sensitivity and specificity of the assay

The RBL SX-38 cells, when sensitized with sera from patients with large amounts of anti-peanut IgE, degranulate when exposed to very dilute preparations of CPE. In Fig. 2a, cells were sensitized with serum D17 (44 IU/ml of anti-peanut IgE and no detectable IgE to walnuts or grass) and exposed to dilutions of crude peanut extract (CPE) or crude extracts of walnut or timothy grass pollen. A 1-million-fold dilution of the CPE gave a signal almost as large as that seen with anti-IgE and a detectable signal is present with a 10-million-fold dilution. A 1-million-fold dilution of the CPE contains approximately 600 pg/ml of Ara h 1 and 150 pg/ml of Ara h 2. Exposure to a $1:10^3$ dilution of two other crude extracts (walnut and grass pollen) did not give significant degranulation. The concentration of Phl p 5 in the $1:10^3$ dilution of the grass pollen extract is 750 ng/ml. A similar level of sensitivity to grass pollen extract was seen with serum B18 (Fig. 2b). This serum had very little anti-peanut IgE (1.0 IU/ml) but had 42 IU/ml of grass-specific IgE. As can be seen, cells sensitized with serum B18 react well to a $1:10^5$ dilution of grass pollen extract but there is minimal reactivity to a $1:10^3$ dilution of the CPE.

3.3. Activity of peanut sensitive sera correlates well with amount and percent allergen-specific IgE

Table 1 catalogues the clinical history of our 18 subjects, the total IgE levels, anti-peanut IgE levels and the activity for their sera in this assay. The sera are listed in descending order of their ability to sensitize the RBL SX-38 cells for degranulation upon exposure to CPE. This activity is expressed as a percentage of the degranulation with anti-IgE. Fig. 3a demonstrates that there is a close correlation ($r=0.95$, $p<0.001$) between the amount of anti-peanut IgE in these sera and the ability of these sera to effectively sensitize the RBL SX-38 cells. The inset in Fig. 3a reveals a sharp transition of activity with sera that contain between 10 and 20 IU/ml of anti-peanut IgE. There is also a strong association between the percent of IgE that is anti-peanut IgE and the ability of these sera to effectively sensitize the cells ($r=0.62$, $p=0.01$; Fig. 3b). In this figure, there is one outlier (serum B37). This serum contained 4.4 IU/ml of anti-peanut IgE and 33 IU/ml

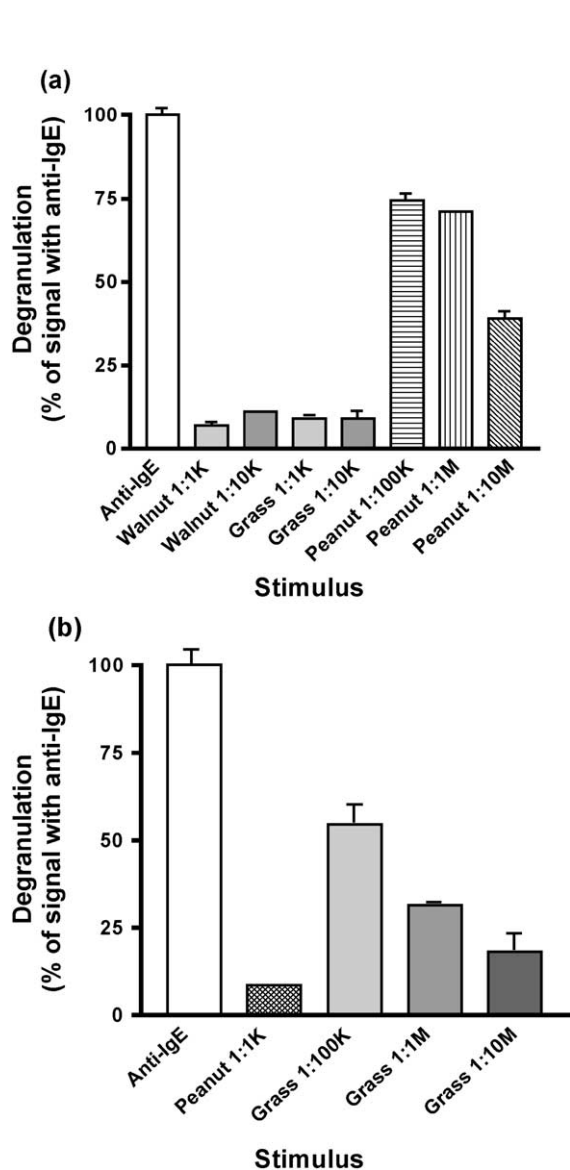


Fig. 2. RBL SX-38 cells sensitized with sera from allergic individuals can be triggered with dilute extracts of allergens. Cells were sensitized with sera from donors who were allergic to peanut (a) or grass (b). After excess IgE was washed away, the cells were triggered with anti-IgE (3 μ g/ml) or with diluted extracts of peanuts, grass pollen or walnuts. Extracts were added at the dilutions shown, where 1K refers to a dilution of 1:10³ and 1M refers to a dilution of 1:10⁶. The net degranulations seen with anti-IgE were 74 $\pm$ 25% (a) and 55 $\pm$ 5% (b). The release with the allergen extracts is shown as a percent of the release with anti-IgE. Two additional experiments with sera from two different peanut-sensitive subjects and one additional experiment with serum from a second grass-sensitive subject gave similar results (data not shown).

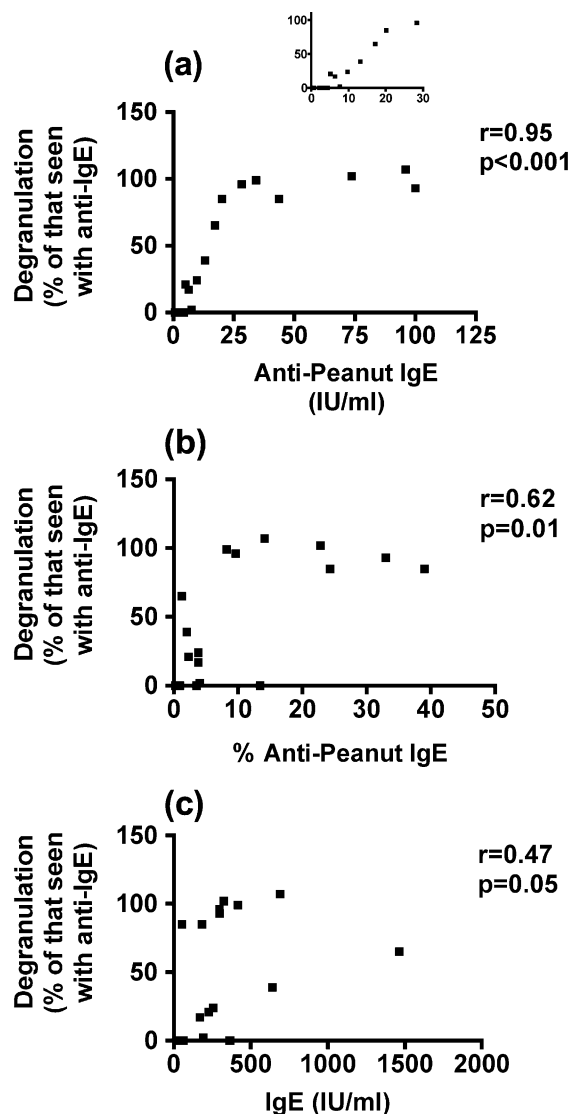


Fig. 3. Correlation of activity with IgE. The data from Table 1 are shown in graphic form. (a) The relative activity of each serum (compared with anti-IgE) is plotted against the absolute amount of anti-peanut IgE. The inset shows the data in more detail for sera containing 0 to 30 IU/ml of anti-peanut IgE. (b) The relative activity is plotted against the percentage of IgE that is anti-peanut IgE. (c) The relative activity is plotted against the amount of total IgE. The Spearman Rank Correlation Coefficient r -value and the associated p -value are shown for each panel.

of total IgE. Finally, there is a poorer correlation between the total amount of IgE and the activity of these sera (Fig. 3c).

4. Discussion

RBL cells expressing the human α , β and γ chains of the Fc ϵ RI receptor provide a convenient cell culture system for analysis of functional IgE–allergen interactions. These cells have distinct advantages over primary cultures of human mast cells and human basophils. The former are inconvenient to culture whereas the latter must be harvested immediately before using and are nonfunctional in 8–20% of subjects (Saito et al., 1996; Kepley et al., 1999).

We sensitized RBL SX-38 cells with sera from patients who have had systemic reactions to peanuts in order to explore the parameters of this cell-based assay. We found that sera must contain approximately 200 IU/ml of total IgE so that after dilution at 1:10, the IgE will optimally occupy the available Fc ϵ RI on these cells (Fig. 1). With highly active sera, the assay is a very sensitive method to detect peanut allergen. It is able to detect a 1:10⁷ dilution of CPE (containing approximately 60 pg/ml of Ara h 1 and 15 pg/ml of Ara h 2) (Fig. 2). Preliminary data with two subjects allergic to grass pollen (one shown in Fig. 2; the other not shown) reveals that, for selected patients, this assay has similar sensitivity for grass allergen as for peanut allergen. The assay is also very specific. We assessed specificity by exposing cells sensitized with sera from three subjects with IgE specific for peanut but not grass pollen or walnut. In these experiments, there was greater than a 10⁴ difference in sensitivity to the peanut and grass pollen extracts which had similar concentrations of major allergens. A representative experiment is shown in Fig. 2a. Furthermore, serum from a subject with substantial anti-grass IgE and very little anti-peanut IgE did not respond to a 10⁴ higher concentration of peanut extract (Fig. 2b). Serum from a second grass-allergic subject without clinical peanut allergy (total IgE=1265, anti-grass IgE=17 IU/ml, and anti-peanut IgE=2 IU/ml) behaved similarly (data not shown).

Sera with >25 IU/ml of peanut-specific IgE fully sensitized the RBL SX-38 cells for degranulation with CPE. The extent of degranulation with CPE was indistinguishable from that seen with anti-IgE (Table 1 and Fig. 3). This is likely due to the large amount of the IgE that is peanut specific. Experiments with monoclonal IgE showed have shown that cross-linking of 20% of the available receptors on RBL cells is

sufficient to produce full degranulation of RBL cells (Maeyama et al., 1988).

Analysis of sera from these 18 peanut-sensitive patients reveals that this assay is not a sensitive method for identifying patients with symptoms produced by peanut allergy and cannot distinguish among patients with different patterns of clinical reactivity (Table 1). For example, there are patients who clinically are exquisitely sensitive to peanuts and have anti-peanut IgE but whose sera are not able to sensitize the RBL SX-38 cells for degranulation. This was also seen with subjects allergic to grass pollen (data not shown). Typically, these are subjects with either low amounts of total IgE and/or of allergen-specific IgE. The most likely explanation for this is that the number of Fc ϵ RI on the RBL SX-38 cells may be limiting. Naturally occurring basophils in atopic individuals have as many as 500,000 receptors per cell (MacGlashan et al., 1997; MacGlashan et al., 1998). The RBL SX-38 cells are reported to contain approximately 100,000 receptors per cell (Wiegand et al., 1996). In this case, given a serum with a set proportion of IgE specific for peanut, fewer receptors on the RBL SX-38 cells compared to naturally occurring basophils will lead to fewer receptors that can be cross-linked by allergen and lesser sensitivity to allergen. Preparation of RBL cells expressing larger numbers of Fc ϵ RI should make these cells more useful for a broader range of sera.

The data in Fig. 3a demonstrate that the absolute amount of peanut-specific IgE is an important determinant of the ability of a serum to effectively sensitize these cells ($r=0.95$; $p<0.001$). The peanut-specific IgE must be relatively high (>10 IU/ml) for the cells to respond to a commercially available crude peanut extract with a plateau of reactivity being seen at >25 IU/ml. The effective concentration for 50% of the maximal activity is approximately 15 IU/ml (inset, Fig. 3a). Interestingly, Sampson (2001) and Sampson and Ho (1997) have identified an ImmunoCAP value of 15 IU/ml to have a high positive predictive value for anaphylactic reactions to peanuts. The percentage of the total IgE that is specific for peanut is another predictor of the ability of a given serum to adequately sensitize the cells. If a serum contained >10% peanut-specific IgE, it usually gave excellent allergen-specific degranulation (Fig. 3b). As expected, the amount of total IgE was a relatively poor predictor of the ability

of these sera to sensitize the cells for allergen-dependent degranulation (Fig. 3c).

In conclusion, RBL cells expressing human FcεRI are a useful model system to explore IgE–allergen interactions. We have analyzed sera from peanut-sensitive subjects and have defined the parameters that adequately sensitize these cells. In addition, previously published data and data in this report demonstrate that sera from individuals with other sensitivities (e.g. grass pollen (Wiegand et al., 1996) and latex (Williams et al., 1996)) can also effectively sensitize these cells. Given sera from subjects with adequate amounts of total IgE and of anti-peanut IgE, this assay is very sensitive and specific. In its current form, this assay has potential usefulness for standardizing allergens and for detecting functional allergen in the environment.

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