

## Allergen-induced histamine release in rat mast cells transfected with the $\alpha$ subunits of Fc $\epsilon$ RI

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### Abstract

A rat mast cell histamine assay (RMCHA) has been developed to quantitate the biological activity of a recombinant humanized, monoclonal anti-IgE antibody (rhuMAbE25). Rat mast cells (RBL 48), transfected with the  $\alpha$  subunit of the high affinity human IgE receptor (Fc $\epsilon$ RI), were presensitized for 2 h with human plasma containing IgE specific for ragweed and challenged with ragweed allergen in the presence of 50% D<sub>2</sub>O. Histamine release plateaus at 0.1  $\mu$ g/ml of ragweed. The release of histamine was time, temperature and Ca<sup>2+</sup> dependent. This ragweed-induced histamine release could be inhibited by rhuMAbE25 in a dose-dependent fashion with an IC<sub>50</sub> of  $1.19 \pm 0.31$   $\mu$ g/ml ( $n = 25$ ). Other humanized MAbs and recombinant human growth factors neither trigger histamine release nor inhibit ragweed-induced histamine release. This RMCHA correlates well with the human basophil histamine assay (HBHA) (Fei et al., 1994) with a correlation coefficient of 0.93 ( $n = 59$ ,  $p < 0.0001$ ). Histamine was also released when the cells were presensitized with human plasma containing the respective allergen-specific IgE and then challenged with standardized mite, *D. farinae*, house dust mix, standardized cat pelt, or *Alternaria tenuis*. Comparison of allergen-induced histamine release showed a good correlation between RMCHA and HBHA with a correlation coefficient of 0.69 ( $n = 37$ ,  $p = 0.0001$ ). We conclude that RMCHA provides a useful tool to confirm allergen-specific IgE in allergic patients and can be used to evaluate the biological activity of any anti-IgE monoclonal antibody. Moreover, RMCHA provides a unique opportunity to study the mechanism of IgE-mediated histamine release in the absence of interfering proteins and growth factors normally present in whole blood.

Abbreviations: HBHA, human basophil histamine assay; RMCHA, rat mast cell histamine assay; rhuMAbE25, recombinant humanized anti-human IgE monoclonal antibody; RSHP, ragweed specific human plasma; PBS, phosphate-buffered saline; BSA, bovine serum albumin; HRP, horseradish peroxidase; OPD, *o*-phenylenediamine dihydrochloride; ELISA, enzyme-linked immunosorbent assay; sIMDM, supplemented Iscove's modified Dulbecco's medium; HBSS, Hanks' balanced salt solution; HRB, histamine release buffer.

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**Keywords:** Monoclonal antibody; Recombinant humanized anti-human IgE; Anti-IgE; Fc $\epsilon$ RI; Ragweed; House dust; Cat pelt; *Alternaria tenuis*; Mite, *Dermatophagoides farinae*; Histamine; Basophil; RBL 48; Rat mast cell; D<sub>2</sub>O; Allergy; Immunotherapy

## 1. Introduction

The high affinity receptor for IgE (Fc $\epsilon$ RI) on mast cells and basophils plays a key role in initiating allergic response (Siraganian, 1981; Metzger et al., 1986; Haydik and Ma, 1988; Kinet, 1990; Ravetch and Kinet, 1991). The receptor is a tetramer composed of an  $\alpha$  chain which binds IgE, a  $\beta$  chain with four transmembrane domains, and a homodimer of disulfide-linked  $\gamma$  chains, each with one transmembrane domain (Kinet, 1990). The gene for the human Fc $\epsilon$ RI  $\alpha$  chain, which consists of five exons and four introns, and spans 5889 bp of genomic DNA, has been cloned (Pang et al., 1993). Hakimi and coworkers have demonstrated that the  $\alpha$  chain of the human Fc $\epsilon$ RI is sufficient for high affinity IgE binding (Hakimi et al., 1990). In allergic patients, immunoglobulin E binds to the Fc $\epsilon$ RI via its Fc region (Metzger et al., 1986; Haydik and Ma, 1988). Subsequent cross-linking of receptor-bound IgE with multivalent allergens can induce degranulation of basophils and mast cells resulting in release of histamine, leukotrienes, serotonin, and platelet activating factor which are responsible for the manifestation of allergic symptoms (Ishizaka et al., 1970; Ishizaka et al., 1978; Ishizaka et al., 1983; Metzger et al., 1986).

Various forms of immunotherapy aimed at blocking IgE responses have been proposed as treatments for allergic disease. Agents which prevent IgE from binding to its receptor have been suggested as possible treatment modalities by various groups (Lichtenstein et al., 1968; Levy, 1972; Davis et al., 1993). Likewise, murine anti-IgE monoclonal antibodies that bind to specific epitopes on IgE-secreting human B cells and down regulate IgE synthesis have also been developed (Chang et al., 1990; Davis et al., 1991). These investigators propose that anti-IgE antibodies could be used to eliminate IgE-secreting human B cells in allergic patients as a means of ameliorating

IgE-mediated allergies. Our approach has been to develop a humanized monoclonal antibody (rhuMAbE25) directed against the Fc region of human IgE which blocks binding of IgE to its receptor (Presta et al., 1993). We have recently reported a novel bioactivity assay using whole blood (HBHA) to quantitate the biological activity of anti-IgE antibodies based on the ability of the antibody to block histamine release from allergen-sensitized blood cells of healthy donors (Fei et al., 1994). To circumvent the problems of donor to donor variability and for ease of cell preparation, in this report we describe the development of a cell line-based assay (RMCHA) to quantitate the biological activity of anti-IgE antibodies. The correlation of rat mast cell histamine assay (RMCHA) to human basophil histamine assay (HBHA) was also investigated.

## 2. Materials and methods

### 2.1. Reagents

Monoclonal antibodies produced by conventional hybridoma techniques were humanized and isolated as described (Carter et al., 1992; Presta et al., 1993) at Genentech, South San Francisco, CA. These included recombinant humanized anti-human IgE MAb (rhuMAbE25), recombinant humanized anti-HER2 MAb (rhuMAbHER2), recombinant humanized anti-CD18 MAb (rhuMAbCD18), and recombinant humanized anti-ICAM MAb (rhuMAbICAM). Goat anti-human IgE( $\epsilon$ ) HRP conjugate was purchased from Kirkegaard and Perry Laboratories, Gaithersburg, MD. Recombinant humanized nerve growth factor (rhuNGF), tumor necrosis factor (rhuTNF), and interferon- $\gamma$  (rhuIFN- $\gamma$ ) were generated, cloned and sequenced at Genentech (South San Francisco, CA). Ragweed antigens E-B and E-C (lot A-601-903A-185) were

obtained from the National Institute of Health, Bethesda, MD. Standardized mite, *Der-matophagoides farinae* (*D. farinae*) (cat. #6720UP, lot #E53L3533) and house dust mix allergen (cat. #4701ED, lot #C63J8308) were purchased from Miles, Elkhart, IN. Standardized cat pelt (lot #3E00202) and *Alternaria tenuis* (lot #3J17242) were from Center Laboratories, Port Washington, NY. Human plasmas were obtained from North American Biological company, Miami, FL. Hanks' balanced salt solution (HBSS, 1 × ) and glutamine were purchased from Gibco BRL, Gaithersburg, MD. Bovine serum albumin (BSA, fraction V), *o*-phenylenediamine dihydrochloride (OPD) substrate and Triton X-100 were purchased from Sigma, St. Louis, MO. Fetal bovine serum was purchased from Hyclone, Logan, UT. Deuterium oxide (D<sub>2</sub>O, 99.996% purity) was from Aldrich Chem. Company, Milwaukee, WI. Sodium heparin (cat. #367673) was purchased from Becton Dickinson, Rutherford, NJ.

## 2.2. Blood donors

Heparinized whole blood was collected from a group of healthy, non-allergic or allergic individuals who were prescreened prior to being used as donors for the HBHA. The screening protocol involved presensitizing donors' whole blood with 10% RSHP in the absence or presence of rhuM-AbE25 (10 µg/ml) for 2 h at 37°C followed by challenge with ragweed allergen (0.1 µg/ml). Those donors with basophils that released greater than 10% of the total cellular histamine were selected for further study. No volunteers were on medication and informed consent was obtained in all cases.

## 2.3. Determination of histamine concentration

The concentration of histamine in the supernatant was determined by a histamine enzyme immunoassay kit (AMAC, Westbrook, ME) that has a high assay sensitivity of 0.2 nM and a low interassay variability (%CV) of 9.1% (n = 24). Briefly, histamine was acylated and allowed to compete with histamine acetylcholinesterase for binding to antibody coated onto microtiter wells.

After 18 h, each well was rinsed and the bound enzymatic activity was measured by the addition of a chromogenic substrate (acetylthiocholine, dithionitrobenzoate). The intensity of the color developed at 405 nm is used to calculate the concentration of histamine in the sample using a standard curve obtained with standards furnished by the kit.

## 2.4. Human basophil histamine assay (HBHA)

Heparinized whole blood was diluted 1/7 with HBSS/1% BSA and mixed with 10% (v/v) allergen sensitized human plasma (eg. RSHP). The mixture was incubated with or without rhuM-AbE25 (prepared in PBS/0.01% BSA) for 2 h at 37°C in a humidified 5% CO<sub>2</sub> incubator. After incubation, samples were transferred to a 96-well plate (Costar #3797) containing either HBHA histamine release buffer (HBHA HRB) (HBSS/1% BSA/33% D<sub>2</sub>O/0.8% NaCl/1.3 mM CaCl<sub>2</sub> · 2H<sub>2</sub>O) or allergen (0.1 µg/ml in HBHA HRB) and further incubated for 30 min at 37°C. Plates were centrifuged at 900 × g for 5 min at 4°C and the supernatants were harvested for histamine determination. For each blood sample, total cellular histamine was determined by mixing 50 µl whole blood with 950 µl distilled water followed by two 15 min cycles of freezing and thawing. The concentration of histamine in the supernatant was determined as described above.

## 2.5. Cell culture

The RBL 48 rat mast cell line was kindly provided by Dr. Jarema Kochan of Hoffmann-La Roche, Nutley, NJ. This RBL 48 cell line was derived from the parental rat mast cell line RBL 2H3 that has been subsequently transfected with the human α subunit of the high affinity IgE receptor FcεRI (Gilfillan et al., 1992). Cells were grown in sIMDM, Iscove's modified Dulbecco's media supplemented with 10% fetal bovine serum, 2 mM glutamine, and 500 µg/ml of active geneticin (Gibco BRL #11811-031) in a T175 tissue culture flask (Falcon #3028) at 37°C in a humidified 5% CO<sub>2</sub> incubator. The cells were harvested by exposure to 4 ml of PBS/0.05%

trypsin/0.53 mM EDTA (Gibco BRL #15400-013) for 2 min at 37°C followed by centrifugation ( $400 \times g$  for 10 min) and resuspension in fresh sIMDM. The cells were optimally split at 1:5 ratio after confluence was attained.

#### 2.6. Human plasma IgE binding ELISA assay

RBL 48 cells, seeded at 40 000 cells per well in a 96-well tissue culture plate (Linbro #76-042-05), were cultured overnight at 37°C/5% CO<sub>2</sub> in a humidified incubator. After three washes with PBS/0.05% Tween 20 in a plate washer, the cells were fixed for 2 min with 200 µl per well of absolute alcohol at ambient temperature followed by six washes to remove residual alcohol. Cells were incubated with either sIMDM, sIMDM/10% RSHP, or sIMDM/10% RSHP/10 µg/ml rhuMAbE25 for 1 h at 37°C. After the incubation, cells were rinsed six times with PBS/0.05% Tween 20. The captured IgE were then reacted for 30 min at 37°C with goat anti-human IgE(ε) HRP (1/10 000 dilution of 1.0 mg/ml stock) followed by 30 min incubation with OPD substrate at ambient temperature. Reaction was stopped by 100 µl/well of 6 N H<sub>2</sub>SO<sub>4</sub>. Absorbance at 492 nm was measured.

#### 2.7. Rat mast cell histamine assay (RMCHA)

RBL 48 cells were grown to confluence and then trypsinized as described above. Cells in suspension were counted with a hemacytometer (Reichert-Jung Company, Buffalo, NY) and the density was adjusted to  $0.4 \times 10^6$  cells/ml. Cells were then seeded at 100 µl/well (40 000 cells per well) in a 96-well tissue culture plate and cultured for 24 h at 37°C in a humidified 5% CO<sub>2</sub> incubator. After being washed once with 200 µl/well of sIMDM, the cells were preincubated for 2 h with 100 µl/well of fresh sIMDM/3 U/ml sodium heparin/10% (v/v) of allergen sensitized human plasma either in the presence or absence of rhuMAbE25 standards (final concentrations ranging from 0.078 to 10 µg/ml) at 37°C. After incubation, cell culture medium in the well was aspirated and washed three times with sIMDM. The cells were then incubated with 100 µl/well

of either RMCHA histamine release buffer (RMCHA HRB) (sIMDM/50% D<sub>2</sub>O/0.8% NaCl/1.3 mM CaCl<sub>2</sub> · 2H<sub>2</sub>O), allergen (0.1 µg/ml in RMCHA HRB), or 0.5% Triton solution (for total histamine release) for 30 min at 37°C. Plates were centrifuged at  $900 \times g$  for 5 min at 4°C and the supernatants were harvested and diluted 1000 times in PBS for total histamine release and 80 times for allergen-induced histamine release. The concentration of histamine in the supernatant was determined as described above.

### 3. Data analysis

Histamine concentration was determined from the histamine standard curve using a least square, non-linear 4 parameter fit program. All statistics were analyzed in StatView 4.0 program.

### 4. Results

#### 4.1. Binding of IgE to RBL 48 cells

The binding of IgE to RBL 48 cells was evaluated in an IgE binding ELISA which uses an alcohol-fixed RBL 48 monolayer to determine whether human IgE in the RSHP (total IgE measured at 1.64 µg/ml) could bind to the α subunit of the FcεRI receptors expressed on the surface of the RBL 48 cells. Fig. 1 demonstrates that the IgE present in the RSHP does indeed bind to the FcεRI receptors of the RBL 48 cells. In addition, this binding is specific to IgE since the addition of a blocking anti-IgE monoclonal antibody, rhuMAbE25, completely abolishes the binding.

#### 4.2. Specificity of the RMCHA

The data in Table 1 demonstrate that neither recombinant human monoclonal antibodies nor recombinant human cytokines would induce mast cell degranulation alone. Moreover, preincubation of ragweed specific human plasma with 10 µg/ml of rhuMAbE25 completely abolished histamine release induced by 0.1 µg/ml of ragweed

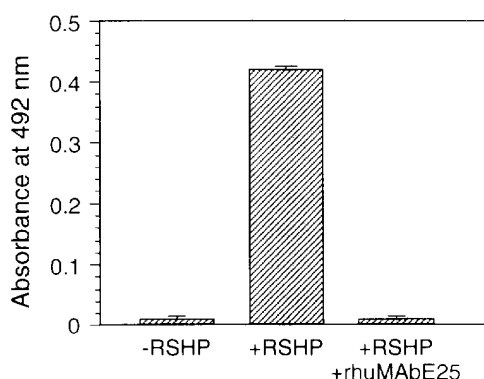


Fig. 1. Inhibition of human IgE binding to RBL 48 cells by rhuMAbE25 in an IgE binding ELISA. Each bar and error bar represents the mean and the range of duplicate determinations respectively.

in the presence of 50% D<sub>2</sub>O. No inhibition of histamine release was observed when samples were preincubated with 10 µg/ml of the aforementioned antibodies and cytokines.

Table 1  
Specificity of RMCHA for rhuMAbE25

| Treatment  | % Total cellular histamine in supernatant |                     |
|------------|-------------------------------------------|---------------------|
|            | – Ragweed challenge                       | + Ragweed challenge |
| Buffer     | 2.0 ± 0.2                                 | 22.0 ± 0.1          |
| rhuMAbE25  | 2.7 ± 0.5                                 | 2.1 ± 0             |
| rhuMAbHER2 | 2.0 ± 0.3                                 | 23.6 ± 0.1          |
| rhuMAbCD18 | 2.3 ± 1.2                                 | 22.9 ± 0.7          |
| rhuMAbICAM | 1.7 ± 0                                   | 23.7 ± 1.4          |
| rhuIFN-γ   | 2.3 ± 0.9                                 | 23.3 ± 1.1          |
| rhuTNF     | 1.6 ± 0.1                                 | 23.9 ± 0.2          |
| rhuNGF     | 2.0 ± 0.5                                 | 22.9 ± 0.7          |

RBL 48 cells were preincubated for 2 h with either buffer (sIMDM), rhuMAbE25 (10 µg/ml), rhuMAbHER2 (10 µg/ml), rhuMAbCD18 (10 µg/ml), rhuMAbICAM (10 µg/ml), rhuIFN-γ (10 µg/ml), rhuTNF (10 µg/ml), or rhuNGF (10 µg/ml) in the presence of 10% RSHP at 37°C. Cells were then challenged with 0.1 µg/ml of ragweed allergen for 30 min at 37°C. In addition, RSHP-sensitized cells were directly challenged with either buffer (sIMDM), rhuMAbE25 (10 µg/ml), rhuMAbHER2 (10 µg/ml), rhuMAbCD18 (10 µg/ml), rhuMAbICAM (10 µg/ml), rhuIFN-γ (10 µg/ml), rhuTNF (10 µg/ml), or rhuNGF (10 µg/ml) alone for 30 min at 37°C. Each value represents the mean ± SD of triplicate determinations.

#### 4.3. Effect of temperature and calcium

Histamine release induced by ragweed in the RMCHA was dependent on the concentration of ragweed, temperature, and calcium ions (Fig. 2). When the ragweed challenge step was incubated in the presence or absence of 50% D<sub>2</sub>O at 37°C, a bell-shaped dose-dependent histamine release curve that reaches maximal release at 0.1 µg/ml was observed. The release of histamine elicited by ragweed was attenuated or abolished if the incubation was performed either at ambient temperature or in the presence of 2.5 mM EDTA which chelates free calcium ions. These data indicate that the optimal condition for histamine release is at 37°C and in the presence of calcium.

#### 4.4. Time course of histamine release

The data presented in Fig. 3 demonstrate that ragweed-induced histamine release at 37°C is time-dependent and reaches maximal release at 30 min incubation. When the incubation time was

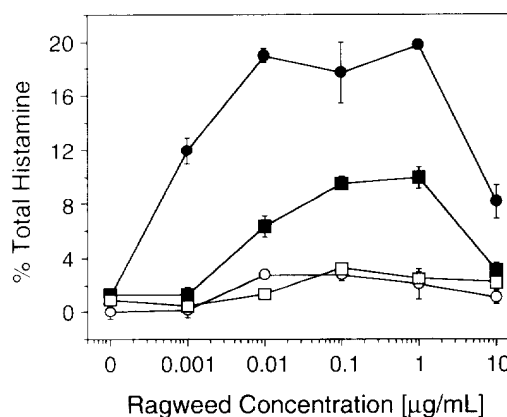


Fig. 2. Effect of temperature and calcium ion on ragweed-induced histamine release in RBL 48 cells. Cells were preincubated for 2 h with sIMDM/3 U/ml sodium heparin/10% RSHP at 37°C before challenge for 30 min with ragweed allergen (at 0, 0.001, 0.01, 0.1, 1, and 10 µg/ml) in either RMCHA HRB (sIMDM/50% D<sub>2</sub>O/0.8% NaCl/1.3 mM CaCl<sub>2</sub>·2H<sub>2</sub>O) at room temperature (open circle), RMCHA HRB at 37°C (filled circle), sIMDM at 37°C (filled square), or RMCHA HRB spiked with 2.5 mM EDTA at 37°C (open square). Each point and error bar represents the mean and the range of duplicate determinations respectively.

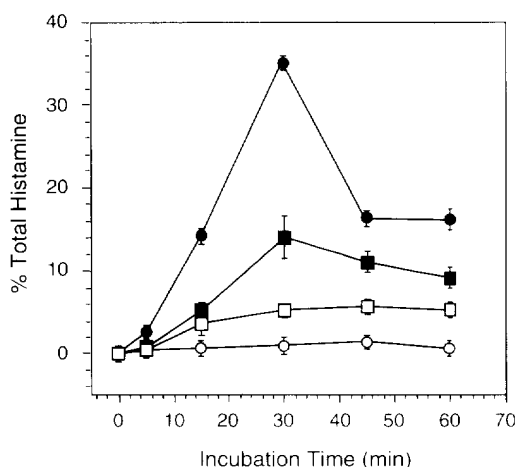


Fig. 3. Time course of histamine release by RMCHA HRB (open circle), or RMCHA HRB/0.1 µg/ml ragweed allergen alone (filled circle), in the presence of 0.5 µg/ml (filled square) or 1 µg/ml (open square) rhuMAbE25. Each point and error bar represents the mean and the range of two determinations respectively.

increased to 45 min or 60 min, the concentration of histamine in the supernatant dropped sharply from 36% to 16% of total histamine. In addition, preincubation of ragweed specific plasma with either 0.5 or 1 µg/ml of rhuMAbE25 inhibited the release of histamine induced by ragweed allergen. A typical standard curve for quantitating the biological activity of rhuMAbE25 is shown in Fig. 4. Inhibition of ragweed-induced histamine release by rhuMAbE25 has a mean  $IC_{50}$  of  $1.19 \pm 0.31$  µg/ml ( $n = 25$ ).

#### 4.5. Correlation of RMCHA with HBHA

Experiment was run in parallel to evaluate the ability of 15 samples of rhuMAbE25 (stored at  $-70^{\circ}\text{C}$ ,  $2-8^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$ , and  $40^{\circ}\text{C}$  for 1–18 months) to inhibit ragweed-induced histamine release in both the HBHA and the RMCHA. Two concentrations (0.625 and 1.25 µg/ml for RMCHA, 0.25 and 0.5 µg/ml for HBHA) of each sample were used in the assays as described in methods. Protein concentration of samples was determined from the rhuMAbE25 standard curve. The recovered concentrations (after correction of dilution

factor) of samples were calculated. A good correlation was demonstrated between the RMCHA and the HBHA with a correlation coefficient of 0.93 ( $n = 59$ ,  $p < 0.0001$ ) (Fig. 5).

In a separate study, a panel of 5 allergens (prepared in 50% glycerin) that included mite, *D. farinae*, house dust mix, ragweed, cat pelt, and *Alternaria tenuis* were used to screen a panel of eight human plasmas (seven allergic individual plasma samples from North American Biological company, Miami, FL and one pooled plasma sample from Genentech, South S.F., CA) in both RMCHA and HBHA. Both RBL 48 rat mast cells and blood basophils were first sensitized for 2 h at  $37^{\circ}\text{C}$  with 10% (v/v) human plasma in the presence or absence of rhuMAbE25 (1 µg/ml, prepared in sIMDM/3 U/ml sodium heparin for RMCHA and in PBS/0.01% BSA for HBHA), then challenged with histamine release buffer (for HBHA, HBSS/1% BSA/33%  $\text{D}_2\text{O}$ /0.8% NaCl/1.3 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; for RMCHA, sIMDM/33%  $\text{D}_2\text{O}$ /0.8% NaCl/1.3 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) or a single dose of allergen (at final concentration of 0.1 µg/ml) in histamine release buffer. Histamine concentration in the super-

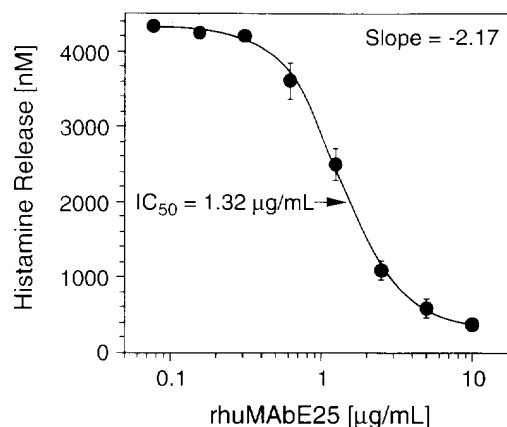


Fig. 4. Inhibition of ragweed-induced histamine release in RBL 48 cells by rhuMAbE25. Cells were preincubated for 2 h with rhuMAbE25 at 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, and 10 µg/ml in the presence of 10% (v/v) RSHP at  $37^{\circ}\text{C}$ . After  $3 \times$  washes with sIMDM, cells were challenged with 0.1 µg/ml of ragweed allergen in the presence of 50%  $\text{D}_2\text{O}$  for 30 min at  $37^{\circ}\text{C}$ . Each point and error bar represents the mean and the range of duplicate determinations respectively.

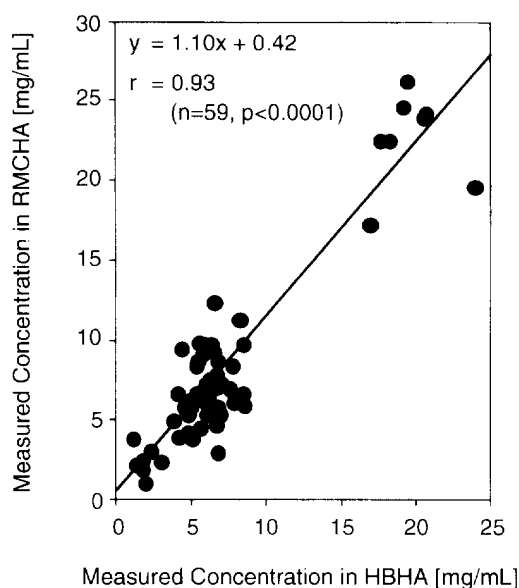


Fig. 5. Correlation between HBHA and RMCHA. 15 samples of rhuMAbE25 were each tested at two doses in both the HBHA and RMCHA for their ability to inhibit ragweed-induced histamine release. Protein concentration of samples was read from the rhuMAbE25 standard curve of respective assay. The recovered concentrations (after correction of dilution factor) of samples were plotted. Simple regression analysis with 95% confidence interval was performed using the StatView 4.0 program.

natant was determined as described in methods. A representative bar graph of four individual plasma samples for the RMCHA is shown in Fig. 6. When cells were sensitized with plasma sample one (P1), only mite, *D. farinae* and ragweed allergen elicited histamine release above the baseline (HRB). However, preincubation with a second plasma sample (P2) caused histamine release above the baseline when induced by mite, *D. farinae*, house dust, ragweed, cat pelt, and *Alternaria tenuis* allergens. Likewise, preincubation with a third plasma sample (P3) caused histamine release above the baseline when induced by mite, *D. farinae*, house dust, ragweed, and cat pelt. In contrast none of the five allergens stimulated histamine release above the baseline when cells were preincubated with a fourth plasma sample (P4). In all cases, this allergen-induced histamine release could be blocked by 1  $\mu$ g/ml of rhuMAbE25. Histamine release data obtained from

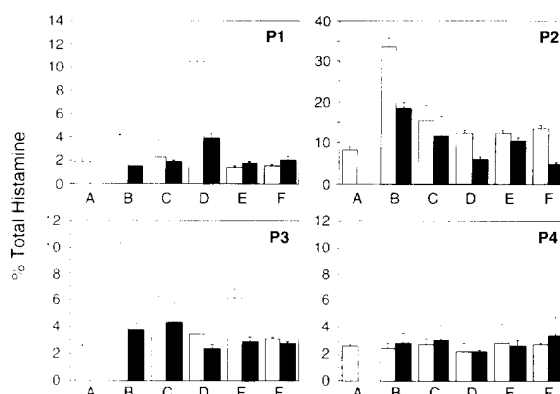


Fig. 6. Screening of human plasma samples from allergic subjects with allergen panel in RMCHA. Cells were separately sensitized with four plasmas (P1, P2, P3, and P4) isolated from allergic donors and challenged with HRB (A), standardized mite, *D. farinae* (B), house dust mix (C), ragweed antigens E-B and E-C (D), standardized cat pelt (E), or *Alternaria tenuis* (F) as described in method. Percent total histamine was determined in the absence (open bar) and presence (filled bar) of 1  $\mu$ g/ml rhuMAbE25. Each bar and error bar represents the mean and the range of duplicate determinations.

both blood and cell assays were analyzed with simple regression statistics. Table 2 shows the correlation of the two assays for house dust allergen ( $r = 0.84$ ,  $p = 0.0013$ ,  $n = 11$ ), ragweed allergen ( $r = 0.86$ ,  $p = 0.0133$ ,  $n = 7$ ), cat pelt allergen ( $r = 0.67$ ,  $p = 0.0165$ ,  $n = 12$ ), and all allergens ( $r = 0.69$ ,  $p = 0.0001$ ,  $n = 37$ ).

Table 2  
Correlation between HBHA and RMCHA in the allergen-induced histamine release

| Allergens     | Correlation Coefficient ( $r$ ) | $n$ | $p$    | Slope |
|---------------|---------------------------------|-----|--------|-------|
| House dust    | 0.84                            | 11  | 0.0013 | 3.52  |
| Ragweed       | 0.86                            | 7   | 0.0133 | 1.07  |
| Cat pelt      | 0.67                            | 12  | 0.0165 | 3.93  |
| All allergens | 0.69                            | 37  | 0.0001 | 2.03  |

Seven individuals and one pooled human plasma samples were obtained from allergic subjects for this study. These samples were screened for histamine release induced by a panel of allergens in both assays as described in method. Concentration of histamine was determined and plotted for house dust, ragweed, cat pelt, or all allergens. Simple regression analysis with 95% confidence interval was performed using the StatView 4.0 program.

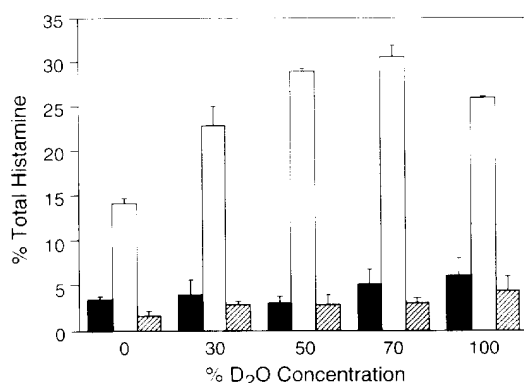


Fig. 7. Effect of D<sub>2</sub>O on ragweed-induced histamine release in RBL 48 cells. Histamine release upon challenge with either RMCHA HRB (filled bar), or RMCHA HRB/0.1 µg/ml ragweed allergen in the absence (open bar), or presence (hatched bar) of 10 µg/ml rhuMAbE25 in the presence of various concentration of D<sub>2</sub>O (0, 30, 50, 70, and 100%). Each point and error bar represents the mean and the range of duplicate determinations respectively.

#### 4.6. Effect of deuterium oxide (D<sub>2</sub>O)

Earlier studies (Fei et al., 1994) have demonstrated that incorporation of 33% D<sub>2</sub>O in the challenge medium of HBHA significantly enhances ragweed-induced histamine release. We therefore investigated the effect of D<sub>2</sub>O on the RMCHA. As expected, D<sub>2</sub>O enhances ragweed-induced histamine release in RBL 48 cells in a dose-dependent fashion reaching a maximal release at 50% D<sub>2</sub>O (Fig. 7). However, D<sub>2</sub>O neither stimulates histamine release by itself nor interferes with the inhibition of histamine release by rhuMAbE25 from RBL 48 cells.

### 5. Discussion

A bioassay was developed to quantitate allergen-induced histamine release and rhuMAbE25 activity using a rat mast cell line (RBL 48) transfected with the  $\alpha$  subunit of the Fc $\epsilon$ RI receptor. In the assay, RBL 48 cells were sensitized with human plasma containing allergen specific IgE which binds to Fc $\epsilon$ RI receptors expressed on the surface of these cells (Fig. 1). Subsequent chal-

lenge with ragweed allergen resulted in histamine release (Fig. 2) that was inhibited by the anti-IgE monoclonal antibody (rhuMAbE25) in a dose-dependent manner (Fig. 4). Since rhuMAbE25 can inhibit any allergen-induced histamine release, the format of this assay can be applied to all allergens. The assay can be modified by using a panel of allergens to screen human plasma samples for diagnosing IgE-mediated allergic conditions (Fig. 6).

The principle of the current cell-based assay (RMCHA) is identical to the human basophil histamine assay (HBHA) (Fei et al., 1994). The HBHA assay, however, has the requirement of identifying a pool of suitable human donors whose basophils have enough free Fc $\epsilon$ RI sites to allow binding of the specific IgE from the test sample. Although the donor pool can be substantially increased for the HBHA by incorporating 33% D<sub>2</sub>O in the incubation medium (Hopkins et al., 1994) this is not without consequence. It has been reported that some donors, especially patients with allergic rhinitis and asthma, may have a high background histamine release when high concentrations of D<sub>2</sub>O (Tung and Lichtenstein, 1982) are used.

In developing the RMCHA, the effect of D<sub>2</sub>O on histamine release was carefully investigated. As shown in Fig. 7, histamine release was not observed even when RBL 48 cells were challenged with high concentrations (up to 100%) of D<sub>2</sub>O. However, D<sub>2</sub>O enhances significant ragweed-induced histamine release in these cells. The mechanism whereby D<sub>2</sub>O facilitates histamine release is unknown. It has been suggested that D<sub>2</sub>O causes histamine release from basophils of atopic asthmatic subjects (Tung and Lichtenstein, 1982). Moreover, D<sub>2</sub>O stimulates glucose uptake, lactate production and CO<sub>2</sub> generation in rat hepatocytes (Wals and Katz, 1993), and induces a biphasic increase in intracellular calcium concentration in endothelial cells (Wang et al., 1993). It has been reported that D<sub>2</sub>O enhances antigen-induced histamine release from human leukocytes by stabilizing the formation of microtubules and does not alter the time course of release (Gillespie and Lichtenstein, 1972). Regardless of the mechanism, addition of D<sub>2</sub>O in

the assay clearly enhances histamine release from the RBL 48 cells.

Human myeloma IgE binds to the  $\alpha$  subunit of the human Fc $\epsilon$ RI receptor of 3D10 cells with high affinity (Hakimi et al., 1990). Furthermore, immobilization of the Fc $\epsilon$ RI receptor of the RBL 2H3 cells by wheat germ agglutinin, a lectin that binds to the  $\alpha$  subunit of the Fc $\epsilon$ RI receptor, inhibits signal transduction and secretion stimulated by multivalent antigen, covalent IgE oligomers, anti-IgE, or anti-Fc $\epsilon$ RI antibody (McCloskey, 1993). As expected, the IgE binding ELISA assay clearly demonstrated that the human IgE, which is present in ragweed sensitized human plasma, binds to the Fc $\epsilon$ RI receptor expressed on the surface of RBL 48 cells (Fig. 1). Furthermore, addition of rhuMAbE25 blocks the binding of IgE to the receptor (Fig. 1) and subsequent histamine release (Figs. 3, 4, 6, 7). Since preincubation with rhuMAbE25 and RSHP inhibits ragweed-induced histamine release in a dose dependent fashion (Fig. 4), the relative blocking activity of any anti-IgE monoclonal antibodies could be determined from the rhuMAbE25 standard curve. Specificity of the assay was demonstrated by the fact that other recombinant human monoclonal antibodies and human cytokines do not stimulate histamine release by themselves. Nor do these reagents inhibit ragweed-induced histamine release (Table 1).

Several articles reported that histamine release is dependent on Ca<sup>2+</sup> ions (Tedeschi et al., 1992; MacGlashan and Botana, 1993) and temperature (Haydik and Ma, 1988; Pottosin et al., 1993). Our data confirm these findings since the release of histamine elicited by ragweed depends on the concentration of ragweed (Fig. 2), temperature (Fig. 2), Ca<sup>2+</sup> ions (Fig. 2) and time (Fig. 3). It was reported that D<sub>2</sub>O-induced histamine release in human basophils reaches maximum in 30 min, followed by a significant decay of histamine in supernatant over several hours (Tung and Lichtenstein, 1982). These investigators reported that the catabolism of histamine can be blocked by histamine *N*-methyltransferase inhibitors, amodiaquine and SKF 91488. We also observed that in the rat mast cells, when the incubation was extended to 45 min, the concentration of

histamine in the supernatant decreased by almost 50% (Fig. 3). Presumably histamine *N*-methyltransferase inhibitors would inhibit the decay of histamine from these rat mast cells as well. The release of histamine induced by ragweed can be completely abolished if the incubation was performed either with 2.5 mM EDTA or at ambient temperature (Fig. 2) indicating the importance of calcium and temperature in regulating histamine release in these transfected rat mast cells.

The format of RMCHA may be readily modified to determine the effectiveness of rhuMAbE25 or other anti-IgE monoclonal antibodies in inhibiting histamine release induced by a variety of allergens as shown in Fig. 6. Since the RMCHA correlates well with the HBHA in evaluating the biological activities of 15 humanized anti-IgE monoclonal antibodies (Fig. 5) and in histamine release induced by various allergens (Table 2), it could be used as an additional tool of diagnosing IgE-mediated allergies. This assay can detect at least 1 ng/ml of IgE specific to ragweed (data not shown). The sensitivity of RMCHA in detecting IgE specific to other allergens is currently being evaluated. Since several studies have clearly demonstrated that IgE levels correlate with allergic disease (Bahna, 1989; Mascia et al., 1989; Platts-Mills, 1992), this RMCHA can also be used to explore IgE-mediated pathway as well as to predict the likely efficacy of immunotherapy aimed at blocking of binding of IgE to Fc $\epsilon$ RI.

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