

Down-Regulation of Human Basophil IgE and FcεRIα Surface Densities and Mediator Release by Anti-IgE-Infusions Is Reversible In Vitro and In Vivo¹

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Previously, infusions of an anti-IgE mAb (rhumAb-E25) in subjects decreased serum IgE levels, basophil IgE and FcεRIα surface density, and polyclonal anti-IgE and Ag-induced basophil histamine release responses. We hypothesized that these effects would be reversed in vivo by discontinuation of infusions and in vitro by exposing basophils to IgE. Subjects received rhumAb-E25 biweekly for 46 wk. Blood samples taken 0–52 wk after rhumAb-E25 were analyzed for serum IgE and basophil expression of IgE, FcεRIα, and CD32. Basophil numbers were unaffected by infusions. Eight weeks after infusions, free IgE levels rose in vivo but did not reach baseline. Basophil IgE and FcεRIα rose in parallel with free IgE while CD32 was stable. FcεRI densities, measured by acid elution, returned to 80% of baseline, whereas histamine release responses returned to baseline. Basophils cultured with or without IgE or IgG were analyzed for expression of IgE, FcεRIα, and CD32. By 7 days with IgE, expression of IgE and FcεRIα rose significantly, whereas cultures without IgE declined. IgE culture did not effect CD32. IgG culture did not effect expression of any marker. The present results strongly suggest that free IgE levels regulate FcεRIα expression on basophils. *The Journal of Immunology*, 1999, 162: 5624–5630.

Approximately 25% of the U.S. population suffers from some form of atopic disease (1). As a result of genetic and environmental factors, these individuals synthesize allergen-specific IgE that attaches to circulating basophils and tissue mast cells via high-affinity receptors (FcεRI). Binding of multivalent allergens by surface-bound specific IgE cross-links FcεRI and triggers cellular events resulting in the release of preformed mediators, such as histamine, and newly synthesized mediators, such as 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosanoic acid (LTC₄) and IL-4 (2). These and other mediators produce the clinical symptoms of allergic disease and, with repeated allergen exposure, can promote chronic airway inflammation, the hallmark of diseases such as allergic rhinitis and asthma. Therefore, the removal of IgE may offer a specific therapy for control of essentially all allergic diseases.

Although chronic allergic airway diseases, such as rhinitis and asthma, have been managed effectively with topical antiinflammatory therapies, newer treatments directed at IgE have entered human trials. A humanized monoclonal anti-IgE Ab (rhumAb-E25)³ has been developed to complex-free IgE at its receptor binding

site, competitively inhibiting its interaction with FcεRIα on mast cells and basophils, thereby preventing sensitization (3). Early clinical trials of rhumAb-E25 infusions in allergic asthmatics demonstrated suppression of both early and late responses to inhaled allergen (4, 5). In our hands, in vivo treatment with rhumAb-E25 led to rapid and dramatic falls in free serum IgE levels, and of IgE and FcεRIα receptors on circulating basophils (6). Reductions in allergen-specific basophil histamine release were also seen. In more recent work, the presence or absence of IgE in cultures of peripheral blood basophils determines whether surface expression of FcεRIα increases or decreases (7). The modulation noted in total FcεRIα surface expression supports the concept that free serum IgE levels influence the number of FcεRI receptors on the basophil surface. Indeed, a correlation between total serum IgE levels and the number of high-affinity IgE surface receptors on peripheral blood basophils has been known for 20 years (8). Thus, if removal of IgE is related to down-regulation of its receptor on basophils, conversely, IgE exposure may exert a positive effect on FcεRI receptor expression by circulating basophils. Indeed, accumulating evidence from cultured mouse mast cells and human basophils suggests a positive effect of IgE exposure on its own receptor expression (7, 9, 10).

The aim of the present study was to determine whether rhumAb-E25-induced reductions in basophil IgE and FcεRIα expression, and histamine release responses, were reversible. This was tested in two ways. First, we examined the kinetics of the reappearance of free IgE and surface-bound IgE and FcεRIα on circulating basophils in individuals completing a total of 46 wk of rhumAb-E25 infusions. Subjects were followed for up to 1 yr by monitoring levels of free IgE in the serum, as well as basophil surface levels of IgE and FcεRIα. At the termination of the follow-up, total IgE receptor number as well as basophil histamine release to both anti-IgE and Ag (*Dermatophagoides farinae*) were examined. Second, basophils from these subjects obtained within 8 wk of discontinuation of rhumAb-E25, and therefore expressing markedly reduced

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³ Abbreviations used in this paper: rhumAb-E25, a humanized monoclonal anti-IgE Ab; MFI, mean fluorescence intensity; BPO, benzylpenicilloyl.

Table I. Baseline characteristics of study subjects

Characteristic	Mean $\pm$ SD (range)
Age	29 $\pm$ 6 yr (19-38)
Male/female	9/6
Weight	89 $\pm$ 16 kg (62-112)
Baseline serum IgE	624 $\pm$ 153 ng/ml (199-1255)

levels of surface IgE and Fc ϵ RI α , were exposed in vitro to IgE in short-term cultures and examined for effects on basophil IgE and Fc ϵ RI α surface levels. Using these approaches, we demonstrate a reversal of rhumAb-E25-induced effects on basophil phenotype both in vitro and in vivo.

Materials and Methods

Subjects and protocol

Fifteen adult rhinitic subjects with dust mite sensitivity by skin prick test were enrolled into a phase I safety and tolerance trial of rhumAb-E25 therapy (Table I). Nine subjects also had asthma. Baseline enrollment serum IgE levels (obtained in 1995) were between 83 and 523 IU/ml (199–1255 ng/ml). These subjects were randomized to receive two dosing schedules for rhumAb-E25 based on initial total serum IgE measurements, either 0.015 mg/kg/IU/ml or 0.03 mg/kg/IU/ml, with three initial weekly i.v. loading infusions followed by maintenance infusions at biweekly intervals (11). At week 28, subjects were randomized to receive a new maintenance dose of either 0.005 mg/kg/IU/ml or 0.0015 mg/kg/IU/ml, representing a 3- to 20-fold dose reduction. Patients gave informed written consent for the rhumAb-E25 study as approved by the Johns Hopkins Bayview Medical Center Institutional Review Board. Exclusion criteria were as previously described (6).

Venous blood samples were obtained from these subjects before and during the first 3 mo of therapy (6) and again beginning at the conclusion of rhumAb-E25 infusions (i.e., after 46 wk of therapy) and at weekly or monthly intervals for up to 1 yr, as listed in the text. For in vitro basophil culture experiments (described below) blood was obtained at 0, 2, 4, or 8 wk posttreatment. For other experiments, blood samples were obtained at the above time points and then monthly for serum IgE monitoring until termination. Basophil surface phenotyping, receptor measurements, and histamine release studies were also performed at termination. Subjects were terminated from the study when they met one of the following criteria: 1) circulating total rhumAb-E25 levels became undetectable (<16 ng/ml) and total serum IgE was >50% of baseline; 2) free serum IgE (not complexed with rhumAb-E25) reached $\geq$ 50% of their enrollment level; or 3) the subjects reached 1 yr post rhumAb-E25 infusions.

Buffers

Piperazine-*N,N'*-bis-2-ethanesulfonic acid (PIPES) obtained from Sigma (St. Louis, MO) was used in a stock buffer composed of 25 mM PIPES containing 110 mM NaCl, 5 mM KCl, and 40 mM NaOH, adjusted to pH 7.3. PAG buffer consisted of PIPES buffer containing 0.003% human serum albumin obtained from Miles Laboratories (Elkhart, IN) and 0.1% glucose; PAGCM buffer consisted of PAG buffer with 1 mM MgCl₂ and 1 mM CaCl₂; PAG-EDTA buffer consisted of PAG buffer with 1 mM EDTA.

Basophil enrichment

Basophils for culture were enriched from 60 ml of venous blood using a Percoll-based density gradient centrifugation technique as previously described, except that all leukocyte isolations were performed in a sterile manner using 0.45- μ m sterile-filtered reagents (12). Basophil purity ranged from 1 to 16% (4 $\pm$ 1%, n = 17, mean $\pm$ SEM) as enumerated by Alcian blue staining and light microscopic counts (13). Total cell counts and viability (98 $\pm$ 0.4%, n = 17, mean $\pm$ SEM) were determined by light microscopy and erythrosin B dye exclusion.

Basophils for immediate flow cytometric analysis were isolated from nonsterile 10-ml samples using the same technique (6). For receptor measurements by acid elution and histamine release studies, blood was separated on a single-step Percoll gradient (specific gravity, 1.080), and the interface monolayer was harvested and washed as described (14).

Basophil culture

Enriched basophils were cultured for up to 7 days in Iscove's modified Dulbecco's medium from Life Technologies (Gaithersburg, MD) with 2%

heat-inactivated FCS, 10 μ g/ml gentamicin (Life Technologies) and 10 ng/ml rIL-3 from R&D Systems (Minneapolis, MN) at a final concentration of $1-2 \times 10^6$ cells/ml. Cultures were supplemented with or without 50–500 ng/ml myeloma IgE-PS, (15), or 500 ng/ml human IgG (Sigma), containing <0.6 ng/ml IgE as determined by ELISA, Johns Hopkins Dermatology, Allergy, and Clinical Immunology Laboratory. Doses of myeloma IgE used for culture experiments were based on the range of original serum IgE levels of the 11 subjects and preliminary culture studies. Cells were incubated at 37°C in 5% CO₂ in 6-well tissue culture plates from Costar (Cambridge, MA) and harvested after 3 or 7 days. After 7 days of culture, viability was 80% $\pm$ 4%, n = 17 (mean $\pm$ SEM) and total cell recovery was 64% $\pm$ 6%, n = 16 (mean $\pm$ SEM).

Flow cytometry

Enriched basophil preparations were labeled for direct and indirect immunofluorescence and flow cytometry in the presence of 4 mg/ml human IgG to minimize nonspecific binding to Fc γ R, as previously described (6). Abs utilized for these studies included an irrelevant mouse IgG1 control from Coulter (Hialeah, FL); FITC-conjugated polyclonal goat anti-human IgE and its control FITC-conjugated normal goat IgG from Kirkegaard and Perry (Gaithersburg, MD); mouse anti-human Fc ϵ RI α subunit (22E7, IgG1 unaffected by Fc ϵ RI occupancy, kindly provided by J. Kochan (Roche Pharmaceuticals, Somerville, NJ) (16) and mouse anti-human Fc γ RII (CD32) IgG1, mAb IV.3 from Medarex (West Lebanon, NH); or mAb 2E1 from AMAC (Westbrook, ME). Samples were analyzed on a Coulter EPICS profile flow cytometer. At least 5000 events per experimental condition were counted. Data is expressed as net mean fluorescence intensity (MFI) (actual MFI – MFI of irrelevant IgG control (4 $\pm$ 1, mean $\pm$ SEM). Standard microbeads (Sigma) were run with each group of samples to monitor for day-to-day machine variability (average bead MFI 49 (47.5, 51.2); 95% confidence intervals).

Serum-free IgE analysis

Standard total serum IgE assays would capture E25-bound IgE, therefore, serum-free IgE levels were measured using a solid-phase ELISA (17) to avoid binding IgE complexed to rhumAb-E25. High-binding polystyrene plates (Costar) were coated overnight at 2–8°C with 100 ng of human IgE receptor α -chain IgG chimera in 100 μ l PBS (pH 7.2), washed, and stored frozen. Samples were then analyzed by standard ELISA methods. After washing, captured IgE was detected with biotinylated monoclonal anti-human IgE and the color reaction generated with streptavidin- β -galactosidase and 4-methylumbelliferyl- β -D-galactoside. The detection limit of the assay was 0.8 ng/ml with an upper limit of 226 ng/ml. For samples containing <40 ng/ml of total E25, dilutions of serum samples were possible for free IgE measurements. A second assay for a higher range of free IgE detection was the same, except plates were coated in carbonate buffer (pH 9.6) and processed with a streptavidin-horseradish peroxidase and o-phenylenediamine/H₂O₂ (other assay differences, e.g., diluent, not discussed). The higher range assay had a range of 24–396 ng/ml. The high-range and low-range assay differ systematically in quantitation, with the high-range assay giving higher free IgE estimates. Exploratory analysis showed that the percent difference in quantitation was approximately constant over the assay range, $\pm$ 3.6% higher than the low-range assay (estimate $\pm$ SE). To compare high- and low-range assay results in this study, the high-range assay results were multiplied by an adjustment factor of 1/1.600 = 0.625.

Measurements of open and occupied Fc ϵ RI on basophils

To determine both the total and unoccupied receptor densities, isolated mononuclear cells were first sensitized with benzylpenicilloyl (BPO)-specific IgE (5 μ g/ml) for 20 min at 37°C in RPMI 1640 medium (Life Technologies) containing 1 mM EDTA and 10 μ g/ml heparin (6). Previous studies demonstrated that sensitization with this concentration of BPO-specific IgE for this length of time effectively saturates unoccupied receptors and, therefore, allowed measurement of total receptors (by measuring the total IgE eluted from saturated cells) as well as the measurement of unoccupied receptor density (by measuring the total BPO-specific IgE eluted from saturated cells; see below) (18). After washing once, the cells were layered over 1 ml of EDTA-chelated FCS (5 mM EDTA in heat-inactivated FCS) and centrifuged to separate the cells from their diluted sensitization buffer. The cells were further washed twice in PAG, then resuspended in PAG buffer and incubated for 60 min at 37°C. After centrifugation, the pellets were resuspended in 1 ml of saline. After a final centrifugation, duplicate samples were removed for cell counts. Supernatants from sensitized cells are also analyzed to ensure a lack of IgE carry-over from the sensitization step.

The pellets were resuspended in ice-cold acetate buffer (pH 3.7) and incubated in an ice bath for 10 min. After a brief centrifugation (15,000 $\times$

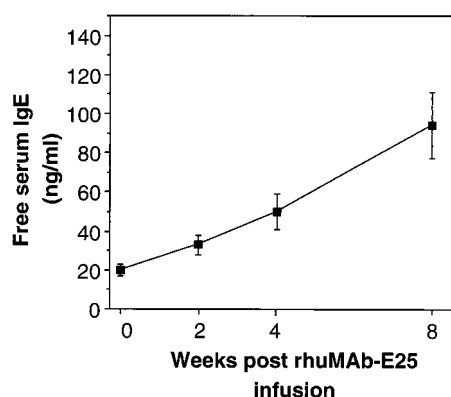


FIGURE 1. Levels of free serum IgE within 8 wk after discontinuation of rhumAb-E25. Mean values $\pm$ SEM for 11 subjects are shown.

g), the supernatant was removed and neutralized with 1 N NaOH. The eluted IgE was measured in either a total IgE RIST (total receptor measurement) or BPO-RAST (unoccupied receptors) (17). Cell counts (Alcian blue-positive cells) obtained before elution allowed a calculation of the receptor density to be made (the amount of IgE measured by RIST or RAST divided by cell count with the result expressed as IgE molecules per basophil).

Histamine measurements

The histamine content of whole blood leukocytes was analyzed by automated fluorometric analysis of perchloric acid lysates of 100- μ l aliquots of whole blood as performed previously (6, 19).

Aliquots of mononuclear cells destined for receptor analysis were also analyzed for maximal histamine release by examination of dose response curves for polyclonal goat anti-human-IgE Ab (0.01–10 μ g/ml). In addition, two doses of *D. farinae* (10 and 0.5 PNU/ml) from Miles (Spokane, WA), previously shown to be optimal and suboptimal for release, were included for Ag-specific basophil histamine release (6). Briefly, cells were suspended in PAGCM and challenged with the stimulus for 45 min at 37°C at a final volume of 1 ml. All reactions were performed in duplicate and stopped by centrifugation, and the supernatant was removed for histamine analysis.

Statistical methods

All values are mean $\pm$ SD, unless otherwise noted. A nonparametric Wilcoxon signed rank statistic was used to determine differences in the data sets. Correlations were calculated using simple regression analysis.

Table III. Percentages of basophils in mononuclear cell preparations and whole blood lysate histamine content during and post rhumAb-E25 infusions

Study Day	n ^a	% Alcian Blue Positive	Histamine Content (ng/100 μ l whole blood) ^b
During infusion			
70	9	1.7 $\pm$ 1.5	6.3 $\pm$ 2.2
140	11	3.5 $\pm$ 2.5	8.2 $\pm$ 3.5
322*	11	4.8 $\pm$ 3.4	7.7 $\pm$ 2.5
Postinfusion			
56	11	3.5 $\pm$ 2.3	6.8 $\pm$ 2.2
>278	6	3.4 $\pm$ 2.3	5.6 $\pm$ 1.2

^a Values (mean $\pm$ SD) are provided at each time point only for those donors where matched samples of percent Alcian blue positive and histamine content were available for analysis. Of the 11 subjects in the post rhumAb-E25 study, a maximum of 9 were available for these analyses at infusion day 70. The $n = 6$ at the >278 day time point is due to the loss of subjects 1–4 and 8 in Table II.

^b Duplicate aliquots (100 μ l) of EDTA-anticoagulated whole blood were lysed in perchloric acid and histamine content measured, as described in *Materials and Methods*.

* $p > 0.05$ for all time points as compared to day 322 (last rhumAb-E25 infusion) by paired t test.

Results

Effect of discontinuation of rhumAb-E25 infusions on free serum IgE levels

A total of 11 of the original 15 subjects participated in the post rhumAb-E25 portion of the study. Of these, four subjects were terminated from the follow-up study by meeting the first criteria, four others the second criteria, and three subjects completed the entire 1-yr follow-up period (see *Materials and Methods*). During the follow-up period after the final infusion with rhumAb-E25, subjects slowly regained levels of detectable serum-free IgE. Shown in Fig. 1 is the rise of free serum IgE in the 11 subjects who completed the follow-up period. Mean free IgE increased from 20 ± 3 ng/ml 1 h after last rhumAb-E25 infusion (time 0) to 94 ± 17 ng/ml at 8 wk post rhumAb-E25, reaching 16% of the pre-rhumAb-E25 infusion mean of 583 ± 106 ng/ml. At the termination from the follow-up portion of the study, serum-free IgE reached different levels depending on the reason for termination (Table II). Subjects who received the highest dosing regimen of rhumAb-E25 (0.03 mg/kg/IU/ml the first 28 wk and subsequent 6-fold dose reduction for the remaining 18 wk) had the longest

Table II. Free serum IgE levels at termination from the study

Subject	Day ^a	Reason for Termination	Preinfusion (postinfusion) IgE (ng/ml) ^b	Termination IgE (ng/ml)	% of Baseline IgE ^c
1	146	Free IgE >50% baseline	329 (24)	>150	ND
2	190	Free IgE >50% baseline	419 (14)	>150	ND
3	140	Free IgE >50% baseline	195 (11)	138	71
4	168	Free IgE >50% baseline	309 (34)	>150	ND
5	365	Completed follow-up	523 (5)	238	46
6	370	Completed follow-up	515 (19)	316	61
7	362	Completed follow-up	676 (34)	492	73
8	224	E25 undetectable	658 (22)	342	52
9	335	E25 undetectable	485 (6)	385	79
10	308	E25 undetectable	1492 (31)	760	51
11	308	E25 undetectable	810 (23)	530	65

^a Number of days after the last infusion of rhumAb-E25.

^b Preinfusion refers to serum IgE at subjects enrollment; Postinfusion refers to free IgE levels measured 1 hr after last rhumAb-E25 infusion.

^c % = termination IgE/ preinfusion IgE.

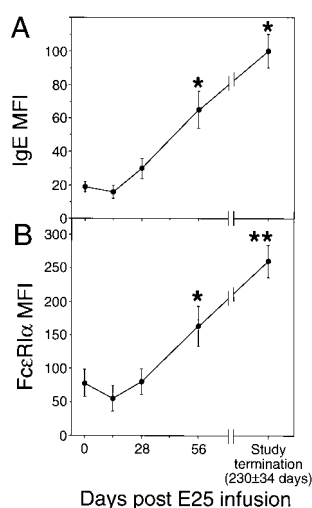


FIGURE 2. Levels of basophil surface IgE (A) and FcεRIα (B) after discontinuation of rhumAb-E25. Flow cytometric analysis of blood basophils by FITC-conjugated goat anti-human IgE (A) and by FcεRIα Mab 22E7 plus phycoerythrin-conjugated goat anti-mouse IgG (B). Values represent mean $\pm$ SEM for 11 subjects (preinfusion mean levels are 216 ± 5 MFI for IgE). *, $p < 0.01$ or **, $p < 0.001$, as compared with day 0.

follow-up in comparison to other dosing regimens (343 ± 13 days, $n = 4$ (subjects 5, 7, 9, 11; Table II) vs 218 ± 32 days, $n = 7$).

Effect of discontinuation of rhumAb-E25 on circulating basophil numbers, histamine content, and surface IgE, FcεRIα, and FcγRII expression

As shown in Table III, the relative percentage of basophils in mononuclear cell preparations as measured by Alcian blue staining during and after the rhumAb-E25 infusion period remained stable. Likewise, the histamine content of whole blood lysates remained stable during and after rhumAb-E25 infusions. Thus, rhumAb-E25 infusions did not alter the percentage or the average histamine content of circulating basophils.

In parallel with a rise in serum-free IgE levels in subjects after discontinuation of rhumAb-E25, levels of IgE and FcεRIα on basophils also increased (Fig. 2). Basophils obtained within 4 wk of the last rhumAb-E25 infusion demonstrated either low ($n = 9$) or undetectable ($n = 2$) surface labeling for IgE (Fig. 2A). The limit of detection is $\sim 8,000$ – $10,000$ surface IgE molecules (20). During the observation period in this study, IgE surface intensity returned to baseline levels in only a single subject (subject 3, 24 wk post rhumAb-E25 after a $\geq 50\%$ return of free IgE). Median MFI of surface IgE staining rose significantly by 8 wk after the last rhumAb-E25 infusion ($p < 0.01$, $n = 11$, 2.7-fold increase), and continued to rise, so that by the time of termination, a median 4.4-fold increase had occurred ($p < 0.01$, $n = 11$). These termination values represent 47% of the enrollment, pre-rhumAb-E25 infusion values while free serum IgE levels had reached an average of 62% ($n = 8$, three subjects indeterminable) of the enrollment values (6).

A similar rise in basophil FcεRIα expression was also noted (Fig. 2B) with a median rise in MFI from 60 at day 0 to 118 by day 56 ($p < 0.01$, $n = 11$, ~ 2 -fold increase). By the end of the study, median expression reached 266 MFI ($p = 0.001$, $n = 11$, 4.4-fold increase). Unfortunately, enrollment levels of FcεRIα were not analyzed (6) so no comparison is available. In contrast, expression of another surface Ig receptor, FcγRII (CD32), was not significantly altered during the same period (data not shown).

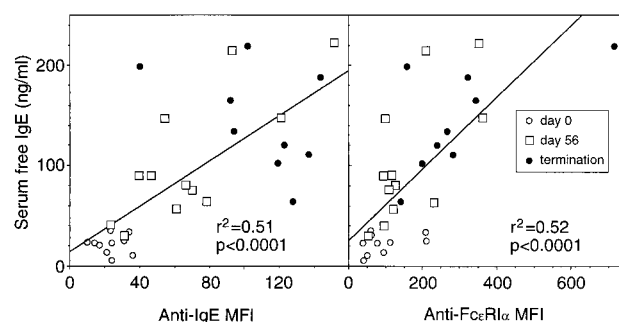


FIGURE 3. Correlation of serum free IgE with basophil surface IgE and FcεRIα. Data are from three different time points as indicated in the figure legend. Values of p were calculated using simple regression analysis.

In support of the hypothesis that free serum IgE levels influence basophil FcεRIα expression, significant correlations were seen between free serum IgE levels and basophil surface phenotype at three time points during the follow-up period (Fig. 3). Receptor occupancy appeared to be fairly consistent at these time points given that the intensity of surface IgE was correlated to the intensity of IgE receptor ($r^2 = 0.49$, $p = .0001$).

Effect of discontinuation of rhumAb-E25 on basophil total IgE receptor number and IgE-dependent histamine release responses

Shown in Fig. 4 are the results of additional basophil receptor numbers as measured by the lactic-acid elution method. Fig 4A shows mean pretreatment levels of total basophil receptors to be $\approx 230,000$ per basophil. After 12 wk of rhumAb-E25 infusion, mean receptor levels were dramatically reduced to $< 10,000$ receptors per basophil, as previously described (6). By the time of discontinuation of rhumAb-E25 (i.e., 18 wk after institution of rhumAb-E25 dosage reductions; see *Materials and Methods*), receptors had risen to nearly 35,000 per basophil. At the termination of the follow-up study (see Table II), the receptor numbers had almost returned to pretreatment levels ($\approx 214,000$ per basophil).

The functional consequences of IgE and IgE receptor modulation by rhumAb-E25 treatment are depicted in Fig. 4, B and C. The mean basophil histamine release responses to an optimal dose of *D. farinae* (10 PNU/ml) were reduced by $\approx 90\%$ after 12 wk of rhumAb-E25 treatment, whereas anti-IgE-mediated release was reduced to a lesser extent ($\approx 40\%$), as previously reported (6). However, by the final rhumAb-E25 infusion (46 wk), the 3-fold rise in receptor number (Fig. 4A) and rising free IgE levels (Fig. 1) were associated with an almost complete return of *D. farinae*-specific histamine release and a complete return of anti-IgE-induced histamine release.

Effect of culture with IgE or IgG on basophils from rhumAb-E25-treated subjects

Studies of human basophils have demonstrated that IgE induces up-regulation of FcεRIα (7). Given the markedly reduced IgE surface expression present on basophils at the completion of rhumAb-E25 infusions, we took advantage of this unique opportunity to see whether basophils from these subjects would display similar regulation of FcεRIα by IgE. Basophils obtained within 8 wk of discontinuation of rhumAb-E25 were cultured with IgE (or IgG, as a control) and effects on basophil surface expression of IgE, FcεRIα, and FcγRII (CD32, as a control) were examined. No significant enhancement of surface IgE and FcεRIα expression was detectable at 3 days of culture with myeloma IgE (data not shown); however, 7 days of culture with myeloma IgE resulted in significant 1.6- to

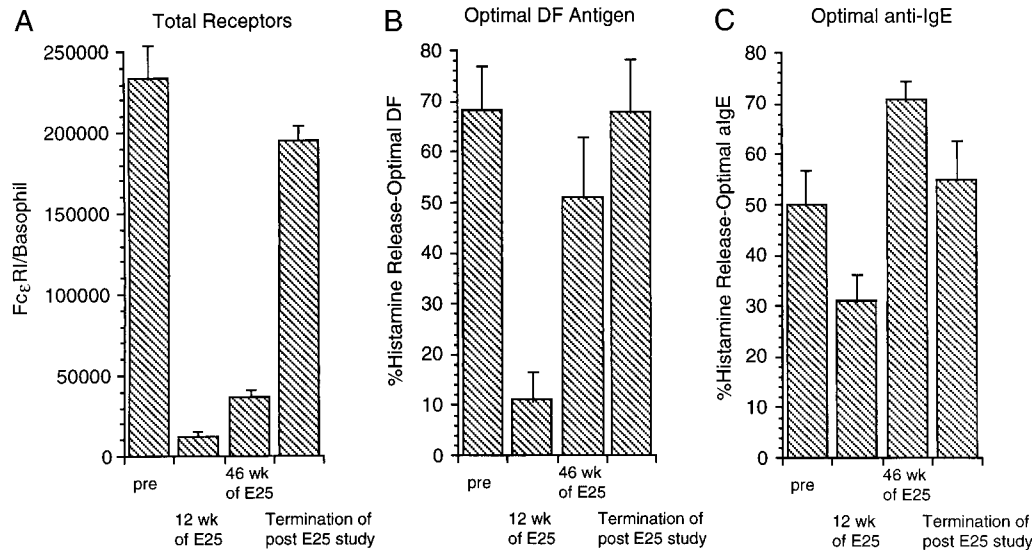


FIGURE 4. Quantitation of basophil total IgE receptors by lactic acid elution (A) and *D. fariniae* (DF) and anti-IgE-induced histamine release responses (B and C). A, Mean RIST values for total IgE eluted from subjects' basophils ($n = 11$) before infusion of E25 (pre), after 12 wk of rhumAb-E25 treatment (12 wks of E25), 1 h after last infusion of E25 (46 wks of E25), and at termination from the postinfusion follow-up study (termination of post E25 study). Values are expressed as the mean $\pm$ SEM number of receptors per basophil. B and C, Samples obtained at the same time points as in A were examined for histamine release responses to optimal doses of *D. fariniae* or anti-IgE. Results are expressed as mean percent of histamine release.

2-fold enhancement of basophil FcεRIα and surface IgE staining, respectively (Fig. 5). The enhancement in both surface IgE and FcεRIα expression was dose-dependent in that parallel cultures using 500 ng/ml demonstrated greater enhancement than 50 ng/ml IgE cultures ($n = 5-6$; data not shown). Culture in medium alone led to a slight but significant decline in surface IgE, whereas FcεRIα did not change. Basophils cultured in the presence of 500 ng/ml IgG resembled basophils cultured with medium alone in that no significant increases in expression of IgE or FcεRIα were observed. As a control, expression of another Ig receptor on basophils FcγRII (CD32) was also assessed and did not change sig-

nificantly under either of the Ig culture conditions (data not shown). Cell recovery and viability did not differ among each of the culture conditions.

The relative increase in receptor density observed in basophils cultured with IgE (Fig. 5B) was related to starting FcεRIα expression (Fig. 6). Basophils cultured within 2 wk of rhumAb-E25 completion, and hence lower surface expression of FcεRIα, showed larger increases than later cultures, as previously demonstrated (7). Increases ranged from 15- to 0.7-fold for starting FcεRI densities ranging from 15 to 250 MFI. Basophils cultured within 2 wk of completion of rhumAb-E25 rose from a median of 50 on day 0 to

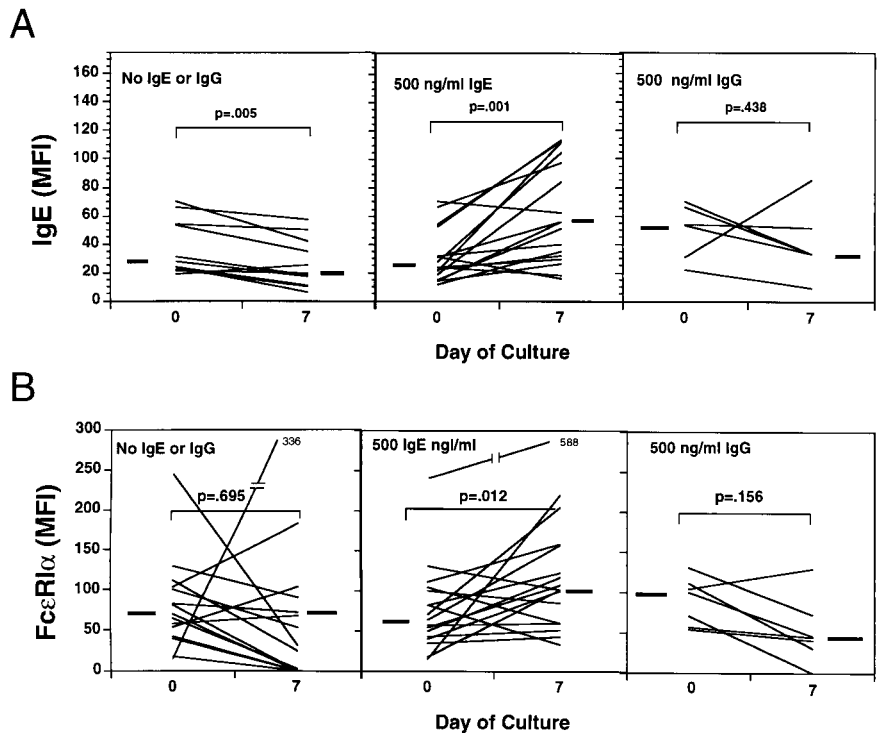


FIGURE 5. Levels of basophil expression of surface IgE (A) and FcεRIα (B) after 7 days of culture with or without 500 ng/ml IgE ($n = 17$) or IgG ($n = 6$). Mononuclear cells were obtained from all eleven subjects (5 studied once, 6 studied at two different time points) within 0–8 wks of discontinuation of rhumAb-E25 and cultured for 7 days as indicated. Horizontal bars represent median MFI values for each time point. Staining was performed as in Fig. 2. Values of p were calculated using Wilcoxon signed rank statistic.

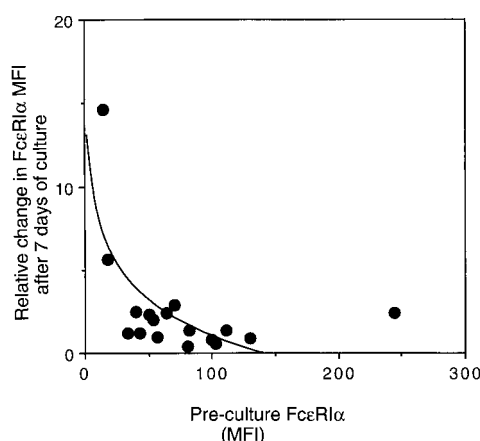


FIGURE 6. Relationship between levels of FcεRIα on freshly isolated basophils from rhumAb-E25 recipients and levels achieved after 7 day culture with 500 ng/ml IgE. Relative change in receptor expression = net MFI of 22E7 day 7 divided by the net MFI of 22E7 day 0 of culture. Curve generated by logarithmic regression analysis. ($r^2 = 0.42$).

a median of 122 on day 7 ($n = 9$, $p = .004$, 2.4-fold increase). A single culture using basophils from a patient in the midst of rhumAb-E25 infusions (week 36 of 46) yielded a 3.8-fold rise over the 7 day culture period (data not shown).

Discussion

We have shown that discontinuation of rhumAb-E25 in atopic subjects leads to a rise in free serum IgE levels and a repopulation of the basophil surface with both IgE and FcεRIα. The relative strength of the correlation ($r^2 > 0.5$) supports the existence of a relationship between serum IgE levels and basophil phenotype for IgE and FcεRIα density. A separate measurement of total basophil receptor number (acid elution) also demonstrated a return in basophil FcεRI receptor levels to nearly pretreatment levels by the termination endpoints from the follow-up study. Basophil surface enhancement of IgE and FcεRIα after rhumAb-E25 occurred without effects on numbers of isolated basophils from the circulation. Furthermore, whole blood histamine content, another marker of blood basophil presence, also remained stable during and after rhumAb-E25 infusions. The effect on basophil surface phenotype was selective, because levels of an additional basophil Ig receptor (FcγRII, CD32) remained unaltered during the same period. Thus, discontinuation of rhumAb-E25 therapy led to a reversal of its effects on both free serum IgE levels and basophil surface expression of IgE and FcεRIα.

Unlike the situation after 3 mo of rhumAb-E25 treatment when Ag-specific histamine release was reduced by ~90% (6), by the time of final rhumAb-E25 infusion, Ag-specific release was nearly restored despite only a modest rise in receptor number. Thus, functional effects of receptor down-regulation by rhumAb-E25 were restored at receptor levels well below pretreatment levels. This restoration can be interpreted as a consequence of both receptor repopulation as well as rising free serum IgE levels seen in subjects after conclusion of rhu-MAb-E25. Given that the threshold number of IgE-cross-links by Ag needed to cause maximal basophil histamine release has been reported to be several thousand, this setpoint appears to have been partially reestablished as a result of the rhumAb-E25 dose reduction protocol. Furthermore, Ag-induced histamine responses were fully restored within weeks of rhumAb-E25 discontinuation. The clinical implications of this phenomena of restored basophil histamine release in terms of therapy with rhumAb-E25 remains to be established.

In vitro experiments with basophils isolated from former rhumAb-E25 recipients and exposed in culture to myeloma IgE also showed enhancement of surface expression of IgE and FcεRIα receptors. The low level of FcεRIα and surface IgE expression induced in vivo on basophils from rhumAb-E25-treated subjects was nearly doubled by short-term in vitro culture with myeloma IgE. Parallel cultures with equal amounts of IgG or medium alone failed to show enhancement, supporting the specificity of IgE in altering basophil surface expression. The increase in the density of cell surface FcεRI in vitro was more rapid than observed in vivo, suggesting that 1) there is no alteration in the ability of the basophils to up-regulate FcεRI since these results are similar to those found with rhumAb-E25-naïve donors in in vitro studies, and 2) that the slower rise in vivo probably results from the slower rise in free IgE levels. In vitro cultures also showed dose dependence on IgE for high-affinity receptor enhancement. As before, starting levels of receptor expression appear to predict the degree of receptor enhancement. The lower the initial starting level of FcεRIα, the greater the resulting enhancement seen in basophil cultures as shown in basophils cultured from rhumAb-E25-naïve donors (6), where the fold increase of FcεRIα expression after 1 wk of culture with myeloma IgE (2.5-fold) was similar to that observed in Fig. 5 in our basophil cultures from E25 recipients. Thus, free IgE levels appear to regulate the levels of FcεRIα and IgE expression on cultured human blood basophils extending the activity of free IgE to both in vitro and in vivo conditions.

Evidence for receptor regulation by free IgE levels is not limited to human basophil studies. Work in other cultured basophil systems, including basophil cell lines (21), or mouse bone marrow basophil cultures (21), in vitro and in vivo mast cell studies in the mouse (9), and cultured human fetal liver mast cells (22), all have shown FcεRI regulation by ambient IgE levels. However, this is the first report that human peripheral blood basophils demonstrate up-regulation of the high-affinity receptor in association with rising serum levels of free IgE in vivo.

The mechanism for this IgE-dependent effect, whether direct or indirect, remains unclear. Experiments using protein synthesis inhibitors in murine mast cells (9) suggested that up-regulation of the high-affinity receptor could not be completely explained by pre-formed receptors that are stabilized under the influence of free IgE, as was suggested by studies using rat basophilic leukemia cells (23). Furthermore, the kinetics of receptor loss seem to follow a time course measured in days, despite a rapid fall in free IgE levels that occurred over hours, such as with the first infusions of rhumAb-E25 Ab. Conversely, in vitro restoration of the basophil surface receptors required days to repopulate both IgE and FcεRIα, without detectable enhancement present at 3 days (as measured by the limits of flow cytometer).

A similar scenario could be invoked for the in vivo results after decay of excess rhumAb-E25; however, the in vivo enhancement was complicated by levels of free IgE that were the net effect of ongoing IgE synthesis in the face of declining rhumAb-E25 levels. Unlike murine systems demonstrating a suppression of IgE synthesis by treatment with anti-IgE mAbs (24), the in vivo experience with rhumAb-E25 did not appear to suppress on-going IgE synthesis. Whether the subjects free IgE level will return to baseline levels is not yet established.

The in vitro up-regulation of receptor occurred in the presence of rIL-3, which was necessary to maintain basophil viability for 1 wk. IL-3 is known to prime basophils for inflammatory activities (25), as well as support bone marrow precursor development toward a basophilic lineage (26). However, in the presence of IL-3 alone, we failed to detect basophil FcεRIα receptor enhancement

and, in fact, observed a slight but consistent loss of receptor. Furthermore, the specificity for IgE to cause receptor enhancement in cultures suggested that binding of IgE to the basophil is needed to achieve receptor induction. Recent in vitro studies by MacGlashan (6) show that a mixture of an excess of an anti-IgE mAb with IgE in basophil cultures prevented basophil receptor up-regulation, supporting a need for IgE binding to FcεRIα for enhancement. While others have shown a role for IL-4 in further enhancing induction of FcεRIα (22, 27, 28), this was not examined in our studies.

Given the mixed leukocyte composition of our culture preparations, we needed to exclude expression and measurement of high-affinity receptor on contaminating cells. Other circulating leukocytes such as monocytes may bear high-affinity IgE receptors in certain atopic conditions (29, 30). The use of gating by cellular scatter characteristics allowed exclusion of most monocytes from FcεRIα analysis by flow cytometry. Furthermore, preliminary experiments designed to preferentially detect monocyte FcεRIα expression enhancement under identical culture conditions have failed to demonstrate such an IgE-dependent effect. Previous reports of monocyte FcεRIα expression (29, 30) have been generally limited to individuals with atopic dermatitis and at considerably lower levels of intensity (~2 logs) than basophil as measured by flow cytometry.

Infusions of an IgE-binding mAb in human subjects have reduced basophil FcεRIα expression and histamine release responses to Ag, both of which are reversed after discontinuing infusions. Restoration of Ag-triggered histamine release responses occurred more rapidly than receptor number and implies a lower threshold for basophil activation through Ag interaction with specific IgE bound to its receptor. Furthermore, basophil FcεRIα receptor enhancement occurred under the influence of IgE, both in vivo and in vitro, and suggests a regulation by free IgE of basophil receptor expression. Further studies to examine the exact mechanism of IgE-dependent regulation of the high-affinity receptor on human basophils are needed.

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