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Research Article

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Recombinant Soluble Form of the Human High-affinity Immunoglobulin E (IgE) Receptor Inhibits IgE Production through Its Specific Binding to IgE-bearing B Cells

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Abstract

A recombinant soluble form of the α subunit of the human high-affinity receptor for IgE (rsFcϵRIα), one of the potent IgE-binding molecules, was tested for its ability to regulate IL-4–induced IgE synthesis by human lymphocytes. Addition of rsFcϵRIα to cultures induced a dose-dependent inhibition of the T cell–dependent and independent synthesis of IgE. The suppression of IgE synthesis was observed at the protein and the mRNA levels, and it was IgE class specific. By flow cytometry, specific binding of rsFcϵRIα was detected on surface IgE-bearing B cells as well as on U266 cells, and it was completely blocked by preincubation with IgE. rsFcϵRIα bound to the cell surface IgE could be effectively dissociated not only by a large excess of IgE, but also by an anti-rsFcϵRIα mAb that competes with IgE for the binding to rsFcϵRIα. This mAb abolished the rsFcϵRIα-mediated suppression of IgE synthesis. These data suggest that rsFcϵRIα may have a function in selectively suppressing IgE synthesis through its interaction with the membrane-bound form of IgE. (*J. Clin. Invest.* 1994. 94:2162–2165.) Key words: IgE • IgE-binding molecule • IgE-bearing B cells • IL-4 • ϵ mRNA

Introduction

It is well-documented that IgE-binding factors derived from T cells can selectively modulate the IgE response, possibly by regulating the differentiation of IgE-bearing B cells to IgE-producing cells (1–3). Although the induction of IgE synthesis is also controlled by several cytokines such as IFN-α, IFN-γ, TGF-β, IL-8, and IL-12 (4–7), IgE-binding factors play an important role in the isotype-specific regulation of the IgE response. The high-affinity receptor for IgE (FcϵRI) mainly expressed on mast cells and basophils has a tetrameric structure ($\alpha\beta\gamma_2$), of which the α subunit binds IgE with high affinity. A recombinant soluble form of the α subunit of human FcϵRI (rsFcϵRIα)[1] is shown to bind IgE with an affinity as high as that of native FcϵRI (8). Moreover, rsFcϵRIα has the ability to inhibit the IgE-mediated allergic reaction, exclusively by interfering with IgE binding to mast cells and basophils. On the basis of the finding that rsFcϵRIα is a potent IgE-binding molecule, we examined whether it may regulate the human IgE response in vitro. Here we demonstrate that rsFcϵRIα inhibits IgE synthesis by IL-4–stimulated human lymphocytes and that this suppression is mediated by its specific binding to IgE-bearing B cells.

Methods

Reagents. Human rIL-4 was purchased from Genzyme Corp. (Boston, MA). Anti-CD40 mAb (B-B20) was obtained from Serotec Ltd. (Kidlington, Oxford, England). Hydrocortisone (HC) and pokeweed mitogen (PWM) were purchased from Sigma Chemical Co. (St. Louis, MO). FITC-conjugated anti–human IgE mAb was obtained from Nordic Immunological Laboratories (Tilburg, The Netherlands), and phycoerythrin (PE)-conjugated streptavidin was from Becton Dickinson (Mountain View, CA). Human IgE myeloma protein and U266 cells were the same preparations as previously described (9).

Human rsFcϵRIα and mAbs to rsFcϵRIα. The cDNA encoding the leader peptide and 95% of the human FcϵRIα exodomain (residue numbers 1–172) was introduced into an eukaryotic expression vector, pcDL-SRα (8). The resulting vector and a vector inserted with dihydrofolate reductase (DHFR) coding sequence were cotransfected into DHFR-deficient CHO cells. Secreted rsFcϵRIα was purified from the culture supernatant of the cells through mouse IgE-coupled Sepharose columns. Two mouse anti-rsFcϵRIα mAbs (CRA-1 and -2) were prepared and characterized as described elsewhere (8). The former mAb (IgG2b) showed no competition with IgE in binding to rsFcϵRIα, whereas the latter (IgG1) competed with IgE. Mouse IgG1 mAb specific for keyhole limpet hemocyanin (×40; Becton Dickinson) and purified myeloma mouse IgG2b (Cappel Labs, West Chester, PA) were used as isotype-matched controls.

Culture conditions for immunoglobulin production. Peripheral blood mononuclear cells (PBMC) were obtained from healthy adult donors, and purified B cells (80–85% $CD20^+$ B cells and 5–7% $CD14^+$ monocytes) were separated from PBMC as previously described (10). Cytophilic IgE was removed by treatment with acetate buffer (0.05 M, pH 4.0) as described elsewhere (11). After acid treatment, viable cells at a density of 1×10^6/ml were suspended in Iscove's modified Dulbecco's medium (GIBCO-BRL, Grand Island, NY) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml

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1. *Abbreviations used in this paper:* rsFcϵRIα, recombinant soluble form of the α subunit of high-affinity IgE receptor; HC, hydrocortisone.

streptomycin, and then cultured in round-bottomed microwells (each 0.2 ml) in a 5% CO_2 incubator. PBMC were stimulated with PWM (0.1 μg/ml) for 7 d or with IL-4 (100 U/ml) for 14 d. Purified B cells were costimulated with IL-4 and anti-CD40 mAb (1 μg/ml) or HC (1 μM) for 14 d. To estimate the passive release of preformed immunoglobulins into the culture supernatant, both cycloheximide and puromycin were added to some cultures as described (10).

Solid phase RIAs for the measurement of immunoglobulins. Since FcεRI has been shown to trap human IgE via a peptide fragment of 76 amino acids (residue numbers 301–376) in the Cε2 and Cε3 domains (12), an attempt was made to establish an RIA that displays no inhibition of the IgE measurement by rsFcεRIα. To this end, several combinations of antibodies against IgE were tested for the exact measurement of IgE in the presence of rsFcεRIα. The best result was obtained when clone 89, which had been obtained from American Type Culture Collection (Rockville, MD), was used to coat the solid phase, and clone 5G.11.11.7 (anti-Cε1 mAb; Immunotech S. A., Marseille, France) was used as the radioiodinated second antibody. Although the epitope specificity of 89 was not determined in detail, this mAb could not bind to the Fcε epitopes recognized by at least two mAbs against Cε1 and Cε2. This preliminary observation indicates that 89 may recognize either the Cε3 (but not IgE-binding site) or Cε4 domain. Nevertheless, rsFcεRIα at 0.1–1,000 ng/ml, regardless of its concentration, showed ~ 30% inhibition of the RIA. However, no significant differences were observed among these five concentrations of rsFcεRIα when the standard curve was constructed by calculating least-square lines. For example, they were log y = 1.23 log x + 2.43 (r = 0.999) in the control vs log y = 1.22 log x + 2.25 (r = 0.998) in 0.1 ng/ml of rsFcεRIα and log y = 1.24 log x + 2.25 (r = 0.999) in 1,000 ng/ml of the agent, respectively. To determine IgE levels in the culture supernatants containing rsFcεRIα, rsFcεRIα at a final concentration of 100 ng/ml was usually added to the control culture supernatant before the assay. IgG, IgM, and IgA concentrations in the culture supernatants were measured by sandwich RIAs exactly as described (10), and each assay was not affected by adding rsFcεRIα at any concentration.

Northern blot analysis of Cε mRNA. Total RNA was extracted from cultured cells by the single step method as previously described (13). 10 μg of total RNA was electrophoresed in 1% agar gel containing 6% formaldehyde, and then transferred onto nylon membrane. The membrane was hybridized with ^{32}P-labeled human Cε probe at 42°C for 12 h. Human β-actin control was also performed on the membrane transferred from gels prepared in parallel from the same RNA samples. Membranes were washed, air-dried, and then exposed to Kodak XAR-films (Eastman Kodak Co., Rochester, NY) with intensifier screens for 3 d (actin mRNA) or 10 d (Cε mRNA) at −70°C. The cDNA probe specific for human β-actin was obtained from Clontech Laboratories (Palo Alto, CA). The 1.2 kb EcoRI–PstI fragment of the pTB 145 corresponding to the human Cε2-Cε4 region was a kind gift of Takeda Pharmaceutical Co. (Osaka, Japan).

Flow cytometric analysis. For the analysis of rsFcεRIα-binding cell surface molecules, cells were incubated with 10 ng of rsFcεRIα for 2 h at 4°C, washed thoroughly, and then suspended in 50 μl of PBS containing 1 μg of biotinylated CRA-1 (bi-CRA-1), followed by incubation with PE-conjugated streptavidin for 30 min at 4°C. In some experiments for two color analysis, the cells were further incubated with FITC-labeled anti-CD3, anti-CD14, anti-CD16, anti-CD20, anti-CD64, or anti-IgE mAb for 30 min at 4°C. The stained cells were analyzed by FACScan (Becton Dickinson) equipped with an argon ion laser running at 488 nm.

Results

Since some factors having affinity for IgE are known to modulate the IgE response both in rodent and human systems (1–3), we first examined whether rsFcεRIα, a potent IgE-binding factor, may affect the synthesis of IgE by human lymphocytes stimulated with IL-4 or with the combination of IL-4 and anti-CD40 mAb or HC. As summarized in Fig. 1, addition of rsFcεRIα at 1–100 ng/ml could induce a concentration-dependent inhibition of IgE synthesis by PBMC stimulated with IL-4 as well as by purified B cells costimulated with IL-4 and anti-CD40 mAb or HC, and the suppression of IgE synthesis by rsFcεRIα (10 and 100 ng/ml) in three such systems was statistically significant ($P < 0.05$). This suppression does not result from the inhibition of the IgE assay by rsFcεRIα, because IgE levels in the control culture supernatant supplemented with rsFcεRIα just before IgE measurement were compared with those in the samples. Since IL-4 is shown to have no ability to induce the production of IgG (except for IgG4), IgM or IgA (14), we next studied whether rsFcεRIα may affect the production of IgG, IgM, and IgA by PWM-stimulated PBMC. As a result, rsFcεRIα at 1–100 ng/ml showed no suppressive effects on the production of these isotypes in three different donors. For example, the amounts of immunoglobulins produced in the absence and presence of 100 ng/ml of rsFcεRIα were 2.41±0.20 vs 2.40±0.15 (mean±1 SEM) μg/ml for IgG, 1.52±0.48 vs 1.65±0.26 μg/ml for IgM, and 0.67±0.08 vs 0.69±0.10 μg/ml for IgA, respectively. These results indicate that the suppression of immunoglobulin production by rsFcεRIα is IgE class specific.

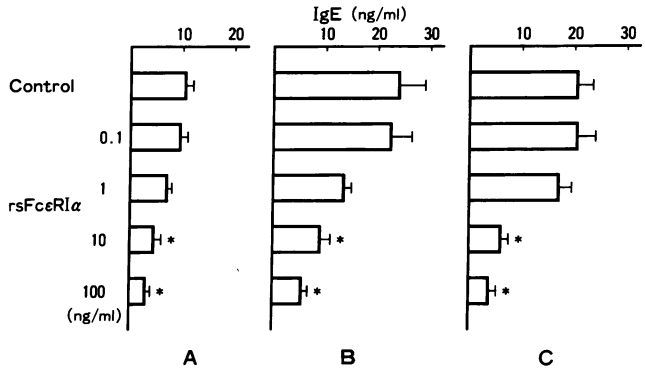

Figure 1. Effect of rsFcεRIα on IgE synthesis by PBMC stimulated with IL-4 (*A*) and by purified B cells costimulated with IL-4 and anti-CD40 mAb (*B*) or HC (*C*). After acid treatment, cells were stimulated in the absence or presence of rsFcεRIα (0.1–100 ng/ml) with IL-4 alone (100 U/ml) or with combination of IL-4 and anti-CD40 mAb (1 μg/ml) or HC (1 μM) for 14 d. The control culture supernatant was supplemented with 100 ng/ml of rsFcεRIα before the IgE assay. The net IgE synthesis was determined by subtracting IgE values passively released in the cultures containing both cycloheximide and puromycin from those in untreated cells. The data represent the mean±1 SEM of results obtained in three or four donors; *statistically significant difference from the control at $P < 0.05$ (paired t test).

The IgE-suppressive activity of rsFcεRIα was further investigated at the transcriptional level, using purified B cells stimulated with IL-4 alone or with the combination of IL-4 and anti-CD40 mAb. Three independent experiments were performed for each Northern blot analysis, and one representative result is presented in Fig. 2. rsFcεRIα at 10 and 100 ng/ml dose-dependently inhibited the expression of the mature (productive and membrane-bound) Cε transcripts in B cells costimulated with IL-4 and anti-CD40 mAb, whereas the expression of the germline Cε transcript in IL-4–stimulated B cells was not suppressed by rsFcεRIα at any concentration. With regard to the inhibition of the expression of mature Cε mRNA by rsFcεRIα, similar

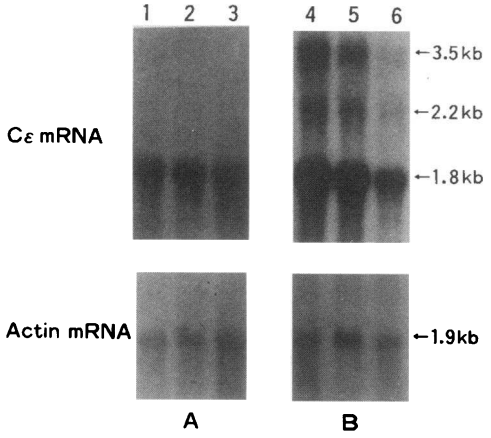

Figure 2. Effect of rsFcεRIα on the expression of germ-line and mature Cε mRNAs in purified B cells stimulated with IL-4 alone (*A*) or with combination of IL-4 and anti-CD40 mAb (*B*). Cells were stimulated in the absence (lanes *1* and *4*) or in the presence of rsFcεRIα (10 ng/ml, lanes *2* and *5;* 100 ng/ml, lanes *3* and *6*) with Il-4 alone for 3 d or with combination of IL-4 and anti-CD40 mAb for 10 d. Total RNA was extracted from cultured B cells, and Northern blot analysis was performed by using ^{32}P-labeled probes specific for the Cε2–Cε4 region or for the β-actin.

results were obtained both in PBMC stimulated with IL-4 and B cells costimulated with IL-4 and HC (data not shown). These findings indicate that the suppression of IgE synthesis by rsFcεRIα occurs at the protein and the mRNA levels.

The observation that rsFcεRIα suppressed the T cell–dependent and independent synthesis of IgE prompted us to examine the possibility that B cells may be targets for the action of rsFcεRIα. As shown in Fig. 3, rsFcεRIα as well as anti-IgE

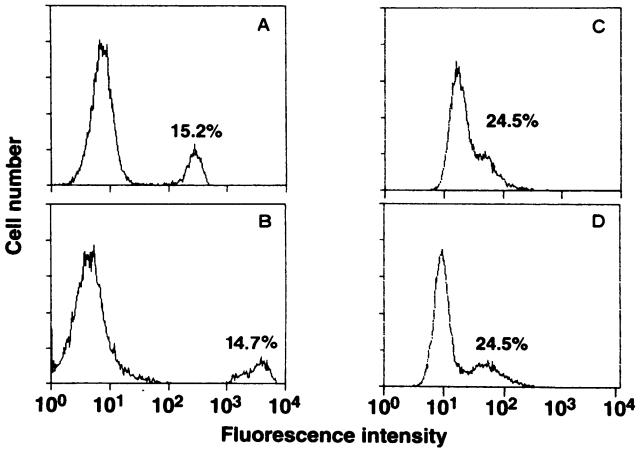

Figure 3. Binding of anti-IgE mAb (*A* and *C*) or rsFcεRIα (*B* and *D*) to surface IgE$^+$ B cells and U266 cells. Surface IgE$^+$ B cells (*A* and *B*) were induced by costimulation of purified B cells with IL-4 and anti-CD40 mAb for 5 d. U266 cells (*C* and *D*) were used as a positive control. Stimulated B cells or unstimulated U266 cells were incubated either with FITC-labeled anti-IgE mAb (3 μg) or with rsFcεRIα (10 ng). B cells trapping rsFcεRIα via their surface IgE were further reacted with bi-CRA-1 (1 μg), followed by incubation with PE-conjugated streptavidin. Stained cells were analyzed by means of single-color FACS.

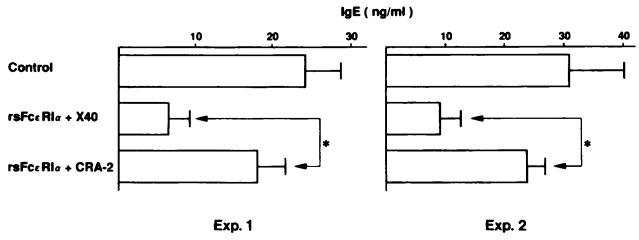

Figure 4. Effect of CRA-2 on rsFcεRIα-mediated suppression of IgE synthesis by B cells. Purified B cells were costimulated with IL-4 and anti-CD40 mAb for 14 d. A mixture of rsFcεRIα and CRA-2 or isotype-matched control mAb (×40), which had been preincubated at 4°C for 2 h, was added at the initiation of the culture. Final concentrations of rsFcεRIα and CRA-2 or ×40 in the culture were 100 ng/ml and 5 μg/ml, respectively. The data represent the mean±1 SEM of triplicate cultures; *statistically significant difference from the culture containing ×40 at $P < 0.05$ (Student's *t* test).

mAb could bind to both surface IgE$^+$ B cells induced by costimulation with IL-4 and anti-CD40 mAb for 5 d and some populations of U266 cells. Interestingly, this binding was completely blocked by preincubation of rsFcεRIα with IgE (10 μg) at 4°C for 2 h, whereas the same concentration of IgG did not inhibit the binding of rsFcεRIα (data not shown). Approximately 50% inhibition of rsFcεRIα binding to the cells was also induced by preincubation with CRA-2 (5 μg) that shows a competitive inhibition of the binding of IgE to rsFcεRIα. In addition, rsFcεRIα bound to surface IgE$^+$ B cells induced by costimulation with IL-4 and anti-CD40 mAb could be effectively dissociated not only by a large excess of IgE, but also by CRA-2 (data not shown). Moreover, CRA-2 but not isotype-matched control mAb abolished the rsFcεRIα-mediated suppression of IgE synthesis by B cells costimulated with IL-4 and anti-CD40 mAb when preincubated with rsFcεRIα (Fig. 4), and this abrogation was statistically significant ($P < 0.05$). On the other hand, rsFcεRIα had no ability to bind to T cells (CD3$^+$), surface IgE$^-$ B cells (CD20$^+$), monocytes (CD14$^+$ and CD64$^+$) and NK cells (CD16$^+$) of both freshly isolated and IL-4–stimulated PBMC (data not shown). These results indicate that rsFcεRIα specifically binds to IgE-bearing B cells.

Discussion

Our data demonstrate that rsFcεRIα having high affinity for IgE inhibits IL-4–induced IgE synthesis by human lymphocytes. Indeed, rsFcεRIα at 1–100 ng/ml suppressed in a dose-dependent fashion the induction of IgE synthesis by PBMC stimulated with IL-4 as well as by purified B cells costimulated with IL-4 and anti-CD40 mAb or HC. This suppression, however, is not due to the interference with the RIA for the detection of IgE, because the control culture supernatant was usually supplemented with 100 ng/ml of rsFcεRIα before the assay. Although ~ 30% inhibition of the IgE assay was induced by addition of rsFcεRIα, regardless of its concentration, there were no significant differences among various concentrations of the agent. It should be noted that the suppression of IgE synthesis by rsFcεRIα was observed at the protein and the mRNA levels. Nevertheless, B cell proliferation induced by costimulation with IL-4 and anti-CD40 mAb was not inhibited by rsFcεRIα (data not shown). On the other hand, the induction of IgG, IgM, and

IgA synthesis by PWM-stimulated PBMC was not affected by rsFcεRIα at any concentration. These observations indicate that the suppression of immunoglobulin production by rsFcεRIα is IgE class-specific.

rsFcεRIα was effective in inhibiting the T cell–dependent and independent expression of the mature Cε transcripts in IL-4–stimulated lymphocytes, whereas it did not affect the T cell–independent expression of germ-line Cε mRNA in IL-4–stimulated B cells. The finding that rsFcεRIα could specifically bind to surface IgE^+ B cells induced by costimulation with IL-4 and anti-CD40 mAb reveals that B cells expressing membrane-bound IgE are targets for the action of rsFcεRIα. In fact, the binding of rsFcεRIα to both IgE-bearing B cells and U266 cells was completely inhibited by preincubation with IgE. Moreover, rsFcεRIα bound to the cell surface IgE could be effectively dissociated not only by anti-rsFcεRIα mAb, CRA-2, that shows a competitive inhibition of the IgE binding, but also by a large excess of IgE. Interestingly, rsFcεRIα capable of binding to surface IgE^+ B cells in which the switching to IgE had been completed (15) displayed the ability to inhibit the expression of both membrane-bound and productive Cε transcripts. This result suggests that membrane-bound IgE on activated B cells, which interacted with rsFcεRIα, may provide a negative signal(s) responsible for the inhibition of the further accumulation of mature Cε transcripts. This assumption is supported by the previous reports that T cell–derived IgE-binding factors as well as some antibodies against IgE can modulate the IgE response through their binding to surface IgE molecules on B cells (1–3, 16–18).

IgE-immune complexes are shown to supress IL-4–induced IgE synthesis by cross-linking of CD23 on B cells (19). This finding suggests that in certain conditions, rsFcεRIα capable of dissociating the cell-fixed IgE from $CD23^+$ B cells (Ra, C., unpublished observations) may enhance IgE synthesis through the cancellation of IgE-mediated negative feedback regulation. However, it must be underlined that IgE-bearing B cells are devoid of CD23 expression (20). Therefore, it is obvious that rsFcεRIα inhibits IgE synthesis at a later stage by binding with high affinity to IgE-bearing B cells.

Although the function and role of membrane-bound IgE on B cells with respect to the regulation of IgE synthesis is not clear, the present study suggests that membrane-anchoring IgE molecules by themselves may lead to the suppression of IgE synthesis through their interaction with rsFcεRIα, one of the potent IgE-binding factors. It is currently in progress to clarify the signal transduction mechanism by which rsFcεRIα inhibits IgE synthesis via its interaction with the membrane-bound form of IgE, in particular the Cε3 domain.

Acknowledgments

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