

Recombinant soluble form of the human high-affinity receptor for IgE prevents anaphylactic shock in mice

Koji Naito, PhD,^a Minoru Hirama, PhD,^a Ko Okumura, MD, PhD,^b
and Chisei Ra, MD, PhD^b Osaka and Tokyo, Japan

The ectodomain of the alpha subunit of the human high-affinity receptor for IgE (FcεRI) has been engineered as a recombinant soluble protein (sFcεRIα) and purified in large quantity from the supernatant of transfected mammalian cells. The sFcεRIα was apparently heterogeneous in terms of molecular weight (40 to 60 kd), and this heterogeneity was largely due to N-linked carbohydrates on the molecule. The amino terminal sequence was homogeneous and identical to the sequence predicted from the complementary DNA sequence. The amino acid composition of the sFcεRIα was in agreement with the values expected from the cDNA sequence. The sFcεRIα formed a complex with IgE, and gel filtration analyses of the complex of the sFcεRIα and human IgE supported the idea that the stoichiometry of the IgE binding was 1:1. Passive anaphylactic shock in mice was suppressed when the sFcεRIα was intravenously injected after sensitization with anti-trinitrophenyl IgE and challenged with the appropriate antigen. In this model the sFcεRIα was effective when administered 48 hours before the antigen challenge, but not 24 hours before the challenge. These results suggest that the sFcεRIα facilitated the dissociation of IgE from FcεRI on mast cells in vivo and suppressed type I allergic reactions. (J ALLERGY CLIN IMMUNOL 1996;97:773-80.)

Key words: High affinity IgE receptor (FcεRI), IgE, allergy, anaphylactic shock

The high-affinity receptor for IgE (FcεRI) consists of four polypeptides: one α chain, one β chain, and a homodimer of γ chains.^{1,2} The binding site for IgE is localized on the ectodomain of the alpha subunit.^{3,4} The ectodomain of the human alpha subunit (sFcεRIα), which contains 95% of the extracellular region (172 amino acid residues), can be secreted as a soluble protein by Chinese hamster ovary (CHO) cells transfected with engineered complementary DNA, and the sFcεRIα purified from the supernatant was shown to bind to IgE with the same dissociation constant as the natural FcεRI on the cell surface.⁵

The sFcεRIα has been shown to block the binding of IgE to FcεRI on mast cells and thus to

Abbreviations used

CHO:	Chinese hamster ovary
FCS:	Fetal calf serum
HPLC:	High-pressure liquid chromatography
HSA:	Human serum albumin
PBS:	Phosphate-buffered saline
PCA:	Passive cutaneous anaphylaxis
SDS:	Sodium dodecyl sulfate
SDS-PAGE:	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sFcεRIα:	Recombinant soluble form of the human FcεRI alpha subunit
TNP:	Trinitrophenyl

From ^athe Research Division, The Green Cross Corporation, Osaka; and ^bthe Department of Immunology, Juntendo University, School of Medicine, Tokyo.

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Reprint requests: Chisei Ra, MD, PhD, Department of Immunology, Juntendo University, School of Medicine, 2-1-1, Hongo, Bunkyo-Ku, Tokyo 113, Japan.

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suppress the IgE-dependent degranulation of both rat and human basophils in vitro.⁶ Passive cutaneous anaphylaxis (PCA) reaction in rats was also blocked completely when the sFcεRIα was administered before sensitization with specific IgE. The sFcεRIα could also facilitate dissociation of IgE from mast cells, resulting in suppression of PCA

reaction even when administered after IgE sensitization.

Intracutaneous administration of the sFcεRIα, however, was required to achieve high concentrations of the sFcεRIα at the sensitized sites, and the suppression was partial.⁶

In this report the sFcεRIα was purified in large quantity from the supernatant of the stable transfectants and further characterized biochemically. We were also interested in its therapeutic potential for type I allergic responses and have analyzed its effects on another model of type I allergy, passive anaphylactic shock model in mice that had already been sensitized.

METHODS

Expression of sFcεRIα

The cDNA for the sFcεRIα was constructed by polymerase chain reaction and cloned into pKC3 vector driven by the SV40 early promoter according to the method of Blank et al.⁵ Transfection of the vector containing the cDNA for the sFcεRIα and co-amplification of the sFcεRIα cDNA along with dehydrofolate reductase gene were performed as previously described^{5,7} with slight modifications. Briefly, the pKC3 vector containing the sFcεRIα cDNA and the pTT06 plasmid containing dehydrofolate reductase gene driven by the single-chain urokinase-type plasminogen activator gene promoter⁸ were cotransfected to dehydrofolate reductase-deficient CHO cells by electroporation. The transfectants were selected in Eagle's minimum essential medium (alpha modification without deoxyribonucleosides and ribonucleosides; Gibco BRL, Grand Island, N.Y.) supplemented with 10% fetal calf serum (FCS) (Flow Laboratories, Irvine, Scotland), and resistant colonies were obtained by micromanipulation. The individual colonies were subjected to stepwise increases of methotrexate ((+)amethopterin; Sigma Chemical Co., St. Louis, Mo.) up to 0.01 mmol/L. The culture supernatant was obtained at each concentration of methotrexate, and the sFcεRIα secreted in the waste medium was quantitated by radioreceptor assay as previously described.⁵ The cell population was cloned twice by limiting dilution at 0.3 cells/well.

Purification of the sFcεRIα

The confluent monolayer of the transfectants was cultured in 250 ml of Eagle's minimum essential medium (alpha modification without deoxyribonucleosides and ribonucleosides) supplemented with 1% FCS in roller bottles (F3027; Becton Dickinson Labware, Franklin Lakes, N.J.). The medium was changed every 3 days, the waste medium was collected by centrifugation and concentrated 10- to 20-fold with 10 kd cut ultrafiltration membranes (Filtron Technologies Co., Northborough, Mass., or Nihon Millipore Ltd., Tokyo, Japan). The concentrate was passed through a mouse IgE-AffiGel-10

column (Bio-Rad, Richmond, Calif.), and the column was washed with phosphate-buffered saline (PBS) containing 0.02% NaN₃. The bound materials were eluted with 0.2 mol/L acetic acid, 0.2 mol/L NaCl, 0.02% NaN₃ (pH 2.8) and immediately neutralized with 1.0 mol/L Tris-HCl (pH 8.0). The eluate was dialyzed against 0.05 mol/L Tris-HCl (pH 7.5), 0.5 mol/L NaCl and further purified by gel filtration through a Sephacryl S-200 column (Pharmacia Fine Chemicals, Uppsala, Sweden) (100 cm × 1.6 cm) equilibrated with the same buffer. The sFcεRIα was extensively dialyzed against PBS. IgE-AffiGel-10 was prepared by coupling mouse IgE to AffiGel-10 in 0.1 mol/L NaHCO₃ overnight at 4° C.

Purification of IgE

The IGEL b4 hybridoma (ATCC TIB 141) that secreted anti-trinitrophenyl (TNP) IgE was maintained in Dulbecco's minimum essential medium (Nissui Seiyaku Co. Ltd., Tokyo, Japan) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 0.01 mol/L N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid, and 10% FCS.⁹ The culture supernatant was passed through a TNP-coupled bovine serum albumin Sepharose column as previously described,¹⁰ and the column was washed with 0.05 mol/L Tris-HCl (pH 8.0), 1.0 mol/L NaCl, 0.1% NaN₃. The anti-TNP IgE was eluted with 0.2 mol/L glycine (pH 2.5), 0.1% NaN₃ and immediately neutralized with 1.0 mol/L Tris-HCl (pH 8.0). Monomeric IgE was obtained by gel filtration through a Sephacryl S-200 column (100 cm × 1.6 cm) equilibrated with 0.05 mol/L Tris-HCl (pH 7.5), 0.5 mol/L NaCl. TNP-coupled bovine serum albumin that contained 16 TNP molecules per one albumin molecule was prepared with 2,4,6-trinitrobenzenesulfonic acid¹¹ and coupled to activated CH Sepharose (Pharmacia) according to the manufacturer's instructions.

U266 myeloma cells were cultured in RPMI-1640 medium (Nissui) supplemented with 10% FCS. The supernatant was passed through a monoclonal anti-human IgE antibody column; the column was washed with PBS containing 0.02% NaN₃; and the human IgE was eluted with 0.2 mol/L glycine (pH 2.5), 0.1% NaN₃. The eluate was immediately neutralized with 1.0 mol/L Tris-HCl (pH 8.0) and dialyzed against PBS. The monoclonal anti-human IgE antibody was purified from the culture supernatant of the E5BB3IIA2 hybridoma (ATCC HB121) with the use of a protein A Sepharose (Pharmacia) column and coupled to AffiGel-10.

Concentration of purified IgE was determined by reading the absorbance at 280 nm ($E_{1\text{ cm}}^{1\%} = 14$). Molar concentration was calculated assuming molecular weights of 180 kd.

Gel filtration by high-pressure liquid chromatography (HPLC)

The samples containing the sFcεRIα and IgE were applied to a TSK G3000 SW_{XL} column (Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan) equilibrated

with 0.05 mol/L sodium phosphate, pH 7.2, 0.15 mol/L NaCl, 0.01% polyoxyethylene (23) lauryl ether (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and eluted with the same buffer at a flow rate of 0.4 ml/min. Absorbance at 210 nm was recorded.

Determination of amino acid composition and an amino terminal sequence

The sFcεRIα in 0.01 mol/L NH_4HCO_3 was dried in a centrifugal concentrator, and hydrolyzed in 6N HCl vapor in the presence of 1% phenol for 24 hours at 110°C under vacuum. The hydrolyzed samples were derivatized with phenyl isothiocyanate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and analyzed with HPLC as described by Cohen and Strydom.¹² To quantify the sFcεRIα, a known amount of α-amino-n-butyric acid was added as an internal standard to the sample before the hydrolysis. The amounts of phenylalanine and leucine were compared with the standard, and the quantity of the analyzed sFcεRIα was calculated assuming that 1 mol of the sFcεRIα contained 7 mols of phenylalanine and 13 mols of leucine.

An amino terminal sequence was determined with an automated sequencer (470A Protein Sequencer; Applied Biosystems, Foster City, Calif.).

Treatment of the sFcεRIα with N-glycanase

The sFcεRIα was denatured with 0.5% sodium dodecylsulfate (SDS) and 0.1 mol/L 2-mercaptoethanol and treated with 10 U/ml of N-glycanase (Genzyme Co., Boston, Mass.) in the presence of 1.25% Triton X-100 and 100 mmol/L 1,10-phenanthroline hydrate (Nakalai Tesque Inc., Kyoto, Japan) at pH 8.6 overnight at 37°C.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was performed according to the method of Laemmli¹³ under reducing or nonreducing conditions with gradient gel (TEF Co., Nagano, Japan).

Passive anaphylactic shock

Female ICR mice (aged 7 to 8 weeks) were purchased from Japan SLC (Shizuoka, Japan). Two hundred microliters of pertussis vaccine (10^{10} /ml) was intraperitoneally injected on day -5. Pertussis vaccine was provided by The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan. The mice were sensitized intraperitoneally with anti-TNP IgE on day -3. They were treated intravenously with various doses of the sFcεRIα either on day -2 or day -1 and challenged intravenously with 50 μg of TNP-coupled human serum albumin (HSA) (Miles Scientific, Naperville, Ill.) on day 0. Mortality of the mice was determined at 1 hour after the challenge. TNP-coupled HSA was prepared as described above¹¹ and contained 18 to 20 TNP molecules per one HSA molecule. Statistical significance was determined by chi square tests.

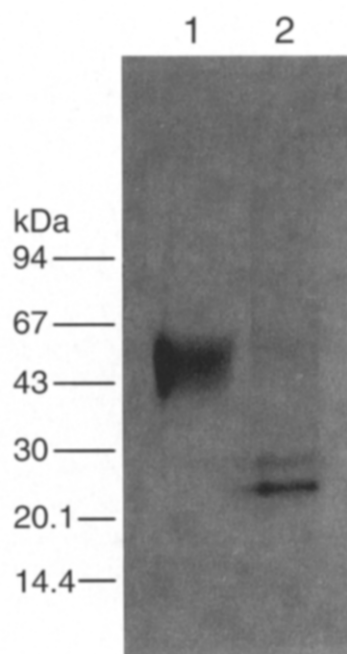


FIG. 1. SDS-PAGE analysis of the purified sFcεRIα. The purified sFcεRIα, either untreated or treated with N-glycanase, was analyzed in 4% to 20% gradient gel under reducing conditions. About 8 μg of the proteins was applied to each lane, and the gel was stained with Coomassie brilliant blue (Wako Pure Chemical Industries, Ltd.). Lane 1, The sFcεRIα; lane 2, the sFcεRIα treated with N-glycanase. Positions of molecular weight markers are shown on the left margin.

RESULTS

Expression and purification of the sFcεRIα

The cDNA for the sFcεRIα was transfected to CHO cells, and a gene amplification technique was applied to enhance production of the protein as described in the Methods section. The best clones secreted 5 mg/L of the sFcεRIα in roller-bottle culture for 3 days. The sFcεRIα purified from the supernatant migrated to a position of 40 to 60 kd as a broad band on SDS-PAGE under reducing conditions (Fig. 1) and nonreducing conditions (data not shown) as previously described.⁵ After removal of N-linked oligosaccharides with N-glycanase, the sFcεRIα migrated to the position of 25 kd as a sharp band (Fig. 1).

Determination of the amino terminal sequence and amino acid composition of the sFcεRIα

Sequencing of the amino terminus of the sFcεRIα (about 300 pmol) yielded a single sequence of Val-Pro-Gln-Lys-Pro-Lys-Val-Ser-Leu-Asn. This sequence was identical to the one predicted from the cDNA sequence, starting from the

TABLE I. Amino acid analysis of the sFcεRIα

Amino acid	Residues/mol	Amino acid	Residues/mol
Asx	23.5 (22)	Pro	7.3 (7)
Glx	23.6 (23)	Tyr	9.7 (10)
Ser	13.3 (14)	Val	14.1 (15)
Gly	9.9 (9)	Met	0.9 (1)
His	4.1 (4)	Ile	6.6 (7)
Arg	2.8 (3)	Leu	13.5 (13)
Thr	8.7 (9)	Phe	7.0 (7)
Ala	7.2 (6)	Lys	10.2 (11)

Residues/mol is based on seven Phe. Values expected from the cDNA sequence are shown in parentheses.

twenty-sixth codon.^{14, 15} Thus the mature sFcεRIα comprised a homogenous peptide in terms of its amino terminus. The amino terminus of the mature human sFcεRIα seemed not to be blocked, unlike the natural alpha subunit of rat FcεRI.¹⁴

To determine the amino acid composition, 100 μl of the sFcεRIα in 0.01 mol/L NH₄HCO₃, the absorbance of which was 0.490 at 280 nm in a cuvette with 1 cm path length, was dried with a centrifugal concentrator, and hydrolyzed in 6N HCl vapor for 24 hours in the presence of 5 nmol of α-amino-n-butyric acid as an internal standard. The observed ratio of each amino acid residue agreed well with the expected values (Table I). Compared with the internal standard, the concentration of the sFcεRIα in 0.01 mol/L NH₄HCO₃, the absorbance of which was 0.490 at 280 nm, was 9.4 μmol/L, assuming that the sFcεRIα contained the expected numbers of leucine and phenylalanine. Thus the molar extinction coefficient of the sFcεRIα at 280 nm was 5.2×10^4 (mol/L)⁻¹ cm⁻¹.

Binding stoichiometry of the sFcεRIα and human IgE

The sFcεRIα at various concentrations was incubated with human myeloma IgE under physiologic conditions and analyzed by gel filtration with HPLC. The complex of the sFcεRIα and IgE was eluted slightly before free IgE (Fig. 2, A). When increasing amounts of the sFcεRIα were added to the constant amount of IgE (0.22 μmol/L), the peak heights of the complex increased until an equimolar amount of the sFcεRIα was added and reached a plateau thereafter (Fig. 2, B). The peaks of the free sFcεRIα were observed after the peak heights of the complex reached maximum, and the peak heights of the free sFcεRIα increased along with the amounts of the added sFcεRIα. The peak heights of free IgE became shorter with increasing

TABLE II. Anaphylactic shock in mice sensitized with various doses of IgE

Dose of IgE (μg)	Alive/total (% survival)
0.08	10/10 (100)
0.4	12/12 (100)
2.0	7/12 (58)
10	2/10 (20)

Mice sensitized with indicated doses of anti-TNP IgE were challenged with TNP-HSA.

amounts of the sFcεRIα and seemed to disappear when an equimolar amount of the sFcεRIα was added (Fig. 2, B). These results were consistent with the idea that the stoichiometry of the binding between the sFcεRIα and human IgE was 1:1.

The sFcεRIα suppresses passive anaphylactic shock in mice

The sFcεRIα had been shown to prophylactically suppress PCA reaction in rats, and we further investigated the effect of the sFcεRIα on anaphylactic shock induced in IgE-sensitized mice. Mice were treated with pertussis vaccine to enhance sensitivity to histamine^{16, 17} and sensitized with monoclonal anti-TNP IgE 2 days later. When challenged with TNP-coupled HSA on day 3 after sensitization, systemic anaphylaxis was elicited in the mice: all of the mice exhibited dyspnea, cyanosis, and loss of mobility; and some were dead within 1 hour. The mortality was dependent on the dose of sensitizing IgE (Table II), and sensitization with the specific IgE was a prerequisite, because the mice sensitized with anti-phenylarsonate IgE did not have shock on injection of TNP-coupled HSA (data not shown). The mice were sensitized with 2 μg of IgE in subsequent experiments.

To examine the effects of the sFcεRIα on shock, the sFcεRIα at various doses was administered intravenously on day 2 before the challenge, and the survival rate of the treated mice on antigen challenge was compared with that of the control mice that received PBS. As shown in Fig. 3, the survival rate of the treated mice was consistently higher than that of the control mice. There was a correlation between the dose of the sFcεRIα and the improvement in the survival rate.

The sFcεRIα was administered to the sensitized mice, either at 48 hours or 24 hours before the antigen challenge. The sFcεRIα injected at 48 hours before the challenge suppressed the anaphylactic shock, but not at 24 hours before the challenge (Table III). Thus a longer period between

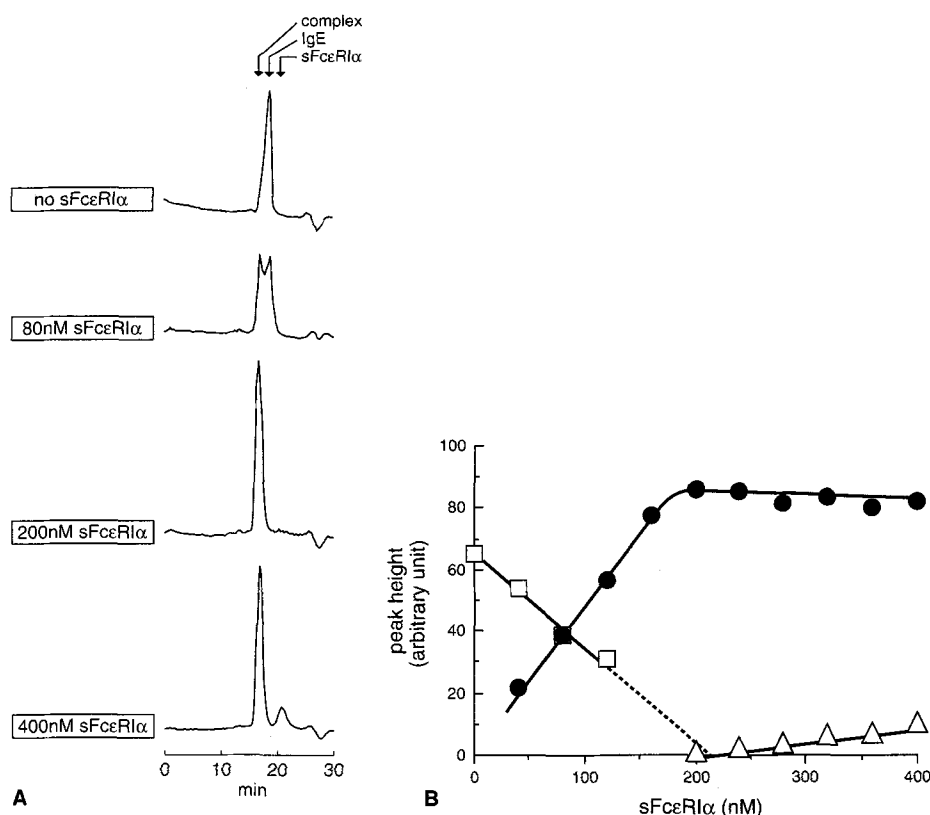


FIG. 2. HPLC analysis of the complex of the sFcεRIα and human IgE. **A**, Human IgE (0.22 μmol/L) was incubated with the sFcεRIα at the indicated concentration in a mixture of 3 volumes of PBS and 1 volume of the HPLC buffer for more than 50 minutes at room temperature, and the mixture was analyzed by gel filtration through a TSK G3000 SW_{XL} column at a flow rate of 0.4 ml/min in 0.05 mol/L sodium phosphate (pH 7.2), 0.15 mol/L NaCl, 0.01% polyoxyethylene (23) lauryl ether. Absorbance at a wavelength of 210 nm was monitored. The peaks of the complex of the sFcεRIα and IgE, free IgE, and the free sFcεRIα are indicated. **B**, The mixture of human IgE (0.22 μmol/L) and the sFcεRIα at the indicated concentration was incubated and separated by gel filtration as in A. Peak heights of the complex (filled circles), free IgE (open squares), and the free sFcεRIα (open triangles) were measured, respectively, on the obtained charts and plotted against the concentration of the sFcεRIα in arbitrary units.

injection of the sFcεRIα and antigen challenge seemed to be important to suppress the anaphylactic shock more efficiently.

DISCUSSION

The sFcεRIα, which contains 95% of the extracellular region, has been shown to bind IgE with the same dissociation rate constant as the natural FcεRI on mast cells or basophils.⁵ Ra et al.⁶ demonstrated that the sFcεRIα prevented sensitization of mast cells and basophils in vitro and inhibited PCA reaction in vivo. In this report the sFcεRIα purified from the supernatant of newly established CHO transfectants was further characterized biochemically, and its effects on another allergic reaction, passive anaphylactic shock in mice, were assessed.

The purified sFcεRIα migrated as a broad band in SDS-PAGE in accordance with previous reports,^{5,6} and it was thus difficult to assess the homogeneity of the protein in the analysis. Because treatment of the sFcεRIα with N-glycanase yielded a sharp band of about 25 kd, as well as some faint bands (Fig. 1), apparent heterogeneity of the sFcεRIα seemed to be due to N-linked oligosaccharides as previously reported.⁵ Blank et al.⁵ demonstrated that the sFcεRIα treated with N-glycanase exhibited a band slightly smaller than 20 kd. Because the calculated molecular mass of the peptide core is 19.931 kd, we cannot rule out the possibility that the sFcεRIα produced by the new transfectants might contain some post-translational modification, such as O-glycosylation. Faint bands larger than 25 kd might be generated

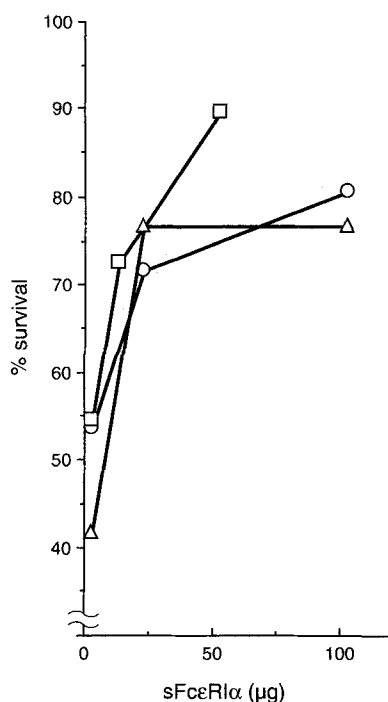


FIG. 3. Effect of the sFcεRIα on passive anaphylactic shock. ICR mice treated with pertussis vaccine were passively sensitized with monoclonal anti-TNP IgE (2 μg per mouse) and injected intravenously with the sFcεRIα at the indicated doses at 24 hours after sensitization. Control mice received PBS. Mice were challenged with intravenous injection of 50 μg of TNP-coupled HSA at 72 hours after sensitization and checked for survival. Each symbol represents a separate experiment performed independently (circle, experiment 1; triangle, experiment 2; square, experiment 3). Percent survival was calculated as: number of live mice at 1 hour after challenge ÷ total number of mice in the experiment × 100. Total numbers of mice were 14 to 22 for each point.

by incomplete removal of N-linked oligosaccharides with N-glycanase treatment. The amino terminal sequencing further demonstrated the homogeneity of the purified sFcεRIα. The signal sequence that was predicted from the cDNA sequence was correctly removed by signal peptidase from the recombinant polypeptide expressed in the transfectants. Amino acid composition of the purified sFcεRIα was almost identical to the one expected from cDNA sequence (Table I).

With HPLC gel filtration, we could clearly detect the complex of the sFcεRIα and IgE and quickly separate the complex, the free IgE, and the free sFcεRIα (Fig. 2, A). The results obtained from these analyses indicated that the IgE-binding stoichiometry was 1 sFcεRIα/1 IgE (Fig. 2, B). Earlier studies suggested that FcεRI, whether on mast cells or in solubilized form, was monovalent in

TABLE III. Effect of the sFcεRIα on survival of mice after anaphylactic shock

Time of injection (hours before challenge)	Dose of sFcεRIα (μg)	Alive/total (% survival)
24	0	7/17 (41)
	20	9/17 (53)
	100	7/18 (39)
48	0	9/22 (41)
	20	13/17 (76)*
	100	13/17 (76)*

The sFcεRIα at the indicated doses was administered intravenously to the mice at the indicated time points.

* $p < 0.05$ (chi square test).

terms of the binding for IgE,¹⁸⁻²¹ and our results support the previous data. Robertson²² also suggested that sFcεRIα expressed in *Escherichia coli* bound IgE with the stoichiometry of 1:1.

The sFcεRIα facilitated dissociation of IgE from the cell surface FcεRI in vitro and was able to suppress PCA reaction partially when administered after sensitization with specific IgE, though local administration of the sFcεRIα to the sensitized sites was a prerequisite.⁶ We have now assessed the effects of the sFcεRIα on another model of type I allergy, anaphylactic shock in mice sensitized passively with anti-TNP IgE. The shock was elicited in the sensitized mice on antigen challenge, and mortality was dependent on the dose of sensitizing IgE (Table II). Our results showed that the sFcεRIα suppressed the shock in a dose-dependent fashion, even when administered after the sensitization (Fig. 3). Therefore treatment with the sFcεRIα could, in general, be effective in dampening the subsequent allergic responses in sensitized patients, though much more sFcεRIα than IgE in the molar ratio appeared to be required. Thus 50 μg of the sFcεRIα was about 90 times more than the amount of anti-TNP IgE used for the sensitization in Fig. 3 (2 μg).

The time point when the sFcεRIα was injected appeared to be critical: the sFcεRIα injected at 48 hours before the antigen challenge suppressed the shock, whereas the sFcεRIα injected at 24 hours before the challenge had almost no effect (Table III). These results indicate that a longer interval between administration of the sFcεRIα and the antigen challenge made the treatment more effective. The sFcεRIα facilitates the dissociation of IgE from the cell surface FcεRI by trapping the dissociated IgE and suppresses the activation of the sensitized mast cells. It is likely to take a long time for the sFcεRIα to "desensitize" the mast

cells because of the slow dissociation of IgE from the cell surface FcεRI.²³⁻²⁶

The ectodomain of the alpha subunit could be displayed on the surface of a filamentous bacteriophage, and the ectodomain could be secreted by transfected *E. coli*.²² In this case any oligosaccharides would not be attached to the soluble form of the receptor, and removal of glycosylation may cause quick degradation of the protein when administered in vivo. The chimeric molecule that contained the α chain ectodomain and human Fcγ1 portion was also created and shown to block PCA reaction²⁷ and passive sensitization of human and rhesus monkey lung parenchyma.²⁸ Although such chimeras might have a longer plasma half-life than the pure soluble extracellular domain alone,²⁹ there seemed to be no requirements for generating such chimeras, because the sFcεRIα in this report had an unusually long plasma half-life in mice (manuscript in preparation). The FcεRI-IgG chimera formed a homodimer through disulfide bonds, making this chimera bind two IgE molecules, which could then be cross-linked by multivalent antigens to cause some unwanted responses by the aggregation of Fcγ receptors on inflammatory cells.

Now the recombinant sFcεRIα can be produced in large quantity, and the one-to-one complex between the sFcεRIα and IgE has become a feasible target for crystallization. Determination of the three-dimensional structure of the complex would make it possible to design low-molecular-weight competitors for IgE binding.³⁰

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