

Analysis of Murine Soluble FcεRII Sites of Cleavage and Requirements for Dual-Affinity Interaction with IgE¹

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The low-affinity receptor for IgE (FcεRI/CD23) is a type II integral membrane protein with an extracellular C-terminal sequence homologous to C-type animal lectins. Between this region and the membrane is a repetitive sequence predicted to form an α -helical coiled-coil and is termed the stalk region. The FcεRII is proteolytically cleaved when at the cell surface in this stalk region. Both the 38 K_d and 28 K_d major released fragments were isolated from culture media and N-terminal sequencing demonstrated that the cleavage sites were in the third and fourth repeat domains, respectively. The identified sites show no apparent similarity with the cleavage sites previously identified in human FcεRII. Recent studies have demonstrated that the intact FcεRII interacts with IgE with a dual-affinity, resulting from a multivalent interaction with the IgE Fc region; mutant FcεRII that have a disruption of the α -helical coiled-coil have a single low-affinity interaction consistent with a monomeric interaction with IgE. The soluble FcεRII were shown to interact with IgE with an affinity similar to these mutant FcεRII. Preparation of a chimeric FcεRII in which the transmembrane and cytoplasmic regions were replaced with sequences from Ly-49 revealed that these regions played no role in the multimeric association of the FcεRII necessary for dual-affinity interaction with IgE. In addition, a full-sized soluble FcεRII construct was expressed, and this molecule demonstrated increased capacity to interact with IgE. *The Journal of Immunology*, 1995, 154: 4240–4246.

The low-affinity receptor for IgE (FcεRI),⁴ also known as CD23 (1), has been known for some time to exist as both a membrane bound and soluble form in both the human (2) and mouse (3) systems. Cloning studies (see Refs. 4, 5 for review) demonstrated that the FcεRII was a type II membrane protein and was a member of the C-type animal lectin family. Recent studies have indicated that what is termed the stalk region of FcεRII, i.e., the region between the carboxyl terminal lectin homology domain and the membrane surface, contains a heptad periodicity that is characteristic of an α -helical

coiled-coil (6). Saturation analysis of IgE binding by the mouse FcεRII indicated a dual-affinity interaction and was consistent with chemical crosslinking studies that demonstrated that the membrane form of mouse FcεRII existed both as an oligomer and a monomer. The higher affinity interaction is then caused by an interaction of two lectin domains with symmetrical sites on the IgE Fc region (6). The oligomeric structure is consistent with the coiled-coil model mentioned above. Analysis of engineered FcεRII molecules that had deletions in the stalk region indicated that formation of the oligomeric structure was dependent on this region (6).

The objective of the present study was to analyze the structural requirements needed for the higher affinity interaction with IgE, namely the affinity seen when the FcεRII exists as an oligomer. The studies demonstrated that mouse sFcεRII was cleaved within the stalk region of the molecule, analogous to the human situation (7), and that this sFcεRII had a measurable affinity for IgE, in contrast to our previous report (8), in which a less sensitive analysis was used. The studies further demonstrate that the transmembrane and cytoplasmic region are not necessary for the dual-affinity interaction with IgE and that a full-length construct demonstrates increased capacity to block the membrane FcεRII-IgE interaction.

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⁴ Abbreviations used in this paper: FcεRII, low affinity receptor for IgE; sFcεRII, soluble FcεRII; EC-FcεRII, soluble FcεRII construct consisting of entire extracellular region; Δ 109–144, FcεRII deletion mutant where numbers refer to deleted aa; Affi, Affi-gel; CHO, Chinese hamster ovary.

Materials and Methods

Abs, immunosorbents, and cell culture

Mouse IgE from H1-DNP- ϵ -26 (9) ascites and monoclonal anti-Fc ϵ RII (B3B4) were purified as previously described (3). These Abs, as well as BSA, were coupled to Affigel-15 (Bio-Rad Laboratories, Richmond, CA) at a concentration of 1–2 mg/ml. Purified proteins were radiolabeled with carrier-free ^{125} I (New England Nuclear, Boston, MA) as described previously (10). COS7 cells used for transient protein expression were cultured in RPMI 1640 supplemented with 10% serum, pyruvate, 2-ME, glutamine, penicillin, streptomycin, and gentamicin (3). CHO-K1 cells used for stable protein expression were cultured in DMEM-GS media (11) when using both the pEE6.hcmv-GS (Celltech, Berkshire, UK) vector system and for neo selection. The CHO cells that expressed both the native Fc ϵ RII as well as the mutant Fc ϵ RII in which two of the stalk repeat domains were missing (termed Δ 109–144 cells, where the numbers refer to the amino acids that were deleted) were prepared as described previously (12) and were maintained in GS media.

Preparation of chimeric and full-sized Fc ϵ RII constructs

The construct containing the Ly-49 transmembrane region was prepared as follows. The Ly-49 cDNA in the plasmid vector pCDM8 was provided by Dr. Wayne Yokoyama (Mt. Sinai Medical School, New York City, NY). cDNA fragments encoding the N-terminal cytoplasmic and transmembrane region of Ly-49 (primers: 5'TACGACTCACTATAGGGAGACGGC (sense) and 5'TGTTTAACTTTTATTGCCAACAC) and the entire extracellular region of Fc ϵ RII (primers: 5'GATCCGGGTGGGAAACGAGAAG (sense) and 5'ACAAAGATCCTCTAGAGTCGCGGC) were produced by PCR, and after cutting with *Hind*III, *Hpa*I, *Sma*I and *Not*I, the construct was produced by triple ligation into the pCDNAI vector that had been previously cut with *Hind*III and *Not*I. Sequence analysis, using dideoxy method and an Applied Biosystems 373A Automated DNA Sequencing System (Foster City, CA), was performed by the Nucleic Acid core laboratory using the 5' PCR primer; this confirmed that the junction between the Ly-49 and Fc ϵ RII was in frame.

The construct for expression of the complete extracellular region of the Fc ϵ RII as a soluble molecule (EC-Fc ϵ RII) for the soluble extracellular (EC) sFc ϵ RII construct was produced in a similar manner. The cDNA encoding the leader peptide for the Fc ϵ R1 α protein (Fc ϵ R1 α cDNA in pSVL was a gift of Dr. Jean-Pierre Kinet, National Institutes of Health, Bethesda, MD) produced by PCR using the following oligos: 5'CCACCAAGCTTGGCCTATATTGAAG (sense) and 5'GATGTTAACGACTGCTAACACGCCATC. The cDNA for the extracellular region of the Fc ϵ RII was produced by PCR in the same manner as used for the Ly-49/Fc ϵ RII chimera above. After restriction enzyme cutting of the appropriate PCR products using the enzyme pairs indicated for the chimera and isolation of the appropriate PCR fragments by agarose gel electrophoresis, the EC-Fc ϵ RII construct was produced by triple ligation into pCDNAI. After sequencing analysis as above to confirm in-frame ligation at the junction of the leader and Fc ϵ RII sequence, the appropriate plasmid was isolated by double banding on CsCl and used for both transient and permanent transfections.

Preparation of transient and permanently transfected cells

COS7 cells used for transient expression experiments were collected in log phase growth, and electroporation was as described previously (12). Electroporated cells were cultured for 48 h and then analyzed for surface expression of chimeric protein by ^{125} I-labeled ligand binding analysis. Analysis of EC-Fc ϵ RII production was accomplished by collecting the transfected cells at 36 h, washing by centrifugation, and continuing the culture for an additional 12 h in methionine-free RPMI (Life Technologies, Inc., Grand Island, NY) containing 5% dialyzed FCS and the same additives as complete RPMI above. [^{35} S]Methionine was added to a concentration of 100 μ Ci/ml after 30 min of culture, giving a total pulse of 8 h. For isolation of larger quantities of EC-Fc ϵ RII, permanently transfected CHO-K1 cells producing EC-Fc ϵ RII were prepared by calcium phosphate co-transfection of the EC-Fc ϵ RII construct in pCDNAI along with the pSV2-neo vector (a gift of Dr. Eric Westin, Virginia Common-

wealth University, Richmond, VA) at a 5:1 molar ratio. Selection was accomplished by addition of G418 (Sigma Chemical Co., St. Louis, MO) to a concentration of 700 μ g/ml. After one month, colonies producing EC-Fc ϵ RII were identified by the capacity of supernatants from isolated colonies to inhibit the binding of ^{125} I-labeled B3B4 to CHO cells expressing the native Fc ϵ RII (12). EC-Fc ϵ RII was isolated from these cells as ^{35} S-labeled protein as above, or larger amounts were isolated as described below.

SDS-PAGE analysis and analysis of purified Fc ϵ RII fragments

For amino acid analysis experiments, supernatants from $\approx 1 \times 10^9$ CHO-K1 cells permanently transfected with Fc ϵ RII cDNA (sFc ϵ RII) or the EC-Fc ϵ RII construct were collected after cell growth had reached maximum density. Respective supernatants were cycled initially through BSA-Affi and subsequently through B3B4-Affi, using a 0.5×10 cm disposable column containing 1 ml of absorbent and a peristaltic pump. The B3B4-Affi was washed with 50 bed volumes of PBS and eluted with 3.5 M sodium thiocyanate. Preliminary experiments had demonstrated that this method of elution was superior in yield and purity to acid elution. Eluted samples having the most protein (OD_{280}) were pooled and dialyzed exhaustively against water and lyophilized. Samples were dissolved in SDS-PAGE sample buffer, and SDS-PAGE analysis utilized the protocol described by (13), which minimizes artifactual N-terminal blocking. After electrophoresis, samples were transferred to PDVF membranes, and Coomassie blue staining was used to reveal appropriate bands for sequencing. Amino acid sequencing was performed on the PDVF strip containing the stained band by the amino acid core laboratory for a minimum of 8 cycles. Samples containing [^{35} S]methionine-labeled protein were subjected to the same type of affinity chromatography, except that absorption was by batch incubation rather than continuous recycling (preabsorption with BSA-Affi was for 30 min), and 0.30 ml of absorbent was used. Elution was by boiling for 90 s in SDS-PAGE sample buffer, and analysis was performed on 8 to 18% gradient gels (3). Gels were set up for fluorography by using Fluoro-Hance (RPI, Mount Prospect, IL) following the protocol recommended by the manufacturer.

Binding analysis

The capacity of purified EC-Fc ϵ RII and/or sFc ϵ RII to inhibit ^{125} I-labeled IgE binding was performed by first incubating the indicated amounts of inhibitor and labeled IgE for 30 min on ice. Subsequently, 1×10^6 CHO cells expressing either the native Fc ϵ RII or Δ 109–144 mutant Fc ϵ RII were added (final volume 0.5 ml), and culture continued for 60 min at 4°C. Cell-bound radioactivity was then determined using the phthalate-oil cushion procedure previously described (14).

Results

Site of cleavage for mouse Fc ϵ RII

In an effort to assess the structure and biologic function of the soluble forms of mouse Fc ϵ RII (sFc ϵ RII), the sites of cleavage were determined. CHO-K1 cells that express high levels of Fc ϵ RII were surface iodinated and cultured overnight to allow for production of ^{125} I-labeled sFc ϵ RII in the supernatant. This supernatant was used to "spike" 1.5 liters of unlabeled spent medium from the transfected CHO cells and the sFc ϵ RII was purified by affinity chromatography. After chaotropic elution, the fractions positive for ^{125} I were prepared for electrophoresis and transferred to PDVF membranes. The 38 K_d and 28 K_d sFc ϵ RII band was identified by Coomassie blue staining of the blot and confirmed by autoradiography. After excision from the blot, automated N-terminal sequence analysis was performed. Figure 1 shows the identified cleavage sites, as indicated by the N-terminal sequence analysis; the 38 K_d site lies between Asp93 and Leu94, whereas the 28 K_d site is

FIGURE 1. Identification of the cleavage sites for murine sFcεRII and comparison with the human FcεRII. Amino acid sequences for mouse (*top*) and human FcεRII are shown aligned for homology; data are taken from (37, 38) and a solid line represents identity, whereas two and one dots represent conservative and semiconservative substitutions, respectively; no symbol is a nonconservative aa substitution. Human FcεRII has three repeat domains compared with four in the mouse (39), resulting in the 21 aa gap shown. Eight aa were sequenced from the N-terminus of both major fragments (38 K_d and 28 K_d, respectively) and the sequences found are indicated in bold italics. The cleavage sites determined for the major FcεRII fragments seen in humans, reported by Letellier et al. (7) are indicated by bold underline. In all cases the cleavage occurs just N-terminal of the indicated fragment.

Demonstration that sFcεRII has the capacity to interact with IgE

Fold Excess of Inhibitor	% Inhibition of Binding (Parent Compound)	% Inhibition of Binding (2-Oxide Compound)
0.01	0	0
0.05	0	0
0.1	0	2
0.5	0	8
1	0	10
10	0	30
50	0	56
100	0	70
200	10	-
400	18	-

inhibiting binding to native FcεRII, and two orders of magnitude more sFcεRII are required to inhibit binding to the intact FcεRII. We also attempted to ascertain the affinity of interaction of the sFcεRII with IgE by coating microtiter wells with purified sFcεRII and performing a saturation analysis. In three separate experiments, a low level of specific binding was obtained giving an affinity in the 10^5 to 10^6 M⁻¹ range (data not shown).

Besides the loss of part of the stalk region, the sFcεRII has lost the entire transmembrane and cytoplasmic sequences. To ascertain if these regions played any role in the receptor-receptor association necessary to achieve multivalent interaction with IgE, a chimeric construct was prepared that utilized the amino-terminal transmembrane and cytoplasmic sequences of Ly-49 (15) linked to the predicted entire extra-cytoplasmic region of the FcεRII beginning with Trp49. This chimeric construct was placed into the pCDNA1 expression vector and used in transient transfection analyses in COS cells. Expression levels of the chimera were confirmed by ¹²⁵I-labeled B3B4 binding. Figure 3 shows a saturation analysis of the binding of ¹²⁵I-labeled IgE to COS cells transfected 48 h earlier with the chimera-containing vector. Clearly the same dual-affinity binding observed previously (12) for IgE interaction with the native FcεRII is seen. The two affinities observed.

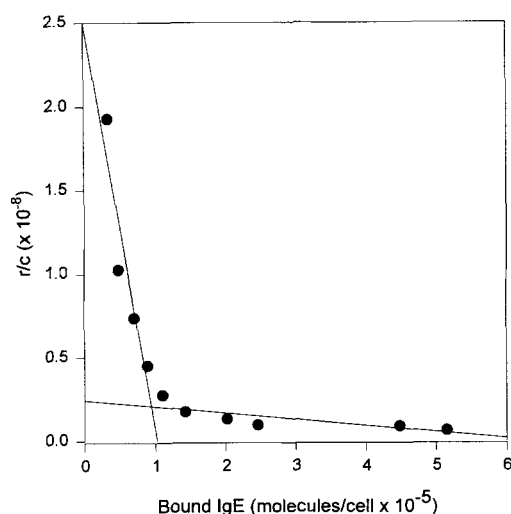


FIGURE 3. Dual-affinity binding of ^{125}I -labeled IgE to the Ly-49/Fc ϵ RII chimera. The construct containing the transmembrane and cytoplasmic domain of Ly-49 and the extracellular region of the Fc ϵ RII was transfected into COS7 cells by electroporation and ^{125}I -labeled IgE binding was determined after 48 h. Scatchard analysis of one experiment representative of three are shown.

1.45×10^8 and $2.29 \times 10^6 \text{ M}^{-1}$, respectively, compare well with the previous results with the native Fc ϵ RII in which values of 4 to 7×10^7 and 4 to $8 \times 10^6 \text{ M}^{-1}$ were reported for the high and low affinity binding, respectively (12). Thus, the extra-cytoplasmic region of Fc ϵ RII is sufficient to allow receptor-receptor oligomerization and the dual-affinity interaction with IgE.

Analysis of a sFc ϵ RII containing the entire extracellular region of Fc ϵ RII

We next wished to determine if a stable sFc ϵ RII could be produced using the extracellular region of Fc ϵ RII. A chimeric construct was developed using the Fc ϵ RI α leader sequence spliced in frame to the cDNA coding for the extracellular portion of Fc ϵ RII. This construct (termed EC-Fc ϵ RII for extracellular sFc ϵ RII construct) was placed into the pCDNA1 expression vector, and transient transfection into COS7 cells was performed. Supernatants from EC-Fc ϵ RII-transfected COS cells were purified by affinity chromatography using B3B4 columns, and SDS-PAGE analysis of the purified EC-Fc ϵ RII indicated two bands (data not shown), suggesting that the EC-Fc ϵ RII remains susceptible to proteolytic cleavage. Confirmation that the major 40 K_d band is EC-Fc ϵ RII was seen by performing N-terminal analysis of the 40 K_d band. The N terminal sequence obtained was VPGWHWETEK; the N-terminal Val is from the Fc ϵ RI α leader, whereas the Pro and Gly are from PCR-mediated insertion of a *Sma*I restriction site. The remaining 9 amino acids are exactly as predicted from the Fc ϵ RII sequence, beginning with the Trp49 that is just

beyond the predicted membrane spanning domain (see Fig. 1).

Subsequently, a permanently transfected CHO line was prepared. Demonstration that this EC-Fc ϵ RII would interact with IgE was seen by comparing affinity chromatography on IgE vs anti-Fc ϵ RII columns. Figure 4 demonstrates the results of such an experiment using [^{35}S]methionine-labeled EC-Fc ϵ RII containing supernatant from the permanently transfected cells. Note that whereas, as previously reported (8), the native sFc ϵ RII binds only to the anti-Fc ϵ RII absorbent, the EC-Fc ϵ RII bound to both the anti-Fc ϵ RII and the IgE Affi-gel. Thus, these studies demonstrate that significant IgE binding requires the stalk region and the lectin domain. To explore the relative affinity of the purified EC-Fc ϵ RII, the capacity to inhibit ^{125}I -labeled IgE binding to both the native membrane-bound Fc ϵ RII as well as the modified ($\Delta 109$ – 144) deletion mutant Fc ϵ RII was determined (Fig. 5). For comparison purposes, the inhibition data for the normally shed sFc ϵ RII shown in Figure 2 are also plotted here. The EC-Fc ϵ RII exhibited the same capacity as the native sFc ϵ RII with respect to inhibition of ^{125}I -labeled IgE binding to the $\Delta 109$ – 144 deletion mutant; however, about a 10-fold increase in capacity, when compared with native sFc ϵ RII, to inhibit binding to the intact Fc ϵ RII was observed. Note that the EC-Fc ϵ RII continued to inhibit ^{125}I -labeled IgE binding more effectively to the $\Delta 109$ – 144 deletion mutant indicating that the soluble construct does not bind IgE as effectively as the membrane-bound form of the Fc ϵ RII (see Discussion).

Discussion

The early finding that the Fc ϵ RII exists in two forms in both the mouse (3) and the human (16) has prompted a search for functions for both the membrane-bound and soluble molecule. This search was further encouraged by the finding in humans that sFc ϵ RII could exhibit cytokine-like activities in that growth factor activities for both myeloid and T cell precursors (17–19) were seen, as well as the capacity to enhance basophil histamine release (20, 21) and inhibit germinal center B cell apoptosis (22). In addition, studies with Fc ϵ RII deletion mutants demonstrated that many of these novel biologic activities were mediated by an epitope distinct from the IgE binding site (23). This has lead to the identification of a new ligand for the Fc ϵ RII, namely CD21 (24, 25). While it remains uncertain whether the above biologic activities are mediated by the new ligand/receptor interaction, the observation that certain anti-CD21 mAbs would exhibit a similar activity with respect to modulation of IgE synthesis, inhibition of germinal center B cell apoptosis, and basophil histamine release (21, 25, 26) is suggestive in this regard.

In the mouse system, cytokine-like activities of the sFc ϵ RII were not observed (27). In addition, mice that have no Fc ϵ RII expression because of homologous recombination of a defective gene have no reported defects in hematopoiesis (28–30). Indeed, the only defect found was

FIGURE 4. Demonstration of IgE binding by the EC-FcεRII construct. CHO-K1 cells permanently transfected with EC-FcεRII (A) or FcεRII (B) were biosynthetically labeled with [³⁵S]methionine, the EC-FcεRII and sFcεRII were isolated from the supernatant by affinity chromatography as described in *Materials and Methods*. (A) Lane 1, EC-FcεRII isolated by rabbit IgG-Affi (control); lane 2, EC-FcεRII isolated on B3B4-Affi; lane 3, EC-FcεRII isolated by IgE-Affi; (B) lane 1, sFcεRII isolated by B3B4-Affi; lane 2, sFcεRII isolated by IgE-Affi. m.w. marker migration position is shown at left of each gel. Experiment is representative of three of similar design.

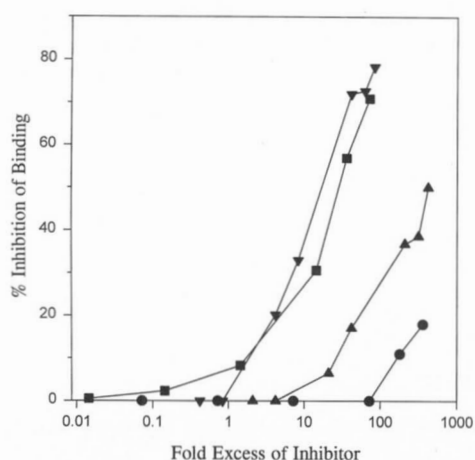
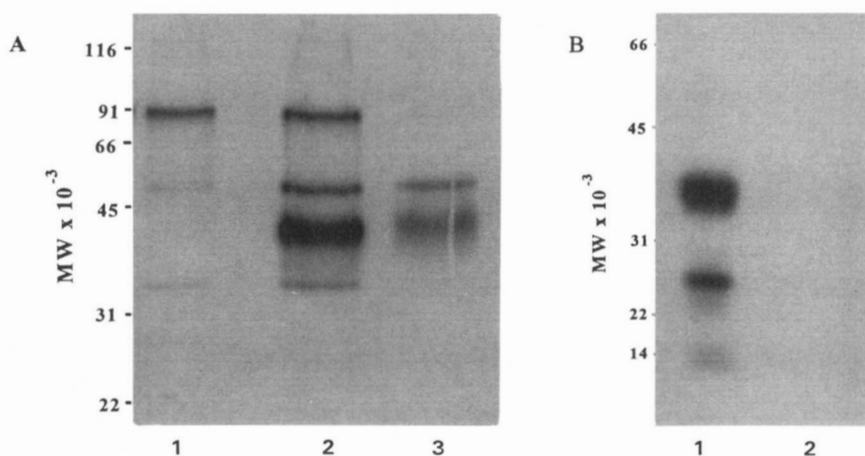


FIGURE 5. Comparison of the capacity for EC-FcεRII vs sFcεRII to inhibit ¹²⁵I-labeled IgE binding. Increasing amounts of purified EC-FcεRII were incubated with 1 μg of ¹²⁵I-labeled IgE and subsequently binding to CHO cells expressing either the Δ109–144 mutant FcεRII (▼) or native FcεRII (▲) was determined. For comparison, the data plotted in Figure 2 is also shown in which the ability of native sFcεRII to inhibit IgE binding to mutant FcεRII (■) or native FcεRII (●) was determined. Fold increase of inhibitor is determined as in Figure 2, m.w. of sFcεRII, 38,000; EC-FcεRII, 43,000. Experiment is representative of two others of similar design.

with the IgE system; these mice do not exhibit the IgE-antigen-complex-induced immune enhancement found in normal mice (29, 31). Interestingly, they evidently exhibit a significant increase in both the total and Ag-specific IgE response in response to an Ag/adjuvant regimen, but not to a strong IgE stimulus such as a parasite infection or anti-IgD injection (30) (E. Studer and D. Conrad, unpublished observations). Of note also is that mice that are transgenic for the FcεRII and overexpress the FcεRII on (primarily) T cells have the opposite result, namely, IgE synthesis is inhibited even in a parasite model. These results suggest

that the FcεRII plays a modulatory role with respect to IgE synthesis, and related studies in the rat (32) and human systems (33) where anti-FcεRII was shown to inhibit IgE synthesis are supportive for such a role. Whether both the membrane and soluble forms of the FcεRII are involved in the IgE synthesis modulation seen is unknown, but the fact that a mouse transgenic for the sFcεRII exhibited a normal IgE response is suggestive for the membrane form (34).

These studies were initiated to determine the structural requirements for interaction of the murine sFcεRII with IgE. Analysis of the sites of cleavage for sFcεRII demonstrated that both the initial and penultimate proteolytic sites were in the stalk region of the molecule (see below). While the cleavage sites bear a relationship to the sites found in human FcεRII (7) in that similar sized fragments are generated, there were no obvious homologies between the cleavage sites. The protease responsible for this breakdown has yet to be identified, although Letellier et al. (35) reported that the protease responsible either is very tightly associated with the FcεRII or is the FcεRII itself.

In early studies this laboratory reported that the mouse sFcεRII did not bind IgE (8). However, we and others recently reported that the higher affinity exhibited for the intact membrane form of the FcεRII was due to the dual affinity for IgE exhibited by the coiled-coil FcεRII trimer present in the membrane (6, 12). The α-helical coiled-coil part of the FcεRII molecule consisted of the "stalk" region comprising the sequence from the carboxyl terminal lectin domain down to the membrane surface. Interestingly, FcεRII stalk deletion mutants, while losing the ability to self-associate, remained capable of binding IgE, albeit with a 10-fold lower affinity (12). These findings lead us to reevaluate the interaction of the sFcεRII with IgE and further determine whether a soluble form could be produced that would interact with IgE in a manner similar to the native membrane FcεRII. The results reported here clearly show an interaction of the sFcεRII with IgE; the reason for

the failure to find such an interaction earlier is related to the methods used. Thus, both the murine and human sFcεRII (36) interact with IgE, although the affinity of the sFcεRII is reduced by at least an order of magnitude. In view of the above mentioned findings that the membrane form is most likely involved in IgE regulation, development of a sFcεRII that has a similar affinity as the intact trimeric FcεRII has an attractive potential as an agent to further delineate FcεRII functions. With regard to the cytokine-like activities that have been reported in the human system, a trimeric FcεRII would be anticipated to work more effectively due to avidity considerations. With regard to IgE synthesis, the membrane form of the FcεRII is at present the form implicated (28, 33); a trimeric construct would be an effective antagonist for IgE binding to membrane-bound FcεRII. Thus, if interaction with membrane-bound FcεRII is required to modulate IgE synthesis, enhancement of the same would result; alternatively, if the control mechanism is related to the interaction of FcεRII, either membrane bound or soluble, with IgE, e.g., surface IgE of switched or memory B cells, then the soluble construct would be expected to inhibit IgE synthesis. Studies are currently underway to evaluate which of the above is operative.

Development of a chimeric construct in which only the transmembrane and cytoplasmic regions of the FcεRII were replaced demonstrated that the extracellular region was still capable of exhibiting the same dual-affinity binding shown by the intact FcεRII (Fig. 3 and Ref. 12), indicating that these regions of the molecule are not important in this aspect. In a previous study from this laboratory (12), an Ly-49/FcεRII construct was made in which only the head group of the FcεRII was attached to the stalk plus transmembrane and cytoplasmic regions of Ly-49. Such a construct did not exhibit IgE binding. Thus, the combined data indicate that the FcεRII stalk, but not the transmembrane and cytoplasmic regions of the FcεRII, are important for IgE binding. Subsequently, a full length sFcεRII construct, consisting of the entire extracellular region was prepared (EC-FcεRII). Examination of the expressed construct demonstrated expression of the expected molecule; however, in some experiments (data not shown) lower mw forms were also evident indicating that, as with the membrane form, the full length construct is subject to proteolysis (Fig. 4); while other bands were seen in this figure, the control IgG absorbent indicates lack of specificity. This construct clearly exhibits increased capacity to interact with IgE when compared with the native sFcεRII, as evidenced both by an increased capacity to inhibit ¹²⁵I-labeled IgE binding to FcεRII⁺ cells and its ability to bind to IgE-Affi-gel. In contrast, the EC-FcεRII construct exhibited a similar capacity to inhibit ¹²⁵I-labeled IgE binding to the FcεRII deletion mutant; this is explained in that both constructs would be expected to exhibit the same capacity to inhibit monomeric FcεRII/IgE interactions. However, the relatively high levels of purified construct re-

quired for inhibition of IgE binding, combined with the proteolysis seen for this construct, indicates that stability is a problem. In this study we did not attempt to determine when the breakdown of the EC-FcεRII construct occurred, i.e., before or after purification of the construct. In studies with human FcεRII, Letellier et al. (35) reported evidence for intrinsic proteolytic activity by the FcεRII, indicating that the proteolysis observed would be expected to continue to occur even in highly purified EC-FcεRII preparations. Currently we are preparing chimeric soluble constructs that replace the FcεRII stalk with other structurally related sequences, with the objective of obtaining a more stable sFcεRII that interacts with IgE in the multivalent fashion of the intact membrane bound molecule. We anticipate such a construct should be of use both with respect to studying the mechanism via which the FcεRII modulates the IgE response and be useful in the search for other potential ligands for the FcεRII.

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