The high affinity receptor for IgE (FceRI) plays an integral role in triggering IgE-mediated hypersensitivity reactions. The IgE-interactive site of human FceRI has previously been broadly mapped to several large regions in the second extracellular domain (D2) of the α-subunit (FceRIα). In this study, the IgE binding site of human FceRIα has been further localized to subregions of D2, and key residues putatively involved in the interaction with IgE have been identified. Chimeric receptors generated between FceRIα and the functionally distinct but structurally homologous low affinity receptor for IgG (FcγRIIa) have been used to localize two IgE binding regions of FceRIα to amino acid segments Tyr129–His134 and Lys154–Glu161. Both regions were capable of independently binding IgE upon placement into FcγRIIa. Molecular modeling of the three-dimensional structure of FcεRIα-D2 has suggested that these binding regions correspond to the “exposed” C′-E and F-G loop regions at the membrane distal portion of the domain. A systematic site-directed mutagenesis strategy, whereby each residue in the Tyr129–His134 and Lys154–Glu161 regions of FceRIα was replaced with alanine, has identified key residues putatively involved in the interaction with IgE. Substitution of Tyr131, Glu132, Val155, and Asp159 decreased the binding of IgE, whereas substitution of Trp136, Trp156, Tyr166, and Glu161 increased binding. In addition, mutagenesis of residues Trp113, Val115, and Tyr116 in the B-C loop region, which lies adjacent to the C′-E and F-G loops, has suggested Trp113 also contributes to IgE binding, since the substitution of this residue with alanine dramatically reduces binding. This information should prove valuable in the design of strategies to intervene in the FceRIα-IgE interaction for the possible treatment of IgE-mediated allergic disease.

FceRI binds monomeric IgE with high affinity ($K_a = 10^{10} \text{ M}^{-1}$) and is expressed on mast cells, basophils (1, 2), Langerhans cells (3, 4), peripheral blood dendritic cells (5), eosinophils (6), and monocytes (7). The receptor can exist in two functionally distinct but structurally homologous low affinity receptors generated between FceRIα-D2, each capable of independently binding IgE upon placement into FcγRIIa. Molecular modeling of the three-dimensional structure of FcεRIα-D2 has suggested that these binding regions correspond to the “exposed” C′-E and F-G loop regions at the membrane distal portion of the domain. A systematic site-directed mutagenesis strategy, whereby each residue in the Tyr129–His134 and Lys154–Glu161 regions of FceRIα was replaced with alanine, has identified key residues putatively involved in the interaction with IgE. Substitution of Tyr131, Glu132, Val155, and Asp159 decreased the binding of IgE, whereas substitution of Trp136, Trp156, Tyr166, and Glu161 increased binding. In addition, mutagenesis of residues Trp113, Val115, and Tyr116 in the B-C loop region, which lies adjacent to the C′-E and F-G loops, has suggested Trp113 also contributes to IgE binding, since the substitution of this residue with alanine dramatically reduces binding. This information should prove valuable in the design of strategies to intervene in the FceRIα-IgE interaction for the possible treatment of IgE-mediated allergic disease.

The binding of IgE by FceRI on mast cells and basophils is a fundamental step in the cascade of events that lead to allergic disease. The interaction of multivalent allergens with FceRI-bound IgE results in cross-linking of the receptor, which triggers a range of biological sequelae that ultimately leads to the release of inflammatory mediators and the onset of the type I hypersensitivity response (1, 2). Approaches that intervene in the binding of IgE by FceRI may prove useful in the treatment of allergic disease. Clearly, understanding the molecular basis of the interaction of FceRI with IgE would provide valuable information for such a therapeutic strategy.

Studies from our group and others using chimeric receptors together with the epitope mapping of anti-FceRIα monoclonal antibodies have identified the second extracellular domain of FcεRIα as the principle IgE interactive domain (9–12). The first extracellular domain has not been demonstrated to have a direct IgE binding role; however, it does appear to make an important structural contribution in the maintenance of the high affinity IgE binding of the receptor (9, 11). Multiple regions of FcεRIα-D2 have been implicated in the binding of IgE. In a series of “gain of function” experiments using chimeric FcεRIα/FcγRIIa receptors, we identified three relatively large regions of FcεRIα-D2, each capable of independently binding IgE (9). The FcεRIα regions encompassed by residues Trp87–Lys128, Tyr129–Asp145, or Ser146–Val168 when inserted into FcγRIIa were each able to impart IgE binding to the receptor. Mallamaci et al. (10) have used a similar approach with chimeric FcεRIα/FcγRIII receptors, however, in “loss of function” experiments and identified four regions of FcεRIα-D2 that putatively contribute to IgE binding. The replacement of each of the FcεRIα regions encompassed by residues Ser169–Phe104, Arg111–Glu125, Asp123–Ser137, and Lys154–Ile167 with the corresponding regions of FcγRIII, was found to result in reduced IgE binding. In addition, a recent study by McDonnell et al. (13) has demonstrated that residues Ile119–Tyr129 of FcεRIα-D2, when synthesized as a conformationally constrained peptide, can inhibit the binding of IgE to FceRI. Despite the localization of multiple binding regions in FcεRIα-D2, the interaction of FceRI with IgE at the level of individual residues has not been defined. In this study, we have identified small IgE binding subregions of FcεRIα-D2, which have been analyzed by site-directed mutagenesis, and residues putatively involved in the interaction with IgE have been determined. These findings have enabled the development of a model of how FcεRIα binds IgE and contribute to our understanding of the interaction of the leukocyte FcR family with their ligands.
**Experimental Procedures**

**Generation of Chimeric FcRIα/FcγRIα and Mutant FcRIα Receptor CDNA Expression Constructs**

Chimeric FcRIα/FcγRIα or mutant FcRIα CDNAs were constructed by splice overlap extension PCR (14) using an expressible form of the FcRIα chain (15) or FcγRIα (16) as templates. The expressible form of the FcRIα chain consists of the extracellular region of FcRIα linked to the transmembrane and cytoplasmic tails of FcγRIα and is expressed on the cell surface and binds monomeric IgE with an affinity comparable with that of the wild-type FcRIα chain, as described previously (15). Splice overlap extension PCR was performed as follows. Two PCR reactions were used to amplify the FcRIα/FcγRIα or FcRIα fragments to be spliced together. The reactions were performed on 100 ng of the FcRIα cDNA in the presence of 500 ng of each oligonucleotide primer, 1.25 mM dNTPs, 50 mM KCl, 10 mM Tris-Cl, pH 8.3, 1.25 mM MgCl₂, using 2.5 units of Taq polymerase (Amplitaq; Cetus) for 25 amplification cycles. A third PCR was performed to splice the two fragments and amplify the spliced product. 100 ng of each purified fragment was used with the appropriate oligonucleotide primers under the above PCR conditions.

Chimeric and mutant receptor CDNA expression constructs were produced by subcloning the CDNAs into the eukaryotic expression vector pRK3 (17). Each cDNA was engineered in the PCRs to have an EcoRI site at their 5′-end (the 5′-flanking oligonucleotide primer containing an EcoRI recognition site) and a SalI site at their 3′-end (the 3′-flanking oligonucleotide primer containing a SalI recognition site), which enabled the CDNAs to be cloned into the EcoRI and SalI sites of pRK3. The nucleotide sequence integrity of the chimeric CDNAs were determined by dyeodeoxyxynucleotide chain termination sequencing (18) using Sequence™ (U.S. Biochemical Corp.) as described (19).

**Monoclonal Antibodies and IgG Reagents**

The anti-FcRIα mAb 3B4 and the anti-FcγRIα mAb 8.2 were produced in this laboratory (20). The anti-FcRIα mAb 15A5 was a gift of Dr. J. Kochan (12). The mouse IgE anti-2,4,6-trinitrophenyl mAb (A3) was produced from a hybridoma cell line obtained from the American Type Culture Collection (Rockville, MD); the mouse IgG anti-2,4,6-trinitrophenyl mAb (A3) was produced from a hybridoma cell line that was a gift of Dr. A. Lopez (21). Human IgE myeloma protein was purified from the serum of a myeloma patient. IgE was precipitated with NH₄SO₄, and then IgG was removed by chromatography on protein A, and IgE was purified by size fractionation chromatography on Sephacryl S-300 HR (Amersham Pharmacia Biotech). Purified IgE was analyzed by SDS-polyacrylamide gel electrophoresis and by enzyme-linked immunosorbent assay, and contaminating IgG was estimated at <1%.

**Transfection**

Cos-7 cells (30–50% confluent per 5-cm² Petri dish) were transiently transfected with FcγRIα cDNA expression constructs by the DEAE-dextran method. Cells were incubated with a transfection mixture (1 ml/5-cm² dish) consisting of 5–10 mg/ml DNA, 0.4 mg/ml DEAE-dextran (Amersham Pharmacia Biotech), and 1 µl chloroquine (Sigma) in Dulbecco’s modified Eagle’s medium (Flow Laboratories, Australia) containing 10% (v/v) Nusser (Flow Laboratories, Australia), for 4 h. The transfection mixture was then removed, and the cells were treated with 10% (v/v) dimethyl sulfoxide in phosphate-buffered saline (7.6 mM Na₂HPO₄, 3.5 mM NaH₂PO₄, 145 mM NaCl, pH 7.4, for 2 min, washed, and returned to fully supplemented culture medium for 48–72 h before use in assays. Cos-7 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml gentamicin (Commonwealth Serum Laboratories, Melbourne, Australia), and 0.05 mM 2-mercaptoethanol (Koch Light Ltd., Birmingham, United Kingdom).

**Ig Binding Assays**

The binding of Ig by COS-7 cells following transfection with chimeric or mutant receptor cDNAs was determined using two approaches. Erythrocyte-Antibody Rosetting—COS-7 cell monolayers transfected with FcγRIα expression constructs were incubated with EA complexes, prepared by coating sheep red blood cells with trinitrobenzenesulfonate (Fluka Chemika, Switzerland) and then sensitizing these cells with mouse IgE or IgG, anti-2,4,6-trinitrophenyl mAb (22). Two ml of 2% EAs (v/v) were added per 5-cm² dish of transfected cells and incubated for 5 min at 37°C. Plates were then centrifuged at 500 × g for 3 min and placed on ice for 30 min. Unbound EA were removed by washing with L-15 medium modified with glutamine (Flow Laboratories, Melbourne, Australia) and containing 0.5% bovine serum albumin.

**Direct Binding of Monomeric Human 2g-IgE**—Cos-7 cells transfected with FcγRIα expression constructs were harvested; washed in phosphate-buffered saline, 0.5% bovine serum albumin; and resuspended at 10⁷ cells/ml in L-15 medium, 0.5% bovine serum albumin. 50 µl of cells were incubated with 50-µl serial dilutions of ¹²⁵I-IgE for 120 min at 4°C. ¹²⁵I-IgE was prepared by the chloramine-T method as described (24) and was used to compete equally well with unlabeled IgE binding to FcγRIα receptor expressing COS-7 cells. Cell-bound ¹²⁵I-IgE was determined following centrifugation of cells using following equation: (wild type − mock IgE binding) × (wild type − mock 22E7 binding) (15).

**Generation of FcRIα Domain 2 Model Structure**

Molecular modeling of domain 2 (D2) of human FcRIα was performed using the Homology and Discover modules of the InsightII environment of Molecular Simulations Inc. on a Silicon Graphics Indigo workstation. The model of FcRIα-D2 was constructed by mutation of our previously described model of human FcγRIα-D2 (25), which was based on the published crystal structure of domain 2 of CD4 (protein data base file pbd2cd4.ent; Brookhaven National Laboratory, Upton, NY) (26, 27).

**RESULTS**

**Molecular Modeling of the Extracellular Domains of FcRIα**—The three-dimensional structure of FcRIα has not yet been solved. To aid in the localisation of putative IgE binding regions of FcRIα, we have generated a three-dimensional model of the second extracellular domain (D2) of FcRIα based on the known structure of a related domain, CD4-D2. CD4-D2 belongs to the C2 set of Ig superfamily members, and sequence alignment of FcRIα-D2 with CD4-D2 suggests that this domain will adopt a similar folding pattern (25, 28). The structure of the FcRIα-D2 model is characteristic of the C2 Ig-fold, comprising seven β-sheets (A, B, C, D, E, F, G) that form two antiparallel β-sheets of ABE and CC′G (Fig. 1A).

**Chimeric Receptors Identify Multiple Regions of FcRIα Involved in IgE Binding**—Based on the location of the previously described IgE binding regions (9–13) on our three-dimensional molecular model of FcRIα-D2 and by analogy with mapping studies of the homologous interaction of the FcγRI with IgG (25, 28–31), we targeted the B-C, C-E, and F-G loop regions of FcRIα-D2 as likely to be involved in the binding of IgE. In
order to assess the contribution these three loops made to the binding of IgE by FcεRIα, chimeric receptors were generated, whereby FcγRIIa was used as a scaffold to accept each of these FcεRIα loop regions. The three resultant chimeric receptors consisted of FcγRIIa containing the following regions of FcεRIα-D2: (i) the B-C loop, residues Gly<sup>109</sup>–Tyr<sup>116</sup>; (ii) C'-E loop, residues Tyr<sup>129</sup>–His<sup>134</sup>; and (iii) F-G loop, residues Lys<sup>154</sup>–Glu<sup>161</sup>, designated the γ109–116ε, γ129–134ε, and γ154–161ε chimeric receptors, respectively. COS-7 cells were transfected with expression constructs of these chimeric receptors and tested for their capacity to bind mouse IgE (or IgG<sub>1</sub>) immune complexes by EA rosetting. Cells transfected with the γ154–161ε chimeric receptor bound IgE-EA (Fig. 2A, Table I), and the binding was specific, since mock-transfected cells or cells transfected with FcγRIIa did not bind IgE-EA (Table I). These data indicate that the Lys<sup>154</sup> to Glu<sup>161</sup> region of FcεRIα can directly bind the binding of IgE. As expected, this chimeric receptor was unable to bind IgG<sub>1</sub>, since the previously described IgG binding region, residues Asn<sup>154</sup>–Ser<sup>161</sup> (25), has been replaced with the homologous FcεRIα sequence (Table I). Similar experiments demonstrated that the γ129–134ε chimeric receptor could also specifically bind IgE-EA (Fig. 2B, Table I), indicating that the Tyr<sup>129</sup>–His<sup>134</sup> region also contains an IgE binding site. As expected, this chimeric receptor was able to bind IgG<sub>1</sub>-EA (Table I) due to the presence of the FcγRIIa Asn<sup>154</sup>–Ser<sup>161</sup> IgG binding sequence.

As described above, the segment of FcεRIα-D2 encompassed by residues 87–128 had previously been shown to contain an IgE binding site, which we predicted to be the B-C loop (28). However, when transfected into cells, the γ109–116ε chimeric receptor containing the FcεRIα B-C loop did not bind IgE-EA (Fig. 2C). Since the receptor was clearly expressed on the cell surface, demonstrated by its ability to bind IgG-EA (Table I), these results suggest that the Gly<sup>109</sup>–Tyr<sup>116</sup>ε region is insufficient to bind IgE in its own right and therefore that the IgE binding region in the 87–128 segment is either not the B-C loop or requires the B-C loop in combination with additional surrounding region(s). This was further investigated by site-directed mutagenesis (see below).

Fine Structure Analysis of the FcεRIα IgE Binding Site—To identify the key residues of the FcεRIα binding regions (C'-E loop, residues Tyr<sup>129</sup>–His<sup>134</sup>; F-G loop, residues Lys<sup>154</sup>–Glu<sup>161</sup>) involved in the interaction with IgE, site-directed mutagenesis was used to replace each residue in these regions with alanine. In addition, residues Trp<sup>113</sup>, Val<sup>115</sup>, and Tyr<sup>116</sup> in the B-C loop were also substituted with alanine, since the FcεRIα-D2 model predicts this region is likely to be adjacent to the F-G and C'-E loops and may therefore contribute to IgE binding. The alanine substitution mutants of FcεRIα were expressed in COS-7 cells, and the binding of monomeric human IgE was examined in direct binding assays by titration of <sup>125</sup>I-labeled hIgE (Fig. 3). The levels of cell surface expression of the FcεRIα mutants on...
the COS-7 cell transfectants were determined using the FceRIα mAb 22E7, shown to detect an epitope distant to the binding site (12). Using these results, the binding of hIgE was corrected for variation in expression between the mutant receptors, which ranged from 80 to 120% of wild-type FceRI levels (data not shown).

First, the individual alanine substitution of residues Lys154–Glu161 in the F-G loop indicated that each mutant retained hIgE binding, with the striking exception of the Val155–Ala mutant, where binding of monomeric hIgE was almost totally abolished, this receptor exhibiting only 3.2 ± 2.1% (mean ± S.D.) binding relative to the wild-type receptor (Fig. 3, A and

FIG. 2. IgE complex binding of chimeric Fc receptors. COS-7 cell monolayers were transfected with the following chimeric cDNA constructs: γ154–161e (A), γ129–134e (B), and γ109–116e (C). The binding of IgE immune complexes was assessed directly on monolayers by EA rosetting using mouse IgE-sensitized erythrocytes. The transfections were performed in a transient expression system, resulting typically in 30–50% of cells expressing the chimeric FcR. Cells were considered to be expressing functional receptors if >10 red blood cells were bound per cell.
A schematic representation of the domain 2 composition of the chimeric receptors is shown. Shaded regions are derived from the FceRIα chain, and unshaded regions are derived from FcγRIIIa. The relative positions of the putative β-strands are shown above as labeled solid lines. The binding of mouse IgE and IgG1 (mlgE and mlgG) was assessed by EA rosetting as described under “Experimental Procedures.” +, >10% of cells rosetting; –, no cells rosetting.

| NAME       | CHIMERIC FcR COMPOSITION | Ig COMPLEX BINDING |
|------------|--------------------------|--------------------|
|            | A           | B           | C           | C'         | E           | F           | G           | mlgG | mlgE |
| FcεRIα     |            |             |             |            |             |             |             | +    | –    |
| FcγRII     |            |             |             |            |             |             |             | –    | +    |
| γ154-161ε  |            |             |             |            |             |             | 154–161     | –    | +    |
| γ129-134ε  |            |             | 129–134     |            |             |             |             | +    | –    |
| γ109-116ε  | 109–116     |             |             |            |             |             |             | +    | –    |

TABLE I
Chimeric FcR composition and Ig complex binding

A comparison of the levels of IgE binding with the FcεRI α COS-7 cell transfectants was taken as 100% and mock-transfected cells as 0% binding. Results are expressed as means ± S.E. To control for variable receptor expression between the mutant FcεRI COS-7 cell transfectants, levels of expression were determined using a radiolabeled monoclonal anti-FcεRI antibody 22E7, and IgE binding was normalized to that seen for wild-type FcεRI (see “Experimental Procedures”).

**Fig. 3. Monomeric human IgE binding by FcεRIα alanine point mutants.** Radiolabeled monomeric human IgE was titrated on COS-7 cells transfected with wild-type FcεRIα or FcεRIα containing alanine point mutations. A, F-G loop mutants, wild-type FcεRIα (●), Lys154 → Ala (×), Val155 → Ala (●), Trp156 → Ala (○), Gln157 → Ala (▲), Leu158 → Ala (●), Asp159 → Ala (△), Tyr160 → Ala (●), Glu161 → Ala (□). B, C'-E loop mutants, wild-type FcRIα (●), Tyr129 → Ala (○), Tyr130 → Ala (△), Tyr131 → Ala (□), Glu132 → Ala (▲), Asn133 → Ala (●), His134 → Ala (△). C, B-C loop mutants, wild-type FcRIα (●), Thr133 → Ala (○), Val135 → Ala (●), Tyr136 → Ala (□). A comparison of the levels of IgE binding with the FcεRIα mutants relative to wild-type FcεRIα is shown. D, F-G loop mutants. E, C'-E loop mutants. F, B-C loop mutants. The percentage of binding was calculated from hlgE bound at a concentration of 2 μg/ml. The binding of wild-type FcεRIα was taken as 100% and mock-transfected cells as 0% binding. Results are expressed as means ± S.E. To control for variable receptor expression between the mutant FcεRI COS-7 cell transfectants, levels of expression were determined using a radiolabeled monoclonal anti-FcεRI antibody 22E7, and IgE binding was normalized to that seen for wild-type FcεRI (see “Experimental Procedures”).
D). The loss of hIgE binding by this mutant receptor was not due to decreased cell surface expression as demonstrated by its expression on the cell surface in levels comparable with that of wild-type FcεRI (data not shown). The substitution of Asp<sup>159</sup> with alanine also resulted in diminished IgE binding, this receptor exhibiting 52.7 ± 7.2% binding of the wild-type receptor. The substitution of Lys<sup>154</sup>, Gln<sup>157</sup>, and Leu<sup>158</sup> with alanine had no significant effect on the binding of IgE, these mutants exhibiting binding comparable with wild-type FcεRIa. In contrast, the replacement Trp<sup>156</sup>, Tyr<sup>160</sup>, or Glu<sup>161</sup> with alanine also resulted in diminished IgE binding, this mutant receptor exhibiting binding comparable with that seen for wild-type FcεRIa (data not shown). The localization of IgE binding regions in FcεRI implicated in IgE binding using peptide inhibition studies is highlighted in red. The data are compiled from this paper and Refs. 13, 25, 29, 30, and 31.

Although the chimeric receptor strategy failed to reveal a direct binding role for the B-C loop (residues Gly<sup>109</sup>–Tyr<sup>116</sup>), mutagenesis of residues Trp<sup>113</sup>, Val<sup>115</sup>, and Tyr<sup>116</sup> within this loop suggests that it may also contribute to IgE binding by FcεRIa. This was demonstrated, since the substitution of Lys<sup>154</sup>–Glu<sup>161</sup> in the F-G loop, these results clearly identify the C'-E loop as also playing a role in the binding of IgE by FcεRIa.

**FIG. 4. Ig binding regions of leukocyte FcεRI.** A schematic diagram is shown of model structures proposed for domain 2 of human FcεRI, FcγRIIa, and FcγRIIIa with residues implicated in Ig binding highlighted in red. The models are based on the known structure of CD4 domain 2 (see Refs. 26 and 27) and are shown in ribbon form with β-strands labeled and depicted as arrows. The models are oriented with the four β-strands (C–F) face at the front and adjoin domain 1 at the top and the transmembrane region at the bottom. The predicted positions of amino acids implicated in Ig binding through mutagenesis studies (see “Fine Structure Analysis of the FcεRI IgE Binding Site” for details) are indicated with red circles and labeled in single letter code with their residue number. The C'-C loop region of FcεRI implicated in IgE binding using peptide inhibition studies is highlighted in red. The data are compiled from this paper and Refs. 13, 25, 29, 30, and 31.

**DISCUSSION**

Two approaches have been used to identify and analyze the IgE binding site of FcεRIa. First, to localize IgE binding regions of FcεRIa, a series of chimeric FcεRs were engineered by exchange of segments between the second domain of FcεRIa and FcγRIIa. Second, fine structure analysis of these binding regions, and an additional region likely to be in juxtaposition, was performed by generating 17 point mutants in FcεRIa using alanine scanning mutagenesis. These approaches have enabled the localization of IgE binding regions in FcεRIa to subregions.
of the second extracellular domain and identified key residues putatively involved in the interaction with IgE. Based on a molecular model of FcεRIα-D2, these data suggest that the IgE binding regions comprise the F-G, C'-E, and B-C loops and adjacent strand regions of this domain. Both the F-G and C'-E loops were directly implicated in the interaction with IgE, since insertion of these regions into FcεRIα was able to impart IgE binding to this receptor. In contrast, insertion of the B-C loop was itself insufficient to direct the binding of IgE. However, site-directed mutagenesis of this region identified the residue Trp\textsuperscript{113} as playing an important binding role, which provides evidence to suggest that the B-C loop also contributes to the interaction with IgE.

The molecular model of FcεRIα-D2 suggests that the F-G, C'-E, and B-C loops of FcεRIα-D2 are likely to be juxtaposed at the membrane distal end of the domain at the interface with domain 1. The localization of the FcεRIα-D2 IgE interactive sites to this region, together with the finding that domain 1 also plays a key role in maintaining high affinity binding of the receptor (9, 11), suggests that the interdomain region between domains 1 and 2 comprises an important region of interaction of FcεRIα with IgE. In support of this model, the anti-FcεRIα mAb 15A5, which recognizes an epitope in the B-C loop region of FcεRIα-D2, is able to block the binding of IgE to FcεRI completely (12), suggesting that the multiple IgE binding regions are likely to be situated in close proximity to one another.

Interestingly, a recent study examining the IgE inhibitory capacity of synthetic peptides designed to mimic regions of FcεRIα-D2 has also implicated the C'-C loop (residues Ile\textsuperscript{119}–Tyr\textsuperscript{129} as playing a role in the binding of IgE (13). A peptide encompassing this region and designed to mimic the conformation of the C'-C loop was demonstrated to competitively inhibit IgE binding to FcεRIα and prevent IgE-mediated mast cell degranulation in vitro. Thus, the inclusion of the C'-C loop with the B-C, C'-E, and F-G loops described herein extends the putative region of contact of FcεRIα with IgE. These data therefore suggest that the entire four-stranded β-sheet face of FcεRIα-D2, namely the C'-C'-F-G strands and adjacent loops, may be important in the interaction of FcεRIα with IgE.

The alanine scanning mutagenesis of the F-G, C'-E, and B-C loops of FcεRIα-D2 has identified a number of residues that may contribute to the binding of IgE. The substitution of amino acids Trp\textsuperscript{113}, Tyr\textsuperscript{131}, Glu\textsuperscript{132}, Val\textsuperscript{155}, and Asp\textsuperscript{159} with alanine decreased the binding of IgE, whereas substitution of Trp\textsuperscript{130}, Trp\textsuperscript{156}, Tyr\textsuperscript{160}, and Glu\textsuperscript{161} increased binding. Based on the three-dimensional model of FcεRIα-D2, the side chains of these residues are exposed predominantly on the surface of the domain and contribute to a continuous face in the C'-C'-F-G region (Fig. 1B). The majority of these residues are aromatic (Trp\textsuperscript{113}, Trp\textsuperscript{130}, Tyr\textsuperscript{131}, Trp\textsuperscript{156}, Tyr\textsuperscript{160}) or charged (Glu\textsuperscript{132}, Asp\textsuperscript{159}) and are likely candidates for direct contact with IgE.

Studies examining the binding regions on the Fc portion of IgE for FcεRIα have identified a number of putative interactive sites (32–36). The third constant domain (Cε3) appears to be the principal FcεRIα binding domain, containing major binding sites in the Ce2/Cε3 junction and the Ce3 A-B loop region. Both of these regions contain a number of exposed aromatic and charged residues that may form a complementary surface for interaction with that described herein for FcεRIα. Interestingly, the Ce2/Cε3 junction region is located distally to the Ce3 A-B loop, suggesting a discontinuous binding site in IgE-Fc. This implies that the Ce2/Cε3 and Ce3 A-B loop may interact with different regions of FcεRIα. Since the FcεRIα binding site appears to comprise a single continuous region in the C'-C'-F-G face of domain 2, it is therefore possible that a second binding site distant from this region (e.g. in domain 1) may also exist.

The definition of the precise molecular basis of the interaction between FcεRIα and IgE awaits the elucidation of the structure of FcεRIα-IgE complexes.

The findings described herein for FcεRIα when compared with similar studies of the structurally related FcγRIα, i.e. FcγRI (37), FcγRIλα (25, 29), and FcγRIII (30, 31), reveal a number of similarities in the molecular basis of how these receptors interact with their respective ligands. The two Ig-like domains of the extracellular regions of FcεRIα, FcγRIλα, and FcγRIII have identified common regions of these receptors that are involved in the interaction with their Ig ligands (Fig. 4). The three loop regions identified herein as involved in the binding of IgE by FcεRIα, namely the F-G, C'-E, and B-C, have also been implicated as crucial in the binding of IgG by FcγRIλα (25, 29) or FcγRIII (30, 31) (Fig. 4). The C'-C loop of FcγRIλα and FcγRIII also contributes to Ig binding in both of these receptors (13, 30, 31); however, it does not appear to be involved in FcγRIII (29) (Fig. 4). Thus, the focus of the interaction of FcεRIα and FcγRIII with IgE exhibits some differences to that of FcγRIλα. However, it is clear from all of these studies that the above mentioned loop regions of the second extracellular domain, which contribute to the four-strand β-sheet (C'-C'-F-G) face, constitute the major Ig interactive regions of these receptors. Thus, despite FcεRIα exhibiting a distinctly different specificity and affinity for Ig to the FcγRI, structural similarities are likely to be maintained between these receptors in their interaction with Ig.

Understanding the molecular basis of the interaction of FcεRIα with IgE will assist in the design of therapeutic strategies to treat IgE-mediated allergic disease by blocking the binding of IgE by FcεRIα. The contribution to the definition of the IgE binding site of FcεRIα as described herein represents a step toward the possibility of rational design of such therapeutic agents. The recent demonstration that the structure-based design of a constrained peptide of the C'-C' loop of FcεRIα-D2 can inhibit IgE binding to FcεRI highlights the feasibility of a rational approach (13). The IgE binding loops of FcεRIα-D2 identified herein, i.e. F-G and C'-E, may represent other candidate regions for similar studies. The ability of recombinant soluble FcεRIα to inhibit the binding of IgE to cell surface FcεRI has also been demonstrated (38–40). The engineering of higher affinity forms of soluble FcεRIα, such as the Trp\textsuperscript{156} → Ala, Trp\textsuperscript{160} → Ala, Trp\textsuperscript{160} → Ala, and Glu\textsuperscript{161} → Ala as described in this study, may provide more effective therapeutic agents.

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