Multiple Regions of Human FcγRII (CD32) Contribute to the Binding of IgG*

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The low affinity receptor for IgG, FcγRII (CD32), has a wide distribution on hematopoietic cells where it is responsible for a diverse range of cellular responses crucial for immune regulation and resistance to infection. FcγRII is a member of the immunoglobulin superfamily, containing an extracellular region of two Ig-like domains. The IgG binding site of human FcγRII has been localized to an 8-amino acid segment of the second extracellular domain, Asn154-Ser161. In this study, evidence is presented to suggest that domain 1 and two additional regions of domain 2 also contribute to the binding of IgG by FcγRII. Chimeric receptors generated by exchanging the extracellular domains and segments of domain 2 between FcγRII and the structurally related FcεRI α chain were used to demonstrate that substitution of domain 1 in its entirety or the domain 2 regions encompassing residues Ser109-Val116 and Ser130-Thr135 resulted in a loss of the ability of these receptors to bind hIgG1 in dimeric form. Site-directed mutagenesis performed on individual residues within and flanking the Ser109, Val116 and Ser130-Thr135 domain 2 segments indicated that substitution of Lys113, Pro114, Leu115, Val116, Phe129, and His131 profoundly decreased the binding of hIgG1, whereas substitution of Asp133 and Pro134 increased binding. These findings suggest that not only is domain 1 contributing to the affinity of IgG binding by FcγRII but, importantly, that the domain 2 regions Ser109-Val116 and Phe129-Thr135 also play key roles in the binding of hIgG1. The location of these binding regions on a molecular model of the entire extracellular region of FcγRII indicates that they comprise loops that are juxtaposed in domain 2 at the interface with domain 1, with the putative crucial binding residues forming a hydrophobic pocket surrounded by a wall of predominantly aromatic and basic residues.

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‡ The abbreviations used are: mAb, monoclonal antibody; EA, antibody-sensitized erythrocyte; r.m.s., root mean square; SOE, splice overlap extension; PCR, polymerase chain reaction; SPDP, N-succinimidyl 3-(2-pyridyldithio)propionate.
and C/E loops of FcRII may in addition to the F/G loop also play a role in the binding of IgG by FcRII. Also of interest is that while there is a domain 2 of FcRI in domain 1 of FcRII, not yet clearly defined, a role for domain 1 of FcRII has not been determined. However, domain 1 of FcRII, although demonstrated to not have a direct role in IgE binding, has been shown to play an important role in high affinity binding (18, 26) possibly by maintaining the structural integrity of the receptor or by providing additional contact sites. Since FcRII is structurally related to FcRI, domain 1 of FcRII may also play a similar role.

The possibility that domain 1 and the B/C or C/E loop regions of domain 2 also contribute to the binding of IgG1 by FcRII is addressed herein, using both chimeric receptor and site-directed mutagenesis strategies.

MATERIALS AND METHODS

Generation of Chimeric FcRII/ForcRI and Mutant FcRII Receptor 

cDNA Expression Constructs—Chimeric FcRII/ForcRI α chain or mut-
tant FcRII cDNAs were constructed by splice overlap extension (SOE) 

PCR (27) using the FcRIIdNA (8) as template. SOE PCR was performed as follows. Two PCRs were used to amplify the FcRII or FcRII fragments to be spliced together. The reactions were performed on 100 ng of the FcRIIdNA in the presence of 500 ng of each oligonucleotide primer, 1.25 mM dNTPs, 50 mM KCl, 10 mM Tris-Cl, pH 8.3, and 1.5 mM MgCl₂, using 2.5 units of Taq polymerase (Amplitaq, Perkin-Elmer) for 25 amplification cycles. A third PCR reaction was performed to splice the two fragments and amplify the spliced product and included 100 ng of each fragment (purified by size fractionation through an agarose gel) (28) with the appropriate oligo-

ucleotide primers under the PCR conditions above.

The chimeric FcRII/ForcRI α chain receptors were generated as follows. For chimera y109–116, oligonucleotide pairs NR1 + CHM10 and CHM09 + EG5 were used to produce two fragments, which were spliced together using oligonucleotides (28, 30). For chimera y130–135, oligonucleotide pairs NR1 + PM12 and PM11 + EG5 were followed by NR1 and EG5. The sequences of the oligonucleotides and their positions of hybridization with the FcRIIdNA cDNA are as follows: NR1, 5′-TACGATTTCTATGGAAGCAGGAAATGCTC-3′ (nucleotide positions 10–30); EG5, 5′-TTTGTCCGACATCGTTCGATAACTG-CG-3′ (967-980); CHM09, 5′-CACTCCAGGTCCTCACCACCGG-GACCTCAGAT-3′ (419-437) with nucleotides 442-462 of FcRII α chain; CHM10, 5′-AGGAAGGCTGATGTACAGGACATCCTTGCATC-3′ (462-468 with 446-462 of FcRI α chain); PM11, 5′-GTGTTCTGATCAGAACCTGGGTACCTTGCATC-3′ (473-490 with 492-506 of FcRI α chain); PM12, 5′-GTTGATGAGAACCACCTTTGAC-3′ (515-531 with 490-506 of FcRI α chain). 

Sequential steps involved from ForcRI α chain were underlined, FcRII is not underlined, and nonhomologous sequences including restriction enzyme sites used in cloning of the PCR products are in boldface type. Nucleotide sequences (8, 16).

Two ml of 2% EAs (v/v) were added per 5-cm² dish of transfected cells with mouse IgG1 or IgE anti-trinitrobenzene sulfonate mAb (37). Cells were incubated with antibody-sensitized erythrocytes (EA complex) (36). Cells were incubated with a transfection mixture (1 ml/5 cm²) for 4 h. The transfection mixture was then removed, and cells were treated with 10% (v/v) dimethylsulfoxide in phosphate-buffered saline, pH 7.0, 0.15 M NaCl, which was 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 mglutamine (Commonwealth Serum Laboratories, Australia), and 0.05% 2-mercaptoethanol (Koch Light Ltd., United Kingdom).

Immune Complex Binding—The binding of immune complexes by COS-7 cells following transfection with chimeric or mutant receptor cDNAs was determined using two approaches: erythrocyte-antibody rosetting or direct binding of dimeric hIgG1. For erythrocyte-antibody rosetting, COS-7 cell monolayers transfected with FcR expression constructs were incubated with erythrocytes (EA complexes), prepared by coating sheep red blood cells with trinitrobenzene sulfonate (Fluka Chemika, Switzerland) and then sensitizing these cells with mouse IgG1 or IgG anti-trinitrobenzene sulfonate mAb (37). 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 5 min. Unbound EAs were washed with L-15 medium modified with glutamine (Flow Laboratories) and containing 0.5% bovine serum albumin. For direct binding of dimeric hIgG1, COS-7 cells transfected with FcR 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. Cells in 50-μl aliquots were incubated with 50-μl aliquots of dilutions of 125I-labeled human IgG1 for 2 h at 4 °C. Dimeric Ig was prepared by the chloramine-T method as described (38) and shown to compete equally with unlabeled dimeric Ig in binding to
Fc receptor expressing COS-7 cells. Cell bound 125I-dimeric IgG1 was determined following centrifugation of cells through a 3:2 (v/v) mixture of dibutyl phthalate and dioctyl phthalate oils (Fluka Chemika), and cell bound 125I-dimer was determined. Nonspecific dimer binding was determined by assaying on mock-transfected cells and subtracted from total binding to give specific dimeric IgG1 bound. Levels of cell surface FcRIII expression were determined using the anti-FcRII mAb 8.2, shown to bind distantly to the binding site (32) and used to correct for variable cell surface receptor expression between the mutant FcRII COS-7 cell transfectants. The binding of mAb 8.2 was determined in a direct binding assay as described for the human IgG1 dimer binding assays.

Generation of FcRII Domain 1-Domain 2 Model Structure—Molecular Modeling of the extracellular region of hFcRIIa (domain 1 and 2) was performed using the Homology and Discover modules of the InsightII software package of Biosym Technologies, using the crystal structure of domains 1 and 2 of CD4 (Brookhaven protein data base file pdb2cd4.ent) essentially as described previously for domain 2 (20). Sequence alignments were used to determine the location of β-sheets, with other regions defined as loops. Since the N-terminal A-strand of FcRII-D1 is longer than that of CD4–1, the Bence-Jones protein REI (pdb2rei.ent) V domain was chosen as a template for the first 7 residues after superimposition of REI on CD4–1. A search of the Brookhaven protein crystallographic data base was then carried out using the Loop Search command to find suitable loop templates for the remaining pieces (see below). In some cases, this required a reevaluation of the structurally conserved residues of β-sheets. In two cases, the A/B loop (residues 1–86) and the B/C (residues 109–116) loops were replaced with alanine. The final structure was checked for proper ψ, ϕ, and ω angles and residues of high energy. The loops used in the model are detailed as follows. Domain 1: A/B, (EDS) modeled to GDT from 2cd4 (designated loop); B/C loop (SPRED) modeled to PGTSN of 2mev, starting at residue 169 (including the previous structurally conserved region), deviation 1.25, r.m.s. deviation 2.75; C/C (NGN) modeled to D2 of 2hla, starting at residue 82, deviation 0.45, r.m.s. deviation 0.83; C/C (THTDQ) modeled to KKTKL of 1fd, starting at residue B337, deviation 0.59, r.m.s. deviation 1.76; E/F (NNNDS) modeled to FTDT of 3cla, starting at 159, deviation 0.97, r.m.s. deviation 1.26; F/G (QGTSLS) modeled to VH1GKE of 1bhp, starting at residue B55, deviation 0.49, r.m.s. deviation 1.25. For domain 2: A/B (QTNLEFQEG) modeled to NSDTHLLOGQ of 2zd4 (designated loop); B/C loop (SPRED) modeled to NEHEDE of 2rl1, starting at residue 1222, deviation 0.73, r.m.s. deviation 1.16; C/C (NGKSO) modeled to AATVNV of 2zn, starting at residue A162, deviation 0.22, r.m.s. deviation 0.76; C/E (RLDOP) modeled to 2mev, starting at residue 12, deviation 0.96, r.m.s. deviation 1.98; E/F (ANHSHS) modeled to LELQDS of 2zcd, (designated loop); F/G (NGITYTLF) modeled to AVSDHEA of 2hla, starting at residue A186, deviation 0.67, r.m.s. deviation 1.91, sequence AVSDHEA.

RESULTS

Chimeric Receptors Identify Multiple Regions of FcRII Involved in IgG Binding—In order to determine the role of domain 1 (residues 1–86) and the B/C (residues 109–116) or C/E (residues 130–135) loops of domain 2 in the binding of IgG by FcRII, chimeric receptors were generated whereby each of these regions in FcRII were replaced with the equivalent regions of the FcRI α chain. Chimeric receptor cDNAs were constructed by SOE PCR, subcloned into the eukaryotic expression vector pKc3, and transiently transfected into COS-7 cells. The binding of IgG immune complexes to the chimeric receptors was determined by both EA rosetting and the binding of dimeric IgG1. The distinction between the two assays lies in the nature of the immune complexes; EAs comprise large multivalent immune complexes capable of binding with high avidity to FcRII and were used to qualitatively assess Ig binding of the chimeric receptors, whereas dimeric IgG represent the smallest complexes able to bind FcRII with readily detectable affinity and were used in the quantitation of Ig binding.

The substitution of the FcRII domain 1 with that of the FcRI α chain produced a receptor (designated D1xD2), which as expected retained the capacity to bind the multivalent IgG-EA complexes, as did the wild-type FcRIII (Fig. 1a). However, in contrast to the wild-type receptor the D1xD2 chimeric FcR did not bind dimeric-hlgG1 at any concentration (Fig. 2). This suggests that domain 1 is necessary for optimal Ig binding as demonstrated by the binding of highly substituted but not small dimeric complexes.

The previous analysis of genetic polymorphisms of FcRIII (21–25) in conjunction with our molecular modeling studies described above (20), suggest that the region around residue 114 (human equivalent of polymorphic residue 116 in mouse FcRII) in the predicted B/C loop may be important in Ig binding. To investigate this possibility a chimeric FcR (γ109–116ε) was constructed wherein the B/C loop of FcRIII (residues Ser109, Trp110, Lys111, Asp112, Lys113, Pro114, Leu115, Val116) was replaced with the homologous region of the FcRI α chain (Glu109, Trp110, Arg111, Asn112, Trp113, Asp114, Val115, Tyr116). After transfection into COS-7 cells, this receptor was clearly able to bind Igε in the form of multivalent immune complexes, i.e. erythocyte highly sensitized with IgG (IgG-EA) (Fig. 1b). By contrast, this receptor was unable to bind dimeric hlgG1 at any concentration, implying that the B/C loop is essential for optimal Ig binding (Fig. 2). Similarly, the region surrounding residue 131 responsible for the responder/nonresponder phenotype of FcRIIIa, i.e. the C/E loop (Ser130, Arg131, Leu132, Asp133, Pro134, Thr135) was replaced with the equivalent FcRI α chain sequence (Trp130, Tyr131, Glu132, Asn133, His134, Asn135), generating a chimeric receptor (γ130–135ε) that upon transfection into COS-7 cells was able to bind IgG-EA (Fig. 1c) but not dimeric IgG1 (Fig. 2). As expected COS-7 cells transfected with an expressible form of the FcRI α chain (18) did not bind hlgG1 dimers or IgG-EA (Figs. 1d and 2). Thus the ability of the chimeric FcRIII containing B/C or C/E domain 2 substitutions to bind the highly sensitized EA complexes but not dimeric hlgG1 suggests that these receptors bind IgG less avidly than wild-type FcRII and clearly indicates that the B/C and C/E regions also make a contribution to the binding of Igε by FcRIII.

Fine Structure Analysis of the B/C and C/E loops of FcRIII Domain 2—The contribution of individual amino acids of the B/C and C/E loop regions of FcRIII to the binding of IgG was determined using a point mutagenesis strategy whereby residues in both the B/C (residues 113–117) and C/E (residues 129–134) loops were replaced with alanine. cDNAs encoding the mutant receptors were generated using SOE PCR and subcloned into the eukaryotic expression vector pKc3. The resultant expression constructs were transiently transfected into COS-7 cells, and the Ig binding capacity of the mutant receptors was determined by assessing the binding of dimeric hlgG1. The levels of cell membrane expression of the mutant FcRIII on the COS-7 cell transfectants were determined using the anti-FcRIII mAb B.2 (shown to detect an epitope distant from the binding site) and were comparable with the expression levels of the wild-type receptor (see Fig. 3 legend). The relative capacities of the mutant receptors to bind hlgG1 were determined using the direct binding assay following correction for variation in cell surface expression levels and expressed as a percentage of wild-type FcRIII binding.

The replacement of the B/C loop residues (Lys113, Pro114, Leu115, Val116) in turn with Ala in each case resulted in diminished hlgG1 dimer binding (Fig. 3). The most dramatic effect was seen on substitution of Lys113 or Leu115, which exhibited only 15.9 ± 3.4% (mean ± S.D.) and 20.6 ± 4.0% binding compared with wild-type FcRIII. The replacement of Pro114 or Val116 with Ala had a lesser effect, these receptors displaying 53.5 ± 13.5% and 73.5 ± 7.9% wild-type binding respectively. It is interesting to note that the individual replacement of these
amino acids did not result in the complete abolition of dimer binding seen in chimera 109–116. These results suggest that each of these residues in the B/C loop contribute to the binding of IgG by FcRRII either as direct contact residues or indirectly by maintaining the correct conformation of the binding site. The same approach was used to analyze the role of individual amino acids within the C/E loop (Phe129, Ser130, Arg/His131, Leu132, Asp133, Pro134). In contrast to that observed for residues of the B/C loop, mutation of individual residues of the C/E loop resulted in both loss and enhancement of IgG binding. Substitution of Phe129 and Arg/His131 dramatically decreased hIgG1 dimer binding by approximately 90 and 80%, respectively, to 8.2 ± 4.4 and 21.9 ± 3.9 compared with that seen for wild-type FcRRII (Fig. 3). Interestingly, replacement of residues Asp133 and Pro134 increased binding to 113.5 ± 8.8% and 133.5 ± 3.2% of the wild-type receptor. The substitution of Ser130 or Leu132 had no significant effect on the binding of hIgG1 dimers, since these mutants exhibited binding comparable with that seen for wild-type FcRRII (Fig. 3). These findings suggest that Phe129 and Arg/His131 may play an important role in the binding of hIgG1, and the observation that the substitution of Asp133 and Pro134 increase binding also suggests an important role for these residues, which appears distinct from Phe129 and Arg/His131. Again, a distinction between a possible direct binding role or contribution to structural integrity of the receptor cannot be made; however, these findings clearly identify both the B/C and C/E loops as playing a role in the binding of IgG by FcRRII.

Site-directed mutagenesis was also performed on 3 residues of the C/E loop, a region predicted to be distant from the putative binding region, i.e. the B/C, C/E, and F/G loop regions. The substitution of residues Asn123, Gly124, and Lys125 had no effect on the binding of hIgG1 dimer, since each of these mutants exhibited similar binding to the wild-type receptor (data not shown).

Molecular Modeling of FcRRII Extracellular Region—Molec-
Multiple Binding Sites of FcγRII

**DISCUSSION**

The studies described herein provide evidence to suggest that the interaction of IgG with human FcγRII involves multiple regions juxtaposed to the receptor. Previously, we have described the localization of a single region of FcγRII capable of directing binding to IgG situated in the second extracellular domain between residues Asn^{154} and Ser^{161} (20). Of the entire extracellular region, only the 154–161 segment was demonstrated to directly bind IgG, since placement of only this region in the corresponding region of the human FcRI α chain imparted IgG binding function to the IgE receptor FceRI. Moreover, replacement of this region in FcγRII with that of FceRI resulted in the total loss of IgG binding including large complexes, implying that residues Asn^{154}–Ser^{161} comprise the key IgG interactive site of FcγRII. However, the generation of further chimeric FcγRII/FceRI receptors as described herein indicates that two additional regions of FcγRII domain 2 also influence the binding of IgG by FcγRII. The replacement of the regions encompassing Ser^{109}, Val^{116} (B/C loop) and Ser^{130}, Thr^{139} (C/E loop) of FcγRII with the equivalent regions of the FcRI α chain, produced receptors that, despite containing the putative binding site (Asn^{154}–Ser^{161}) and retaining the ability to bind large complexes (IgG-EA), lost the capacity to bind small complexes (dimeric hIgG1). Indeed, site-directed mutagenesis performed on residues of the B/C and C/E regions identified a number of amino acids that appear to play crucial roles in hIgG1 binding by FcγRII. The replacement of Lys^{113}, Pro^{114}, Leu^{115}, and Val^{116} of the B/C loop and Phe^{129} and Arg/His^{131} of the C/E loop with alanine all resulted in diminished hIgG1 binding. Furthermore, the substitution of Asp^{123} and Pro^{134} of the C/E loop increased hIgG1 binding. Therefore, these findings provide strong evidence to suggest that the B/C and C/E loops of FcγRII, in addition to the F/G loop, also contribute to the binding of IgG.

A number of other studies have provided evidence to support the proposed IgG binding roles of the B/C and C/E loop regions of FcγRII. Studies of genetic polymorphisms of mouse and human FcγRII have implicated residues 114, 131, and 159 in the binding of IgG by human FcγRII. These residues are located in the B/C (residue 114), C/E (131), and F/G (159) loops, respectively. The Ly-17 polymorphism of mouse FcγRII with IgG (24, 25). Furthermore, the high responder/low responder polymorphism of hFcγRII a results in an amino acid substitution at residue 131, which has been shown to influence the binding of mIgG1 and hIgG2 (21–23). The findings described herein also indicate that the nature of the residue at 131 plays a role in the binding of hIgG1, since replacement with alanine results in almost complete loss in bind-
ing of this isotype to FcRII. Thus, although the F/G loop of FcRII is clearly a major region involved in the direct interaction with IgG, as demonstrated by the fact that only this region has been definitively shown to directly bind IgG (20), residue 131 also appears to play a binding role. However, the question of whether residue 131 is directly participating in IgG binding or providing a secondary or indirect influence remains to be answered.

The mutagenesis data clearly implicate a number of distinct regions within FcRII in the interaction with IgG complexes as described above. The spatial relationship of these regions, i.e. residues 109–116 (B/C loop), 129–135 (C/E loop), and 154–161 (F/G loop) is postulated in our model of FcRII (Fig. 4). This model suggests that these regions are juxtaposed to each other in domain 2 at the interface with domain 1 and form a hydrophobic pocket surrounded by a wall of additional residues. The side chains of amino acids implicated in IgG1 binding as described under “Results” are indicated. Those side chains that when substituted result in decreased or increased binding are shown in pale yellow or red, respectively. The bright yellow regions represent the A/B and G strands of domain 1, predicted to be in close proximity to the domain 2 active binding region. B, location of residues putatively involved in the interaction of FcRII with IgG1. Domain 2 and the domain 1 interface region of the FcRII domain 1-domain 2 model is shown to highlight the putative binding region. Residues implicated in IgG1 binding are indicated as described above. The computer model of FcRII domain 1-domain 2 was generated by molecular modeling based on the structure of the related CD4 domains 1 and 2 as described under “Materials and Methods.”
therefore interact with the “active” binding region of domain 2. Further support for the involvement of the B/C and C/E loops of FcRII domain 2 in the binding of IgG has been provided in the cloning and subsequent Ig binding studies of rat FcRIII (40), which is structurally and functionally homologous to FcRII. Two rat FcRIII isofoms, IIA and IIB, which have extensive amino acid differences in their second extracellular domains, have been shown to bind rat and mouse IgG subclasses differently. Both isofoms bind rtIgG1, rtIgG2a, and mlgG1, however, they differ in that only the IIB form binds rtIgG2b and mlgG2b. Significantly, the amino acid differences between rat FcRIIIA and IIIB isofoms are situated predominantly in the predicted B/C and C/E loops of domain 2. However, it should be noted that the F/G loop regions of rat FcRIIIA and IIIB are almost totally conserved, which together with the observation that both forms bind rtIgG1, rtIgG2a, and mlgG1, is consistent with the proposal that the F/G loop region is the major IgG interactive region and that the B/C and C/E loop regions provide supporting binding roles. In addition, a recent mutagenesis study of human FcRIII has also implicated residues in the B/C and C/E loops of this receptor in the binding of IgG (41). It is also interesting to note that in this study the C/C’ region of FcRIII was suggested to play a major role in IgG binding, which is in marked contrast to our findings with FcRII. Indeed, the substitution of 3 residues in the C/C’ loop of FcRII with alanine, namely Asn123, Gly124, and Lys125, did not have any effect on the binding of dimeric IgG1. Therefore, these findings somewhat surprisingly suggest that FcRII and FcRIII, which exhibit substantial amino acid sequence conservation and similar IgG binding affinities and specificities, may interact differently with IgG.

It is interesting to note that a number of parallels are apparent in the molecular basis of the interaction of FcRII with IgG and that of FcRI with IgE. The Ig binding roles of the two extracellular domains of FcRI are similar to FcRII, with domain 2 responsible for the direct binding of IgE and domain 1 playing a supporting structural role (18, 26, 42). Furthermore, as described for FcRIII, we and others have also identified multiple IgE binding regions in domain 2 of FcRI. Using chimeric FcRI/FcRII receptors we have demonstrated that domain 2 of FcRII contains at least three regions, each capable of directly binding IgE, since the introduction of the FcRII regions encompassed by residues Trp87-Lys128, Tyr129-Asp145, and Lys154-Glu161, into the corresponding regions of FcRII was found to impart IgE binding to FcRII (1, 18, 20). A similar study using chimeric FcRII/FcRII receptors has implicated 4 regions of FcRII domain 2 in IgE binding since the regions Ser99-Phe104, Arg111-Glu125, Asp123-Ser137, and Lys154-Ile167 of FcRII when replaced with the corresponding regions of FcRIII resulted in the loss or reduction of IgE binding (42). Taken together, these data suggest that at least four regions of FcRII domain 2 contribute to the binding of IgE, Ser99-Phe104, Arg111-Glu125, Tyr129-Ser137, and Lys154-Glu161. Three of these regions correspond to the three regions identified herein as important in the binding of IgG by FcRII, Arg111-Glu125, Tyr129-Ser137, and Lys154-Glu161, which encompass the B/C, C/E, and F/G loops, respectively. In addition, studies with anti-FcCI a chain mAb have indicated that the region encompassed by residues 100–115 contains an epitope detected by mAb 15A5, which can completely block the binding of IgE to FcRI (43). Thus, these findings implicate the B/C, C/E, and F/G loops juxtaposed in domain 2 at the domain 1 interface as the crucial IgE-interactive region of FcRII. Clearly, the findings described herein for FcRIII together with those discussed for FcRII provide evidence to suggest that the Ig-binding regions of FcRII and FcRI are conserved between the two receptors, with the domain 1–domain 2 interface forming the Ig binding site.

In conclusion, the results presented herein demonstrate that multiple regions of FcRIII are involved in the binding of IgE, with three putative loop regions juxtaposed in the second extracellular domain at the domain 1 interface comprising the IgE binding site. The proposition that the functionally distinct receptor FcRII also interacts with IgE in a structurally similar fashion, in conjunction with the conserved nature of the extracellular regions of the Ig superfamily FcR, strongly suggests that this region will also comprise the key IgE-interactive site of all members of this family.

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