

The Binding Site on Human Immunoglobulin E for Its High Affinity Receptor*

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Immunoglobulin (Ig) E antibodies mediate allergic responses by binding to specific high affinity receptors, FcεRI, on mast cells and basophils. Previous studies have shown that the principal FcεRI binding site is located on the third constant domain, Fcε3, of IgE. Based on a model of the IgE Fcε3 (which is homologous to the second constant domain of IgG), homology scanning mutagenesis and replacement of individual residues were used to determine the specific amino acids of human IgE involved in binding to human FcεRI. The amino acids are localized in three loops, which form a putative ridge on the most exposed side of the Fcε3 domain of IgE and include Arg-408, Ser-411, Lys-415, Glu-452, Arg-465, and Met-469. The preponderance of charged residues suggests that IgE-FcεRI binding is mediated primarily by electrostatic interaction. Furthermore, it is possible to confer FcεRI binding to an IgG molecule by introducing these three IgE loops into the IgG Cγ2 domain.

Immunoglobulin (Ig)¹ E antibodies bind to specific high affinity receptors, FcεRI, on mast cells, basophils, and Langerhans cells (1–4) via their constant, or Fc, region in a one-to-one complex (5). Degranulation of mast cells and basophils triggered by binding of antigen to IgE-loaded FcεRI leads to release of mediators which produce symptoms associated with allergy (6, 7). IgE also binds to low affinity receptors (FcεRII, CD23) on B-lymphocytes and eosinophils (8, 9). A more in-depth review of IgE and its receptors can be gleaned from Ref. 10. Because of the central role of cell-bound IgE in initiation of the allergic cascade, much attention has been focused on localizing the sites on IgE required for receptor binding. Over the last decade a variety of techniques have been used to determine the FcεRI binding site on IgE, including synthetic peptides corresponding to portions of the IgE Fc (11–14), anti-IgE antibodies (15–17), IgE variants (18), chimeras (19–22), and truncated forms (23). However, the exact IgE residues involved in binding have remained undefined. Helm *et al.* (13, 14) reported mapping the FcεRI binding site on human IgE to a sequence of 76 amino acids at the junction of the second (Fcε2) and third (Fcε3) constant domains. In contrast, a recent study reported that only

the Fcε3 domain of murine IgE is involved in binding to rat FcεRI (20), and this domain has also been implicated as important in binding of murine IgE to murine FcεRI and FcεRII (22). In this study, we have now determined the specific amino acid residues involved in human IgE binding to human FcεRI. All of the critical residues are contained in three loops, which, together, form a ridge on the most exposed portion of human IgE Fcε3.

The Fc portion of IgE includes three domains: Fcε2, Fcε3, and Fcε4. The Fcε3 domain has been predicted to be structurally homologous to the IgG Fcγ2 domain (24, 25). Hence, it comprises seven β-strands (denoted A–G) and six connecting loops (denoted by the two β-strands they connect, *e.g.* loop AB), which form a β-sandwich structure representative of all immunoglobulin domains (Fig. 1). Using homology scan methodology (26), the initial variants of human IgE were designed by taking advantage of the fact that human IgG1 does not bind to IgE receptors (19). These included individually replacing five Fcε3 loops with their counterparts from human IgG1 Fcγ2 (Table I). The sixth loop (DE), which contains a conserved, structurally important glycosylation site in both IgE and IgG, was not altered. Additional variants were evaluated in which solvent-exposed residues in Fcε3 β-strands and selected loops in Fcε2 and Fcε4 were changed to alanine, as opposed to IgG1 homology replacements, in order to maximize the effect of altering only 2–3 side chains per variant (Table I). Based on the results from these, a second set of variants assessed individual residues within the regions found to affect the FcεRI-IgE interaction. Residues were changed to alanine or to other amino acids in order to determine if size, charge, hydrogen-bonding capability, or hydrophobicity were important for that particular residue.

EXPERIMENTAL PROCEDURES

Phagemid Constructs—The human IgE gene was isolated from a U266 cell line (27) library prepared at Genentech. Polymerase chain reaction (28) was used to amplify the IgE gene using primers based on previous studies (29, 30). The DNA products were isolated, digested with appropriate restriction enzymes, and purified on acrylamide gels. The gene was then cloned into a previously described phagemid vector (31), replacing the human IgG1 heavy chain with the human IgE heavy chain using the *Bst*EII and *Hind*III sites. This phagemid contains the human cytomegalovirus enhancer and promoter, a 5' intron, and a SV40 polyadenylation signal (32). The sequence of this human IgE heavy chain matched sequences of human IgE reported previously (29, 30).

Mutagenesis was performed by the Kunkel method (33) using buffers and enzymes supplied with a commercially available *in vitro* mutagenesis kit (Bio-Rad). Sequences of the variant IgE DNAs were checked using dideoxynucleotide sequencing.

The phagemid encoding the chimeric protein IgG2/E3 was constructed using a previously described phagemid vector containing the human cytomegalovirus enhancer and promoter, a 5' intron, and SV40 polyadenylation signal (32) encoding a humanized anti-p185^{HER2} IgG1 (31). The nucleotide sequence encoding human IgG1 residues Cys-239–Gly-361 was replaced with DNA encoding IgE Fcε3 residues Cys-357–

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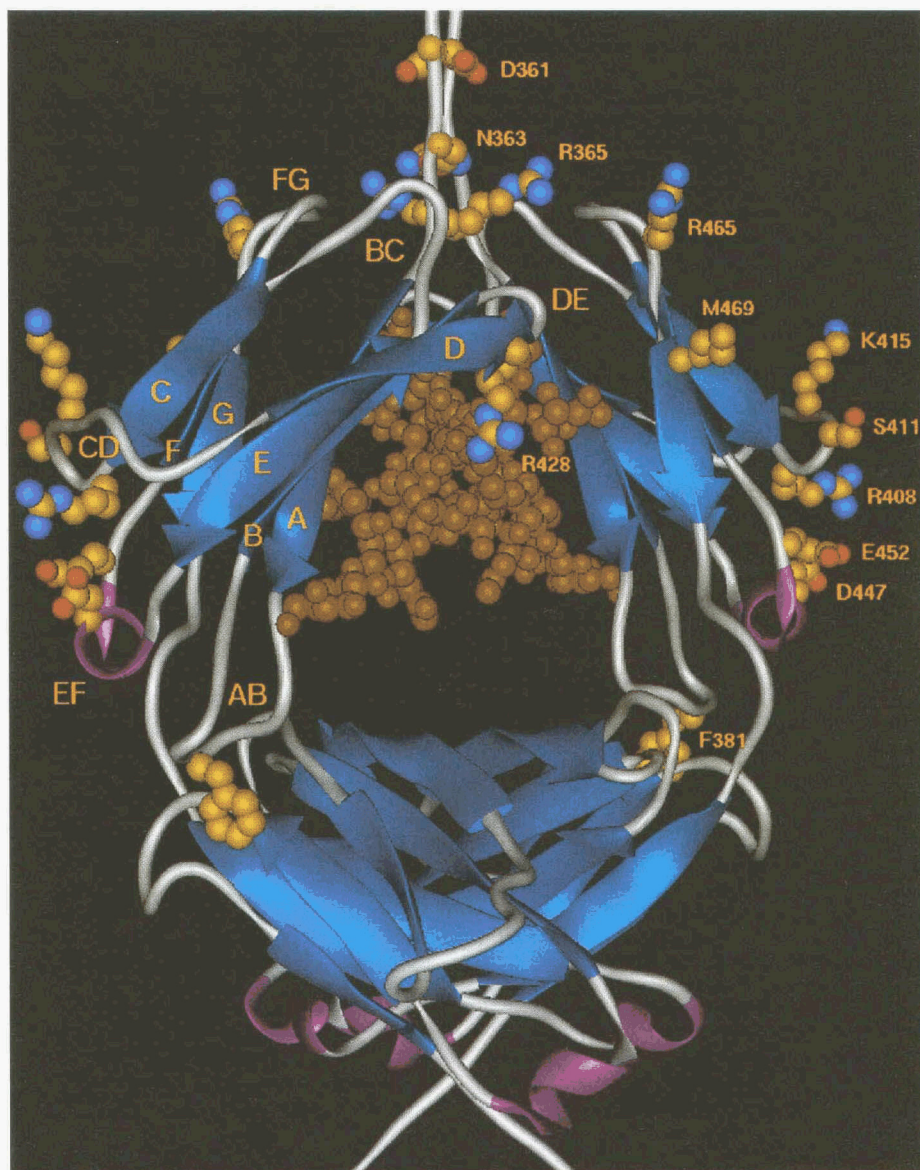
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¹ The abbreviations used are: Ig, immunoglobulin; FcεRI, high affinity IgE receptor; Fc, constant domains of immunoglobulin; ELISA, enzyme-linked immunosorbent assay; CHO, Chinese hamster ovary; FITC, fluorescein isothiocyanate.

FIG. 1. Ribbon diagram of IgE Fc ϵ 3 and Fc ϵ 4. Fc ϵ 3 β -strands A–G, connecting loops, and residues important in IgE binding to Fc ϵ RI are labeled. Coordinates are from the Protein Data Bank (48) model described by Padlan *et al.* (25). The two Fc ϵ 2 domains (not shown) would be at the top of the figure; the two Fc ϵ 4 domains are at the bottom. β -Strands are colored blue, and α -helices are colored purple. Side chains involved in binding to Fc ϵ RI are shown with side chain carbon, nitrogen, and oxygen atoms colored yellow, blue, and red, respectively. Carbohydrate attached at Asn-430 is shown as brown spheres.



Gly-497.² The portion of the phagemid coding for IgG2/E3 was completely sequenced using dideoxynucleotide sequencing. The humanized anti-p185^{HER2} IgG1 phagemid was also used to construct the IgGEL phagemid. Using site-directed Kunkel mutagenesis (33), loop CD (IgE residues 407–420), loop EF (IgE residues 445–453), loop FG (IgE residues 465–469), and Fc ϵ 2–Fc ϵ 3 hinge residues 357–365 were substituted for their homologues in IgG1 (IgG1 residues 291–305, 329–337, 349–352, and 239–249, respectively). The portion of the phagemid coding for IgGEL was completely sequenced using dideoxynucleotide sequencing.

Protein Expression—For each IgE variant, double-stranded phagemid DNA was isolated from *Escherichia coli* strain XL-1 Blue (35) with the Qiagen DNA purification kit (Qiagen Inc., Chatsworth, CA). This DNA was used to transfect the fetal human kidney cell line 293S (36) by a calcium phosphate method (32). The 75% confluent cells were transfected with 10 μ g of phagemid DNA/15-mm cell culture dish and incubated for 15 h in serum-containing medium. The medium was removed and replaced with serum-free PSO4 medium supplemented with 10 mg/liter recombinant bovine insulin, 1 mg/liter transferrin, and trace elements. The supernatant was collected at 48 and 96 h and concentrated approximately 20-fold using a Centrprep 30 unit (Amicon). IgE variants were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to ascertain correct molecular weight and were quantified by ELISA using purified human IgE as the reference standard. A murine anti-human IgE antibody (MaE1, Genentech) was used to capture IgE in the ELISA. IgE was detected with an horseradish peroxi-

dase goat anti-human IgE (Kirkegaard & Perry). IgE concentrations were extrapolated by non-parametric linear regression analysis. Initially, IgE variants were affinity-purified on a murine anti-human IgE antibody (MaE2, Genentech) coupled to CNBr-Sepharose (Pharmacia Biotech Inc.). Since assays using either purified or non-purified IgE gave identical results, the purification step was eliminated for other variants.

In order to assure that all variants were properly folded, each variant was assayed for binding to murine anti-human IgE antibody MaE1, which binds outside domain Fc ϵ 3, and to three murine anti-human IgE antibodies that have been mapped to the Fc ϵ 3 domain: MaE11 (37), MaE13, and MaE17 (Genentech). The binding of all four MaE antibodies to heat-denatured IgE (56 °C for 90 min; Ref. 15) is reduced: 10-fold for MaE1 and at least 100-fold for MaE11, MaE13, and MaE17. All IgE variants bound as well as native IgE to MaE1 and to at least one of MaE11, MaE13, or MaE17 (data not shown).

Binding Assay—Binding of human IgE variants was measured by immunofluorescence using a CHO 3D10 cell line expressing the human Fc ϵ RI receptor α -chain (38). Although the Fc ϵ RI receptor comprises one α -chain, one β -chain, and two γ -chains, a previous study has shown that the α -chain is sufficient for IgE binding (38). 5×10^5 cells in phosphate-buffered saline containing 0.1% bovine serum albumin, 10 mM sodium azide were incubated for 30 min. at 4 °C with 0.1–10 μ g/ml IgE or IgG1 control (humanized anti-p185^{HER2} IgG1; Ref. 31). After washing, the cells were incubated for 30 min. at 4 °C with 20 μ g/ml affinity-purified FITC-conjugated (39) rabbit anti-human IgE (Axell) or goat FITC-F(ab')₂ anti-human IgG (Organon Teknica), respectively. Analyses were

² Residues are numbered according to Ref. 34.

TABLE I
Binding of human IgE variants to human FcεRI

Values are percent binding at 1 μg/ml compared to native human IgE at 1 μg/ml to human FcεRI α-chain expressed on CHO 3D10 cells (38). Homology scan sequences are in italics.

Variant	Kabat residue no.	Human IgE sequence	Variant sequence	Binding to FcεRI α-chain on CHO 3D10 cells		
				Mean	S.D.	n
Fce3 loop AB						
1	377-385	FDLFIRKS	KDTLMISRT	3	1	5
2	377-385	FDLFIRKS	KDLMISRT	8	1	10
3	377	F	A	100		1
4	381	F	A	10	2	7
5	381	F	M	28	8	7
6	382	I	A	91	20	3
7	383-385	RKS	AAA	104	6	5
Fce3 loop BC						
8	396-401	APSKGT	SPEDGQ	77	6	9
9	398-401	SKGT	AAGA	98	10	7
Fce3 loop CD						
10	407-420	SRASGKPVNHS	YVDGVQVHNAK	35	15	8
11	407	S	A	106		1
12	408	R	A	84	7	12
13	408	R	E	12	4	6
14	408	R	Q	50	11	9
15	408	R	K	92	15	6
16	411	S	A	69	1	4
17	411	S	Q	28	12	14
19	415	K	A	118		1
20	415	K	D	66	3	8
21	418	N	A	97	9	5
22	419	H	A	98	4	5
23	420	S	A	97	8	4
Fce3 loop EF						
24	445-453	TRDWIEGET	HQDWLDGKE	11	6	6
25	445	T	A	99	17	10
26	446	R	A	95	7	9
27	446	R	E	74	14	4
28	447	D	A	99	6	21
29	447	D	R	41	21	7
30	447	D	N	95	8	3
31	447	D	E	92	9	5
32	450-453	EGET	AGAA	19	7	8
33	450	E	A	96	8	11
34	450	E	R	104	10	4
35	452	E	A	60	26	19
36	452	E	R	14	7	14
37	452	E	Q	13	3	13
38	452	E	D	103	5	10
39	453	T	R	100	3	5
40	453	T	Y	62	3	5
Fce3 loop FG						
41	465, 469	RALM	AALF	43	7	9
42	465	R	E	27	9	14
43	469	M	A	73	8	9
Fce3 β-strand B						
44	387, 389	TIT	AIA	83	7	5
45	387	T	A	120		2
46	389	T	A	138		1
Fce3 β-strand C						
47	403, 405	NLT	ALA	105	11	5
Fce3 β-strand D						
48	423-428	KEEKQR	PREQQY	2	1	10
49	423-428	KEEKQR	AEAKAR	95	10	5
50	423-428	KEEKQR	KAEAQA	41	17	7
51	422	R	A	99	11	7
52	423-425	KEE	AAA	100	2	8
53	423	K	P	2	1	8
54	426-428	KQR	AAA	60	12	10
55	426	K	A	76	13	5
56	427	Q	A	92	12	7
57	428	R	A	42	13	7
Fce3 β-strand E						
58	438, 440	TST	ASA	84	19	11

performed on a FACScan flow cytometer (Becton-Dickinson) using FAC-Scan LYSIS software. Percent positive cells were calculated using standard one-parameter analysis measuring relative fluorescence intensity compared to the IgG1 control. From the titration curve (0.1–10 μg/ml), binding of variant IgE was determined as the percent of binding relative to human IgE at 1 μg/ml, this concentration being on the steep portion

of the titration curve above the EC₅₀ (Fig. 2). The assay conditions used were not equilibrium conditions, and the short incubation times and wash steps may not have affected all variants in the same way. Hence the kinetics of binding for each variant may have determined the final level of binding observed.

The chimeric proteins IgG2/E3 and IgGEL were quantified by ELISA

TABLE I—continued

Variant	Kabat residue no.	Human IgE sequence	Variant sequence	Binding to FcεRI α-chain on CHO 3D10 cells		
				Mean	S.D.	n
Fcε3 β-strand F						
59	455–459	QCRVT	ACAVA	96	9	15
60	460	H	D	77	9	8
61	462	H	D	90	2	4
Fcε3 β-strand G						
62	471, 473	STT	ATA	107	7	4
Fcε2						
63	329–336	QKHWLSDR	AAAWLAAA	107	8	4
64	361–365	DSNPR	ASAPA	67	19	14
Fcε4						
65	498–501	PRAA	QPRE	89	12	4
66	594–599	ASPSQT	LHNHY	92	1	4
67	595–599	SPSQT	APAAA	80	11	5

using a goat anti-human IgG F(ab) (Organon Teknica) to capture and an horseradish peroxidase-conjugated goat anti-human IgG F(ab) to detect. Humanized anti-p185^{HER2} F(ab) (31) was used as the standard. For IgG2/E3 and IgGEL, FITC-F(ab)₂ goat anti-human IgG1 (Organon Teknica) was used in the immunofluorescence assay.

RESULTS

The homology scan suggested that IgE loops AB, CD, EF, and FG might contribute to binding (variants 1, 8, 10, 24, and 41; Table I). Among the β-strands, only changes in β-strand D significantly reduced binding (variant 48; Table I). Individual residue replacements in loop AB and β-strand D showed that in both cases only a single amino acid reduced binding. In loop AB, only substitutions at Phe-381 had an effect (variants 4 and 5; Table I). Since the crystal structure of IgE is unknown, we cannot discern whether the effect seen for Phe-381 is conformational (Phe buried) or if indeed this side chain is solvent-exposed and interacts with FcεRI. In β-strand D, the single amino acid change Lys-423 → Pro³ abolished binding to the same extent as did replacement of the entire loop (variants 48 and 53; Table I). However, the Lys side chain is not involved in binding since replacing the Lys-423 residue with Ala had no effect on binding (variant 52; Table I). Since Pro is accommodated at position 423 in IgG1 (40) but not in IgE implies that β-strand D in IgE may have a different conformation than in IgG. Another single amino acid change in β-strand D, Arg-428 → Ala, also reduced binding. Arg-428 might be distant in space from the other important residues (Fig. 1) and also precedes the conserved glycosylation site at Asn-430, suggesting that Arg-428 may play a structural role, although direct interaction with FcεRI cannot be ruled out.

In loop CD, Arg-408 is the most important binding residue. Arg-408 → Ala and Arg-408 → Lys had little effect on binding, but Arg-408 → Glu and Arg-408 → Gln reduced binding by 88% and 50%, respectively. While the latter two variants show that a positive charge at this position is important, the Arg-408 → Ala variant is difficult to interpret. While it is conceivable that the Glu and Gln substitutions might cause a local structural perturbation, the binding of the Arg-408 → Ala variant is only slightly reduced relative to IgE and this argues against any local conformational perturbation when Arg-408 is substituted with the other amino acids. In addition, Glu and Gln side chains are smaller than the Arg side chain and would not be likely to prevent IgE binding to FcεRI via steric clash. Replacements at 2 other residues in loop CD also reduced binding. Lys-415 → Asp reduced binding by 34%, much less than for the charge exchange at Arg-408, and at Ser-411, Ala and Gln sub-

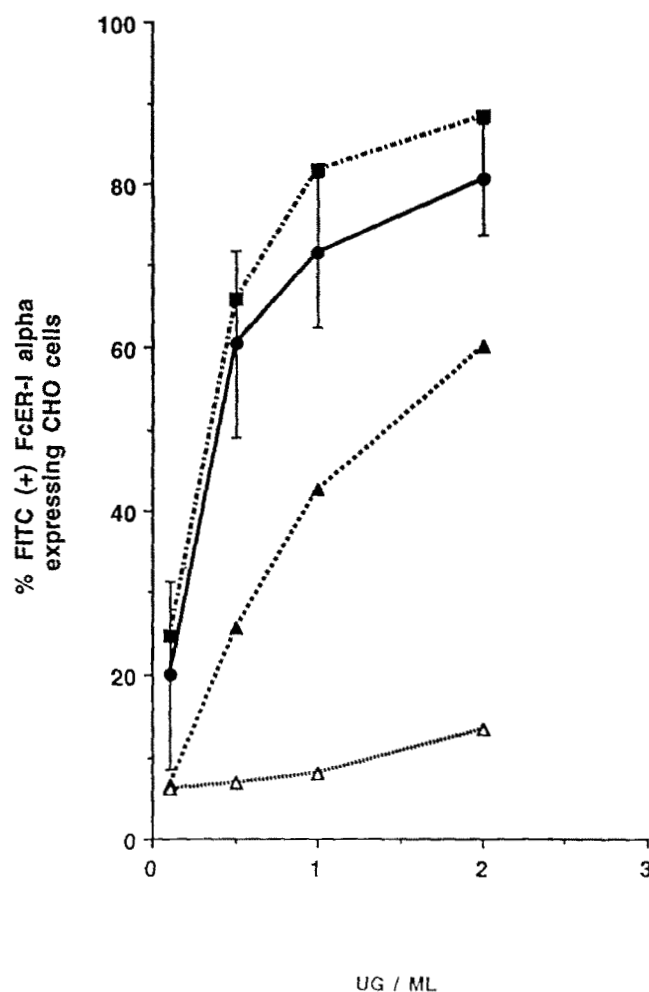


FIG. 2. Binding of human IgE and selected IgE variants to human FcεRI expressed on CHO 3D10 cells. Human IgE (●), variant 37 Glu-452 → Gln (△), variant 38 Glu-452 → Asp (■), and variant 64 Asp-361 → Ala/Asn-363 → Ala/Arg-365 → Ala (▲).

stitutions diminished binding by 31% and 72%, respectively. In murine and rat IgE residue 411 is Lys and Arg, respectively, and both rodent IgEs can bind to human FcεRI (41), providing circumstantial evidence that Ser-411 → Gln did not sterically hinder the human IgE-human FcεRI interaction or perturb the conformation of loop CD.

Loop EF is the only loop for which the conformation is reliably discernible. The crystal structure of IgG domains Cγ2 and Cγ3 (40) shows that in both domains loop EF forms an α-helix

³ The nomenclature for IgE variants is as follows: native amino acid, residue number, substituted amino acid, e.g. Asp-447 → Arg denotes substitution of Arg for Asp at residue 447.

in which a buried Trp anchors the helix and a Gly terminates the helix. In human, murine, and rat IgE, loop EF retains these characteristic amino acids and hence is also likely to be an α -helix. At Asp-447, removal of the side chain (Asp-447 $\rightarrow$ Ala), repositioning the negative charge (Asp-447 $\rightarrow$ Glu), or neutralization of the charge (Asp-447 $\rightarrow$ Asn) had no effect, but charge reversal (Asp-447 $\rightarrow$ Arg) reduced binding by 59%. Human IgG domain C γ 3 contains an Arg at this position and its presence does not disturb the α -helix (40); hence, the Asp-447 $\rightarrow$ Arg variant is not likely to be a structural perturbation. This suggests that the reduced binding of the Asp-447 $\rightarrow$ Arg variant may be due to steric clash between the IgE and Fc ϵ RI. In contrast to residue 447, removal of the side chain at residue 452 (Glu-452 $\rightarrow$ Ala) reduced binding and charge neutralization (Glu-452 $\rightarrow$ Gln) was as deleterious as charge reversal (Glu-452 $\rightarrow$ Arg), suggesting that Glu-452 is involved in an important salt bridge with Fc ϵ RI. Repositioning the negative charge (Glu-452 $\rightarrow$ Asp) had no effect. One other residue in loop EF, at position 453, might be important since Thr-453 $\rightarrow$ Arg had no effect while Thr-453 $\rightarrow$ Tyr reduced binding. This could result from the Arg side chain being able to move out of the way during Fc ϵ RI-IgE binding while the bulkier, less mobile Tyr side chain cannot, thereby sterically hindering the Fc ϵ RI-IgE interaction. The importance of loop EF in rodent systems is uncertain (11, 17).

Two other regions are also involved in IgE binding. In loop FG, the charge reversal Arg-465 $\rightarrow$ Glu significantly reduced binding by 73% while Met-469 $\rightarrow$ Ala reduced binding only 27%, suggesting that Arg-465 plays the dominant role in loop FG. In the hinge region between domains Fc2 and Fc3, 3 residues (at positions 361, 363, and 365) were simultaneously changed to Ala (variant 64; Table I) and binding to Fc ϵ RI was reduced by 33%.

Of the variants that did not alter binding to Fc ϵ RI, a few deserve comment. In addition to the glycosylation site at Asn-430, which corresponds to that in IgG Fc γ 2, human IgE Fc3 contains another glycosylation site at Asn-403 (42). Variant 47 converted Asn-403 to Ala, and the loss of carbohydrate at this position did not alter binding to Fc ϵ RI. One loop in domain Fc2 (variant 63; Table I) and two loops in domain Fc4 (variants 65–67; Table I) were evaluated. These three loops should be adjacent to domain Fc3 according to the published model of IgE (24, 25). In addition, the Fc2 loop was the N-terminal section of a peptide previously reported to bind to Fc ϵ RI (13). However, none of the three variants reduced binding to Fc ϵ RI, suggesting that domains Fc2 and Fc4 may not be involved in IgE binding to Fc ϵ RI, in agreement with previously published results on murine/human IgE and murine IgE/human IgG chimeras (19–21).

To verify that the residues determined in this study are indeed important for binding of IgE to Fc ϵ RI, we constructed two chimeric molecules. In the first, IgG2/E3, the entire IgE Fc3 domain was substituted for the homologous Fc γ 2 domain in an IgG antibody and the Fc2-Fc3 hinge region (including Cys-357, which forms the inter-heavy chain disulfide) replaced the IgG hinge. This human IgG/human IgE chimera is similar, but not identical, to human IgG/murine IgE and murine IgE/human IgE chimeras investigated by other groups (19, 20). In the second chimera, IgGEL, only the IgE loops critical for Fc ϵ RI binding, *i.e.* loops CD, EF, and FG, replaced their homologous loops in an IgG antibody and again the Fc2-Fc3 hinge region replaced the IgG hinge. Although the hinge region (variant 64) contributed minimally to IgE binding, it was included so that the IgGEL chimera would have maximal likelihood of binding to Fc ϵ RI. Fig. 3 shows that IgG2/E3, which contains the entire Fc3 domain, binds only about 4-fold less well (EC_{50} = 1.8 μ g/

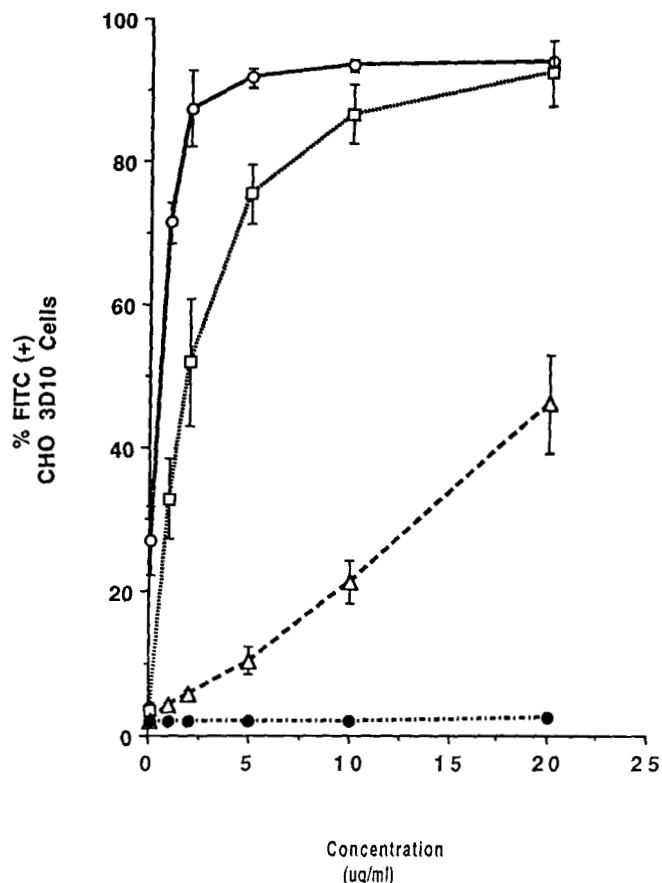


FIG. 3. Binding of human IgE, IgG, and chimeric molecules IgG2/E3 and IgGEL to human Fc ϵ RI expressed on CHO 3D10 cells. $\circ$, human IgE; $\bullet$, human IgG; $\square$, IgG2/E3; $\triangle$, IgGEL.

ml) than native human IgE (EC_{50} = 0.4 μ g/ml). Furthermore, although binding of IgGEL is considerably reduced compared to native IgE, its binding to Fc ϵ RI is significantly better than for the parent IgG, which does not bind at all (Fig. 3).

DISCUSSION

The binding site on human IgE for its high affinity receptor, Fc ϵ RI, is localized in three loops, CD, EF, and FG, which connect β -strands (Fig. 1). According to the model of IgE, these loops form a ridge on the most exposed side of the Fc3 domain of IgE and include Arg-408, Ser-411, Lys-415, Glu-452, Arg-465, and Met-469. The preponderance of charged residues suggests that IgE-Fc ϵ RI binding is mediated primarily by electrostatic interaction. This is supported by the fact that an anti-IgE antibody that blocks binding of human IgE to human Fc ϵ RI utilizes charged residues in its binding to IgE (37).

In addition to the three loops, one other region reduced binding, albeit minimally. Variant 64 consists of simultaneous alteration to alanine of 3 residues (Asp-361, Asn-363, and Arg-365) in the hinge connecting domains Fc2 and Fc3. This variant only reduced binding by 33% (Table I), implying that the Fc2-Fc3 hinge may play only a minor role in IgE binding. However, it has been proposed that IgE free in solution and bound to Fc ϵ RI has a bent and rigid conformation (43). We cannot discern if the 3 residues in variant 64 are involved in binding directly to Fc ϵ RI or if they are involved in maintaining the bent structure of IgE necessary for proper binding to Fc ϵ RI.

It is also interesting to review the data from variants in the 329–409 region, which form the Fc2-Fc3 junction, in light of the report that the Fc ϵ RI binding site on IgE is contained within this 76-amino acid sequence (13, 14). Our variants 63, 64,

2, 44, 8, and 47 covered residues 329–336, 361–365, and 377–405. Of these six, only variants 2 and 64 reduced binding to FcεRI. However, the reduced binding of variant 2 (loop AB) may result from misfolding of loop AB when Phe-381 is substituted with Met, as discussed above. Therefore, of the residues between positions 329 and 409 that have been analyzed in this study, only 3 residues in the Fcε2-Fcε3 hinge (variant 64) reduced binding. None of the important binding determinants found in this study (loops CD, EF, and FG) are contained within positions 329–409. While there may be residues within 329–409 that are involved in binding but have not been covered by our analysis, our data indicate that the critical residues for FcεRI binding to IgE are at sites distant from the Fcε2-Fcε3 junction.

The two chimeric molecules in which the entire Fcε3 domain was exchanged for domain Fcγ2 in IgG (IgG2/3) and in which only loops CD, EF, and FG and the Fcε2-Fcε3 hinge residues were exchanged for their IgG counterparts (IgGEL), both bound to FcεRI (Fig. 3). The reduced binding of IgGEL, relative to IgE and IgG2/E3, may be due to differences in the conformation of the IgE loops when present on the IgG framework. For example, as previously noted, β-strand D probably has a different conformation in IgE than in IgG, and this would influence the presentation of the binding residues in loop CD. In addition, IgG residues outside of loops CD, EF, and FG may interfere with IgGEL binding through steric clash. Nevertheless, the fact that IgGEL binds to FcεRI provides strong support for the importance of IgE loops CD, EF, and FG in the IgE-FcεRI interaction.

Since we have evaluated only two loops in Fcε4 and only one in Fcε2, it is possible that portions of these two domains play a role in IgE-FcεRI binding. However, based on previous studies (20–23) and on the results presented here, we conclude that the Fcε3 domain contains the major, if not complete, site for FcεRI binding of IgE. It is noteworthy that IgE uses domain Fcε3 in binding to its high affinity receptor. This domain is structurally homologous to the Cγ2 domain in IgG (24), which is used in binding to its high affinity receptor, FcγRI (44–47). The IgG-FcγRI system, however, utilizes a more localized region on IgG consisting of the lower portion of the Cγ1-Cγ2 hinge (44, 45) and, possibly, loop FG and β-strands F and G (46). In contrast, the IgE-FcεRI system utilizes a much larger area for binding including not only loop FG and the “hinge” region (as in IgG-FcγRI) but also loops CD and EF. These additional interactions may account for the higher affinity of IgE for FcεRI ($1 \times 10^{10} \text{ M}^{-1}$) (19, 23, 38), as compared to the affinity of IgG for FcγRI ($3 \times 10^8 \text{ M}^{-1}$) (44).

IgE antibodies bind to FcεRI in a one-to-one complex (5). However, the Fc portion of IgE contains two copies of the FcεRI binding site: one on each of the two heavy chains (Fig. 1). Hence, when IgE binds to FcεRI using one site, the second site must be masked so that it cannot interact with another FcεRI. This is supported by the previous observation that the murine monoclonal antibody MaE11, which maps to the Fcε3 domain, binds to free IgE but cannot bind to FcεRI-bound IgE (37). One possibility is that binding of IgE to FcεRI results in a conformational change in IgE that either covers the second site or distorts it so that it cannot be recognized by a second FcεRI.

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