

Fine Specificity of the IgE Interaction with the Low and High Affinity Fc Receptor¹

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ABSTRACT. The characterization of the site(s) on the IgE molecule that accommodate the high (FcεRI) and low (FcεRII) affinity receptors for IgE should allow the design of IgE analogues that can be used to block the onset of the allergic response or to regulate IgE production. To identify the IgE domain responsible for receptor binding, we generated a series of chimeric IgE antibodies in which constant region domains were interchanged between the human and mouse molecules. Binding studies with these chimeras revealed that both the high and low affinity receptor binding-sites reside primarily in the third constant domain of IgE (Cε3). Additional chimeric IgE molecules were generated in which different parts of the human Cε3 domain were exchanged with their murine homologues. Binding experiments with these chimeras suggest that not only the sequence of a particular Cε3 fragment, but the entire Cε3 domain in its native configuration is essential for binding to the FcεRI. The amino acid residues determining the species specificity of the FcεRII are not contained in the first 16 amino acids of the Cε3 domain. *Journal of Immunology*, 1993, 150: 1365.

About 10% of the world population suffers from one or more symptoms of allergy. In the allergic response, IgE binds to specialized receptors on mast cells. Mediators of allergy, including histamine and serotonin, are released as a result of exposure of FcεRI⁵-bound IgE to allergen (1). One of the mechanisms that prevents the allergic response is the maintenance of low serum levels of IgE. This is achieved, in part, by a feed-

back mechanism that suppresses further IgE production by B cells. Although many of the cellular components of this regulatory mechanism are as yet unclear, it was suggested that the low affinity lymphocyte receptor for IgE (FcεRII), also known as CD23, plays a key role in the suppression of IgE production (2, 3). Thus, mapping of the site(s) of the IgE-constant region that interact with both the high and the low affinity FcεR is an important step toward the understanding and possible manipulation of the allergic reaction.

In our previous studies (4–7), we generated mouse/human chimeric IgE molecules and used these chimeras to show that the third constant domain of the IgE heavy chain (Cε3) contains the sequences that react with the FcεRI. In an analogous approach, IgE/IgG chimeric Ig were reported (8, 9). A chimera in which the murine Cε4 domain was replaced with human Cγ3 maintained its ability to bind to the FcεRI, indicating that the Cε4 domain is not directly involved in the IgE-FcεRI interaction. In this system, only chimeras containing both Cε2 and Cε3 were able to bind to the FcεRI.

Several groups have produced IgE peptides representing sequences within Cε3 (10–13). Some of these peptides

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⁵ Abbreviations used in this paper: Cε 1,2,3,4, domains of the IgE constant region; Hu, human; Mu, murine; PCR, polymerase chain reaction; NIP, (4-hydroxy-3-iodo-5-nitrophenyl) acetic acid; SRBC, sheep RBC; FcεRI, high affinity Fc receptor for IgE; FcεRII, low affinity Fc receptor for IgE.

were shown to inhibit binding of native IgE to mast cells (10, 11), and antibodies to other peptides were shown to inhibit the IgE/FcεRI interaction (12, 13). Nevertheless, such associations are of low affinity relative to the binding of the native IgE. However, in one study, fragments corresponding to the Cε2/Cε3 interface produced in bacteria were shown to bind directly to the FcεRI with an affinity close to that of native IgE (14).

Only limited information is available on the region of IgE that interacts with the low affinity FcεRII. Studies with anti-IgE mAb have implicated the Cε3 domain as the site of interaction with the FcεRII. mAb 27, recognizing a 10-amino acid stretch (position 367–376) in the human Cε3 domain, inhibits the IgE-FcεRII interaction (15). Anti-mouse Cε3 mAb R1E4 (that is identical to 84.1C (16)) blocks IgE binding to the FcεRI and FcεRII, and does not bind to receptor-bound IgE on mast cells and B cells (9, 16). The necessity for the Cε3 domain in the binding to the low affinity receptor was confirmed by studies utilizing rIgE. Using bacterially produced fragments of human IgE, it was demonstrated that FcεRII binds to a motif in the Cε3 domain that is formed on dimerization of one or both of the flanking (Cε2 and Cε4) domains (17). Studies with IgE/IgG exon shuffle mutants (9) indicate that mouse Cε3 is the main domain involved in the binding to the murine low affinity receptor, and that the Cε4 domain influences this binding.

In our previous report, we took advantage of the fact that in spite of sequence similarity between mouse and human IgE (18), the latter does not bind to the rodent FcεRI, although both bind equally well to the human receptor (19). Thus, by replacing the human Cε3 with its murine counterpart, we have endowed it with the ability to bind to the rodent FcεRI. To map the extent and precise location of the Fcε receptor binding site of IgE, short subdomain fragments from the murine Cε3 were introduced into the human IgE molecule. Here, we describe the use of such chimeras in an attempt to identify sequences in the Cε3 domain that interact with both the high and low affinity Fcε receptors. Our data suggest that the residues on the IgE molecule that make contact with both the high and low affinity receptors are contained in several regions of the Cε3, and that the conformation of the entire Cε3 domain is crucial for FcεR binding.

Materials and Methods

Construction of hybrid human/mouse Cε3 chimeras

The construction of the rIgE molecules, the chimeric human-mouse domain shuffled IgE and the Cε2 deletion mutants are fully described in our earlier study (5). *CHM3*, *CHM2*, and *CHM2M3* are human-mouse chimeric IgE molecules in which either one or both of the human second and third domains were replaced by their respective mu-

rine counterparts. In the *CSPD* mutant, the entire Cε2 was deleted from *CHM3*, however, a 16-amino acid stretch was retained from the carboxy terminus of Cε2, including Cys328 that takes part in the interheavy disulfide bond.

To construct the human/mouse Cε3 hybrid chimeras, we used the same expression vector described previously (5). To construct the *C3BX* chimera, we used the PCR to regenerate a fragment between bp₁₀₅₈-bp₁₃₈₀ of the human IgE. The following oligonucleotides were used as primers: 5' A CCC AGC CCC CTG GAC CTG TTC ATC CGC A 3'. This primer contains a *Bst*XI linker (*underlined*) and part of the coding sequence of human IgE (*bold*); and 5' CCC TTC CCC CAC GAC CCC 3' containing human sequences b.p₁₃₆₀–1380 of the Cε3-Cε4 intron.

The PCR product was amplified using as a template the human Cε gene except for the first 16 amino acids at the N terminal end of Cε3. The amplified fragment was digested with *Bst*XI and *Nco*I and was then ligated with the *Bgl*III_{Hu-650}-*Bst*XI_{Mu-1400} fragment of the *CHM3* chimera and subcloned into a Hu-pSVCε vector from which the *Bgl*III₆₅₀-*Nco*I₁₃₅₀ fragment was excised.

A similar approach was employed to construct the *C3HD* except that an additional oligonucleotide primer was used: 5' C AAG CTT ACC TGT CTG GTG GTG 3'. This oligonucleotide contains a *Hind*III linker (*underlined*) and human IgE sequences (*bold*). We used oligonucleotides b and c to amplify human Cε3 sequences between b.p₁₀₉₃–1380. This was designed to amplify the entire Cε3 exon except for the N terminal region encoding 26 amino acids. The amplified fragment was digested with *Hind*III and *Nco*I and was ligated to the *Bgl*III_{Hu-650}-*Hind*III_{Mu-1430} fragment of the *CHM3* chimera, and subcloned into the Hu-pSVCε vector from which the *Bgl*III₆₅₀-*Nco*I₁₃₅₀ fragment was deleted.

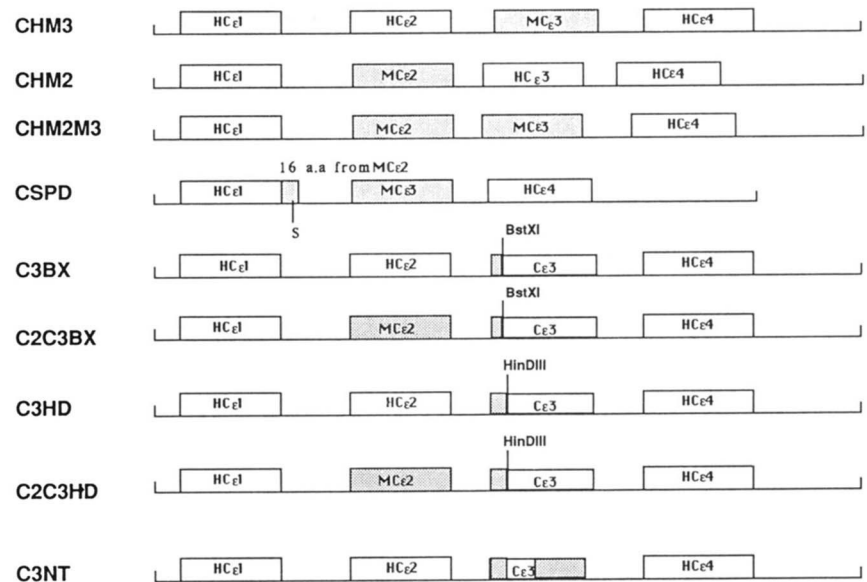
To construct the *C2C3BX* and *C2C3HD* chimeras, the *Sal*I_{Hu-390}-*Bst*XI_{Mu-1400} fragment from the *CHM2 M3* chimera was exchanged with *Sal*I_{Hu-390}-*Bst*XI_{Mu-1400} fragment of *C3BX* and *C3HD*, respectively.

The plasmid encoding the *C3HD* chimera was used to construct the *C3NT* Cε3 hybrid. To this end, a fragment of *Sal*I₃₉₀-*Hpa*I₁₂₂₁ of the *C3HD* was subcloned into the *CHM3* vector from which the *Sal*I_{Hu-390}-*Spe*I_{Mu-1555} fragment was deleted. To maintain the correct reading frame, the *Spe*I site was blunt ended with mungbean nuclease before ligation.

Immunoblot analysis

The details of the immunoblot procedure are as described elsewhere (4, 5). Briefly, affinity-purified IgE preparations were electrophoresed under nonreducing or reducing conditions on 5% and 10% acrylamide gels, respectively.

FIGURE 1. Schematic diagram of the human/mouse IgE chimeras. The hybrid C ϵ 3 domain represents a combination of human ($\square$) and murine sequences ($\square$). *C3BX* and *C3HD* represent hybrids in which human amino acids 330–346 and 330–356, respectively, were replaced with their murine analogues. *C2C3BX* and *C2C3HD* are chimeric C ϵ 3 hybrids in which the human and mouse second domains were also exchanged. The *C3NT* chimera contains mouse sequences at both ends of C ϵ 3 (330–356 and 400–447). The *CHM3*, *CHM2*, *CHM2M3*, and *CSPD* chimeras were previously described (5).



Bands were electroblotted to nitrocellulose filters and reacted with peroxidase-labeled anti-human IgE antibodies (Kpl, Inc. MD).

Immunoassays and binding assays for IgE

The concentration of affinity-purified chimeric IgE was determined by ELISA assay, as described (4, 5), using polyclonal anti-human IgE and anti-mouse ϵ - and $[\lambda]$ -chain antibodies. The protein concentration of purified IgE preparations was also determined by measuring the OD₂₈₀; the values obtained correlated well with the ELISA.

Binding of IgE to Fc ϵ RI-bearing cells was evaluated either directly or by inhibition of ¹²⁵I-IgE binding to the various Fc ϵ R-expressing cells. This test, and the Fc ϵ RI adsorption and the RBL-degranulation assays were performed as previously described (4, 5). The IgE-specific rosette assay was performed according to Rittenberg and Park (20). Briefly, NIP-modified SRBC (NIP-SRBC) were added to the Fc ϵ RII-expressing cells, which were preincubated with different concentrations of anti-NIP rIgE for 1 h at room temperature. Tubes were spun (100 × g, 5 min), and incubated an additional 2 h, then mixed gently, and the percentage of rosette-forming cells was determined by counting. mAb against mouse (21) and human (22) IgE were described previously.

Fc ϵ R expressing cells

As a source of human Fc ϵ RI, we used the CHO-3D10 cells stably expressing a gene composed of the extracellular domain of the human Fc ϵ RI α subunit and the transmembrane-cytoplasmic domains of the IL-2 receptor as a membrane anchor (23) (CHO-3D10 cells, kindly provided by Dr Hakimi, Hoffmann La Roche, Inc., Nutley, NJ). The RBL-2H3 rat mast cell line served as a source of rodent

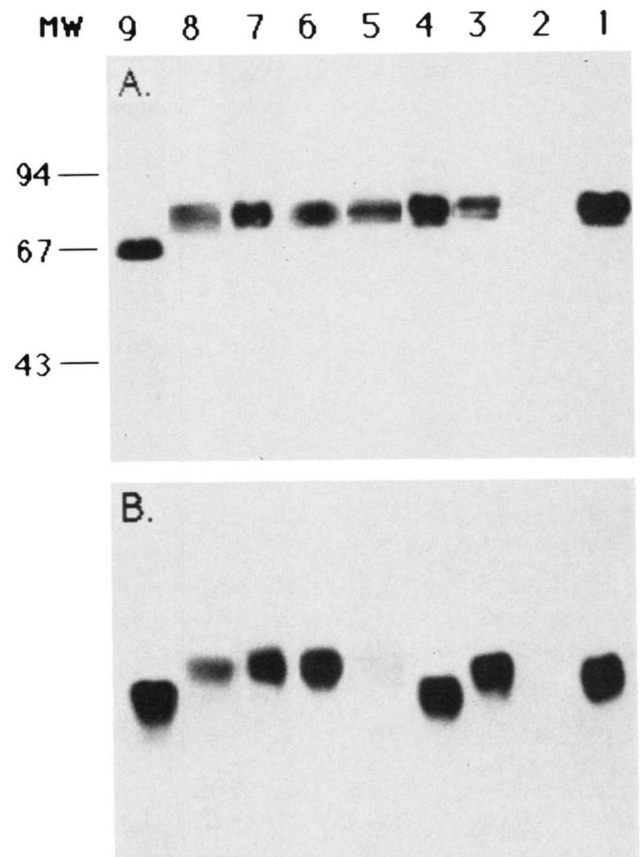


FIGURE 2. Immunoblot analysis of the human/mouse hybrid C ϵ 3 chimeras with anti-human IgE antibodies. Affinity purified IgE preparations were electrophoresed under A, reducing and B, nonreducing conditions, using 10% and 5% acrylamide gels, respectively. Gels were electroblotted to nitrocellulose and hybridized with peroxidase-labeled goat anti-human IgE antibodies. The rIgE were loaded in the following order: human (lane 1), murine (lane 2), *CHM3* (lane 3), *C3BX* (lane 4), *C2C3BX* (lane 5), *C3HD* (lane 6), *C2C3HD* (lane 7), *C3NT* (lane 8) and *CSPD* (lane 9).

Table 1

Recognition of the human/mouse hybrid Cε3 chimeras by anti-mouse and anti-human IgE mAb. Plates were first coated with anti-mouse or anti-human IgE mAb, followed by the addition of IgE mutants and finally, peroxidase-labeled anti-human IgE antibodies were added. Numbers represent O.D. readings at 620 nm. The numbers in parenthesis list additional mAb that were analyzed and show the same pattern of reactivity. The epitope recognized by mAb number 15 resides within the Cε2/Cε3 interface (see also Ref 21)

IgE	NIP	Anti-Mouse mAbs		Anti-Human mAbs		
		84.1C (95.3)	8 (2, 11)	15	13 (14)	10 (9, 12)
Hu-rlgE	0.734	0.000	2.200	0.759	1.371	1.708
CHM3	0.780	0.861	2.200	0.800	0.000	1.632
CHM2	0.773	0.000	2.200	0.165	1.220	1.632
CHM2M3	0.599	0.624	1.900	0.000	0.000	1.023
CSPD	0.614	0.525	1.900	0.000	0.000	1.317
C3BX	0.727	0.000	2.200	0.775	1.410	1.720
C2C3BX	0.393	0.000	1.412	0.000	0.440	0.959
C3HD	0.799	0.000	2.200	0.749	1.360	1.742
C2C3HD	0.754	0.000	2.200	0.030	1.074	1.571
C3NT	0.452	0.000	1.900	0.404	0.000	0.913
Epitope:		Mu-Cε3	Hu-Cε1	Hu-Cε2	Hu=Cε3	Hu-Cε4

mast cells expressing high levels of FcεRI (24). We used the 01.2A3 mouse B cell hybridoma (25) and the human EBV transformed B cell line RPMI-8866 (26) (obtained from Dr. Dan Conrad, Medical College of Virginia, Richmond, VA) as a source of mouse and human FcεRII, respectively.

Results

Construction of the human/mouse hybrid Cε3 chimeras

To construct the human/mouse Cε3 hybrid chimeras we used the same principal expression vector (Hu-pSVCε) described previously (5). Human and murine Cε3 sequences were interchanged, using PCR methodology in combination with convenient restriction sites, as detailed in *Materials and Methods*. A schematic diagram of the different human-mouse hybrid Cε3 chimeras so generated is depicted in Figure 1. In light of the studies implicating the interface between the Cε2 and Cε3 domain as the site of interaction between the IgE and its FcεRI (14, 27), 16 or 26 amino acids from the amino-terminal portion of human Cε3 were exchanged with their murine analogues (amino acids 330–346 for C3BX and 330–356 for C3HD). To study the contribution of Cε2 in the interaction, additional variants (C2C3BX and C2C3HD) were made in which the entire second domain of these chimeric molecules was replaced with its murine counterpart. The involvement of the carboxy end of the Cε3 domain in the FcεR interaction was analyzed using the C3NT mutant, in which two separate stretches from both ends of Cε3 (amino acids 330–356 and 400–447) were replaced with the homologous murine sequences.

The mutated chimeric genes were transfected into the J588L myeloma and expressed as secreted anti-NIP IgE antibodies. Immunoblot analysis of the affinity purified

Cε3 mutants revealed molecular weight identical to that of the nonmutated human IgE (Fig. 2A and B).

Epitope expression on the hybrid Cε3 chimeras

A panel of anti-mouse and anti-human mAb were tested for binding to the IgE chimeras (Table I). None of the Cε3 mutants reacted with the anti-mouse Cε3 mAb, 84.1C and 95.3 (4, 16, 21), presumably because they lack the C terminal mouse sequences recognized by these mAb. C3NT, which contains mouse sequences at both the carboxy and amino ends of Cε3, also did not bind to the 95.3 mAb, which recognizes a conformational epitope at the carboxy end of mouse Cε3 (4). As none of the human/mouse Cε3 hybrid chimeras interact with any of the anti-mouse Cε3 mAb tested, we next used a set of anti-human IgE mAb. As shown in Table I, two mAb (13 and 14) did not bind to human IgE containing the murine Cε3 (CHM3), but retained their ability to bind to the C3BX and C3HD chimeras, in which only the amino terminal sequences of human Cε3 were exchanged with their respective murine homologs. These results, therefore, indicate that mAb 13 and 14 recognize epitopes at the carboxy three-quarters of human Cε3. This group of anti-human mAb was previously shown to recognize conformational epitopes contained within the Fc (mAb 9, 10, 12, 13, 14, and 15) or the Fab (mAb 2, 8, 11, and 15) regions of human IgE (22); the binding of these mAb, and in particular mAb 13 and 14, to the chimeric hybrids indicates that these Cε3 mutants maintain their native protein folding. The C3NT mutant did not react with any of the anti-mouse or anti-human Cε3 antibodies tested. Thus, this mutant seems to have an altered conformation in its Cε3 domain.

mAb inhibition studies

The ability of the various anti-IgE mAb to inhibit the binding of the IgE chimeras to CHO-3D10 and RPMI

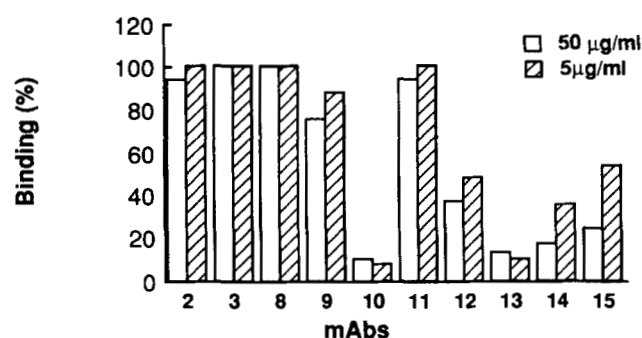
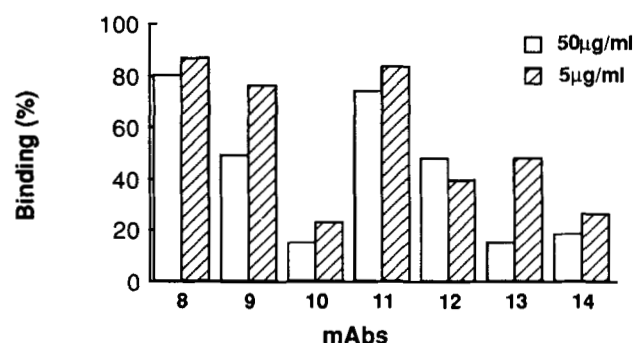
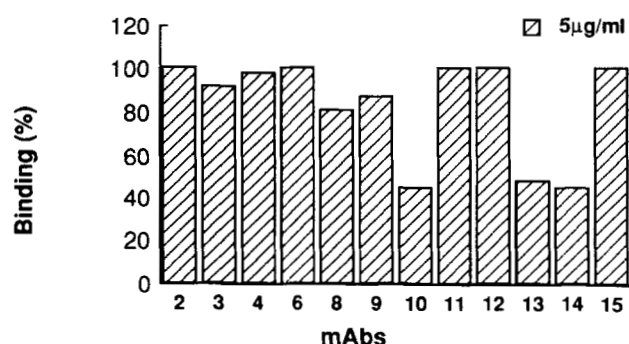
A. inhibition of Hu-rIgE binding to CHO-3D10 cells**B. inhibition of C2C3HD binding to CHO-3D10 cells****C. inhibition of Hu-rIgE binding to RPMI 8866 cells**

FIGURE 3. Inhibition of IgE binding by the anti-human mAb. The ability of the various mAb to inhibit the binding of ^{125}I -human IgE (A) or ^{125}I -C2C3HD (B) to CHO-3D10 cells, or ^{125}I -human IgE to RPMI-8866 cells (C) was measured. Various concentrations of the tested mAb were first incubated with 250 ng of ^{125}I -IgE for 1 h at 37°C. Cells were then added for an additional 2-h incubation at room temperature. The percentage of binding was calculated by the degree of radioactivity bound to cell pellets, using the noninhibited sample as 100% binding.

8866 cells is shown in Figure 3 and Table II. Our data (Fig. 3) indicate that this inhibition is not limited to those mAb that recognize the C ϵ 3 domain (mAb 13 and 14). Rather, mAb directed against the human C ϵ 2/C ϵ 3 junction (mAb 15) and C ϵ 4 (mAb 10 and 12), also inhibited the binding of human IgE. Out of the set of anti-human mAb, only mAb 10 (anti-C ϵ 4) and mAb 13 and 14 (anti-C ϵ 3) inhib-

Table II

Inhibition of rosette formation by mAb 01.2A3. Cells were incubated with 2 µg/ml of rIgE in the presence or absence of the 95.3 or 84.1C anti-mouse C ϵ 3 mAb, or the 51.3 anti-mouse C ϵ 4 mAb (4). The fraction of rosette-forming cells was determined following the addition of NIP-SRBC. N.A., not applicable

IgE	Rosette Forming cells (%)			
	-mAbs	+95.3	+84.1C	+51.3
Mu-rIgE	80	10	0	80
CHM3	30	0	0	N.A.
CHM2	0	0	0	N.A.
CHM2M3	80	0	0	N.A.
CSPD	20	0	0	N.A.

ited the binding of IgE to the human low affinity receptor. However, of this series of mAb, only mAb 13, 14, and 15 did not bind to receptor-bound IgE (22), presumably because the binding of these mAb specifically obscures the contact site. The inhibition by the anti-C ϵ 4 mAb may be attributed to steric hindrance. mAb 84.1C and 95.3 directed against the mouse C ϵ 3 domain inhibited the binding to both the mouse Fc ϵ RI (16) and Fc ϵ RII (Table II). Nevertheless, mAb 51.3 directed against murine C ϵ 4, did not inhibit the binding to 01.2A3 cells. Because antibody-blocking experiments are influenced by steric inhibition, this approach cannot be used to provide conclusive information about specific amino acid residues involved in the IgE-Fc ϵ R interaction. To this end, IgE chimeras were tested directly for their ability to bind to the high and low affinity Fc ϵ Rs.

Binding to the high affinity Fc ϵ RI

The ability of the C ϵ 3-mutated IgE to bind to the murine and human Fc ϵ RI was evaluated. The mutants containing N terminal C ϵ 3 murine sequences on a human background continued to bind to the human Fc ϵ RI, as shown by adsorption of anti-NIP activity, and competitive inhibition of human IgE binding to CHO-3D10 cells (Fig. 4). However, the intra-C ϵ 3 chimeras did not bind to the rodent Fc ϵ RI (data not shown), as determined by competitive inhibition assays, rosetting, mast cell degranulation assays, and the adsorption of anti-NIP activity. Although the entire mouse C ϵ 3 domain enables the human IgE molecule to bind to the rodent Fc ϵ RI (5), swapping of only 16 or 26 amino acids from the amino terminus of C ϵ 3 (C3BX and C3HD mutants) was not sufficient to endow human IgE with the ability to bind to the rodent receptor. Addition of the mouse C ϵ 2 domain to these chimeras (C2C3BX and C2C3HD) was equally ineffective. Swapping of longer stretches from both the carboxy and amino ends of human C ϵ 3 with their murine analogues (C3NT) was not only insufficient to enable binding to the rodent receptor (not shown), but also impaired binding to the human receptor (Fig. 4).

A. Anti - NIP activity removed by CHO - 3D10 cells

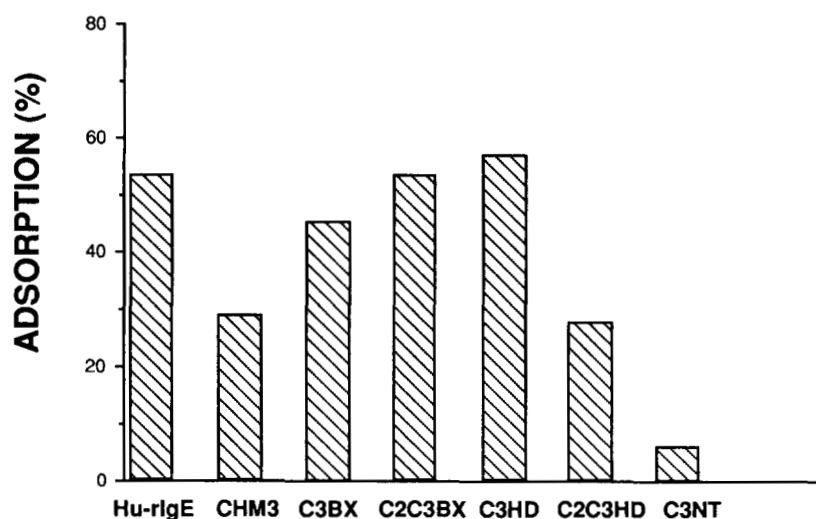
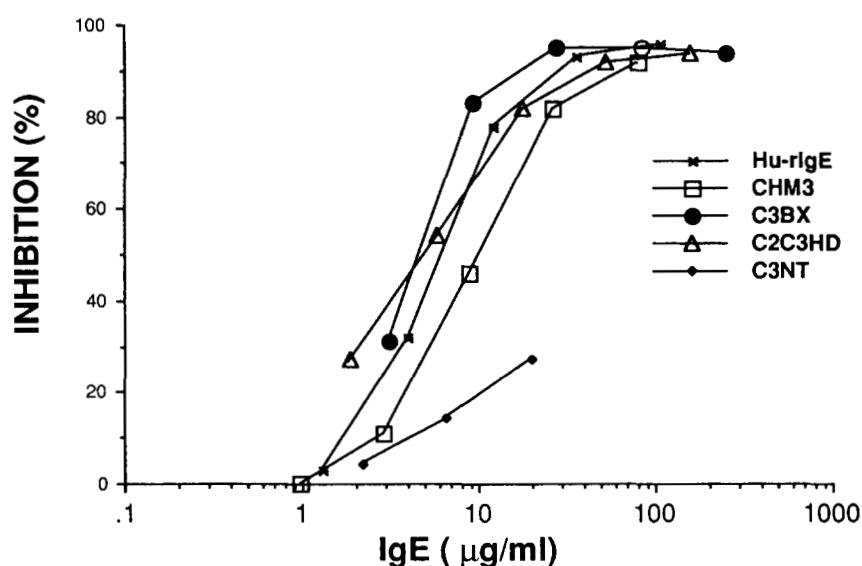


FIGURE 4. Binding of chimeric IgE to the human FcεRI. *A*, The ability of CHO-3D10 cells to adsorb anti-NIP activity. IgE samples were incubated with or without CHO-3D10 cells for 4 h at room temperature. The supernatant was then assayed for remaining anti-NIP activity. *B*, Competitive inhibition assay. The ability of the rIgE to inhibit the binding of 100 ng ¹²⁵I-human rIgE was measured. The percentage of inhibition was calculated by the degree of radioactivity bound to cell pellets, using noninhibited sample as 0% inhibition. Results represent the average of three determinations.

B. Inhibition of Hu-rlgE binding



Binding to the low affinity FcεRII

The various chimeric and mutated IgE molecules were next used to study the site on the IgE molecule that interacts with the murine and human B cell FcεRII. As a source of FcεRII, we used the 01.2A3 mouse B cell hybridoma (25) (expressing moderate levels of FcεRII) and the human EBV-transformed B cell line, RPMI-8866 (26) (expressing high levels of the FcεRII). The ability of the various chimeric IgE to bind to the receptor was determined by the rosette assay, using NIP-derivitized SRBC, and by a competitive inhibition assay. As can be seen in Figures 5 and 6, both human and mouse IgE demonstrate species-specific binding to the FcεRII. Human chimeric IgE molecules containing the murine Cε3 (*CHM3*) bound to the

rodent FcεRII, and lost their ability to bind to the human FcεRII. Thus, the species-specificity observed is contained in the third domain. The binding of *CHM3* to 01.2A3 cells is, however, of lower relative affinity than that of the nonmutated mouse IgE. Exchanging both the Cε2 and Cε3 domains (*CHM2M3*) conferred on human IgE full reactivity toward the mouse FcεRII (Figs. 5A and 6A). The murine Cε2 domain has only a minor role in FcεRII binding, as the *CSPD* Cε2-deletion mutant (5) was at least half as active as native murine IgE in the inhibition assay (Fig. 6A). Swapping of shorter fragments of the amino-terminal portion of human Cε3 with their murine analogues (*C3BX* and *C3HD*) was not sufficient to endow the human IgE with the ability to bind to the mouse FcεRII, even after the

A. Binding to 01.2A3

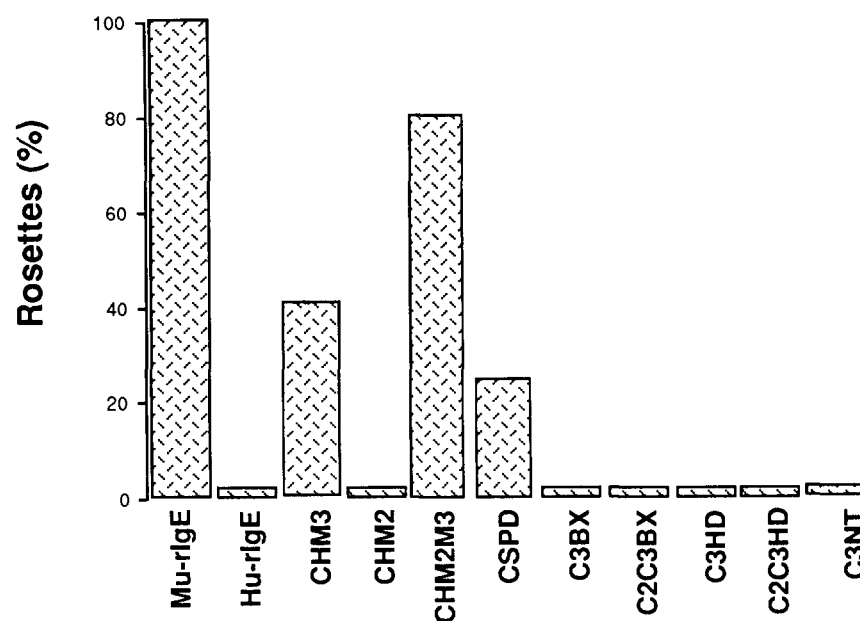
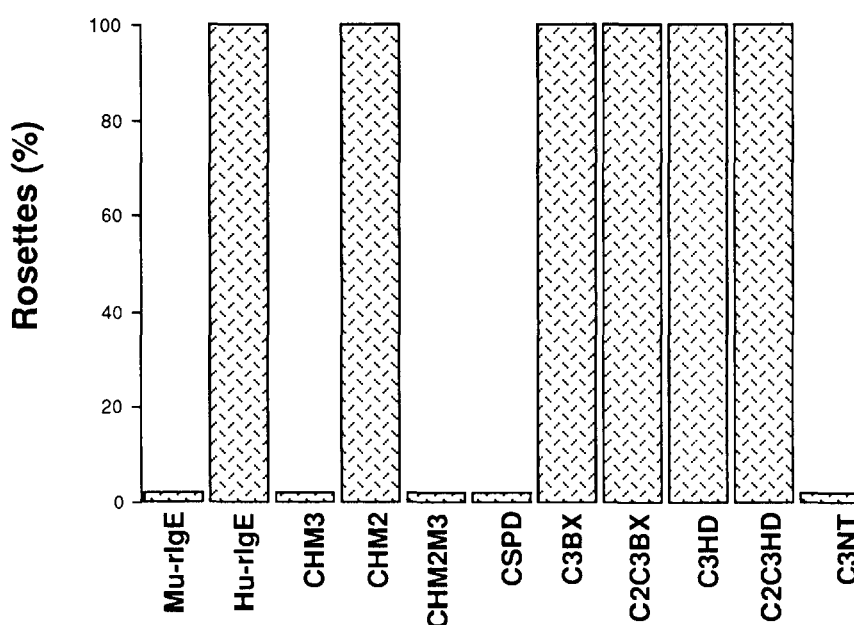


FIGURE 5. Binding to the low affinity FcεRII. A, Mouse B cell hybridoma 01.2A3 cells, and the human EBV-transformed B cell line RPMI-8866 were incubated with either 20 μg/ml (A) or 2 μg/ml (B) of the rlGE, followed by the addition of NIP-derivitized SRBC. The proportion of rosette-forming cells was then determined microscopically.

B. Binding to RPMI 8866



introduction of the murine second domain (*C2C3BX* and *C2C3HD*). This suggests that additional mouse sequences down stream to amino acid 356 at the Cε3 might also contribute to the receptor recognition site. However, as in the case of the high affinity receptor, the *C3NT* mutant failed to bind to the Mu-FcεRII.

The human chimeric molecules that contain the murine Cε3 (*CHM3* and *CHM2M3*) did not bind to the human FcεRII (Figs. 5B and 6B). However, *CHM2*, in which only the second human domain is replaced by its murine ho-

molog, bound with similar relative affinity as the unmutated *Hu-rlgE*. Both formed rosettes (Fig. 5B) and inhibited, to a similar degree, the binding of human IgE to the human FcεRII (Fig. 6B). Thus, it appears that the species specificity of the human FcεRII is determined solely by sequences in Cε3. Furthermore, binding to human RPMI 8866 cells was reduced as mouse sequences were introduced into the human Cε3 backbone. The relative binding of the *C3BX* chimera to the FcεRII is 5-fold lower than that of the native human IgE, as measured by the

A. Mouse FcεRII

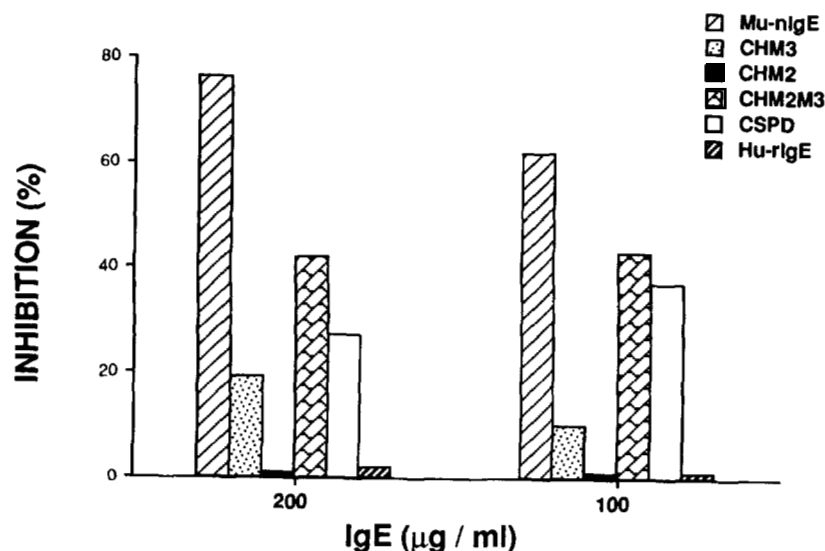
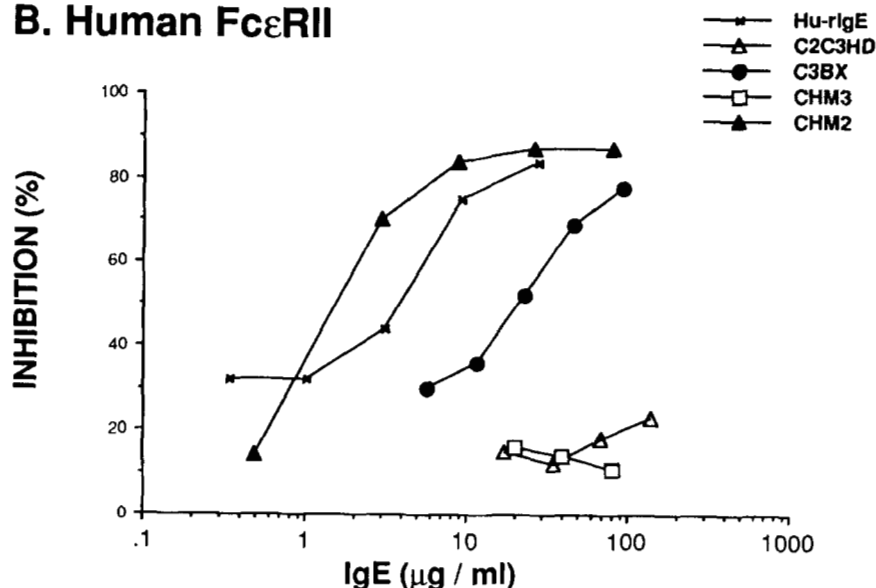


FIGURE 6. Binding of the human/mouse chimeric IgE to the human and mouse FcεRII. Binding was measured by the competitive inhibition assay measuring the ability of the rIgE to inhibit the binding of A, 100-ng ^{125} I-labeled murine native IgE or B, 100 ng ^{125} I-human rIgE. The percentage of inhibition was calculated by the degree of radioactivity bound to cell pellets, using noninhibited sample as 0% inhibition. Results represent the average of three determinations.

B. Human FcεRII



competition assay (Fig. 6B). The binding of the C3HD and C2C3HD chimeras with a somewhat longer murine Cε3 N terminal sequence is dramatically reduced. Because these chimeras formed rosettes but barely inhibited the binding of ^{125} I-Hu-rIgE (Figs. 5B and 6B), we propose that part of the binding energy for the human FcεRII is contributed by residues between amino acids 346–356 (at the amino end of human Cε3). Nevertheless, the avidity of interaction contributed by residues in the remaining three-quarters of the carboxy terminal of the Cε3 was sufficient to form rosettes. The C3NT mutant, which has mouse sequences at both the carboxy and amino ends of Cε3, lost its ability to bind to either the human or mouse FcεRII, presumably because of altered conformation in Cε3.

Discussion

In the present study, we attempted to analyze site(s) within the Cε3 domain that are responsible for Fcε receptor binding. To this end, we replaced various subdomains of the human Cε3 with their murine homologues, testing the ability of the resulting IgE to bind to the rodent FcεRI. As the low affinity FcεRII binds IgE in a strictly species-specific fashion (Figs. 5 and 6), we made use of the same set of human/mouse IgE chimeras to map the FcεRII binding site(s).

Because previous studies implicated the Cε2-Cε3 interface as the site of the IgE-FcεRI interaction (14, 27), we focused our attention on the amino terminal end of Cε3.

Swapping of 16 or 26 amino acids from the amino terminal end of Ce3 (amino acids 330–346 for *C3BX* and 330–356 for *C3HD*), even in the presence of mouse Ce2 (*C2C3BX* and *C2C3HD*), was insufficient to endow the human IgE with the ability to bind to the rodent $\text{Fc}\epsilon\text{RI}$. The exchange of stretches at both the C and N terminal ends of human Ce3 with their mouse counterparts impaired binding to both the rodent and human receptors (*C3NT*, Fig. 4). As the IgE- $\text{Fc}\epsilon\text{RI}$ interaction is sensitive to conformational changes, one explanation for the failure of the N terminal Ce3 hybrid IgE chimeras to bind to the rodent receptor is that the replacement of the human with the murine sequences altered their active conformation. However, two lines of evidence suggest that the native configuration of Ce3 has been maintained in the amino-terminal IgE chimeras, although not in the *C3NT* N and C terminal chimera. First, all the hybrid Ce3 mutants (other than *C3NT*) bind to the human $\text{Fc}\epsilon\text{RI}$ (Fig. 4). In addition, the fact that all the mutants (except *C3NT*) bind to anti- Ce3 mAb (that recognize native IgE, Table I) confirms that these hybrid Ce3 mutants maintain their conformational epitopes. Our results suggest that the contact residues on the IgE molecules that interact with the $\text{Fc}\epsilon\text{RI}$ are not contained in the amino terminus of the third domain. Because the maintenance of the native conformation of the entire domain is necessary for receptor binding, it is very likely that several noncontiguous regions contribute to this interaction.

As is the case for the high affinity $\text{Fc}\epsilon\text{RI}$, site(s) in the Ce3 domain are also required for the binding to the low affinity $\text{Fc}\epsilon\text{RII}$. The lower binding ability of the *CHM3* in comparison to that of the *CHM2M3* (Figs. 5A and 6A) indicates that Ce2 is required for optimal binding to the $\text{Mu-Fc}\epsilon\text{RII}$. As the Ce2 deleted mutant (*CSPD*, Figs. 5A and 6A) binds to the $\text{Fc}\epsilon\text{RII}$ (albeit with lower affinity), and as the mouse Ce2 domain did not enable the human IgE to bind to the mouse $\text{Fc}\epsilon\text{RII}$ (*CHM2*, Figs. 5A and 6A), it is unlikely that Ce2 sequences participate in the actual contact site for the mouse low affinity receptor binding. In contrast, binding to the human $\text{Fc}\epsilon\text{RII}$ is determined exclusively by sites on the human Ce3 domain. *CHM2* and human rIgE bind equivalently to the human $\text{Fc}\epsilon\text{RII}$ (Figs. 5B and 6B).

Our results demonstrating the importance of Ce3 in the binding to the $\text{Fc}\epsilon\text{RII}$, agree with those reported by others (9, 15, 17). Using a recombinant, chimeric mIgE-HuIgG1, Keegan et al. (9) showed the importance of Ce3 in the binding to mouse $\text{Fc}\epsilon\text{RII}$. Our observation that *CHM3* bound more weakly to the murine $\text{Fc}\epsilon\text{RII}$ than *CHM2M3* (Figs. 5A and 6A) suggests that the framework into which the Ce3 is inserted plays an important role in the IgE- $\text{Fc}\epsilon\text{RII}$ interaction. This interpretation may also explain why a $\gamma/\text{Ce3}$ chimera reported by Keegan et al. (9), in which Ce3 was introduced into the human IgG backbone, did not inhibit the binding of mIgE to the $\text{Fc}\epsilon\text{RII}$.

Studies using recombinant human ϵ -chain fragments expressed in bacteria suggest that all three $\text{Fc}\epsilon$ domains are necessary to obtain full reactivity (17). Ce4 and Ce2 are thought to have a role in stabilizing dimerization of the bacterially expressed fragments. A recombinant human ϵ -chain fragment spanning the Ce2-Ce3 junction (Gln301-Arg376), which binds to the $\text{Fc}\epsilon\text{RI}$, failed to bind to the human $\text{Fc}\epsilon\text{RII}$ (17). This could simply be because of the lack of the proper conformation necessary for receptor binding. In fact, our data demonstrate that Ce3 alone determines the species specificity, and that the Ce2 does not have any role in the binding to the human $\text{Fc}\epsilon\text{RII}$. Our approach, using expression of intact IgE molecules, enabled us to study the species and domain specificity of binding and to exclude the participation of residues 330–346 in the binding site. The current set of human/mouse chimeric IgE do not test the involvement of human Ce4 in the binding to the human $\text{Fc}\epsilon\text{RII}$.

An important clinical application for identifying the IgE- $\text{Fc}\epsilon\text{R}$ interaction site is the generation of IgE peptides that could block the binding of IgE to mast cells in vivo. Our findings suggest that the $\text{Fc}\epsilon\text{R}$ binding site on IgE is a conformational determinant that cannot be assigned to a single discrete sequence. It is therefore unlikely that any short IgE fragment will be able to efficiently block the IgE- $\text{Fc}\epsilon\text{R}$ interaction. A more fruitful approach might be the screening of random peptides to identify a sequence (which might not be highly homologous to the primary sequence of IgE) that is able to mimic the putative active conformation of the IgE molecule. Such a peptide, by inhibiting the critical IgE- $\text{Fc}\epsilon\text{R}$ interaction, may offer long-awaited relief of the symptoms of allergy.

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