Identification of the High Affinity Receptor Binding Region in Human Immunoglobulin E*

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We have investigated the capacity of N- and C-terminally truncated and chimeric human (h) IgE-derived peptides to inhibit the binding of [125I]-labeled hIgE, and to engage cell lines expressing high and low affinity receptors (FcεRI/II). The peptide sequence Pro343–Ser353 of the hCε3 domain is common to all h-chain peptides that recognize hFcεRI. This region in IgE is homologous to the A loop in Cγ2 that engages the rat neonatal IgG receptor. Optimum FcεRI occupancy by hIgE occurs at pH 6.4, with a second peak at 7.4. N- or C-terminal truncation has little effect on the association rate of the ligands with this receptor. Dissociation markedly increases following C-terminal deletion, and hFcεRI occupancy at pH 6.4 is diminished. His residue(s) in the C-terminal region of the ε-chain may thus contribute to the high affinity of interaction. Granting the homologous rat ε-chain sequence into hIgE maintains hFcεRI interaction without conferring binding to rat FcεRI. hFcεRI interaction is lost, suggesting that these residues also contribute to hFcεRII binding. h-ε-chain peptides comprising only this sequence do not block hIgE/hFcεRI interaction or engage the receptor. Therefore, sequences N- or C-terminal to this core peptide provide structures necessary for receptor recognition.

Antibodies of the immunoglobulin (Ig)E isotype sensitize target cells expressing the class-specific Fc receptors for antigen-induced mediator release, by binding through residues located in the Fc portion of the molecule (1, 2). The potent pharmacologically active substances that are released in response to this stimulus cause the clinical symptoms of allergy.

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Strategies that block the initial sensitization of target cells with antigen-specific Igε have been explored following the demonstration that human (h)1 myeloma Igε-derived Fcε fragments generated by proteolytic deavage with papain (1, 2), which produces peptides comprising h-chain residues 1–226 and 227–547, can competitively inhibit the binding of Igε to cells expressing high affinity receptor (FcεRI) (1, 2), whereas deavage products of pepsin digestion, which generates ε fragments spanning residues 1–338, 339–349, and 350–547 (3) do not inhibit binding. This observation initiated the quest for progressively smaller peptides as potential Igε antagonists (reviewed in Ref. 4). In early studies, the inhibition of passive cutaneous anaphylaxis in human skin was used to assess the FcεRI-blocking activity of proteolytic fragments or recombinant Igε-derived peptides expressed in Escherichia coli (1, 5, 6). This led to the proposal that sequences N- and C-terminal to Val336 contribute structures necessary for FcεRI interaction (6). More recent studies aimed at the identification of the receptor binding site(s) employed chimeric human/mouse Igε antibodies, ε/γ chimeras, site-specific mutations, anti-Igε antibodies, or Igε-derived peptides (7–15). They indicate that the site(s) in Igε that interact(s) with the Fc receptors depend(s) on structures associated with residues located in the Cε3 domain, although Cε4 involvement has also been invoked (11, 12). Furthermore, it has been suggested that Igε/FcεRII interaction is mediated primarily by electrostatic interactions (14) and dependent on the entire Cε3 in its native conformation (10), while the Cε4 domains are essential for the maintenance of the active conformation of the Cε3 domain (7, 16). Our earlier investigations showed that while Igε/FcεRII interaction is critically dependent on Cε4 or its homologue Cy3 (16), it is possible to delete the entire Cε4 domain and more than 60% of residues in Cε3 and still maintain the FcεRI-blocking capacity of the recombinant ε-chain fragment (6, 17).

Based on our demonstration of the parallel nature of the inter-ε-chain disulfide bonds in hIgε (18), we developed a structural model that predicts that an exposed and probably flexible segment connects the globular portions of the Cε2 and Cε3 domains (18, 19). Subsequently, Gould et al. (20, 21) claimed that the N-terminal 11 residues in Cε3, which are included in this segment, are essential for FcεRII binding. This proposal relied on studies where the biological activity of recombinant ε-chain fragments was tested by blocking the binding of ragweed-specific Igε to mast cells in the skin of the senior investigator conducting the study (6). When the fallibility of the passive cutaneous anaphylaxis reaction in assessing the biological activity of recombinant Igε-derived fragments emerged

1 The abbreviations used are: h, human; r, rat; GST, glutathione S-transferase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.
(17, 22), we re-assessed the biological activity of these and additional truncated fragments using our recently developed receptor binding assay, which allowed us to study direct binding of IgE-derived ligands to rat (r) basophilic leukemia cells (RBL-2/2/C) transfected with the α-chain of hFcrRI (23).

In the present investigation we describe the capacity of a series of overlapping N- and C-terminally truncated and chimeric eγ-chain derived fragments, expressed as glutathione S-transferase (GST) fusion proteins in E. coli to bind directly to and block the binding of hIgE to RBL-2/2/C cells. We show that the peptide sequence spanning amino acid residues Pro343–Ser353 is common to all recombinant e-chain fragments capable of binding to FcεRI. Deletion of this sequence is associated with a complete loss of receptor recognition, confirming earlier observation by others that grafting the homologous sequence from IgG1 into hIgE reduces FcεRI binding by 97% (14). Replacing this sequence in hIgE by the homologous rat sequence maintains binding to hFcrRI, but there is a loss of hFcrRI interaction, confirming earlier observations by others that rodent IgE recognizes only hFcεRI but not hFcrRI (10). Since recombinant GST-e-chain fusion proteins containing this sequence do not block IgE/FcεRI interaction, we conclude that sequences N- or C-terminal to this core peptide are essential for the provision of additional structural scaffolding in order to generate a receptor binding conformation. Viewed in the context of our model structure for IgE (18, 19), this core peptide has been computed to form a loop proximal to the interface between the ε3/4 domains that is homologous to the site in rodent IgG involved in the binding to the groove formed by the α1 and α2 domains of the neonatal FcγRn (24). Interestingly, as for IgGFcyRn interaction, we also observe two pH optima at pH 6.4 and 7.4 for hIgE/FcεRI interaction. While N- or C-terminal truncation has little effect on the association rate, deletion of C-terminal sequences increases the rate of dissociation several hundred-fold and reduces receptor occupancy at pH 6.4. The slow dissociation of IgE from FcεRIα therefore may be due, in part, to the stabilization of the interaction by His residues in the C-terminal region of the ligand.

**EXPERIMENTAL PROCEDURES**

Gene Constructs and Site-Specific Mutagenesis—The numbering scheme for h- and a-chain amino acid residues used in previous publications (6, 16–18) has been maintained. Polymerase chain reaction (PCR) was used to amplify e-chain fragments comprising the entire Fc region (residues 226–547), the Cγ3 from mouse IgG2a, the Cε2 domain (residues 226–329), and the Cε4 domain (residues 440–547). N-terminal deletions of the Fc region were prepared starting at amino acid residue 226 and terminating at residues 361, 357, 354, 353, 352, 345, and 340. The DNA products were purified by agarose gel electrophoresis, digested with appropriate restriction enzymes, and subcloned into the bacterial expression plasmids pGEX-3X and pGEX-KG, which direct the synthesis of foreign polypeptides in E. coli as fusions with the 26-kDa GST (26). Cloning the recombinant e-chain fragments in frame to the 3’ end of the GST gene facilitates the production of large amounts of fusion protein (~500 μg/l). In addition to a versatile multiple cloning site, the vectors have been engineered so that the GST carrier can be cleaved off by digestion with coagulation factor Xa or thrombin. The initial screening for receptor-blocking activity was carried out with partially purified GST-e-chain fusion peptides. Following affinity purification on rabbit anti-GST affinity columns and GST removal with thrombin, e-chains showed identical receptor-blocking capacities when compared with GST-e-chain fusion peptides. Therefore, this step was eliminated, and all assays described in this study were carried out with affinity-purified GST-e-chain fusion peptides. Short GST fusion peptides comprising e-chain residues 250–359 and 340–357 were also generated. Site-specific mutagenesis was performed by overlap extension PCR (25). Bacterial strains used as host for transformation were J109 and MC1061.

For the construction of the chimeric h/r IgE molecule we employed the e-chain expression plasmids pSV6-VhRr (18). A construct where the sequences known to be essential for hFcrRI interaction had been replaced by the homologous rat sequence encoding residues 341–356 was also generated by overlap extension PCR (25). The template for PCR was a 3.4-kilobase pair IgE Ce1-4 genomic DNA cassette cloned into the BamHI site in pUC19 (pH). A 719-base pair fragment essentially for Ce2-3 and Ce2 was generated by PCR. This involved two rounds of PCR and four primers, two external (5′ BglII, CTTGGAACTTCTACCGTGC; 3′ Ncol, CCTGCGCCATGGCTTCCACG) and two internal primers (5′ h-R1, CCTGGGCCCATGCCTTTACCTC; 3′ h-R1, CTTTTCATCGACGGCCTTGAGGTAGTGGGATCCCTTC).

For each PCR, reaction mixtures contained 200 ng of template, 2 μl of each primer, 1 μl dNTPs, 10 μl Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, and 100 μl, following a hot start 1 unit of Taq polymerase was added. An initial denaturation cycle at 96 °C for 6 min, 64 °C for 2 min, 72 °C for 1 min 30 s was followed by 30 cycles at 94 °C for 1 min 30 s, 64 °C for 1 min 30 s, 72 °C for 1 min 30 s. The resultant 719-base pair fragment was digested to pH ris by BglII and Ncol sites to give a chimeric Ce1-4 cassette, which was subcloned, using the BamHI sites, into the mammalian expression vector pSV6-V1 (18). The orientation of this cassette was checked by PCR.

The identity of all gene constructs was confirmed by sequencing the DNA of both strands.

**Expression—** E. coli strains transformed with the expression plasmids were grown overnight at 37 °C, and the overnight culture was diluted 100-fold into LB broth containing 10 μM ampicillin and grown to an absorbance of 0.4 at 600 nm at 37 °C. The inducer isopropyl-p-1-thio-β-D-galactopyranoside (Sigma) was added to a final concentration of 0.1 μM, and the cultures were grown under constant shaking at 37 °C for 4 h. Bacterial cells were harvested by centrifugation at 5,000 × g for 15 min, and the pellets were frozen at −70 °C until purification of the recombinant proteins. Freezing and subsequent thawing of the bacterial cells were essential to obtain effective solubilization of the recombinant proteins, which are expressed as insoluble inclusion bodies.

The chimeric pSV6-V1 construct was linearized using PvuI and electropolished into the J558 plasmacytoma cell line (18). J558 cells were cultured in Dulbecco’s modified Eagle’s medium (10% fetal calf serum, penicillin/streptomycin, gentamicin) and selection medium (Dulbecco’s modified Eagle’s medium, 10% fetal calf serum, penicillin/streptomycin, gentamicin, mycophenolic acid, xanthine, and hypoxanthine) was added 48 h after electroporation (18). High secreting clones were selected by employing linked immunosorbent assay.

**Purification of Recombinant GST-e-chain Fusion Proteins from E. coli Cell Pellets—** This was carried out using procedures described for the purification of recombinant e-chain fragments expressed in E. coli (6). Frozen cell pellets were defrosted on ice before homogenization (50 mg cell pellet volume) in 0.05 M Tris-HCl buffer, pH 7.9, containing 2 mM EDTA, 0.1 mM dithiothreitol, 0.25% NaCl, 0.1% sodium deoxycholate, 25 μM phenylmethylsulfonyl fluoride, 5% glycerol. The homogenates were dispersed by sonication and centrifugation before the addition of 100 μM lysosome and 20 μM DNase I. Homogenates were kept on a rotary shaker for 12–15 h at 4 °C before centrifugation at 10,000 × g for 10 min. The pellets were washed twice in a 20-fold pellet volume of 0.05 M Tris-HCl buffer, pH 7.9, containing 1 mM EDTA, 0.1 mM NaCl, 25 μM phenylmethylsulfonyl fluoride. Inclusion bodies from cell pellets were solubilized in 0.05 M Tris-HCl buffer, pH 7.9, containing 8 mM urea, 1 mM EDTA, 0.1 mM NaCl, 25 μM phenylmethylsulfonyl fluoride and dialyzed for 12 h against a 200-fold volume of the same buffer omitting urea but with the addition of 0.1 M dithiothreitol. Following dialysis, insoluble material was removed by centrifugation, and 30–75% of recombinant e-chain peptides were found in the supernatant fraction. Affinity purification from this fraction was carried out using a rabbit anti-GST antiserum coupled to Sepharose 4B. The chimeric h/r antibody was purified from cell culture supernatants using NP-specific affinity columns and analyzed by polyacrylamide gel electrophoresis (PAGE) and immunoblotting (6, 18).

**GeL Electrophoresis and Immuno blotting—** PAGE and electrophoretic procedures have been described before (6, 18). Blots were developed with a polyiodic peroxidase-conjugated anti-hIgE antibody (Dako).

**Ligand Binding Studies and Cell Culture—** hIgE VhR (18) was iodinated as described previously, and the conditions for ligand binding and cell culture have been published (23). Affinity-purified GST-e-chain fusion peptides were iodinated at 0–4 °C in 0.4 mM phosphate pH 7.4/7.5 using 4.4 μCi of Na125I and 150–300 μg of peptide in tubes coated with...
The FceRI Binding Region in Human IgE

RESULTS AND DISCUSSION

In the present study, we focused on the identification of the site(s) that determine the interaction of hiGE with its cellular receptors. The strategies employed for the expression of an overlapping family of chimeric GST-h-chain fusion proteins are outlined in Fig. 1. Panel A summarizes the receptor-binding capacities of the GST-h-chain fusion proteins, that of a chimeric /γ subunit, and that of a chimeric h/r IgE molecule. The assignment of biological activities is based on (i) competition and (ii) direct binding studies detailed in Fig. 2 and Table I. In Fig. 1, panels C and D show the electrophoretic mobilities of C- and N-terminally truncated recombinant GST-h-chain fusion proteins immunoprecipitated with a rabbit anti-GST antiserum, followed by PAGE analysis under nonreducing conditions and immunoblotting with a horseradish peroxidase-labeled rabbit anti-IgE serum. As shown in Fig. 1, C-terminal truncation yields a number of e-chain peptides for each construct. As judged by PAGE (Fig. 1C) and column chromatography (data not shown), approximately one-third of the peptides in each set corresponds to the full-length fusion peptide as a monomeric fragment. None of these fragments show any propensity to dimerize, although biologically inactive polymers aggregates form at concentrations >1.3 mg/ml. A set of identical fragments is observed following analysis under reducing conditions (data not shown). Most of the smaller e-chain fragments represent proteolytic cleavage fragments that are recognized by monoclonal antibodies specific for the Cε2 domain. In contrast, deletion of N-terminal sequences gives rise to two e-chain fragments and their apparent molecular weight under nonreducing (Fig. 1D) and reducing conditions (data not shown) indicates that they correspond, in almost equal quantities, to monomeric and dimeric GST-h-chain fusion proteins.

The present investigation confirms our previous observations, which show that only those peptides that contain Cε or the homologous Cγ3 domain can engage both FceRI and FcεRII (6, 16), while C-terminal truncation of the e-chain results in elimination of binding to FceRI. As summarized in Fig. 1A, sequences common to all fragments capable of binding to FcεRI comprise residues Pro443, Ser453 in the Cε3 domain. Further deletion from either the C- or N-terminal end beyond these residues is associated with a loss of FceRI binding. As shown in Figs. 2 and Table I, GST-(340–547) and GST-(226–354), which comprise the core peptide, inhibit the binding of hIGE with an 1C50 in the nanomolar range. In contrast, blocking of IGE/FcεRI interaction by the GST control, GST-(226–340), GST-(355–547), and GST-(440–547) is identical and cannot be detected above approximately micromolar concentrations. These results confirm observation by others who find that recombinant IGE-derived fragments comprising residues 355–547 do not block IGE binding to hFcεRI (11) and that substitution of residues 346–353 by the homologous sequence from IγG1 reduces binding of the chimera to background levels (14). As shown in our model structure of hIGE–Fc (Fig. 3) (18), this sequence forms a loop that is homologous to the loop in rIGG shown to bind to the neonatal FcγR (24). A further similarity emerged when we investigated the pH dependence of the binding of hIGE to FceRI. As shown in Fig. 4 two pH optima are observed for the binding of hIGE to FceRI, and occupancy of the receptor is almost twice as high at pH 6.4 as at pH 7.4. Although the significance of this is not known, it is tempting to speculate that hIGE has evolved the lower pH optimum as a result of its physiological importance in the fight against parasitic infestations in the lumen of the intestine at acid pH.

Data summarized in Table I show that N- or C-terminal

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B. Helm, unpublished observations.
truncation has a negligible effect on the rate of association of biologically active e-chain fragments with FcεRI. In contrast, the rate of dissociation increases several hundred-fold following the deletion of residues from the C-terminal end, and, as shown in Fig. 3, this is associated with a concomitant decrease in receptor occupancy at pH 6.4. Taken together, these data suggest that His residues in the C-terminal region of the IgE molecule make a contribution toward the maintenance of the high affinity interaction between IgE and FcεRI since this is largely determined by the slow rate of dissociation of the ligand from the receptor.

Results obtained in the current study differ in one significant respect from those in our previous investigation (6), where the FcεRI-blocking capacity of IgE-derived fragments was evaluated by the senior investigator, who performed passive cutaneous anaphylaxis tests in his own skin. Employing a well defined cellular assay system (23), we demonstrate here that N-terminal IgE sequences can be deleted beyond residue 340 without any significant effect on the kinetics of ligand/receptor interaction. Our data show that the essential determinant for hIgE/FcεRI recognition depends on a consecutive sequence comprising 11 amino acids computed to form a loop at the interface between the Cε3 and Cε4 domain (18). In accord with others (7–10), our observations exclude any direct contribution of Cε-specific residues as proposed by Stanworth et al. (12). Our results confirm and extend those made by Nissim et al. (8–10), who demonstrated that the receptor binding site in IgE is located in the Cε3 domain. They differ from the claims of Hamburger (29) and Gould et al. (20, 21), who propose, respectively, that residues 330–334 and 329–340 in the switch region between Cε2 and Cε3 are essential for IgE/FcεRI binding. As the results of our study clearly demonstrate, these sequences can be deleted without any major influence on the kinetics of hIgE/FcεRI interaction.

It is interesting to note that the active core sequence identified by us corresponds closely to the hIgE-derived peptide generated by Nio et al. (15), who report its capability to block the binding of antigen-specific IgE to cells expressing FcεRI at concentrations in the mM range (15).

Although the results of our study indicate that fragments containing the Cε2 domain show an increased susceptibility to proteolysis (Fig. 1C), the inclusion of the protease inhibitor phenylmethylsulfonfyl fluoride during the isolation procedure facilitates the purification of peptides that engage FcεRI/II. Using our published method, others have been unable to generate e-chain fragments in E. coli that retain FcεRI-binding capacity and have attributed this failure to folding problems (11). At least one other laboratory has expressed e-chain fragments in E. coli which are biologically active (30).

Based on the outcome of FcεRI binding studies with chimeric and mutant hIgE molecules, Presta and co-workers (14) proposed that six amino acid residues located in three loops, C-D, E-F, and F-G, computed to form the outer ridge on the most exposed side of the Cε3 domain, are involved in receptor binding primarily by electrostatic interactions (14). These conclusions were based on the observation that replacement of these...
residues reduced the binding of variant molecules to FcRRIα relative to native hIgE. We disagree with their conclusion in view of the fact that most of the mutations at Arg276 (408), Ser378 (411), Lys380 (415), Glu414 (452), Arg427 (465), and Met429 (469) (Presta et al., 14) numbering scheme in parentheses, which affect IgE/FcRRI interaction to a greater or lesser extent, are invariably due to replacements by residues of opposite charge or by a Pro, changes which could cause structural rearrangements. In contrast, more conservative substitutions of these residues either have little effect or cause an apparent enhancement of binding (Ref. 14, Table I). Our own study shows that a single point mutation involving Cys328, which by itself is not required for either FcRRI or FcRRII binding (18), can have a dramatic effect on the conformation of the IgE molecule. Its substitution by Met, but not Ser, destroys binding to both receptors (18). When we compared the intrinsic fluorescence of Trp residues in the native and IgE Met328 molecule, we found that on average native IgE has 41% of its Trp residues exposed, while IgE Met328 was found to have only 22% of Trp residues exposed, although both molecules were recognized by a conformation-dependent monoclonal antibody directed against the Cε2 domain. This observation shows that the substitution of a single amino acid that is not involved in receptor recognition can induce a significant deformation in structure and profoundly affect ligand/receptor association.

Presta et al. (14) have also claimed that the grafting of loops C-D, E-F, and F-G and the inter-Cε2/3 switch region into hIgG (which they refer to as IgGEL), conferred FcRRI binding to hIgG1. Their own data, however, on the binding of variant IgE do not support this interpretation. It is important to point out that their chimeric IgGEL construct still retains the endogenous IgG1 loop A-B sequence, which when grafted into hIgE (Ref. 14, Table I, variant 1) effected a 97% decrease in binding.

![Figure 2](http://www.jbc.org/)

**FIG. 2.** Percentage of inhibition of 125I-hIgE binding to RBL-2/2/C cells by native and recombinant hIgE-derived ε-chain fragments. To measure the inhibition (IC50) of 125I-hIgE to RBL-2/2/C cells by native IgE and recombinant ε-chain fragments, cells were preincubated at 22°C for 1 h with increasing concentrations (10⁻¹²⁻¹⁰⁻⁴ M) of each of the unlabeled GST fusion peptides in 125I-hIgE binding buffer or, as a negative control, GST or binding buffer. 125I-hIgE was then added (1 nM). After 45 min, the cells were washed twice with 0.5 ml of binding buffer and lysed in the same volume of lysis buffer, and aliquots were removed for γ-counting. The IC50 values for GST-ε(226–547) and GST-ε-(226–257) (data not shown) were identical to those observed for hIgE (△) and GST-ε-(226–354) (△). GST-ε-(226–340) (◯) and GST-ε-(355–547) (■), where the sequence common to all fragments that can engage FcRRI has been deleted by either the N- or C-terminal truncation, show inhibition levels similar to that obtained with GST (□) and all other fragments classified as nonbinders in Fig. 1. ▲, GST-ε-(340–547); ○, GST-ε-(440–547). Data shown represent the means of at least three separate experiments carried out in duplicate.

![Figure 3](http://www.jbc.org/)

**FIG. 3.** Drawing of the α-carbon trace of a model structure (18, 19) for hIgE with various fragments and domains indicated. The light chains are drawn with thin lines and the heavy chains with thick lines, one thicker than the other. The interchain Cys at position 328 is labeled. The 11-amino acid segment 343–353, which is common to all IgE-derived peptides that bind to FcRI, is drawn with bigger circles and wide, empty bonds in both heavy chains.

### Table 1

| Ligand          | k₁ (M⁻¹ s⁻¹) | k⁻¹ (s⁻¹) | Kd (M⁻¹) | IC₅₀ (nM) | n  |
|-----------------|--------------|-----------|----------|-----------|----|
| hIgE            |              |           |          |           |    |
| hIgE + ε (341–356) | 3.14 x 10⁶   | 1.3 x 10⁻⁵ | 4.1 x 10⁻¹⁰ | 14       | 8  |
| hIgE + ε (341–356) | 4.3 x 10⁴    | 1.7 x 10⁻⁵ | 3.9 x 10⁻¹⁰ | ND       | 1  |
| GST-ε-(226–547)  | 3.6 x 10⁹    | 1.6 x 10⁻⁵ | 4.4 x 10⁻¹⁰ | 19       | 2  |
| GST-ε-(326–547)  | 4.1 x 10⁹    | 2.95 x 10⁻⁵| 7.2 x 10⁻¹⁰| 23       | 2  |
| GST-ε-(340–547)  | 4.2 x 10⁹    | 9.8 x 10⁻⁵ | 2.3 x 10⁻¹⁰| 34       | 2  |
| GST-ε-(226–354)  | 4.3 x 10⁹    | 6.02 x 10⁻⁵| 1.4 x 10⁻⁸ | 332      | 3  |

*ND, not determined.*

The kinetics of association and dissociation of the affinity-purified ligands were determined at 22°C as described under "Materials and Methods." Kinetic constants and IC₅₀, which represents the concentration at which 50% inhibition of 125I-hIgE binding was obtained, were calculated on the basis that GST-ε-(226–547), GST-ε-(326–547), and GST-ε-(340–547) form dimers, while GST-ε-(226–354) is a monomer. Nonbinders showed no binding above background at 10⁻⁵ M. (Data shown represent the means of n = number of experiments carried out in duplicate.)
At present, limited structural information is available regarding the interaction between IgE and its receptors. Unlike IgG/FcεRI interaction, where the ligand can engage two receptors, IgE molecules bind to FcεRI in a 1:1 stoichiometry, although bilateral symmetrical protection to proteolysis has been observed when rodent IgE is complexed to the ε-chain (24, 31, 32). This has been explained in terms of a bent conformation of IgE (33), where the second ε-chain becomes inaccessible to an additional copy of the receptor, or to antibodies directed against epitopes in IgE that become masked following receptor engagement. There is little evidence for a beneficial role for IgE antibodies except in parasitic diseases, and such epitopes may therefore have an application as immunogens for the therapy of IgE-mediated allergies, since naturally occurring and monodonal antibodies (33–36) have been described that block the binding of IgE to cells expressing FcεRI but do not trigger mediator release. An improved understanding of the docking of hIgE to its receptors will provide the structural information needed for the rational design of such immunogens. The identification of the binding site constitutes a major step in this direction.

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