

REVIEW

The structural basis of human IgE–Fc receptor interactions

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This article reviews our current understanding of the structural basis of the interaction of hIgE with its class-specific Fc receptors and suggests how these studies can provide the input for the development of rational therapeutic interventions in allergic diseases. Following an introduction into the structural features of human (h) IgE, and its high- (hFcεRI) and low-affinity (hFcεRII/CD23) receptors, an overview of the large body of work, which has been carried out to identify the structural requirements and complementary binding sites on the ligand and its receptors, will be presented. This includes a critical account of the sometimes controversial data and the current consensus regarding the structural basis of the interactions. To conclude, we will evaluate how structure-function studies on the interaction of hIgE with its receptors can assist the development of more effective and safer anti-allergic drugs.

Introduction

The role of IgE antibodies in the initiation and propagation of the allergic cascade has long been established [1,2]. IgE interacts with a high-affinity receptor (FcεRI), initially found on mast cells and basophils, and more recently identified on a variety of cell types including epidermal Langerhan's [3] and eosinophils [4] and peripheral blood monocytes [5] and blood dendritic cells [6]. IgE also binds to a low-affinity receptor (FcεRII/CD23). Again, this receptor is found on a number of cell types including B cells, eosinophils, platelets, natural killer cells, T cells, follicular dendritic cells and Langerhan's cells (reviewed [7,8]). Central to the design of rational intervention strategies is a detailed understanding of the structural basis of the interaction between IgE and its receptors.

Of particular interest is the IgE–FcεRI interaction due to the importance of FcεRI-mediated exocytosis in the initiation of the allergic cascade [9]. *In vivo*, release of cellular mediators is achieved by the initial binding of IgE

(sensitization) to FcεRI, followed by crosslinking of the IgE/FcεRI complex by polyvalent antigens (allergens). In a number of unidentified steps, which include the recruitment of intracellular protein kinases, phospholipases and Ca^{2+} mobilization, a rapid degranulation resulting in the release of preformed and newly synthesized pro-inflammatory mediators. These include, e.g. histamine, leukotrienes, prostaglandins and a number of cytokines including interleukin (IL)-3, 4, 5, 6, 10, 13, tumour necrosis factor (TNF)- α , and granulocyte/macrophage colony-stimulating factor, which causes immediate hypersensitivity reactions. In addition, several of these mediators have a chemoattractant activity and induce, within 6–12 h, the influx of inflammatory cells, which are responsible for the symptoms associated with delayed hypersensitivity reactions. Inhibition of the initial sensitization of cells expressing FcεRI can therefore be expected to confer profound anti-inflammatory benefits since it would prevent both early and late responses and inhibit the ligand induced up-regulation of receptor expression [10].

The IgE–FcεRII/CD23 interaction has been implicated in many IgE-dependent roles, including combating parasitic infestations [11], the induction of IgE synthesis [12] and the maintenance/modulation of the IgE response [13]. Although additional IgE-independent roles have also been suggested for FcεRII [7,8], the role of the receptor in the regulation of the IgE response is of particular interest, since it may provide a target for the design of anti-allergic drugs that help to adjust regulatory circuits in allergic disease.

IgE is one of the five classes of immunoglobulins found in mammals and was the last to be discovered due to its low abundance in serum (50–1000 $\mu\text{g/L}$, compared with IgG (5–15 g/L) [14]. The molecule has a molecular weight of ≈ 192 kDa and is heavily glycosylated (12–15%). It is composed of two identical light chains (λ or κ) and two identical (ϵ) heavy chains arranged in a twofold axis of symmetry. As for other Ig classes, these are composed of Ig domains. IgE, like IgM has four constant domains, in contrast only three constant domains are found in IgG, IgA and IgD. The additional domain (C ϵ 2) is thought to replace the flexible hinge region found in other antibody

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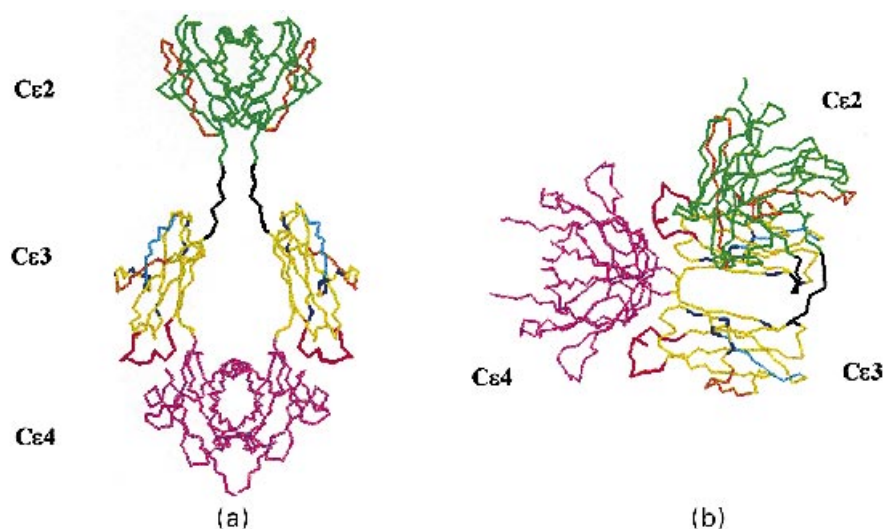


Fig. 1. Structural models of human IgE-Fc in the coplanar (a) and bent conformation (b). The location of regions implicated in the interaction of hIgE with its receptors are shown in the context of structural models of IgE in the coplanar and bent conformation. The predicted structures of hIgE-Fc are based on sequence homology with IgG₁, the demonstration of the parallel nature of the inter-Cε2 disulphide bonds and biophysical data which suggest that IgE bends at the Cε2/3 and/or Cε3/4 interface [15,16,18–21,34]. Colours correspond with: Cε2 (green), Cε3 (yellow), and Cε4 (violet). Sequences thought to influence IgE/FcεRI binding include; the Cε2–3 interface (aa330–335) (black) [26,28,37,38], the A–B loop, aa343–353 (red) [29,30], aa306–313, 311–320, 331–338, 382–391 (orange) [33] and residues R376, S378, K380, D414, R427, M429 (blue) [39]. Regions that have been implicated in FcεRII/CD23 binding include: aa367–376 (cyan) [40,41], the Cε2–3 interface (black) [33] and the A–B loop [30,44].

classes. Papain digestion of IgE yields the Fab fragment that complexes allergen and the Fc region that confers isotype specificity and determines the interaction with class-specific Fc receptors.

In the absence of high resolution structure determinations for any part of the IgE molecule, molecular models have been proposed. Based on sequence homology to the Cγ2 and Cγ3 domains of IgG₁, whose crystal structure has been determined [15], molecular models were developed for the Cε3 and Cε4 domains of hIgE [16,17]. In the absence of an appropriate template for the Cε2 domain, model structures initially projected an arrangement whereby Cys241 in one Cε2 domain binds to Cys328 in the opposite chain [16,17]. This model [16,17] was later revised to incorporate the parallel interchain Cε2 disulphide bond arrangement determined experimentally [18]. The conformation of IgE in solution and when complexed to FcεRI is believed to be bent from the planar position. This was first proposed for rodent IgE based on fluorescence energy transfer (FRET) measurements. A distance of ≈ 7.1 nm between the N- and C-terminal regions of the molecule for IgE in solution and FcεRI bound was observed, indicating a deviation from the planar Y-shape, where the distance between the N-terminal V-regions and C-terminal Cε4 domains was computed to be ≈ 18 nm [19,20]. Further evidence for a bent conformation has also been demonstrated for human IgE from synchrotron X-ray and pulsed neutron scattering data [21]. Therefore, in

solution and when receptor complexed, IgE is a compact, fairly rigid and bent molecule with a limited degree of flexibility at the Cε2–3 and/or Cε3–4 domain interface. These features are depicted in Fig. 1.

Due to the pivotal importance of the IgE–FcεRI interaction (see above) the majority of this work has centred around the elucidation of the FcεRI binding site on human IgE. The identification of the co-ordinates of the interface between IgE and its receptors would provide the optimum information for the design of rational IgE antagonists. In the absence of such data, early molecular models have been refined on the basis of data obtained from the assessment of the biological activities of proteolytic digestion fragments, IgE-derived synthetic and recombinant peptides, human and rodent chimeras and mutant forms of hIgE.

The FcεRI binding site(s) on hIgE

Proteolytic digestion of myeloma IgE (ND) using papain demonstrated that the Fc region of the molecule (residues 227–547) retained FcεRI binding activity [1], while proteolytic digestion with pepsin yielded smaller fragments (residues 1–338, 339–349, 350–547), none of which could block passive cutaneous anaphylaxis (PCA) [2,22]. Further attempts to generate smaller biologically active fragments of IgE Fc were facilitated through the expression of recombinant

ϵ -chain fragments in *Escherichia coli*. The initial conclusion from these studies suggested that sequences N- and C-terminal to residue 340 were required, implicating both the C ϵ 2 and C ϵ 3 domain in Fc ϵ RI interaction [23], as did experiments by Weetall *et al.* [24]. Further studies employing chimeric mouse/human immunoglobulins identified the C ϵ 3 domain as the major binding region for the Fc ϵ RI interaction [25]. Subsequent investigations employing recombinant ϵ -chain fragments expressed in *E. coli* suggested that the Fc ϵ RI binding site on human IgE is contained within a 34 amino acid sequence, comprising residues Cys328-Val 368 of the C ϵ 3 domain (reviewed in [26]). In another study, recombinant fragments of the IgE Fc region generated in mammalian cells identified residues 329–547 (C ϵ 3–4) as the smallest fragment that could bind Fc ϵ RI [27]. IgE-derived synthetic peptides have implicated residues 330–335 in the C ϵ 2/C ϵ 3 interface and residues 345–352 of the C ϵ 3 A–B loop [28,29]. The interpretation of both of these studies has been questioned. The Hamburger study used PCA [28], which can give rise to disputable interpretations [30] and the high concentration of peptide (mmol/L range) and mode of assay employed by Nio and coworkers [29] to show inhibition of IgE effector function (PCA) made meaningful interpretation difficult. Our own studies, based on the generation of a large number of IgE Fc fragments expressed in the form of glutathione-S- transferase (GST) fusion proteins in *E. coli*, led to the identification of a core sequence of 11 amino acids in C ϵ 3, which is common to all N- and C-terminally truncated ϵ -chain fragments that display Fc ϵ RI binding activity [30]. Residues Pro343-Ser353 have been computed to form a loop (Ω) that joins β -strands A and B (A–B loop) in the C ϵ 3 domain, see Fig. 1. The role of additional residues in the C terminal of C ϵ 3 for the maintenance of the high-affinity of the interaction between hIgE and Fc ϵ RI was also identified [30] and has been confirmed more recently by site directed mutagenesis studies of hIgE-Fc [31].

Monoclonal antibodies against human IgE Fc have provided additional evidence for the role of the Fc region and different groups of investigators suggested a role for residues Ser306-Gln313, Thr311-Thr320 of C ϵ 2, Ser331-Ala338 and Val382-Lys391 of C ϵ 3 [32,33]. Whether epitopes recognized by these antibodies identify sequences in close contact with the receptor or inhibit the complementary interaction by steric hindrance remains to be established. We have generated C ϵ 3 A–B loop-derived peptides and constrained the conformation of one of them to mimic more closely the computed Ω loop conformation [34]. The constrained loop, but not the linear peptide, was able to inhibit the IgE–Fc ϵ RI interaction (μ mol/L range) [34]. Moreover, antibodies raised against the loop peptide were able to bind IgE in solution, but not when complexed to the receptor, providing

additional evidence for a role of the A–B loop in IgE/Fc ϵ RI interaction [34,35].

Several protein engineering studies have been conducted in an attempt to identify class-specific effector residues. The C ϵ 2–3 interface comprising of residues 330–335 has been implicated in several studies (see above). Helm *et al.* [18] initially suggested that this region might be a site of interaction due to the homology of this region to the site in IgG₁ (residues 234–237) that interacts with Fc γ RI [36]. Pro333 was of particular interest due to its potential role in the maintenance of a bent IgE conformation. Studies by Beavil *et al.* [26] suggested that its mutation to Glu resulted in a sixfold reduction in the K_A due to an increased dissociation rate, while replacement by Gln was found to have no effect [37]. We also examined the role of Pro333 with regard to Fc ϵ RI recognition and IgE-mediated stimulus secretion coupling. The latter function was not assessed by Beavil and collaborators [26,37]. Although we observed only a minimal change in interaction kinetics, mutation of Pro333 to Ala resulted in a significant reduction, while mutation to Gly led to a complete loss of Fc ϵ RI-mediated effector function [31]. In view of the fact that this region can be deleted without any significant loss of Fc ϵ RI binding activity, we suggested that Pro333 plays a role in the restriction of the conformation required for IgE-mediated stimulus secretion coupling [31,35].

McDonnell and coworkers [37] originally proposed a direct role for Arg334 in this region, and reported that its mutation to Ser resulted in a 34-fold reduction in the K_A when binding to the cellular receptor was assessed. More recently analysis of the effect of this mutation within Fc ϵ 3–4 by Surface Plasmon Resonance (SPR) led to the proposal that Arg334 is a major effector-determinant residue [38]. We re-assessed the conclusions of this study and have presented evidence that the treatment of SPR data in terms of summed exponentials by Henry *et al.* is of questionable validity [31].

Using site-directed mutagenesis we have examined residues in the C ϵ 3 A–B loop. The outcome of this study did not identify any residue as essential for Fc ϵ RI binding, although a role for the entire A–B loop for the correct docking of the ligand into the receptor was postulated [31]. An extensive number of human IgE mutants were generated by Presta and coworkers, a role for six amino acids contained within three proximal C ϵ 3 loops (C-D, E-F and F-G) was suggested [39]. The residues included: Arg376, Ser378, Lys380, Glu414, Arg427 and Met429. Re-evaluation of their data by our group led us to question the significance attributed to these specific residues and we have demonstrated that the data of Presta *et al.* provide direct evidence for the role of the A–B loop in the docking of hIgE to Fc ϵ RI [30,34].

To summarize, the main regions implicated to influence Fc ϵ RI-mediated effector functions of hIgE have been

mapped to the Cε2–3 interface and A–B loop of the Cε3 domain. Other exposed regions in the Cε3 domain, including His residues [30], appear to be essential for the maintenance of the high affinity of interaction between hIgE and FcεRI since deletion of C-terminal residues increases dissociation of the truncated ligand from the receptor by more than three orders of magnitude. These areas are depicted in Fig. 1.

The FcεRII binding site(s) on hIgE

The FcεRII binding region(s) in human IgE have been mapped employing strategies identical to those used for the mapping of the FcεRI binding site. Monoclonal antibodies [32,33,40–43] defined the following regions: Cε3 residues 367–376 [41], Cε3 residues 367–370 [40], Cε3 residues 364–383, 401–415 [42], while IgE–Fc- derived fragments expressed in *E. coli* [30,40], domain swapping and loop grafting experiments [25] identified the Cε3 A–B loop [25,30,44] as the effector determinant site of interaction with FcεRII. In addition, we have also obtained compelling evidence that additional distinct structural requirements must be met for FcεRII, but not FcεRI interaction. For FcεRII interaction the Cε4 domains are required for the formation of an active dimeric molecule. The monomeric IgE fragment generated by mutation of the nucleation site for dimerization site at Phe506 was found to be inactive [40]. However, our observations exclude any participation of Cε4-specific residues since replacement of the Cε4 domain by Cγ3 domains from mouse IgG produces a dimeric fragment which was found to bind FcεRII, although with 10-fold reduced affinity. This confirms the requirement for dimerization and mapping of the binding site to the Cε3 domain [30,40]. This is in agreement with the study of Nissim and coworkers who replaced hIgE residues 330–346 and Cε4 by the homologous rodent residues while maintaining hFcεRII binding [25]. Our recent study of the hIgE–FcεRII interaction identified Lys352 in the A–B loop as the major effector determinant residue and provided additional support for the notion that glycosylation at N371 inhibits IgE/FcεRII interaction [44].

Collectively, these data show that the actual binding region in IgE involved in the interaction with FcεRI and FcεRII are in close proximity, although more rigorous structural requirements are imposed on the interaction between IgE and FcεRII [30,31,44]. These differences are not surprising due to the lack of structural similarities between these two Fc receptors.

The high affinity receptor (FcεRI)

The 'high affinity receptor' (FcεRI) engages IgE with a 1000

higher affinity ($k_A \approx 10^{10} \text{ M}^{-1}$) compared with FcεRII/CD23, which binds IgE with relatively 'low affinity' ($k_A \approx 10^7 \text{ M}^{-1}$). FcεRI is expressed on a variety of cell types (see introduction) and has been implicated in several functions including IgE-mediated hypersensitivity, antigen presentation [45] and IgE-dependent killing of *schistosomes* [4]. The receptor is composed of four transmembrane polypeptides, which are noncovalently attached comprising the FcεRIα chain (232 aa), FcεRIγ chain (244 aa) and two disulphide linked FcεRIγ chains (68 aa). The α chain is a member of the Ig superfamily and its extracellular (EC) domain (45–65 kDa) contains the IgE binding site, the β-(32 kDa) and γ chains (7–9 kDa) are required for surface expression of the receptor complex and transmembrane signalling. The receptor composition is αβγ₂ (Reviewed [46]), but hFcεRI has also been found as αγ₂ complex [47].

The EC domains of FcεRIα contain the IgE binding region [48]. The α-chain is a member of the Ig superfamily subset and contains two small Ig-like domains, D1 and D2, each 86 aa in length and both are heavily N-glycosylated (seven sites total). We have built models of these two Ig-like domains on the basis of sequence similarity with the CL domain of a murine κ-light chain [49,50]. This model is shown in Fig. 2. Others have employed the C-2 set of CD-2 [51] as a template [52,53], although no co-ordinates for this model are available for comparison. Our recent study of the IgE–FcεRII interaction showed that replacement of Lys 352, which is conserved among primate, canine and equine species, by the rodent homologue Gly, destroys human FcεRII recognition [44].

The IgE binding site(s) on FcεRI

The IgE binding site has been mapped to the extracellular (EC) domains of FcεRIα, using soluble(s) forms of the two extracellular domains [55–59]. We have confirmed these observations with α-chain fragments expressed in the methylotrophic yeast *Pichia pastoris* [60,61]. These soluble forms of the EC region retained IgE binding activity similar to the native receptor and EC domains generated by phage display [56]. We have obtained identical interaction kinetics ($k_A \approx 10^{10} \text{ M}^{-1}$) for the EC α-chain fragment generated in yeast when applying pseudo first order kinetics obtained by SPR and these data are in excellent agreement with previous determinations using cellular-based techniques [31].

The IgE binding site(s) has been further localized to the second domain (D2) of FcεRIα using phage display of D2 alone [56]. We have shown that, following expression in *Pichia pastoris*, the second Ig domain of FcεRIα binds IgE with low affinity [58,61]. Several strategies have been used to identify sites in D2. A mouse monoclonal antibody (15 A5) which mapped to residues 100–115 (B–C loop, B

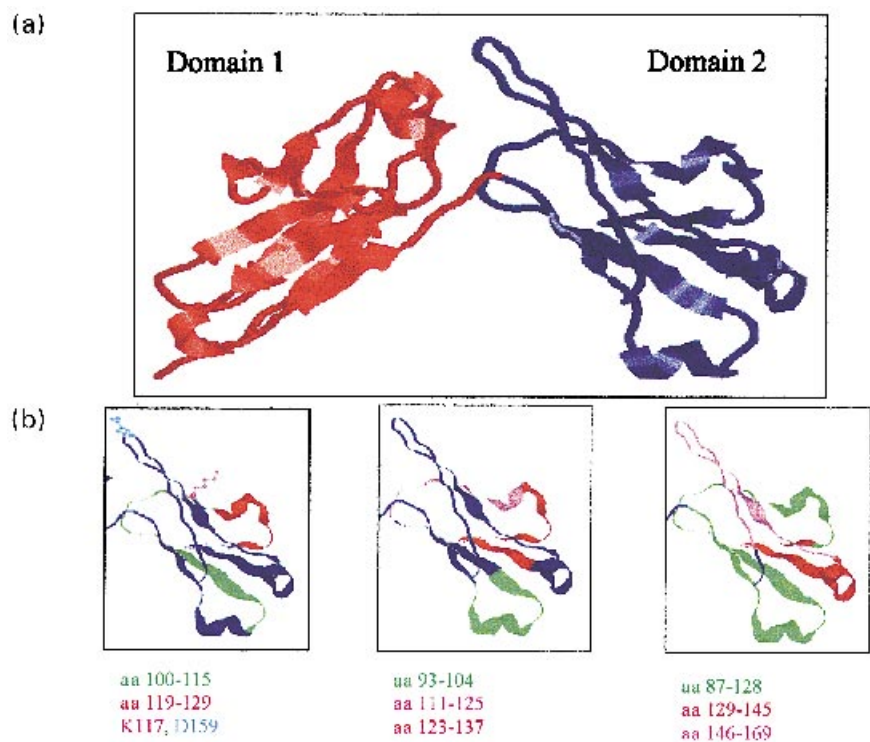


Fig. 2. The high affinity Fc receptor α chain (Fc ϵ RI α). Panel (a) the hFc ϵ RI α -chain extracellular domains (domain 1 aa 1–86, domain 2 aa 87–172) were modelled using homology to with the constant domain of a murine κ -light chain [49]. The predicted domain structure of seven antiparallel β strands, arranged as two β sheets (three and four strands) linked with a single disulphide bond, is clearly visible, the seven sites N-glycosylation, which do not contribute to the complementary interaction, have been omitted. Panel (b) illustrates regions of domain 2 thought to be involved in the interaction with IgE including: aa 100–115 [62], aa 93–104, 111–125, 123–137 [64], aa 87–128, 129–145, 146–169 [63], aa 119–129 [65] and D159, K117 [66].

strand) inhibited the interaction suggesting a role for this region [62], although steric hindrance could not be ruled out. Chimeric Fc ϵ RI/Fc γ RII Fc receptors suggested a role for residues 87–128, 129–145 and 146–169 [63], homologue scanning mutagenesis identified residues 93–104, 111–125 and 123–137 (loops F–G, C'–E, B–C of domain 2) [64]. A constrained receptor-derived peptide has been identified that inhibits the IgE–Fc ϵ RI interaction with an IC₅₀ in the μ molar range, suggesting a role for the C'–C loop [65] in the complementary interaction. More recently, residues Lys117 and Asp159 of domain 2 have been implicated in IgE binding. Mutation of Lys117 to Asp reduced the binding affinity of the interaction 30-fold, while mutation of Asp159 to Lys increased the affinity of the interaction by a factor of seven [66]. In view, however, of the treatment of the data in terms of summed exponentials, we believe that little confidence can be placed in the numerical values of the rate and affinity constants proposed by Cook *et al.* [66].

Although a direct function for domain 2 of Fc ϵ RI α chain has been established, the precise role of domain 1 remains unclear. A role in conferring high affinity for the interaction may occur either through a direct or indirect mechanism. The relative association between the two Ig-like domains of Fc ϵ RI α , which are both required for high-affinity IgE binding, remains uncertain, although we have suggested two possible modes [49]. A more direct involvement for D1 has been proposed for the interaction of rodent IgE with its

receptor. This suggests that the structural requirements for human and rat Fc ϵ RI binding are diverse. It is interesting in view of the fact that rodent IgE can engage the human receptor, but human IgE does not recognize the rodent receptor. The structural basis for this phenomenon, however, remains to be established. Figure 2(b) illustrates the regions of domain 2 implicated in IgE binding.

The human IgE–Fc ϵ RI interaction

The main binding site(s) on the IgE molecule are located within the C ϵ 3 domain and domain 2 of Fc ϵ RI α . The K_A of this interaction has been shown to be $\approx 10^{10} \text{ M}^{-1}$ in numerous studies using cell-based assays [27,67–69]. We have obtained this value using a cellular model system [30,70] and more recently employing SPR and a recombinant soluble form of the Fc ϵ RI α extracellular domains using the simple pseudo first order model [31]. Data analysis obtained from SPR measurements indicated that IgE/Fc ϵ RI interaction is biphasic [38], which was confirmed by our studies [31]. The precise mode of interaction, however, requires further investigation [31]. Glycosylation of either molecule is not required for the interaction [40,55–57]. A pH dependency has been demonstrated for the interaction of hIgE with Fc ϵ RI, with optimum receptor occupancy at 6.4 and 7.4 suggesting a role for His residues in hIgE the in maintenance of the high affinity of interaction [30].

The complementary interaction is thought to involve a

1:1 binding stoichiometry. This was determined using fluorescence studies and hydrodynamic studies [56,71]. It appears paradoxical in view of the fact that the IgE molecule contains two ϵ chains and has therefore two potential binding sites. It has been rationalized by the finding that IgE in solution and when receptor engaged is bent from the planar position [19,20]. The most obvious explanation for the 1:1 stoichiometry would be that the bent conformation renders the second ϵ chain inaccessible, via steric effects involving the C ϵ 2 domain twisting over the face of C ϵ 3, see Fig. 1(b). However, studies by Keown *et al.* [72] appear to rule out this possibility. An alternative explanation is that conformational changes occur during binding and/or that the binding of the α -chain to one site may prevent the interaction with the second site. Interestingly, the removal of structural constraints associated with the mutation of Pro333 to A or G at the C ϵ 2–3 interface dramatically reduces the ability of IgE to mediate stimulus secretion coupling, although the effect on the kinetics of interaction is minimal. This suggests that this residue may play a role in the formation of a bent IgE, and that a decrease in structural constraints computed to accompany the mutation of Pro to Gly inhibits the ability of the ligand to mediate receptor aggregation and secretory responses. Further studies to identify the molecular basis of this unexpected observation are necessary since they hold promise for the design of Fc ϵ RI blocking agents which cannot induce mediator secretion upon crosslinking [31].

The low affinity receptor (Fc ϵ RII/CD23)

Structurally Fc ϵ RII/CD23 is not a member of the Ig superfamily but a type 2 integral membrane glycoprotein. The receptor has an apparent molecular weight of 45–55 kDa (321 aa). The N terminal cytoplasmic tail can exist in either of two forms, Fc ϵ RIIa and Fc ϵ RIIb, which differ only by 6/7 residues in the N terminal cytoplasmic domain, Fc ϵ RIIa-MEEGQYS and Fc ϵ RIIb-MNPPSQ [73]. These forms confer distinctly different functions on the receptor: Fc ϵ RIIa mediates endocytosis by B cells, while Fc ϵ RIIb is involved in IgE-mediated phagocytosis by a number of cells including monocytes and eosinophils. The single TM (24 aa) and large C terminal extracellular region are common to both forms. The extracellular region contains a C-type (calcium dependent) lectin domain, which places the receptor in a family of adhesion proteins known as selectins. This lectin domain is flanked on the C terminal side by a sequence of unknown tertiary structure that contains an inverse RGD (Arg-Gly-Asp) sequence providing further evidence for a role in cell adhesion. On the N terminal side of the lectin domain is a repetitive region composed of five heptadic repeats of hydrophobic Leu/Ileu residues. It is predicted

that these repeats form an α helical stalk involved in trimer formation [74,75]. See Fig. 3.

Auto-proteolysis of Fc ϵ RII produces progressively smaller, soluble receptor fragments including 37 kDa, 33 kDa, 29 kDa, 25 kDa and 16 kDa peptides. These soluble forms of the receptor have been shown to have diverse cytokine-like properties. Expression is under the control of interleukin-4 and interferon- γ , this therefore has obvious implications in allergy, where it has been found that expression of Fc ϵ RII and sFc ϵ RII/CD23 fragments is required for an IgE response [12].

Fc ϵ RII has been shown to bind IgE in a species-dependent manner (monomeric IgE $K_A \approx 10^7 M^{-1}$, dimers and complexes $K_A \approx 10^8 M^{-1}$) [10]. The molecule has been identified on several cell types and is implicated in many diverse functions (see introduction). CD21, a second ligand for CD23, is a membrane protein found on B cells, follicular dendritic cells and basophils and is involved in the modulation of IgE responses [76,77]. More recently, two further ligands for CD23, the β 2-integrins CD18/CD11b and CD18/CD11c, have been identified on monocytes [78].

As no high-resolution structure exists for human Fc ϵ RII, this has led to modelling studies on the basis of homology to rat mannose binding protein (rMBP) [50,79,80]. rMBP is a member of the C-type carbohydrate binding proteins. Its crystal structure has been determined [81] and this provided the template for a structural model of Fc ϵ RII [50,79,80]. See Fig. 3. Recently, these co-ordinates [79] have been used to generate a model of the entire molecule by splicing the lectin head onto a stalk region [82].

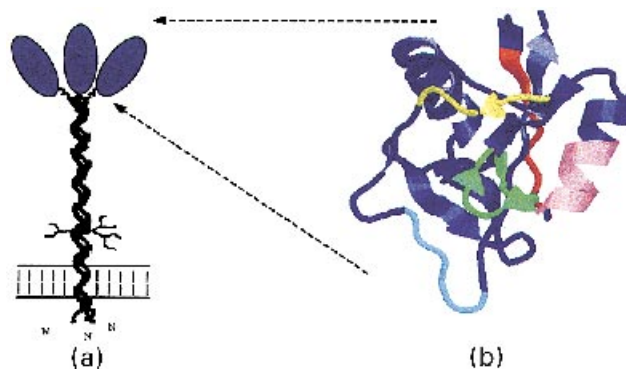


Fig. 3. The low affinity Fc receptor (CD23/Fc ϵ RII). Panel (a) shows a schematic representation of the hFc ϵ RII/CD23 molecule depicting the triple helical coiled coil stalk region that has been computed to connect the extracellular lectin homology domains with the N-terminal cytoplasmic domain. The single N-glycosylation site is also shown. Panel (b), model of lectin-homology domain [79]. Regions involved in IgE binding are coloured; residues 176–183 (red), 185–190 (violet), 224–233 (green), 238–243 (yellow) and 251–256 (cyan) [84].

The interaction between IgE and FcεRII/CD23

In contrast with the IgE–FcεRI interaction the IgE–FcεRII/CD23 complex is thought to involve a 2:1 binding stoichiometry, with two lectin heads interacting with the two ε chains of an individual IgE molecule. It is believed that there are two binding sites on IgE that interact with residues on the dimeric/trimeric FcεRII [83]. As mentioned earlier a residue within the Cε3 A–B loop and a region close to the glycosylation site at N371 play a critical role in the interaction of IgE with the lectin head region of the receptor, see Figs 1 and 3.

The IgE binding site in FcεRII/CD23 has been identified employing a soluble 16 kDa form of the lectin homology domain expressed in NS-O cells (residues 151–288) [83]. Homologue scanning mutagenesis has been used to define the IgE binding region on FcεRII further. These data suggested a role for discontinuous segments contained within residues 165–190 and 224–256 [84]. See Fig. 3(b). There is a distinct region on the molecule to which all the critical segments identified by Bettler *et al.* [84] contribute, while the opposite face is probably involved in the interaction with glycosyl residues. This face of the molecule site has been predicted to be analogous to the carbohydrate binding region of rat MBP and is implicated in the interaction between FcεRII/CD23 and CD21/CR2, which is Ca²⁺-dependent and inhibited by fucose-1-phosphate [85]. In contrast, there is no evidence for a contribution of carbohydrate to the complementary interaction between IgE and FcεRII. In fact enzymatically deglycosylated IgE [40] or IgE variants where the site of carbohydrate attachment has been mutated, engage FcεRII/CD23 with 10-fold increased binding affinity [44].

Concluding remarks

Many approaches have been employed to gain information concerning structural features and molecular forces that govern the interaction between IgE and its class-specific Fc-receptors. Approaches have focused predominantly on the use of novel recombinant proteins or peptides based on the sequence of IgE or FcεRI [34,65,86,87] and monoclonal antibodies against human IgE [88], FcεRII [89] and FcεRI [90]. The identification of binding regions on IgE and FcεRIα has led to the design of constrained peptides and it has been shown that the binding of IgE to the α-chain of FcεRI can be blocked by both IgE- and receptor-derived peptides. The therapeutic utility of these peptides is, however, doubtful in view of the short half life and 10⁴–10⁷-fold lower affinities compared with the native molecules. Furthermore, the administration of receptor-derived peptides carries the inherent danger of eliciting an antipeptide immune response which can activate mast cell degranulation through receptor crosslinking. A phenomenon we

observed recently may also assist the development of more effective and safer anti-allergic drugs. It emerged when Pro333 at the Cε2–3 interface was substituted by Gly. Surprisingly, only a small alteration in the binding kinetics of the FcεRI interaction was evident, although FcεRI-mediated stimulus secretion coupling was completely abrogated. It is tempting to speculate that understanding this phenomenon may assist the design of inhibitors that can actively compete with hIgE for receptor sites while inhibiting FcεRI-mediated function [31].

Investigations leading to the development of nonanaphylactogenic mouse monoclonal (MoAb) antihuman IgE antibodies [88,91] appear particularly promising since these antibodies are potent inhibitors of the IgE–FcεRI interaction. Several MoAbs have now been humanized and are in clinical trials [91]. These intervention strategies can be considered as passive immunotherapy, it may, however, be possible to actively immunize atopic individuals since there is evidence that neonatal or adult immunization with IgE can stimulate the formation of therapeutically useful anti-IgE auto-antibodies in animal systems [92]. Their action suggests that the identification of structural determinants in IgE, which participate directly in IgE–receptor interaction, may form the basis for the development of peptide immunogens to induce the formation of antibodies, which can bind specifically to the receptor binding sites of IgE in the circulation, and to membrane IgE on B cells. Used as vaccines, such peptides could have a direct application in the treatment of all IgE-mediated allergies. This intervention strategy could be particularly effective for the permanent protection of subjects at risk from anaphylactic shock from, e.g. insect stings, food hypersensitivity, and in asthma, where continuous passive immunization may not be practical.

In view of the inherent risk associated with the induction of anaphylactogenic antibodies, high-resolution structural information concerning the complementary site(s) of interaction between the ligand and its receptors is an essential prerequisite for the development of safe anti-allergic vaccines, since the models we have developed can only be considered approximate templates.

Acknowledgements

This work was supported by funds from the Medical Research Council, the Wellcome Trust, the European Union and NATO.

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