



Review

Structural insights into the interactions between human IgE and its high affinity receptor FcεRI

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Abstract

The interaction of IgE antibodies with the high affinity IgE receptor, FcεRI, is a key step in the initiation of anti-parasitic immunity and allergic reactions. Recent structural studies of the receptor, the IgE–Fc and the IgE–Fc:FcεRI complex have revealed how these two proteins interact to prime mast cell responses to antigen. The structures have revealed a novel arrangement for the FcεRI ectodomains that is also observed in homologous members of this antibody receptor family. The crystal structure of the IgE–Fc:FcεRI complex clarified how a 1:1 complex between the antibody and receptor is formed, with the receptor binding each chain of the antibody Fc dimer. The IgE–Fc structure in the absence of the receptor revealed the potential for large conformational rearrangements within the IgE that may affect receptor binding. These studies provide the basis for further investigation of the specificity of antibody:receptor binding and for the development of new treatments for allergic hypersensitivities. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The marshalling of the immune response to pathogenic threats involves the coordination of cellular and molecular defense mechanisms. The stimulation of the antibody response results in the production of antibody isotypes that are tailored to stimulate a variety of cellular responses ranging from the activation of inflammatory pathways, to the engulfment or destruction of pathogens, to the targeted killing of infected or cancerous cells (Daeron, 1997). Soluble antibodies are produced by plasma cells that have terminally switched to the dedicated production of a specific antibody isotype, such as IgG, IgE, IgA, or IgM. Antibodies of different isotypes are thought to be preferentially associated with fighting off infections defined by the type or location of the offending pathogen.

Antibodies can act as a bridge between the humoral and cellular responses of the immune system, in part, by binding to their Fc receptors, which are expressed on a variety of granulocytic and phagocytic cells of the immune system (Daeron, 1997). The types of receptors, their affinities for

various Fc regions, and the cells on which the receptors are located all contribute to the diversity of the immune response to a given antigen. The largest class of Fc receptors belongs to the immunoglobulin (Ig) superfamily and includes three distinct receptors with specificity for IgG: FcγRI, FcγRII and FcγRIII. Also belonging to this receptor family is FcεRI, which is specific for IgE antibodies (Kinet, 1999; Metzger, 1992). FcεRI has the highest affinity of all immunoglobulin receptors, with a binding constant in the 10^{–9} to 10^{–10} M range for its ligand, IgE (Daeron, 1997; Kinet, 1999; Metzger, 1992).

IgE antibodies are associated with the immune response to parasitic infections and are at the core of allergic and asthmatic reactions (Kinet, 1999; Turner and Kinet, 1999). IgE bound to FcεRI expressed on the surface of mast cells and basophils is thought to play a central role in the activation of immediate-type hypersensitivities associated with the allergic response. In IgE-mediated responses, cross-linking of receptor-bound IgE via a multivalent antigen causes the receptor molecules to aggregate, leading to the immediate phosphorylation of the receptor, the rapid release of histamine, and the biosynthesis of leukotrienes and cytokines that act later in the inflammatory cascade (Turner and Kinet, 1999).

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Even in the absence of antigen, the presence of Fc ϵ RI-bound IgE may affect mast cell physiology. Recently, it has been observed that monomeric IgE can stimulate mast cell survival through signaling pathways that are distinct from those initiated by receptor cross-linking (Asai et al., 2001; Kalesnikoff et al., 2001). Thus, the binding of IgE to Fc ϵ RI may contribute to the expansion of mast cell pools in atopic individuals with elevated IgE levels. The mechanisms by which monomeric IgE bound to Fc ϵ RI stimulates mast cell survival remain to be fully clarified.

The serum concentration of IgE is the lowest of all antibodies, found in the range of 50–300 μ g/ml in humans. Most of the IgE antibody appears to be present in the cell-bound state—that is, it is pre-bound to its high affinity receptor, Fc ϵ RI, on the surface of mast cells and basophils. Recent studies in the treatment of allergic asthma have demonstrated the efficacy of anti-IgE therapy. Injections of a humanized monoclonal antibody specific for the IgE–Fc blocked Fc ϵ RI binding and showed promise in decreasing the severity of allergic asthma reactions (Chang, 2000; Milgrom et al., 1999). In addition, IgE levels and Fc ϵ RI levels were observed to be correlated (MacGlashan et al., 1997; Saini et al., 1999), with a decrease in the serum levels of IgE leading to a concomitant down-regulation of Fc ϵ RI expression on cell surfaces (Chang, 2000). The down-regulation of Fc ϵ RI expression may be an important benefit of anti-IgE therapeutic approaches. Reduction in the free IgE concentration and Fc ϵ RI-bound IgE might lead to decreases in mast cell numbers, based on *in vitro* experiments with monomeric IgE and bone-marrow derived mast cells (Asai et al., 2001; Kalesnikoff et al., 2001). Thus, disrupting the IgE:Fc ϵ RI interaction is of considerable therapeutic interest, especially given the steady rise in the number of asthma cases that has been observed in developed countries over the past century.

Recently determined structures of Fc ϵ RI (Garman et al., 1998, 2001), IgE–Fc (Wurzburg et al., 2000), the IgE:Fc ϵ RI complex (Garman et al., 2000), and of the related receptors Fc γ RIIa (Maxwell et al., 1999), Fc γ RIIb (Sondermann et al., 1999) and Fc γ RIII (Radaev et al., 2001; Sondermann et al., 2000; Zhang et al., 2000), have revealed the molecular details underlying antibody:receptor interactions. The IgE-binding domains of Fc ϵ RI form an unusually compact arrangement of two Ig domains that could not have been predicted from the sequence (Garman et al., 1998). The interaction of these two Ig domains with the two chains of the IgE–Fc has been elucidated, showing the surfaces and residues that determine the high affinity of this complex (Garman et al., 2000). The structure of the free IgE–Fc showed that the IgE receptor-binding domains can undergo a large conformational rearrangement. The IgE–Fc has been observed in an open, receptor-bound and a closed, free conformation, revealing a conformational flexibility that has not been observed in IgG antibody structures (Wurzburg et al., 2000). These structural studies suggest how specificity and affinity are generated in the Fc receptors and implicate the IgE–Fc's conformational flexibility in interactions with its

two receptors, Fc ϵ RI and Fc ϵ RII. The crystal structures provide a foundation for the development of new therapies for the treatment of IgE-mediated disease.

2. The high affinity receptor structure

The human high affinity IgE receptor exists as a (hetero-) trimer or tetramer on the surface of mast cells and basophils. The tetramer contains an α -chain, a β -chain and two γ -chains, while the trimer is formed by an α -chain and two γ -chains (Turner and Kinet, 1999). The α - and γ -chains each cross the cell membrane with a single putative helical segment, while the β -chain is predicted to span the bilayer four times. The extracellular domain of the α -chain binds IgE with high affinity (Blank et al., 1991; Hakimi et al., 1990; Ra et al., 1993; Robertson, 1993) and many studies have shown that truncation of the protein upstream of the transmembrane segment yields a soluble protein with high affinity for IgE. The β - and γ -chains contain intracellular tyrosine activation motifs (ITAMs) that are phosphorylated rapidly after receptor cross-linking. The phosphorylated ITAMs recruit and activate cytoplasmic kinases through SH2-domain interactions (Kinet, 1999; Turner and Kinet, 1999). The β -chain has been shown to amplify signal transduction through Fc ϵ RI but is not absolutely required for a functional receptor complex (Dombrowicz et al., 1998; Donnadieu et al., 2000; Lin et al., 1996).

The extracellular region of the receptor α -chain has been crystallized and the structure solved in multiple crystal forms (Garman et al., 1998, 2001; Kinet, 1999). The α -chain is formed by two small immunoglobulin domains (Fig. 1a). A number of unanticipated features of the receptor were observed in the crystal structure. First, the two immunoglobulin domains, D1 and D2, are oriented in a nearly anti-parallel arrangement, placing the N- and C-termini on the same side of the receptor structure. An extensive packing interface was observed between the two domains (Fig. 1b), suggesting that the domain arrangement was stable, forming part of the core fold of the overall structure. This prediction has been borne out by the constancy of the D1–D2 domain arrangements in five different crystal forms of the receptor and in complex with the IgE–Fc (Garman et al., 2001). A similar arrangement of the two domains in the related IgG receptors Fc γ RIIa (Maxwell et al., 1999), Fc γ RIIb (Sondermann et al., 1999) and Fc γ RIII (Radaev et al., 2001; Sondermann et al., 2000; Zhang et al., 2000) has been observed, although there are different residues at the D1–D2 interface and slightly different angles formed between the D1 and D2 domains.

In addition to the arrangement of the D1 and D2 domains, the receptor structure revealed an unusual grouping of four surface-exposed tryptophans at the top of the D1–D2 interface—the “tryptophan ridge” (Fig. 1a)—suggesting that these might be involved in antibody-binding interactions (Garman et al., 1998). Two of the four tryptophans are completely conserved in antibody receptors related to Fc ϵ RI α

(W87 and W110) forming a common functional motif in antibody recognition. Many mutational studies had implicated the D2 domain and its FG and C' loops as important in antibody binding (Cook et al., 1997; Hulett et al., 1993–1995, 1999; Mallamaci et al., 1993; Robertson, 1993; Scarselli et al., 1993). The FG and C' loops lie adjacent to the surface-exposed tryptophans. In addition, mapping of potential N-linked carbohydrate attachment sites on the Fc ϵ RI α structure revealed that none of these sites were located on the top of the receptor (near the tryptophan ridge) or along the C'-strand region of the D2 domain. Instead, they were dispersed over the remaining surface of the D1 and D2 domains (Garman et al., 1998). This analysis pointed to a large interaction surface for the antibody formed by the top of the D1–D2 interface and one side of the D2 domain. The solution of the Fc ϵ RI α structure in multiple crystal forms and glycoforms has supported these initial observations and interpretations of the receptor structure (Garman et al., 2001).

3. Structure of the IgE–Fc:Fc ϵ RI α complex

The IgE–Fc is symmetric—that is, there are two identical heavy chains that comprise the Fc region. If each IgE antibody were able to bind to two receptors, the receptors could potentially be cross-linked by a single antibody and initiate cellular signaling pathways in the absence of antigen. However, the observed binding stoichiometry of the IgE to Fc ϵ RI α is 1:1 (Kanellopoulos et al., 1980; Keown et al., 1995, 1997), raising the question of how this binding stoichiometry is achieved. Binding of two receptors to a single Fc might be blocked by: (1) the binding of a receptor to the Fc in such a way as to eliminate or block a second binding site, (2) the induction of a conformational change in the Fc that sterically blocks or eliminates a second receptor binding site, (3) an inherent asymmetry in the overall shape of the Fc that causes it to have two non-equivalent faces/sides, or (4) a combination of these.

The crystal structure of the IgE–Fc C ϵ 3–C ϵ 4 in complex with Fc ϵ RI α showed that the Fc binds to the receptor asymmetrically (Garman et al., 2000). The Fc ϵ RI α binds at the top of the two C ϵ 3 domains near the IgE–Fc two-fold axis, wedged between the two chains of the Fc (Fig. 2). The IgE C ϵ 2–C ϵ 3 linker residues adopt an asymmetric, arch-like structure over the top of the receptor, while the lower C ϵ 4 domains point away from the interaction site and possibly away from the membrane surface. The general features in the arrangement of this complex are also conserved in the complex of IgG with its low affinity receptor, Fc γ RIII (Radaev et al., 2001; Sondermann et al., 2000).

The two C ϵ 3 domains of the IgE–Fc interact with two different surfaces of Fc ϵ RI α , referred to as Binding Site 1 and Binding Site 2 (Fig. 2). The interactions of the two IgE chains with the Fc ϵ RI α are distinct. IgE residues involved in binding are located either in Ig-domain loop regions or in the C ϵ 2–C ϵ 3 linker. Loop residues from the two chains

as well as from the C ϵ 2–C ϵ 3 linker appear to adapt to the receptor surface. This flexibility may allow accommodation of the IgE residues to either binding surface of Fc ϵ RI α and enhance the receptor:IgE affinity. Twenty-two residues of the Fc are involved in the two binding sites, however, only four residues are common to both. Binding Site 1 interactions involve four contiguous segments and 12 residues from the first chain, while Binding Site 2 is formed by two segments and 10 IgE residues from the second chain.

The observed 1:1 binding stoichiometry is established as follows. First, an equivalent second receptor binding site does not exist in the complex. The contact of each C ϵ 3 domain with the receptor involves four identical IgE residues, along with additional site-specific interactions. Second, an additional receptor would be sterically blocked from binding to the Fc because the Fc ϵ RI α binds across the Fc two-fold symmetry axis. Third, the C ϵ 2–C ϵ 3 linkers are bound asymmetrically by the receptor. In addition, it is possible that in an intact IgE, the Fc, the C ϵ 2 domains might sterically block one face of the Fc, effectively leaving only one receptor binding site.

In Binding Site 1, the Fc ϵ RI α residue Y131 sits in a shallow pocket formed by residues from one IgE chain (R334, V336, D362, A364, and H424). The C ϵ 3 domain loops that interact with Y131 are structurally analogous to CDR loops of the variable domains in antibodies and T cell receptors. The C ϵ 3 domain loops form a binding pocket for Y131 of the receptor and similar interactions are observed between the IgG C γ 2 domains and Fc γ RIII in the crystal structures. However, the receptor interaction surface varies among members of the Fc-receptor family. In particular, the Binding Site 1 surface is quite variable even within the IgG receptor family (Garman et al., 2000; Radaev et al., 2001; Sondermann et al., 2000), providing a mechanism for fine-tuning receptor affinities for IgG subclasses. Thus, Binding Site 1 interactions appear to establish specificity and affinity differences across the receptor family.

Binding Site 2 is dominated by interactions of the four Fc ϵ RI α surface-exposed tryptophans. The two conserved tryptophan residues (W87 and W110) form a pocket for the conserved IgE residue Proline 426, forming a “proline sandwich”. This “proline sandwich” is also observed in the IgG–Fc γ RIII complex (Radaev et al., 2001; Sondermann et al., 2000). The conservation of the receptor tryptophans and the Fc proline across antibody classes marks this sub-site interaction of Binding Site 2 as the major conserved interaction in this receptor family. These tryptophans are buried in crystal packing interactions in all crystal forms of the free Fc ϵ RI α (Garman et al., 2001). In every case, the “sandwich” is filled with either a proline or other residue donated from a crystallographic symmetry mate. Burial of the large hydrophobic surface area associated with the exposed tryptophans likely provides a significant energetic driving force for both crystal packing and antibody interactions. The two non-conserved tryptophans in Binding Site 2 form a platform that interacts with the IgE C ϵ 2–C ϵ 3 linker. These

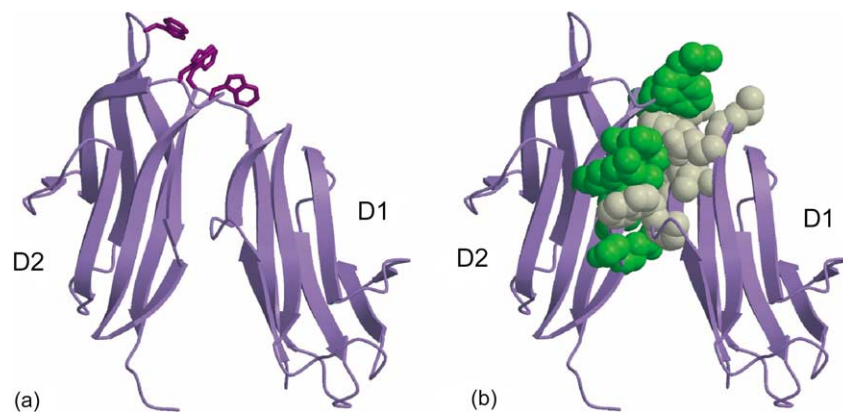


Fig. 1. Ribbon diagram of the structure of FcεRIα: (a) the four tryptophans at the top of the receptor are highlighted in violet; (b) the interface between D1 and D2 is characterized by extensive interactions between the domains. Residues buried from domain D2 are shown in green CPK and from domain D1 are shown in off-white CPK. Figs. 1–3 were made using Molscript (Kraulis, 1991) and Raster3D (Merritt and Bacon, 1997).

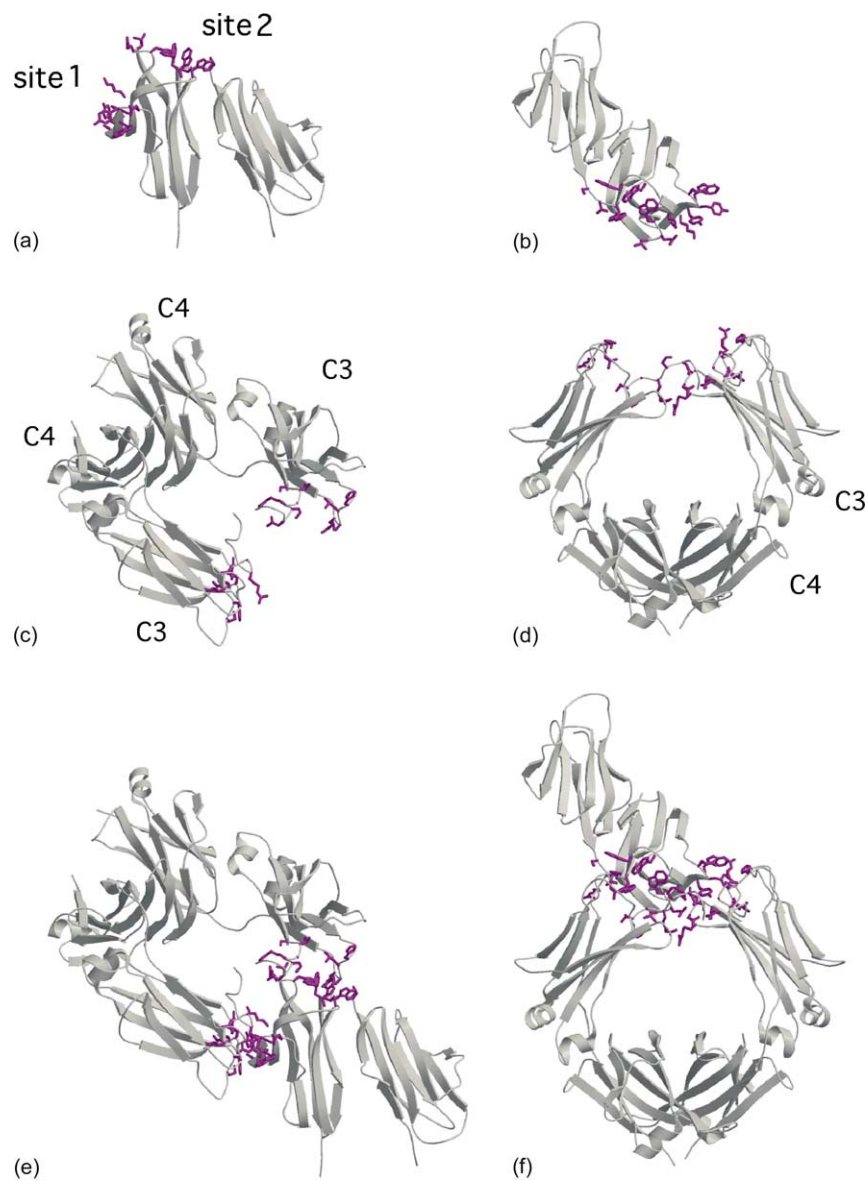


Fig. 2.

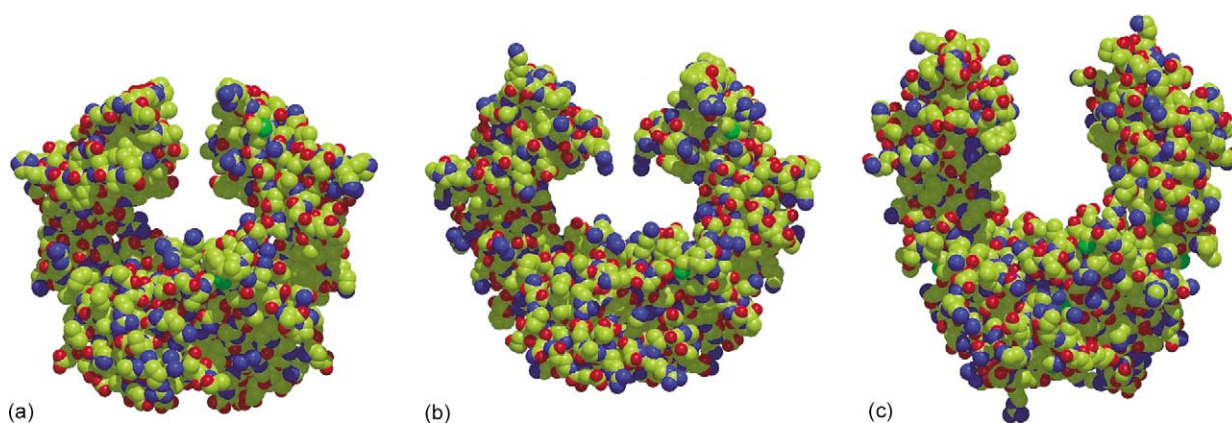


Fig. 3. CPK representations of the IgE-Fc C ϵ 3–C ϵ 4 and an IgG-Fc: (a) the closed form of the IgE-Fc has only a narrow groove between the tops of the C ϵ 3 domains; (b) in the open form, the C ϵ 3 domains move upward and away from each other, leaving a wide, concave surface accessible for receptor binding. The C ϵ 2–C ϵ 3 linker region is not shown. The domain arrangement of the open Fc more closely resembles the structure of a typical IgG Fc (Harris et al., 1997) shown in (c).

interactions are not conserved across receptors and antibodies, consistent with a role for these residues in the specificity of antibody binding.

Analysis of the structure of Fc ϵ RI α in multiple crystal forms suggests that adaptability of the receptor may play a role in the IgE interaction (Garman et al., 2001). While the overall structure and arrangement of the Fc ϵ RI α domains does not change significantly in different crystal forms or upon complex formation with the IgE-Fc, one segment of the receptor at Binding Site 1 shows unusual flexibility in different crystal packing environments. Residues in the C'–E crossover region of the D2 domain have been observed in a variety of conformations, ranging from a canonical C'-strand to a D-strand on the opposite side of the Ig domain, to various conformations in between. While the predominant conformation of this segment in the various receptor crystal forms is the same as in the IgE-Fc complex (a C'-strand), the differences in conformation observed among the various crystal forms is unusual for Ig domain structures and may be indicative of adaptability that allows optimal interactions with IgE.

The crystal structure of the IgE-Fc:Fc ϵ RI α complex was obtained in the presence of the detergent CHAPS, and a cluster of partially ordered CHAPS molecules are observed at the IgE-Fc:Fc ϵ RI α interface near Binding Site 2. One of the CHAPS molecules occupies a position near the IgE-Fc C ϵ 3 domain that is similar to receptor residue Y131 in Binding Site 1. Although the CHAPS interactions do not appear to be highly complementary, this observation provides a potential starting point for the development of inhibitors of IgE binding.

4. Structure of the IgE-Fc

Most crystallographic studies of antibodies have been limited to the IgG isotype and have formed the basis for our understanding of antibody effector domain structure. Structures of IgG, including intact antibodies (Harris et al., 1992, 1997, 1998, 1999; Rajan et al., 1983), Fc fragments (Deisenhofer et al., 1976; Huber et al., 1976), and Fc fragments bound to proteins and ligands (Burmeister et al., 1994; Corper et al., 1997; Deisenhofer, 1981; DeLano et al., 2000; Radaev et al., 2001; Sauer-Eriksson et al., 1995; Sondermann et al., 2000) have consistently shown the IgG-Fc to be in an open conformation, with the upper C γ 2 domains well separated from each other and projecting upward, away from the lower C γ 3 domains. Slight variations observed in the exact position of the upper C γ 2 domain relative to the lower C γ 3 domain present an ensemble of related IgG structures. However, these variations, representing different functional states—free, bound to receptor, bound to other proteins—always show the IgG-Fc to be in an open conformation.

The structure of the receptor-bound IgE-Fc C ϵ 3–C ϵ 4 (Garman et al., 2000) is overall very similar to that of the IgG-Fc bound to the Fc γ RIII receptor and other IgG-Fc structures (Wurzburg et al., 2000)—it is in an open conformation. The structure of the IgE-Fc alone, however, revealed an unexpected arrangement of the C ϵ 3 domains relative to the C ϵ 4 domains (Wurzburg et al., 2000; Fig. 3). The structure is closed, with the upper C ϵ 3 domains lying closer to each other and nearer to the lower C ϵ 4 domains. The space between the C ϵ 3 domains is drastically

Fig. 2. Ribbon diagrams of the structures of the Fc ϵ RI α and the IgE-Fc C ϵ 3–C ϵ 4 from the complex. Fifteen residues from the receptor and 22 residues from the Fc are involved in binding (highlighted in violet). Two views are shown—the left panels show a view from the receptor perspective, the right panels show a view from the Fc perspective; (a and b) the structure of the Fc ϵ RI α from the complex; (c and d) the structure of the IgE-Fc C ϵ 3–C ϵ 4 from the complex; (e and f) the structure of the Fc ϵ RI α :IgE-Fc C ϵ 3–C ϵ 4 complex. The total surface area buried upon complex formation is 1850 Å².

reduced—it becomes a long, narrow groove instead of the large concave bowl that accommodates binding of the high affinity receptor. This closed conformation of the IgE–Fc is novel among antibody structures.

Unlike the IgG, the Fc regions of the IgE and IgM are dimers of three constant domains instead of two. The role of each of the Fc domains (Cε2, Cε3, Cε4) in FcεRIα binding has been studied. The Cε3–Cε4 domains of IgE–Fc retain the high affinity binding to the FcεRIα and a variety of functional studies have shown that the majority of residues involved in binding to the receptor are present in this construct. Most mutagenesis studies identified residues in the Cε3 domain as being critical to the binding of the receptor (Basu et al., 1993; Henry et al., 1997; Presta et al., 1993), while the role of the Cε2 domain has been disputed (Nissim et al., 1991; Weetall et al., 1990). Recent studies suggest that the Cε2 domain may play a role in stabilizing FcεRI-bound IgE from dissociation (McDonnell et al., 2001).

5. Role of Cε3–Cε4 domain residues

Early studies demonstrated that the Cε4 domains could be replaced by the homologous Cγ3 domains and that the chimeric Fc retained binding affinity, kinetics, and ability to mediate degranulation of RBL cells similar to native IgE (Baird et al., 1989; Weetall et al., 1990). These data suggested that the Cε4 domains were not directly involved in binding to FcεRIα but were acting as a scaffold, allowing the Cε3 domains to assume an appropriate orientation for FcεRIα binding. The X-ray crystal structure of the IgE–Fc:FcεRIα complex showed that the Cε4 domains make no direct contacts to the receptor. Recent studies of monomeric Cε3 domains suggest that these may bind FcεRI with micromolar affinity (Henry et al., 2000; Vangelista et al., 1999). However, the monomeric Cε3 expressed in bacteria was characterized by CD and fluorescence spectroscopy and found to be unfolded (Henry et al., 2000). Thus, the affinity measurements may reflect the interaction of a particularly dominant linear segment of the IgE with FcεRI, such as the N-terminal Cε2–Cε3 linker (Garman et al., 2000).

Mutagenesis studies have implicated many residues of Cε3 as directly involved in or important for binding to the FcεRIα (Basu et al., 1993; Henry et al., 1997; Nissim et al., 1991; Presta et al., 1994; Sayers et al., 1998). The X-ray crystal structure of the complex revealed that not all of the residues that affect binding interact directly with the receptor. The residues that affect receptor binding fall into several categories: (1) residues in the receptor-binding loops of the IgE–Fc, (2) residues in the AB helix of Cε3, (3) residues in the Cε2–Cε3 linker, and (4) residues located elsewhere in the structure. Of the residues that directly contacted the receptor, those identified by mutagenesis as having the most profound effect on binding also buried the greatest surface area in the complex. Of the residues that did not contact the receptor directly, most were in the Cε3 AB helix.

5.1. Residues in the receptor-binding loops

The structure of the complex revealed that residues from the BC, DE and FG loops contacted the receptor (D362, L363, A364, P365, R393, N394, G395, T396, H424, L425, P426 and R427). Only one residue—H424—was used by both Fc chains. Four residues in particular formed a significant fraction of the contact surface area for the receptor—N394, P426, R427, and linker residue R334. Mutagenesis studies had previously implicated residues in the BC, DE and FG loops in general as being involved in receptor binding (Presta et al., 1994), but only two mutations—R427E (Presta et al., 1994) and N394T (Sayers et al., 1998) showed any significant effect on binding. Arginine 427 buried $\sim 120 \text{ \AA}^2$ upon complex formation—more than any other single residue, while Asparagine 394 and associated carbohydrate buried $\sim 100 \text{ \AA}^2$.

5.2. Residues in the Cε2–Cε3 interdomain linker

Residues in the Cε2–Cε3 linker region were disordered in the free IgE–Fc structure but became ordered upon complex formation with the receptor (N332, P333, R334, G335 and A-strand residue V336; Garman et al., 2000). R334, G335 and V336 from both chains contact the receptor, with the two arginine 334 residues burying the greatest surface area of all the residues ($\sim 160 \text{ \AA}^2$ total). In fact, mutation of arginine 334 to serine caused a 120-fold decrease in the affinity of the Fc for the receptor (Henry et al., 1997).

5.3. Residues in the Cε3 AB helix

Although the Cε3 AB helix (P345–K352) lies far from the FcεRI binding sites, mutation of helix residues can profoundly affect receptor binding. Given the observation of conformational flexibility in the Cε3–Cε4 crystal structures, the AB helix is of particular interest in understanding IgE function. The Cε3 AB helix likely affects receptor binding by influencing the flexibility of the upper domain. The AB helix has very limited interactions with other residues in its domain, unlike the homologous helix in IgG, and this may allow the Cε3 AB and EF helices to move independently, giving the Cε3 domain greater flexibility.

The Cε3 AB helix lies at the junction between the Cε3 and Cε4 domains and mediates most of the contacts between the two domains (Wurzburg et al., 2000). The AB helix makes most of its contacts to the lower Cε4 domain unlike the homologous helix in IgG that makes most of its contacts within the upper Fc domain. In IgG, a single residue insertion after the AB helix (an isoleucine) forms part of a pocket for a completely conserved histidine from the EF helix. No similar pocket or packing interaction was observed in the IgE–Fc or complex structures. Substitution of the Cε3 AB helix with the longer IgG2 Cγ2 AB helix disrupted receptor binding as did the mutation F349A (Presta et al., 1994). The substitution of the entire helix may disrupt the Fc structure or may

create new contacts within the upper domain, particularly with the EF helix. When more conservative single residue substitutions were made—P345A, D347N, D347E, L348I, R351K, K352G—no substantial effect on receptor binding was observed (Sayers et al., 1998). Of these residues, only L348 and K352 contact the EF helix and the mutations made would be predicted to decrease the contact area between the AB and EF helices. Thus, the closer association of the IgE C ϵ 3 AB helix with the lower domain may allow its AB and EF helices to move relatively independently of each other, while in IgG the two helices remain closely associated in all available structures and may limit the flexibility of the IgG–Fc. Interestingly, substitution of the entire lower IgE C ϵ 4 domain with the homologous IgG C γ 3 domain did not affect Fc ϵ RI binding (Baird et al., 1989; Weetall et al., 1990), suggesting that the contacts of the C ϵ 3 AB helix to the upper C ϵ 3 domain are the critical interactions of the helix that determine receptor binding activity.

5.4. Other residues

Two residues were also observed to affect receptor binding without being in direct contact with the receptor. The mutations R376E and E414R (Presta et al., 1994) both decrease the receptor binding activity of the Fc. R376 is at the end of the C-strand in C ϵ 3 and forms salt bridges to two residues—directly to E414 and indirectly through a water molecule to D409 (Wurzburg et al., 2000). D409 is in the EF helix while D414 is the first residue after the helix. Either of the mutations, R376E or E414R, could cause a repulsive steric interaction at this site and might push the EF helix away from the CD loop towards the AB helix and interdomain region.

6. Role of the C ϵ 2–C ϵ 3 linker and C ϵ 2 domain

The construct used for the X-ray crystal structure of the free Fc contained 10 residues N-terminal to the first β -strand residue of the C ϵ 3 domain (V336). However, there was no electron density for these residues, which included the cysteine that forms the interchain disulfide (C328). Density for most of these residues became apparent in the X-ray structure of the complex, suggesting that the residues became ordered upon binding to the receptor. Linker residues N332, P333, R334, and G335 were directly involved in binding to the receptor, however, the structure of some of the linker residues and even the full extent of the linker region are unknown.

The NMR structure of a monomeric C ϵ 2 domain was recently published (McDonnell et al., 2001). The protein included residues S226 to D330 with the cysteines that form the interchain disulfides mutated to serine (C241S and C328S) to circumvent refolding problems. Although the protein was predicted to dimerize, based on homology modeling with other C-type Ig domains (Padlan and

Davies, 1986; Padlan and Helm, 1993), analytical ultracentrifugation and NMR data suggested that the C ϵ 2 domain remained monomeric (McDonnell et al., 2001). It is possible that the native interchain disulfide (C328–C328), which was not present in this construct, is required to stabilize a C ϵ 2 dimer. For example, the production of a soluble CD3 ϵ γ dimer for structural studies required the presence of an interchain disulfide bond (Sun et al., 2001).

While the structure of the free Fc indicated that V336 was the beginning of regular secondary structure for the C ϵ 3 domain, the C-terminus of the C ϵ 2 domain is not well defined (McDonnell et al., 2001) and therefore the extent of the linker between the two domains remains unclear. It has been proposed that this linker region exists in a flexible, extended conformation (Padlan and Helm, 1993; Zheng et al., 1991, 1992), and the complex and free Fc structures support this proposal. Unfortunately, none of the structures published to date encompasses the intact Fc sequence from C ϵ 2 through C ϵ 4. Arguments for and against the involvement of the C ϵ 2 domain in binding to the high affinity receptor often invoked residues now obviously located in the interdomain boundary region. Crystals of the intact IgE–Fc (C ϵ 2–C ϵ 4) have been reported (Young et al., 1995) and progress on this structure may define the extent of the linker region. Even then, the linker may be flexible, changing depending on whether the Fc is free or in a receptor-bound state.

Because the interactions of the intact Fc (C ϵ 2–C ϵ 4) and the shorter Fc (C ϵ 3–C ϵ 4) with the high affinity receptor are thermodynamically indistinguishable (ΔH° and ΔC_p° are virtually identical), the mode of binding to the high affinity receptor is likely to be very similar (Henry et al., 1997; Keown et al., 1998). However, the various Fc constructs have very similar, though non-identical binding constants to the high affinity receptor, with the shorter Fc construct (C ϵ 3–C ϵ 4) having a slightly lower affinity for the receptor (largely attributable to a faster dissociation rate). Surface plasmon resonance experiments have revealed faster association and dissociation kinetics for the shorter Fc compared to the whole IgE or the intact Fc (C ϵ 2–C ϵ 4) (Cook et al., 1997; McDonnell et al., 2001). These observations suggest a role for the C ϵ 2 in interfering with the association of the IgE–Fc with Fc ϵ RI as well as in stabilizing the complex with the receptor. The structural basis of the C ϵ 2 domain in slowing the dissociation from the receptor has yet to be elucidated but may involve direct, though limited, contacts with the receptor (McDonnell et al., 2001). Biophysical studies have suggested that bending of the IgE molecule takes place between the C ϵ 2 and C ϵ 3 domains (Beavil et al., 1995; Zheng et al., 1991, 1992). Bending would render the two faces of the Fc non-equivalent despite the fact that the Fc is a homodimer (Fig. 4). Even if the bend is dynamic, with each face alternating between concave and convex forms, the non-equivalence of faces in the intact Fc (with one favored for binding of the receptor) might account for the slightly slower on rate observed for the whole IgE and intact Fc (C ϵ 2–C ϵ 4) compared to

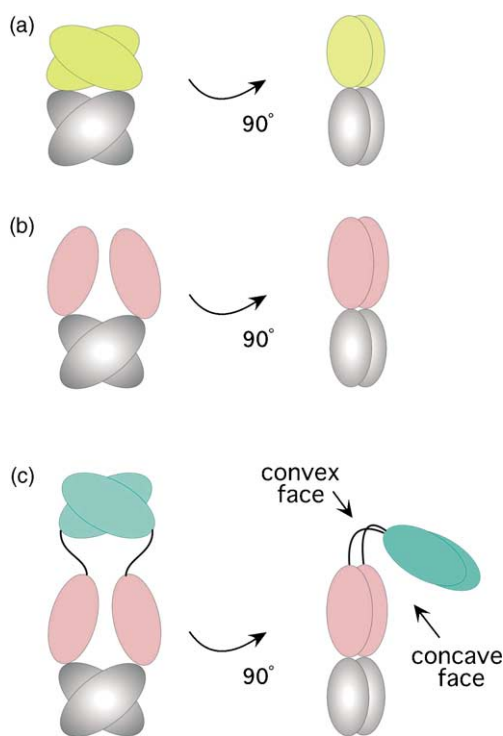


Fig. 4. A cartoon representation of the IgE–Fc C ϵ 3–C ϵ 4 and IgE–Fc C ϵ 2–C ϵ 4 illustrating the faces that each might present to the Fc ϵ RI α . Two views 90° apart are shown. The bend in the IgE antibody likely occurs in the C ϵ 2–C ϵ 3 interdomain linker. The shorter Fc C ϵ 3–C ϵ 4, lacking the C ϵ 2 domains, presents two virtually equivalent faces to the receptor; (a) the closed form of the Fc C ϵ 3–C ϵ 4 with the C ϵ 3 domains in yellow and the C ϵ 4 domains in gray; (b) the open form of the Fc C ϵ 3–C ϵ 4 with the C ϵ 3 domains in pink; (c) although the disposition of the C ϵ 2 domains relative to each other and to the rest of the Fc is unknown, their presence in the larger Fc may render the two faces of the Fc asymmetric. The C ϵ 2 domains may be tightly associated with each other or may be independent and flexible as suggested by McDonnell et al. (2001). For simplicity, the C ϵ 2 domains are shown here (green) as being tightly associated and the C ϵ 3 domains are shown in the open form.

the smaller Fc (C ϵ 3–C ϵ 4). In the C ϵ 3–C ϵ 4 protein, the absence of the C ϵ 2 domains leaves two equivalent faces of the free Fc available for binding of the receptor (Wurzburg et al., 2000). Presumably, both faces would be equally favored.

7. Biphasic binding kinetics

Multiple binding and dissociation steps for the interaction of the IgE–Fc with Fc ϵ RI α have been observed using surface plasmon resonance methods. Two association and dissociation steps—a fast on/off and a slow on/off—have been detected and investigated with substantial experimental controls, leading to the conclusion that the binding of the Fc to the Fc ϵ RI α is best represented using a biphasic model (Cook et al., 1997; Henry et al., 1997; Sayers et al., 1998). This observation applies to all forms of the Fc—whole

IgE, intact Fc (C ϵ 2–C ϵ 4), and the smaller Fc construct (C ϵ 3–C ϵ 4)—although the exact binding constants differ slightly. Since all forms of Fc display similar biphasic binding kinetics, the fast and slow kinetic rates are due to intrinsic properties of the interaction of the C ϵ 3–C ϵ 4 domains with Fc ϵ RI α .

The mechanism underlying the biphasic kinetics remains to be clarified. The kinetics are consistent with two distinct pathways for the assembly and disassembly of the IgE–Fc:Fc ϵ RI complex. The kinetics may reflect the observations that the IgE–Fc interacts with two distinct surfaces of Fc ϵ RI and that the IgE–Fc may adopt both binding and non-binding conformational states in solution. For example, in solution the Fc ϵ RI-binding loop residues would be inaccessible in the closed IgE–Fc conformation, which could interfere with receptor binding. Initial interactions might be primarily through the flexible C ϵ 2–C ϵ 3 linker residues in this IgE conformation. In the open IgE conformation, the receptor binding loops could engage the receptor directly, leading to a distinct kinetic rate. Alternatively, the binding of IgE sequentially to Binding Site 1 and then to Binding Site 2 may have a distinct kinetic rate as compared to sequential binding to Binding Site 2 and then to Binding Site 1. The two dissociation rates of the IgE–Fc may also result from distinct disassembly pathways in which bound C ϵ 3 domains are released sequentially, with individual microscopic rate constants. The observed flexibility of the IgE–Fc structure and the interaction sites on the receptor for each chain of the Fc are consistent with dynamic models for the local dissociation of each antibody chain in the complex. If the two C ϵ 3 domains bind independently, with transient exposure of each site in the complex, inhibitors for either Site 1 or Site 2 could potentially accelerate the dissociation of receptor-bound IgE.

8. Conclusions

The crystal structures of the high affinity IgE receptor, the IgE–Fc and the complex of these two proteins have clarified their interactions at the atomic level. The mechanisms by which specificity and affinity are achieved in this antibody receptor family can now be addressed by more directed experimental approaches, focussed on the residues and interactions identified by the structural analyses. Such approaches may lead to the engineering of more effective therapeutic antibodies by the targeted manipulation of the Fc specificity and affinity for receptors. The structural results will also guide new approaches to developing inhibitors of the IgE:Fc ϵ RI interaction that have the potential to block IgE-mediated allergic reactions to a wide universe of allergens. Identification of small molecules that interact with either the IgE–Fc or the receptor may be further developed in combination with these structural tools.

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References

- Asai, K., Kitaura, J., Kawakami, Y., Yamagata, N., Tsai, M., Carbone, D.P., Liu, F.-T., Galli, S.J., Kawakami, T., 2001. Regulation of mast cell survival by IgE. *Immunity* 14, 791–800.
- Baird, B., Shopes, R.J., Oi, V.T., Erickson, J., Kane, P., Holowka, D., 1989. Interaction of IgE with its high-affinity receptor. Structural basis and requirements for effective cross-linking. *Int. Arch. Allergy Appl. Immunol.* 88, 23–28.
- Basu, M., Hakimi, J., Dharm, E., Kondas, J.A., Tsien, W.H., Pilson, R.S., Lin, P., Gilfillan, A., Haring, P., Braswell, E.H., Nettleton, M.Y., Kochan, J.P., 1993. Purification and characterization of human recombinant IgE–Fc fragments that bind to the human high affinity IgE receptor. *J. Biol. Chem.* 268, 13118–13127.
- Beavil, A.J., Young, R.J., Sutton, B.J., Perkins, S.J., 1995. Bent domain structure of recombinant human IgE–Fc in solution by X-ray and neutron scattering in conjunction with an automated curve fitting procedure. *Biochemistry* 34, 14449–14461.
- Blank, U., Ra, C.S., Kinet, J.P., 1991. Characterization of truncated α chain products from human, rat, and mouse high affinity receptor for immunoglobulin E. *J. Biol. Chem.* 266, 2639–2646.
- Burmeister, W.P., Huber, A.H., Bjorkman, P.J., 1994. Crystal structure of the complex of rat neonatal Fc receptor with Fc. *Nature* 372, 379–383.
- Chang, T.W., 2000. The pharmacological basis of anti-IgE therapy. *Nat. Biotechnol.* 18, 157–162.
- Cook, J.P., Henry, A.J., McDonnell, J.M., Owens, R.J., Sutton, B.J., Gould, H.J., 1997. Identification of contact residues in the IgE binding site of human Fc ϵ RI α . *Biochemistry* 36, 15579–15588.
- Corper, A.L., Sohi, M.K., Bonagura, V.R., Steinitz, M., Jefferis, R., Feinstein, A., Beale, D., Taussig, M.J., Sutton, B.J., 1997. Structure of human IgM rheumatoid factor Fab bound to its autoantigen IgG Fc reveals a novel topology of antibody–antigen interaction. *Nat. Struct. Biol.* 4, 374–381.
- Daeron, M., 1997. Fc receptor biology. *Annu. Rev. Immunol.* 15, 203–234.
- Deisenhofer, J., 1981. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8-Å resolution. *Biochemistry* 20, 2361–2370.
- Deisenhofer, J., Colman, P.M., Epp, O., Huber, R., 1976. Crystallographic structural studies of a human Fc fragment. II. A complete model based on a Fourier map at 3.5 Å resolution. *Hoppe Seylers Z. Physiol. Chem.* 357, 1421–1434.
- DeLano, W.L., Ultsch, M.H., de Vos, A.M., Wells, J.A., 2000. Convergent solutions to binding at a protein–protein interface. *Science* 287, 1279–1283.
- Dombrowicz, D., Lin, S., Flamand, V., Brini, A.T., Koller, B.H., Kinet, J.P., 1998. Allergy-associated Fc β is a molecular amplifier of IgE- and IgG-mediated in vivo responses. *Immunity* 8, 517–529.
- Donnadieu, E., Jouvin, M.H., Kinet, J.P., 2000. A second amplifier function for the allergy-associated Fc ϵ RI- β subunit. *Immunity* 12, 515–523.
- Garman, S.C., Kinet, J.P., Jardetzky, T.S., 1998. Crystal structure of the human high-affinity IgE receptor. *Cell* 95, 951–961.
- Garman, S.C., Wurzburg, B.A., Tarchevskaya, S.S., Kinet, J.P., Jardetzky, T.S., 2000. Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc ϵ RI α . *Nature* 406, 259–266.
- Garman, S.C., Sechi, S., Kinet, J.P., Jardetzky, T.S., 2001. The analysis of the human high affinity IgR receptor Fc ϵ RI α from multiple crystal forms. *J. Mol. Biol.* 311, 1049–1062.
- Hakimi, J., Seals, C., Kondas, J.A., Pettine, L., Danho, W., Kochan, J., 1990. The α subunit of the human IgE receptor (Fc ϵ RI) is sufficient for high affinity IgE binding. *J. Biol. Chem.* 265, 22079–22081.
- Harris, L.J., Larson, S.B., Hasel, K.W., Day, J., Greenwood, A., McPherson, A., 1992. The three-dimensional structure of an intact monoclonal antibody for canine lymphoma. *Nature* 360, 369–372.
- Harris, L.J., Larson, S.B., Hasel, K.W., McPherson, A., 1997. Refined structure of an intact IgG2a monoclonal antibody. *Biochemistry* 36, 1581–1597.
- Harris, L.J., Skaletsky, E., McPherson, A., 1998. Crystallographic structure of an intact IgG1 monoclonal antibody. *J. Mol. Biol.* 275, 861–872.
- Harris, L.J., Larson, S.B., McPherson, A., 1999. Comparison of intact antibody structures and the implications for effector function. *Adv. Immunol.* 72, 191–208.
- Henry, A.J., Cook, J.P., McDonnell, J.M., Mackay, G.A., Shi, J., Sutton, B.J., Gould, H.J., 1997. Participation of the N-terminal region of C ϵ 3 in the binding of human IgE to its high-affinity receptor Fc ϵ RI. *Biochemistry* 36, 15568–15578.
- Henry, A.J., McDonnell, J.M., Ghirlando, R., Sutton, B.J., Gould, H.J., 2000. Conformation of the isolated C ϵ 3 domain of IgE and its complex with the high-affinity receptor, Fc ϵ RI. *Biochemistry* 39, 7406–7413.
- Huber, R., Deisenhofer, J., Colman, P.M., Matsushima, M., Palm, W., 1976. Crystallographic structure studies of an IgG molecule and an Fc fragment. *Nature* 264, 415–420.
- Hulett, M.D., McKenzie, I.F., Hogarth, P.M., 1993. Chimeric Fc receptors identify immunoglobulin-binding regions in human Fc γ RII and Fc ϵ RI. *Eur. J. Immunol.* 23, 640–645.
- Hulett, M.D., Witort, E., Brinkworth, R.I., McKenzie, I.F., Hogarth, P.M., 1994. Identification of the IgG binding site of the human low affinity receptor for IgG Fc γ RII. Enhancement and ablation of binding by site-directed mutagenesis. *J. Biol. Chem.* 269, 15287–15293.
- Hulett, M.D., Witort, E., Brinkworth, R.I., McKenzie, I.F., Hogarth, P.M., 1995. Multiple regions of human Fc γ RII (CD32) contribute to the binding of IgG. *J. Biol. Chem.* 270, 21188–21194.
- Hulett, M.D., Brinkworth, R.I., McKenzie, I.F., Hogarth, P.M., 1999. Fine structure analysis of interaction of Fc ϵ RI with IgE. *J. Biol. Chem.* 274, 13345–13352.
- Kalesnikoff, J., Huber, M., Lam, V., Damen, J.E., Zhang, J., Siraganian, R.P., Krystal, G., 2001. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 14, 801–811.
- Kanellopoulos, J.M., Liu, T.Y., Poy, G., Metzger, H., 1980. Composition and subunit structure of the cell receptor for immunoglobulin E. *J. Biol. Chem.* 255, 9060–9066.
- Keown, M.B., Ghirlando, R., Young, R.J., Beavil, A.J., Owens, R.J., Perkins, S.J., Sutton, B.J., Gould, H.J., 1995. Hydrodynamic studies of a complex between the Fc fragment of human IgE and a soluble fragment of the Fc ϵ RI α chain. *Proc. Natl. Acad. Sci. U.S.A.* 92, 1841–1845.
- Keown, M.B., Ghirlando, R., Mackay, G.A., Sutton, B.J., Gould, H.J., 1997. Basis of the 1:1 stoichiometry of the high affinity receptor Fc ϵ RI–IgE complex. *Eur. Biophys. J.* 25, 471–476.
- Keown, M.B., Henry, A.J., Ghirlando, R., Sutton, B.J., Gould, H.J., 1998. Thermodynamics of the interaction of human immunoglobulin E with its high-affinity receptor Fc ϵ RI. *Biochemistry* 37, 8863–8869.
- Kinet, J.P., 1999. The high-affinity IgE receptor (Fc ϵ RI): from physiology to pathology. *Annu. Rev. Immunol.* 17, 931–972.
- Kraulis, P.J., 1991. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Cryst.* 24, 946–950.
- Lin, S., Cicala, C., Scharenberg, A.M., Kinet, J.P., 1996. The Fc ϵ RI β subunit functions as an amplifier of Fc ϵ RI γ -mediated cell activation signals. *Cell* 85, 985–995.
- MacGlashan Jr., D.W., Bochner, B.S., Adelman, D.C., Jardieu, P.M., Togias, A., McKenzie-White, J., Sterbinsky, S.A., Hamilton, R.G.,

- Lichtenstein, L.M., 1997. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J. Immunol.* 158, 1438–1445.
- Mallamaci, M.A., Chizzonite, R., Griffin, M., Nettleton, M., Hakimi, J., Tsien, W.H., Kochan, J.P., 1993. Identification of sites on the human FcεRIα subunit which are involved in binding human and rat IgE. *J. Biol. Chem.* 268, 22076–22083.
- Maxwell, K.F., Powell, M.S., Hulett, M.D., Barton, P.A., McKenzie, I.F., Garrett, T.P., Hogarth, P.M., 1999. Crystal structure of the human leukocyte Fc receptor FcγRIIa. *Nat. Struct. Biol.* 6, 437–442.
- McDonnell, J.M., Calvert, R., Beavil, R.L., Beavil, A.J., Henry, A.J., Sutton, B.J., Gould, H.J., Cowburn, D., 2001. The structure of the IgE Cε2 domain and its role in stabilizing the complex with its high-affinity receptor FcεRIα. *Nat. Struct. Biol.* 8, 437–441.
- Merritt, E.A., Bacon, D.J., 1997. Raster3D: photorealistic molecular graphics. *Methods Enzymol.* 277, 505–524.
- Metzger, H., 1992. The receptor with high affinity for IgE. *Immunol. Rev.* 125, 37–48.
- Milgrom, H., Fick Jr., R.B., Su, J.Q., Reimann, J.D., Bush, R.K., Watrous, M.L., Metzger, W.J., 1999. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAB-E25 Study Group. *N. Engl. J. Med.* 341, 1966–1973.
- Nissim, A., Jouvin, M.H., Eshhar, Z., 1991. Mapping of the high affinity Fcε receptor binding site to the third constant region domain of IgE. *EMBO J.* 10, 101–107.
- Padlan, E.A., Davies, D.R., 1986. A model of the Fc of immunoglobulin E. *Mol. Immunol.* 23, 1063–1075.
- Padlan, E.A., Helm, B.A., 1993. Modelling study of IgE/receptor interactions. *Biochem. Soc. Trans.* 21, 963–967.
- Presta, L.G., Lahr, S.J., Shields, R.L., Porter, J.P., Gorman, C.M., Fendly, B.M., Jardieu, P.M., 1993. Humanization of an antibody directed against IgE. *J. Immunol.* 151, 2623–2632.
- Presta, L., Shields, R., O'Connell, L., Lahr, S., Porter, J., Gorman, C., Jardieu, P., 1994. The binding site on human immunoglobulin E for its high affinity receptor. *J. Biol. Chem.* 269, 26368–26373.
- Ra, C., Kuromitsu, S., Hirose, T., Yasuda, S., Furuichi, K., Okumura, K., 1993. Soluble human high-affinity receptor for IgE abrogates the IgE-mediated allergic reaction. *Int. Immunol.* 5, 47–54.
- Radaev, S., Motyka, S., Fridman, W.H., Sautes-Fridman, C., Sun, P.D., 2001. The structure of a human type III Fcγ receptor in complex with Fc. *J. Biol. Chem.* 276, 16469–16477.
- Rajan, S.S., Ely, K.R., Abola, E.E., Wood, M.K., Colman, P.M., Athay, R.J., Edmundson, A.B., 1983. Three-dimensional structure of the Mcg IgG1 immunoglobulin. *Mol. Immunol.* 20, 787–799.
- Robertson, M.W., 1993. Phage and *Escherichia coli* expression of the human high affinity immunoglobulin E receptor α-subunit ectodomain. Domain localization of the IgE-binding site. *J. Biol. Chem.* 268, 12736–12743.
- Saini, S.S., MacGlashan Jr., D.W., Sterbinsky, S.A., Togias, A., Adelman, D.C., Lichtenstein, L.M., Bochner, B.S., 1999. Down-regulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. *J. Immunol.* 162, 5624–5630.
- Sauer-Eriksson, A.E., Kleywegt, G.J., Uhlen, M., Jones, T.A., 1995. Crystal structure of the C2 fragment of streptococcal protein G in complex with the Fc domain of human IgG. *Structure* 3, 265–278.
- Sayers, I., Cain, S.A., Swan, J.R., Pickett, M.A., Watt, P.J., Holgate, S.T., Padlan, E.A., Schuck, P., Helm, B.A., 1998. Amino acid residues that influence FcεRI-mediated effector functions of human immunoglobulin E. *Biochemistry* 37, 16152–16164.
- Scarselli, E., Esposito, G., Traboni, C., 1993. Receptor phage. Display of functional domains of the human high affinity IgE receptor on the M13 phage surface. *FEBS Lett.* 329, 223–226.
- Sondermann, P., Huber, R., Jacob, U., 1999. Crystal structure of the soluble form of the human Fcγ-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 Å resolution. *EMBO J.* 18, 1095–1103.
- Sondermann, P., Huber, R., Oosthuizen, V., Jacob, U., 2000. The 3.2-Å crystal structure of the human IgG1 Fc fragment–FcγRIII complex. *Nature* 406, 267–273.
- Sun, Z.J., Kim, K.S., Wagner, G., Reinherz, E.L., 2001. Mechanisms contributing to T cell receptor signaling and assembly revealed by the solution structure of an ectodomain fragment of the CD3εγ heterodimer. *Cell* 105, 913–923.
- Turner, H., Kinet, J.P., 1999. Signaling through the high-affinity IgE receptor FcεRI. *Nature* 402, B24–B30.
- Vangelista, L., Laffer, S., Turek, R., Gronlund, H., Sperr, W.R., Valent, P., Pastore, A., Valenta, R., 1999. The immunoglobulin-like modules Cε3 and α2 are the minimal units necessary for human IgE–FcεRI interaction. *J. Clin. Invest.* 103, 1571–1578.
- Weetall, M., Shopes, B., Holowka, D., Baird, B., 1990. Mapping the site of interaction between murine IgE and its high affinity receptor with chimeric Ig. *J. Immunol.* 145, 3849–3854.
- Wurzburg, B.A., Garman, S.C., Jardetzky, T.S., 2000. Structure of the human IgE–Fc Cε3–Cε4 reveals conformational flexibility in the antibody effector domains. *Immunity* 13, 375–385.
- Young, R.J., Owens, R.J., Mackay, G.A., Chan, C.M., Shi, J., Hide, M., Francis, D.M., Henry, A.J., Sutton, B.J., Gould, H.J., 1995. Secretion of recombinant human IgE–Fc by mammalian cells and biological activity of glycosylation site mutants. *Protein Eng.* 8, 193–199.
- Zhang, Y., Boesen, C.C., Radaev, S., Brooks, A.G., Fridman, W.H., Sautes-Fridman, C., Sun, P.D., 2000. Crystal structure of the extracellular domain of a human FcγRIII. *Immunity* 13, 387–395.
- Zheng, Y., Shopes, B., Holowka, D., Baird, B., 1991. Conformations of IgE bound to its receptor FcεRI and in solution. *Biochemistry* 30, 9125–9132.
- Zheng, Y., Shopes, B., Holowka, D., Baird, B., 1992. Dynamic conformations compared for IgE and IgG1 in solution and bound to receptors. *Biochemistry* 31, 7446–7456.