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Structure and dynamics of IgE–receptor interactions: FcεRI and CD23/FcεRII

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Summary: Immunoglobulin E (IgE) is well known for its role in allergic disease, the manifestations of which are mediated through its two Fc receptors, FcεRI and CD23 (FcεRII). IgE and its interactions with these receptors are therefore potential targets for therapeutic intervention, and exciting progress has been made in this direction. Furthermore, recent structural studies of IgE-Fc, the two receptors, and of their complexes, have revealed a remarkable degree of plasticity at the IgE–CD23 interface and an even more remarkable degree of dynamic flexibility within the IgE molecule. Indeed, there is allosteric communication between the two receptor-binding sites, which we now know are located at some distance from each other in IgE-Fc (at opposite ends of the Cε3 domain). The conformational changes associated with FcεRI and CD23 binding to IgE-Fc ensure that their interactions are mutually incompatible, and it may be that this functional imperative has driven IgE to evolve such a dynamic structure. Appreciation of these new structural data has revised our view of IgE structure, shed light on the co-evolution of antibodies and their receptors, and may open up new therapeutic opportunities.

Keywords: *immunoglobulin E, antibody, Fc receptor, FcεRI, CD23*

Introduction

IgE was the last of the five classes of human antibodies to be discovered, almost 50 years ago (1, 2). It has the same four-chain structure as the other antibody isotypes, but shares with the polymeric IgM subunit a domain architecture comprising three constant (C) domains in each heavy chain of the Fc (*Fig. 1A*), in contrast to IgG with its hinge region and only two C-domains in each Fc chain. IgE appears to have evolved in order to effect the clearance of parasitic infections, but it is more commonly known today for its key role in the allergic response (3), particularly as allergic disease has increased dramatically in recent decades, most notably in the developed world. The first receptor for IgE to be identified was FcεRI, expressed on tissue mast cells and blood basophils as a tetrameric complex of three chains with the stoichiometry $\alpha\beta\gamma_2$, the IgE-binding activity residing entirely in the α-chain (4, 5) (*Fig. 1B*). The interaction

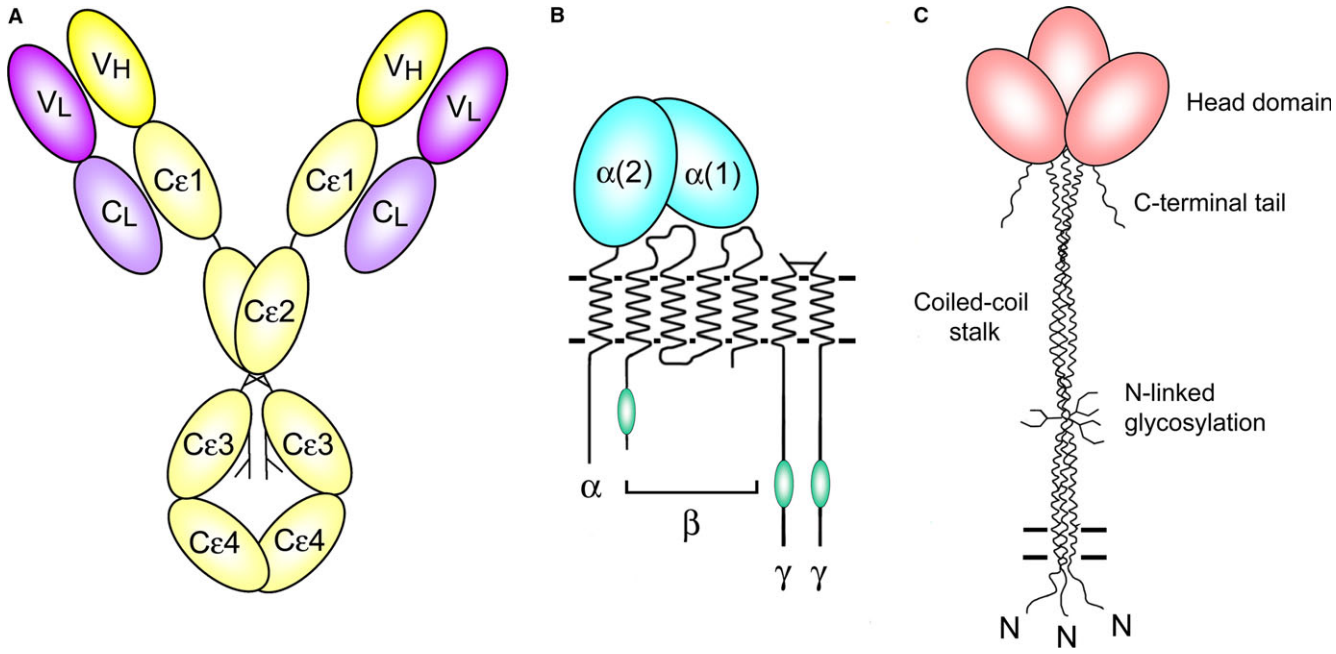

***Fig. 1.* Schematic representations of IgE and its receptors.** (A) Domain architecture and nomenclature of IgE. Inter-heavy chain disulfide bonds in Cε2 and the conserved glycosylation sites at Asn394 in Cε3 only are shown. (B) Arrangement of the four chains of FcεRI, showing the two extracellular IgE-binding domains of FcεRIα, and the ITAMs (green ellipses, refer text) in the β- and disulfide-linked γ-chains. Glycosylation sites in α(1) and α(2) are not shown. (C) The CD23 trimer, with three identical C-type lectin-like, IgE-binding, 'head' domains, glycosylated 'stalk' and C-terminal 'tails'.

between IgE and FcεRI on these cells is critical for the immediate hypersensitivity response that is so characteristic of many allergic reactions. Crosslinking of receptor-bound IgE on mast cells or basophils by multivalent allergen leads to degranulation and the release of a range of inflammatory mediators. The nature of the IgE–FcεRI interaction, and in particular its thermodynamic and kinetic features, determine these physiological consequences. FcεRI is also expressed in either a trimeric form, $\alpha\gamma_2$, or the tetramer, on a range of other cell types: various antigen-presenting cells (APCs), dendritic cells, Langerhans cells, monocytes, macrophages, neutrophils, eosinophils, and platelets (5), as well as human airway smooth muscle (6), bronchial epithelial (7) and intestinal epithelial cells (8), contributing to IgE-mediated allergic pathophysiology at these sites. The affinity of IgE for FcεRI is remarkably high for an antibody–FcR interaction, with $K_a = 10^{10} - 10^{11}\ M^{-1}$(4); it is thus commonly known as the 'high-affinity' receptor for IgE.

A second IgE receptor, FcεRII, also known as CD23, by which name it will be referred to here, was first discovered on B cells (9, 10). It is known as the 'low-affinity' receptor, as the affinity of a single chain of CD23 for IgE has $K_a = 10^6 - 10^7\ M^{-1}$, but in fact the molecule commonly exists as a homo-trimer (*Fig.* 1C), and avidity can enhance the strength of the interaction with IgE immune complexes to render it comparable to, if not greater than that of FcεRI (11, 12). CD23 is unusual as an FcR in several respects: the IgE-binding domain belongs to the C-type lectin superfamily, which is connected to the membrane by a triple α-helical coiled-coil stalk region; furthermore, it exists in membrane-bound form (mCD23) as well as soluble fragments (sCD23) released by cleavage in the stalk region (3, 13). While engagement of mCD23 by IgE immune complexes delivers a downregulatory signal for IgE synthesis (14), sCD23 molecules can exist in monomeric or trimeric states, with opposing effects upon B cells that express mIgE, respectively down- or upregulating IgE synthesis (12, 15, 16). This role of sCD23, together with mCD23, in IgE homeostasis, has been discussed in detail elsewhere (3, 13). CD23 is expressed on several other cell types, including various APCs, and also airway and gut epithelial cells. Its role in transcytosis of IgE–allergen complexes in both the human airway (17) and human intestinal epithelium (18, 19), and presentation to the immune system, is actively being investigated in relation to food allergies and as a potential therapeutic target for allergic airway inflammation in asthma (20).

This review will focus on the structural aspects of these two receptors and their interactions with IgE. This is timely, as we approach the 50th anniversary of the discovery of IgE, recently determined crystal structures, nuclear magnetic

resonance (NMR), and other biophysical analyses have not only identified the binding sites but also elucidated the kinetics and thermodynamics of binding. While this in turn sheds new light on the functions of these receptor interactions, an unexpected outcome of these studies is the revelation of new and unanticipated aspects of the structure and dynamics of the IgE antibody molecule itself.

FcεRI structure

The IgE-binding α-chain of FcεRI consists of two extracellular immunoglobulin (Ig) superfamily domains, α(1) and α(2), attached to a single pass transmembrane region and a short intracellular sequence (4). An immunoreceptor tyrosine-based activation motif (ITAM) is located in the intracellular region of each of the β- and γ-chains, the former chain passing through the membrane four times and the latter once each (5) (*Fig. 1B*). The precise three-dimensional structure of the $\alpha\beta\gamma_2$ receptor with its seven transmembrane helices is unknown, as is the potential role of the extracellular loops of the β-chain in presenting the two Ig-like domains of FcεRIα, although on APCs the β-chain is absent and thus not essential for α-chain expression and functionality. Although the measured affinities of IgE or IgE-Fc for recombinant soluble FcεRIα (sFcεRIα) in solution are comparable to those measured for the complete tetrameric receptor on cells, higher values have been recorded for cell binding, together with evidence for two-step kinetics, suggesting a possible change in conformation within the receptor (21); this may reflect interactions between the α- and β-chains that have yet to be explored in detail.

The two extracellular domains of the α-chain are heavily glycosylated, with seven potential N-linked attachment sites in human sFcεRIα; their presence is required for stability, although not for IgE binding. The crystal structure of sFcεRIα (22, 23) (*Fig. 2A*) revealed that the two domains bend back on each other, exposing a highly hydrophobic 'ridge' devoid of carbohydrate, three chains of which were seen. The relative disposition of the domains and the overall structure are very similar to that of the FcγRII, FcγRIII, and the two N-terminal domains of FcγRI (24, 25), and the only substantial conformational variation between different crystal forms of sFcεRIα was seen in one (CC′) loop region of the second, α(2) domain (23).

IgE binding to FcεRI

The high affinity of this interaction, several orders of magnitude greater than that of IgG for any of its receptors (26), has been studied by a variety of techniques to define the kinetic and thermodynamic profile. The key characteristic is the remarkably slow dissociation rate ($k_{off} \approx 10^{-5}\ s^{-1}$) corresponding to a half-life of days, compared with minutes or even seconds for IgG and FcγR, if measured, e.g. by surface plasmon resonance (SPR) (27, 28). It is here, in the dissociation rate, that the difference in affinity lies, and indeed the half-life of receptor-bound IgE in tissue has been reported as 2–3 weeks (29). (The association rates for IgE and IgG for FcεRI and FcγR are comparable, with $k_{on} \approx 10^5\ M^{-1}\ s^{-1}$). The slow off-rate has the profound consequence that IgE, once bound to a mast cell or basophil, can cause long-term sensitization and account for the immediacy of the inflammatory response when allergen reaches such a cell; the IgE is already in place and only the presence of allergen is required for receptor crosslinking and activation of the cell for degranulation and release of histamine, leukotrienes, and other mediators. This contrasts with the requirement for IgG (or IgA or IgM) immune complex formation with antigen before subsequent interaction with its receptors, i.e. crosslinking of the antibody occurs *before* receptor binding. [Only FcγRI, the highest affinity IgG receptor, is able to capture monomeric IgG, but its dissociation kinetics are still at least an order of magnitude faster than that of IgE (30, 31)].

How does the IgE–FcεRIα interaction achieve this high affinity, and above all, slow dissociation rate? Kinetic and thermodynamic analysis of the binding of IgE-Fc and Fcε3-4, a sub-fragment of IgE-Fc consisting of the dimer of Cε3 and Cε4 domains (*Fig. 1A*), indicated a key role for the Cε2 domains. The presence of the Cε2 domains leads to a slower off-rate (28), and while the binding of Fcε3-4 is accompanied predominantly by a favorable enthalpic change, that of IgE-Fc is entropically driven (32). This may be understood in terms of the structures of the complexes.

The crystal structure of the complex between sFcεRIα and Fcε3-4 was solved in 2000 by Garman *et al.* (33). This landmark study provided the first view of the interaction, and showed that IgE attached to the extracellular α(2) domain and part of the α(1)–α(2) linker region using both of the Cε3 domains, i.e. the receptor interacted with two, non-identical subsites, one on each heavy chain. (A view of this sFcεRIα–Fcε3-4 complex is shown in *Fig. 6*, for later discussion.) The interface is extensive (approximately 1800 $Å^2$), principally hydrophobic, and as it spans both chains, accounts for the known 1:1 binding stoichiometry. Clearly such a stoichiometry is essential, for if IgE could bind to two receptors, there would be the potential for receptor

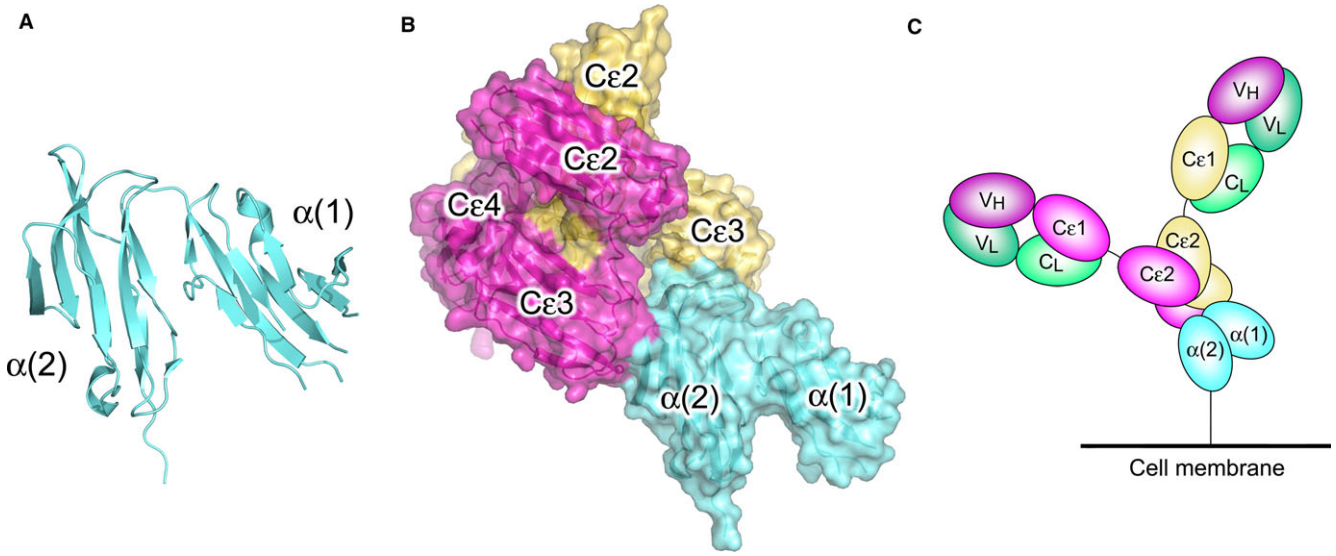

Fig. 2. sFcεRIα and its complex with IgE-Fc. (A) Ribbon trace of the two extracellular domains of FcεRIα. (B) The structure of IgE-Fc bound to sFcεRIα in ribbon representation with translucent surfaces (32). The two chains of IgE-Fc are shown in magenta and yellow, and the receptor is bound between the two Cε3 domains. The Cε2 domains are bent back acutely onto the Fcε3-4 region; one of the Cε4 domains is obscured. (C) Schematic to indicate the disposition of the Fab arms of IgE relative to receptor-bound IgE-Fc. The figure was prepared with PyMOL (100).

crosslinking in the absence of allergen. Published back-to-back in *Nature* with the structure of the Fcε3-4–sFcεRIα complex was that of the IgG1-Fc–sFcγRIII complex (34). This revealed a highly homologous interaction in terms of its three-dimensional structure and mode of engagement, with a similar interfacial area. Both complexes involved a hydrophobic 'proline sandwich' interaction, in which a proline residue of the antibody is packed between the side-chains of two tryptophan residues on the receptor.

Formation of the complex involved virtually no change in the structure of sFcεRIα (apart from a rearrangement in the labile CC′ loop) but in Fcε3-4, the two Cε3 domains moved further apart to accommodate the receptor, compared with their conformation in uncomplexed Fcε3-4 (35). Indeed, a range of 'open' and 'closed' forms of this sub-fragment of IgE-Fc was observed in different crystal forms of the molecule (36), and this range of quaternary structures is now populated by several more free and receptor-bound structures, including those with CD23. In addition to the movement of the Cε3 domains relative to each other, a 'flexing' within each of the domains was also detected when the free and receptor-bound structures were compared (35). The intrinsic flexibility of these domains will be discussed further below.

This first view of the complex did not include the Cε2 domains, which were first seen 2 years later in the crystal structure of the free IgE-Fc fragment (37). Earlier modeling of the intact IgE molecule had placed the $(Cε2)_2$ pair of domains between the two Fab arms and the Fcε3-4 region (38) in a manner similar to the schematic depiction in *Fig. 1A*. However, such an extended molecular conformation was incompatible with fluorescence depolarization studies and also with Förster Resonance Energy Transfer (FRET) analyses carried out with IgE molecules fluorescently labeled in the antigen-binding sites and at their C-termini (39–42). The distances between these two 'ends' of the molecule indicated that IgE must adopt a more compact and bent structure, both free in solution and when bound to FcεRI. A small-angle X-ray scattering (SAXS) study also indicated that IgE-Fc adopts a very compact structure in solution (43), and this was indeed what the crystal structure revealed. In fact, the structure was found to be even more compact than had been expected; the Cε2 domains were acutely bent back onto the Cε3 domains, and in an asymmetric fashion, i.e. the molecule was bent over to one side. In fact, one of the Cε2 domains made extensive contacts with Cε3 and even one of the Cε4 domains (37). This acutely bent structure is shown in *Fig. 3A*.

More remarkably, and contrary to the expectation that there might be an 'unbending' and even an interaction between Cε2 and FcεRIα upon receptor binding (10), when the crystal structure of the IgE-Fc–sFcεRIα complex was later solved (32), the acute bend between Cε2 and Cε3 was found to be even more acute (a reduction from 62° to 54° in the angle between the local twofold axes of the $(Cε2)_2$ domain pair and the Fcε3-4 region), with no direct

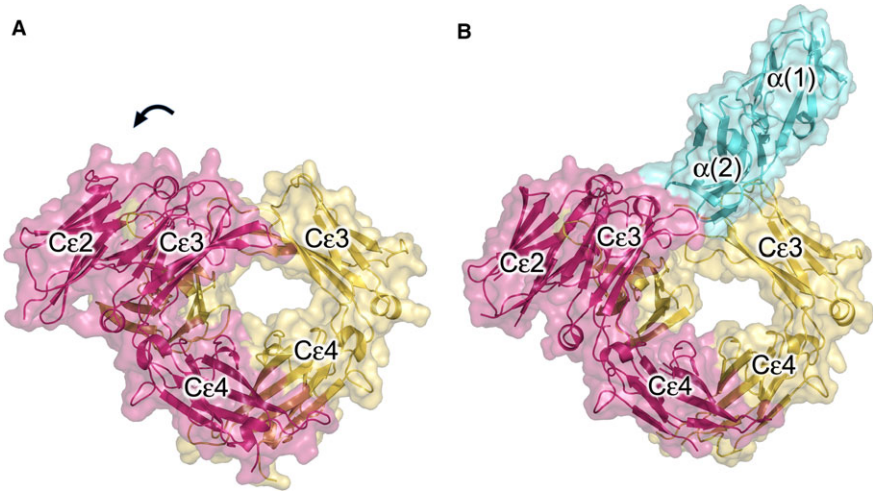

Fig. 3. Conformational change in IgE-Fc upon binding to FcεRIα. (A) The structure of free IgE-Fc showing the two chains (red and yellow) and the acute bend between the Cε2 and Cε3 domains (37). (B) The IgE-Fc complex with sFcεRIα (blue) (32) showing the opening of the Cε3 domains relative to their positions in free IgE-Fc. The conformational change upon receptor binding may be considered as a movement of the Cε3 (red) together with the $(Cε2)_2$ domain pair, away from the Cε3 (yellow) and the $(Cε4)_2$ domains, i.e. the first three domains move relative to the other three. The figure was prepared with PyMOL (100).

interaction between Cε2 and the receptor at all (*Figs 2B and 3B*). In fact this very result, namely a more compact structure for receptor-bound IgE, had been demonstrated in the earlier FRET study as the range of 'end-to-end' distances for the IgE molecule when receptor-bound was found to be narrower, and with a smaller mean value, than that for free IgE (42). The more acute bend upon receptor binding was further confirmed, in solution, by the enhanced FRET signal from an IgE-Fc molecule fluorescently labeled at its N- and C-termini, when bound to sFcεRIα (44).

The conformational change that occurs in the six domains of the IgE-Fc upon binding to sFcεRIα can best be considered as a movement of one of the Cε3 domains together with the $(Cε2)_2$ domain pair, away from the other Cε3 domain together with the $(Cε4)_2$ domain pair (32) (*Figs 3A, B*). In free IgE-Fc, one of the Cε3 domains is already in the 'open' state (as defined above in relation to the Fcε3-4–sFcεRIα structure), while the other is in the 'closed' state (37). Both adopt the open state in the IgE-Fc–sFcεRIα complex. These structures offer an explanation for the effect of the Cε2 domains on the thermodynamics of the interaction described above: their presence stabilizes the otherwise flexible Cε3 domains, one of which is already in the receptor-binding open state, reducing the entropic penalty incurred by these domains upon receptor binding (32). The full entropic benefit of the substantial hydrophobic contribution to the interaction with sFcεRIα can thus be realized for IgE-Fc, which together with the conformational changes that involve three of the six domains moving relative to the other three, contributes to a larger barrier to dissociation and slower off-rate for IgE than for IgG bound to any of its receptors. [For a more detailed explanation refer to Holdom *et al.* (32).]

Alongside the structure of the complex in *Fig. 2B* is a schematic showing the expected disposition of the Fab arms in receptor-bound IgE (*Fig. 2C*). While the Fab arms are clearly presented in a manner suited to allergen binding, a detailed modeling of the range of mobility of the two Fab arms showed that in contrast to IgG with its flexible hinge regions, the two arms of IgE sweep out distinct regions of space, one arm pointing roughly parallel to the membrane and the other pointing away (44). This relative restriction of Fab arm mobility (compared with IgG) may be important for the disposition of epitopes on allergens that can cause crosslinking of receptor-bound IgE; note that while antigens crosslink IgG in solution prior to receptor engagement (with the caveat of IgG binding to FcγRI, mentioned above), allergens crosslink receptor-bound IgE.

Neither of these structures show any direct interaction between sFcεRIα and the carbohydrate component of IgE-Fc or Fcε3-4 that is N-linked at Asn394 in Cε3 (32, 33). The multiply branched, high-mannose carbohydrate moiety with heterogeneity in composition (45–47) occupies the space between the Cε3 domains (37). This asparagine residue is conserved in other antibody isotypes, although at Asn297 in the structurally homologous Cγ2 domain of IgG-Fc, the carbohydrate is of the complex, rather than the high-mannose type; it similarly occupies the space between the Cγ2

domains (48). However in IgG1-Fc, there is evidence of limited interaction between carbohydrate and FcγR in some of the crystal structures [e.g. (30)], and in general, full deglycosylation abrogates or seriously compromises FcγR binding activity (48); it has long been known that IgE-Fc lacking carbohydrate, either produced by bacterial expression (49, 50) or deglycoslation following mammalian expression (51, 52), will bind FcεRI (and CD23). The aglycosylated molecules expressed in bacteria require refolding. In mammalian expression *in vitro* or *in vivo*, as both an earlier (53) and a recent report demonstrate (47), glycosylation at Asn394 is essential for FcεRI binding and IgE functional activity, but this appears to be a critical requirement for correct folding, assembly, and secretion, rather than for FcεRI binding activity *per se*. [There are three other potential sites for N-glycosylation in IgE-Fc, at Asn265 in Cε2, and Asn371 and Asn383 in Cε3, but these are not fully glycosylated in all molecules and lie at the surface, distant from the FcεRI binding site; they are not essential for folding and/or activity (47, 54)].

IgE dissociation from FcεRI

The explanation for the uniquely slow dissociation rate of IgE from FcεRI thus lies partly with the Cε3 domains, but also within the Cε2 domains, the presence of which further decreases the off-rate for IgE-Fc compared with Fcε3-4, as described above (28). Although the Cε2 domains make no direct contact with receptor, they play an indirect role through the Cε3 domains, which themselves have unique properties when compared with other Ig C-domains. As will be described in the following section, a remarkable feature of the Cε3 domains is their considerable intrinsic flexibility, and by stabilizing them, the Cε2 domains thus serve to reduce the entropic gain that would otherwise favor disengagement.

The blocking of IgE binding to FcεRI is clearly a promising therapeutic goal, achieved recently with the introduction into clinical use of the anti-IgE monoclonal IgG1 antibody omalizumab (Xolair™, Novartis, Basel, Switzerland) (55). Omalizumab binds to the Fc region of free IgE, preventing receptor binding, both FcεRI and CD23. It has proved effective for many people with severe asthma, and trials have shown efficacy in other allergic conditions (55). However, a remarkable observation was reported last year, namely that omalizumab can, at very high concentrations, *in vitro*, actively dissociate IgE from the receptor (56). This phenomenon was in fact first reported for another molecule, a designed ankyrin repeat protein or DARPin that binds to the Cε3 domains of IgE and facilitates or accelerates the dissociation of IgE from FcεRI (57). The DARPin is a small protein comprising four short (approximately 30 residue) repeats; it was this result, and the demonstration that it could not only dissociate IgE from basophils *in vitro* but also show efficacy *in vivo* (56) that led to the investigation *in vitro* of the effect of omalizumab, albeit with concentrations considerably higher than those used clinically. The prospect of accelerating the dissociation of pre-bound IgE from mast cells or basophils is clearly very promising for therapeutic intervention in allergic disease (56).

The mechanism of this accelerated dissociation remains unclear, but the effect of omalizumab binding to free IgE-Fc has been investigated using the N- and C-terminally fluorescently labeled molecule referred to earlier. While binding of sFcεRIα enhanced the FRET signal, indicative of more acute bending, omalizumab binding reduced the signal, suggesting perhaps a degree of 'unbending' (44). This may offer a clue, implicating the remarkable dynamic properties of the IgE molecule. Some of these have been mentioned already. Other, more extreme dynamic properties will be described in the following section.

Flexibility and dynamics of IgE-Fc

The bend in the IgE-Fc is not only acute but also asymmetric (37), i.e. the $(Cε2)_2$ domain pair can be envisaged as bending over either to one side of the Fcε3-4 region or the other (*Figs 3A and 4A*). In doing so, they distort the Cε3 domains and break the symmetry of the Fcε3-4 region, although bending either to one side or the other presumably generates identical, and identically stable, molecular structures. But ever since the discovery of this bent conformation, the tantalizing question exists: might the Cε2 domains be able to 'unbend', pass through an extended conformation or conformations, and bend over onto the other side of the Fcε3-4 region? Analysis of IgE-Fc in solution by SAXS (43), as mentioned above, is consistent only with a bent structure, which suggests that any extended conformations, if they exist at all, are only transiently populated.

It was the crystal structure of a complex between an anti-IgE antibody and IgE-Fc that provided the answer to this question, and yet another surprise, when it revealed a near perfectly extended conformation for the $(Cε2)_2$ domain pair relative to the Fcε3-4 region (58). The complex, with two Fab fragments bound on either side of the IgE-Fc (*Fig. 4B*), demonstrates that the IgE molecule can indeed adopt a fully

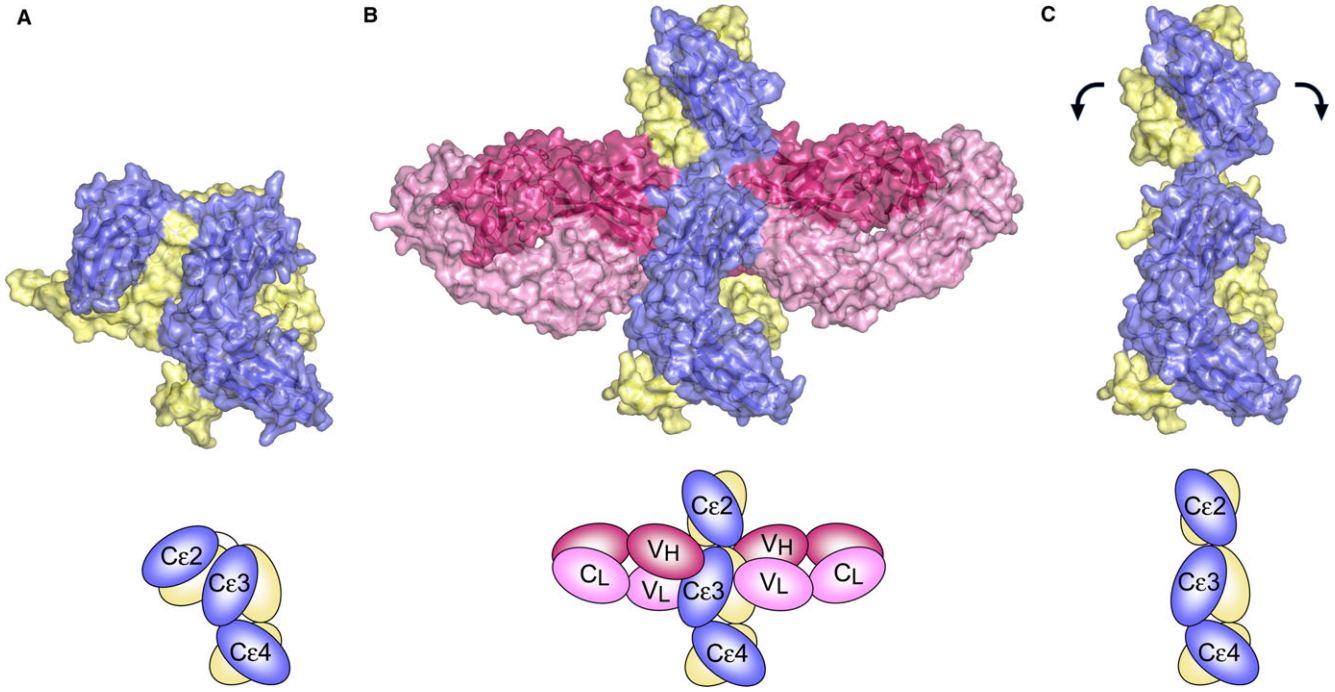

Fig. 4. IgE-Fc and its complex with an anti-IgE Fab. (A) The structure of free IgE-Fc (with cartoon to indicate the domains) showing the two chains (blue and yellow) and the acute bend between the Cε2 and Cε3 domains (37). (B) The 2:1 Fab:IgE-Fc complex showing the fully extended conformation of the IgE-Fc (with cartoon to indicate the domains) (58). (C) The extended structure of IgE-Fc extracted from the Fab complex, indicating the likely flexibility of such a conformation of free IgE-Fc. The figure was prepared with PyMOL (100).

extended conformation (Fig. 4C), one in which the local twofold axis of rotational symmetry of the $(C\varepsilon2)_2$ domains is virtually coincident with the local twofold axis of the Fcε3-4 region. (This symmetry is not imposed by crystal packing, as the entire 2:1 Fab:IgE-Fc complex is the asymmetric, or repeat unit, of the crystal.) Given that the extended conformation *can* exist, could IgE-Fc adopt such a structure in the absence of the bound Fabs, i.e. are the Fabs selecting a pre-existing conformation in solution, or inducing the formation of the extended structure? A series of biophysical experiments (including isothermal titration calorimetry and stopped-flow kinetics) demonstrated results consistent with selection from a dynamic population of conformational states (58). Molecular dynamics simulations were carried out to investigate whether the transition from one bent conformation to the other was possible, and to estimate the free energy difference between the bent and extended conformations. The 'flipping' of the $(C\varepsilon2)_2$ domains from one side of the Fcε3-4 region to the other did indeed occur in the simulation, and the energy difference between bent and extended states (approximately 20 kJ mol^{-1} or 8 k_BT) was consistent with an attainable, if rarely populated state. Intriguingly, 10 years earlier, the suggestion that the Cε2 domains might flip from one side of the Fcε3-4 domain to the other was made in order to explain how both oligosaccharide chains could be accessed by the glycan processing enzymes; if the structure was rigidly bent, then one chain would always remain inaccessible (45).

The IgE molecule is thus very different to IgG, in which the two Fab arms and Fc region are rather loosely connected (depending upon the nature of the hinge) and capable of adopting a wide range of conformations. Although IgE appears to be compactly bent most of the time, the flipping of the $(C\varepsilon2)_2$ domains in the IgE-Fc, carrying the Fab arms with them, is a very extreme dynamic capability. The link between each Fab's Cε1 domain and Cε2 is only a few residues long, so that there is rather limited flexibility – compared with IgG – of the Fab arms relative to IgE-Fc; this has been modeled in detail (44, 58). An alternative view of this conformational flexibility might be to consider the Fcε3-4 region flipping relative to the $(C\varepsilon2)_2$ domains and the Fab arms – a motion that might be described as the IgE molecule wagging its tail! However this flexibility is described, the fact that considerable changes in conformation can occur within the domains of the Fc region are important for understanding IgE's receptor interactions.

While much attention has focused on the Cε2 domains and the bending of IgE-Fc, and also the quaternary structural changes between the 'open' and 'closed' conformations of

the Cε3 domains, the Cε3 domains are themselves remarkable, indeed unique in certain respects, among Ig constant domains. Most Ig domains will fold independently when expressed alone, as do Cε2 and Cε4, but the Cε3 domain in isolation has been reported to be incompletely folded, though still capable of binding FcεRI (59, 60). Recently it has been characterized more fully as a 'molten globule' (61, 62). With respect to the packing of the hydrophobic core and its thermodynamic stability, the Cε3 domain is an outlier when compared with other Ig domains (63), including its closest structural homologs such as Cγ2 in IgG or Cμ3 in IgM. On a historical note, it is interesting that within just a few years of its discovery, the remarkably low thermal stability of IgE (T_m of 56°) compared with other antibodies was, correctly as it turns out, attributed to the Cε3 domains (64). When observed in the context of structures containing Cε2 and Cε4 domains, however, Cε3 is always folded, but as described above, flexibility *within* the Cε3 domain has been observed as result of comparing many crystal structures of IgE-Fc and Fcε3-4, both alone and in complex with FcεRI (35, 36) or with CD23. It thus retains a degree of lability within IgE and indeed, as Cε3 is the domain to which both receptors bind, it would appear that IgE has evolved this uniquely responsive Ig domain to permit expression of its Fc-mediated functional activity. Subsequently, this point will be elaborated following a detailed discussion of the low-affinity receptor, CD23.

CD23 structure

This molecule is exceptional among Fc receptors, belonging to the C-type (Ca^{2+}-dependent), lectin superfamily rather than the Ig superfamily. This classification refers to the extracellular IgE-binding 'head' domain, which is connected to the membrane by the trimeric alpha-helical coiled-coil, N-glycosylated, 'stalk' region as described above and illustrated schematically in *Fig. 1C*. In human CD23, there is also a C-terminal 'tail' region attached to the lectin-like head domain. The N-terminus of CD23 is intracellular, and there are two isoforms, CD23a and CD23b, that differ in their first seven (CD23a) or six (CD23b) N-terminal amino acids (13, 65). CD23a is constitutively expressed on antigen-activated B cells, where it contributes to facilitated antigen presentation, whereas CD23b is expressed in response to IL-4 on a range of cells including B cells and also epithelial cells (10, 14, 66).

The structure of the lectin head domain (*Fig. 5A*) has been determined both by X-ray crystallography and NMR, with and without Ca^{2+} (67–70); indeed, there are now almost 40 (crystallographically) independent structures for the domain (excluding those determined in complex with IgE). Comparison reveals little variation throughout most of the domain, but a considerable degree of conformational diversity in two surface loop regions involved in IgE binding; one of these is involved in Ca^{2+} binding (see *Fig. 5A*) and its conformation is sensitive to the presence of Ca^{2+}.

Although CD23 belongs to a lectin, i.e. carbohydrate-binding, superfamily, it was shown early on that it recognizes IgE not through carbohydrate but through polypeptide determinants (50). Similarly it emerged that, at least for human CD23, Ca^{2+} was not a requirement either, although the presence of Ca^{2+} does enhance affinity for human IgE-Fc (67, 69). We now understand this effect of Ca^{2+} in structural terms, as described in the following section. However, there does appear to be a requirement for Ca^{2+} in the case of murine CD23 binding to murine IgE (71), which may be associated with the fact that sequence comparison indicates the presence of a second Ca^{2+}-binding site, whereas human CD23 has only one (68). The presence of a second site in murine CD23 has yet to be confirmed.

The 'anomalous' interaction of the lectin-like CD23 head domain with IgE, requiring neither carbohydrate nor, for human CD23 at least, Ca^{2+}, led to the search for what perhaps might be its evolutionarily more primitive binding partner. CD21 (also known as complement receptor type 2, CR2) was subsequently discovered to be a co-receptor for CD23 (72), an interaction that was at least in part carbohydrate-dependent, although several of the tandem array of domains (termed short consensus repeats) that characterize many complement proteins may be involved (73). The mapping of the interaction on CD21 remains unclear, but on CD23 it involves part of the C-terminal tail sequence that extends beyond the folded C-type lectin domain (67) (*Fig. 1C*), a region that is absent in murine CD23, as is the interaction between murine CD23 and CD21.

The stalk region of CD23 is susceptible to proteolysis at various points, releasing soluble fragments with different lengths of stalk and different tendencies to remain trimeric (3, 13). The endogenous protease ADAM10 has been found to be the principal 'sheddase' for sCD23 (74, 75), although exogenous enzymes also cleave the stalk region, such as the house dust mite protease *Der p* 1 that generates a fragment consisting only of the C-type lectin domain and lacking the tail (76). The recombinant head domain studied crystallographically and by NMR corresponds to this 'derCD23' fragment. To date, no crystal structure of the trimer has been solved, but from the NMR spectra recorded as a function of

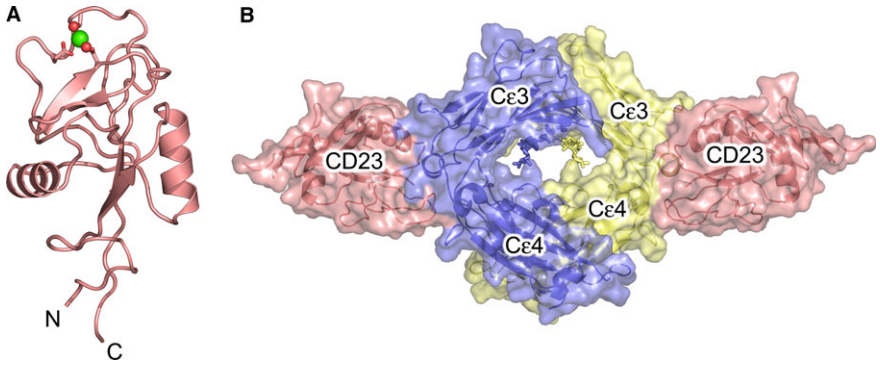

Fig. 5. CD23 head domain and complex with Fcε3-4. (A) Ribbon trace of the lectin-like head domain of CD23 showing the Ca^{2+} ion (green), coordinating side-chains and water molecules (red). (B) The 2:1 CD23:Fcε3-4 complex (82) showing the interaction with the Cε3 and Cε4 domains in each of the two chains (blue and yellow), with the carbohydrate chains attached to Cε3 colored accordingly. No Ca^{2+} is present in this complex, but its location in the virtually identical Ca^{2+}-bound complex may be inferred from the location of the disordered (missing) polypeptide segment in CD23 at each interface, which becomes ordered in the presence of Ca^{2+}. The figure was prepared with PyMOL (100).

concentration, a model for the trimerization of the heads has been proposed (67).

Given the ability to bind to both IgE and CD21, and the known involvement of CD21 in the mechanism of B-cell activation (77), a mechanism in which trimeric sCD23 crosslinks mIgE on a B cell committed to IgE synthesis, and CD21, was proposed to account for the observed mitogenic activity of sCD23 on B cells (3, 13). This hypothesis is supported by the up- and downregulatory activity of trimeric and monomeric sCD23, respectively (12, 15, 16), as the former could crosslink mIgE and CD21, while the latter would only inhibit this interaction. Coupled to earlier reports that mCD23 delivers a downregulatory signal for B-cell proliferation (78–81), it is clear that CD23 plays an important role in the regulation of IgE synthesis, and as such may constitute a potential target for therapeutic intervention. This proposed model of protein–protein interactions involving IgE–CD23–CD21 complexes at the human B cell surface has been described in detail elsewhere (3, 13); while more structural information is required to substantiate it, the recent structure of the complex between sCD23 and Fcε3-4 is an important first step.

IgE binding to CD23: plasticity at the interface

The crystal structure of the complex, shown in *Fig.* 5B, revealed two molecules of the extracellular head region of CD23 (the derCD23 fragment) bound to the Fcε3-4 fragment of IgE-Fc (82). The 2:1 stoichiometry was previously known (83), as was the principal involvement of the Cε3 domains, but given the known mutual inhibition of FcεRI and CD23 binding (11, 84), the location of the CD23 binding site, so distant from that of FcεRI (refer *Fig.* 6) and involving residues from Cε4, was a surprise. NMR analysis of the interaction in solution confirmed the identification of the binding site on CD23 (85). The interface (approximately 870 $Å^2$) is dominated by four salt bridges, in all of which the negative charge resides on IgE, the positive charge on CD23, and four additional hydrogen bonds. The Cε3 domain contributes 63% of the interacting surface of IgE, Cε4 12%, with the remainder coming from the inter-domain linker region.

No Ca^{2+} was seen in this first crystal structure, but a subsequent X-ray analysis of the complex in the presence of Ca^{2+} revealed how the Ca^{2+} ion induces a local conformational change in the loop to which it binds, enabling additional contacts to be made with IgE-Fc (69). This loop was partially disordered in the Ca^{2+} -free complex, but becomes fully ordered in the presence of Ca^{2+}, enabling two additional salt bridges and a net increase of two hydrogen bonds to be made with IgE (69). This accounts well for the observed 30-fold increase in affinity for IgE-Fc (at 37°C) in the presence of Ca^{2+} (67, 69), and it is also consistent with the altered thermodynamic profile of the sCD23–IgE-Fc interaction in the presence of Ca^{2+}. Without Ca^{2+} the interaction is largely driven by a favorable enthalpic change, whereas with Ca^{2+} present, it is accompanied by a favorable entropic component (69). This greater entropic contribution to binding in the presence of Ca^{2+} is likely due to the ordering of the intrinsically flexible Ca^{2+}-binding loop, and consequently reduced entropic penalty upon complex formation. It is particularly intriguing, from an evolutionary perspective, that an aspartic acid residue in CD23 (Asp258) that makes an additional salt bridge with IgE in the presence of Ca^{2+}, is a residue that in many other C-type lectins – and

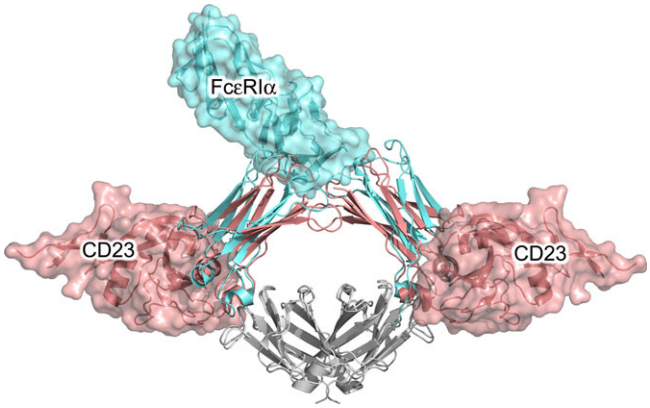

***Fig. 6.* Allosteric communication between the FcεRI and CD23 binding sites.** Complexes between sFcεRIα and CD23 head domains with Fcε3-4 are superimposed on the Cε4 domains (gray). The complexes with FcεRI and CD23 (cyan and pink, respectively) show an 'open' conformation for the Cε3 domains in the former and a 'closed' conformation in the latter. In the open, FcεRI-bound structure, steric clashes occur with CD23 to prevent binding, while in the closed, CD23-bound structure, clashes occur with FcεRI, accounting for the mutual inhibition of FcεRI and CD23 binding to IgE-Fc. The figure was prepared with PyMOL (100).

perhaps also in murine CD23 – is a ligand for Ca^{2+} at a second site. It appears that human CD23, in losing a second Ca^{2+} binding site, has 'co-opted' this residue to gain enhanced binding to IgE.

The physiological significance of this Ca^{2+} dependence remains to be explored, but this modest level of modulation of the interaction with a monomeric CD23 head domain will certainly be accentuated by the avidity effect within a trimer. Ca^{2+} binds to CD23 with a K_d of approximately 1.5 mM (67, 69), which is of the same order of magnitude as the Ca^{2+} concentration at the cell membrane. It has been pointed out that the reduced Ca^{2+} concentration in endosomes (by a factor of 100- to 1000-fold) may contribute in the case of CD23, which undergoes endocytosis and recycling (86) as for other C-type lectin receptors, to a mechanism for releasing a bound IgE–allergen complex following internalization, permitting recycling of receptor to the cell surface (87).

Although the crystal structure shows two CD23 heads binding to a single IgE (Fcε3-4) molecule, it is important to appreciate that these two heads cannot belong to the same CD23 trimer. The N-termini of the CD23 head regions that connect directly to the stalk are located at the left- and right-hand extremes of the complex as shown in *Fig.* 5B, and are 136 Å apart, so that it would be necessary to unravel at least a third of the triple-helical stalk region for this to occur. However, the two heads could belong to two different trimers, and in this way a single IgE molecule could crosslink two CD23 molecules, or a single CD23 trimer could crosslink two (or perhaps even three) IgE molecules. The high avidity of the CD23–IgE interaction, reported to be comparable to that of the 'high-affinity' FcεRI interaction, has always been measured in one or other of these situations, with either more than one CD23 trimer, or more than one IgE molecule, available for crosslinking on the surface of a biosensor, in SPR analysis, or a cell (11, 12, 15, 88–92).

The interaction between CD23 and IgE is characterized by conformational changes or 'plasticity' in both components. As indicated, many structures of the CD23 head domain have been determined, revealing conformational variability in the loop regions that constitute the IgE-binding site (67–70), and similarly the many crystal structures of IgE-Fc and Fcε3-4 structures have shown variation in the angle between the Cε3 and Cε4 domains (35,36), where the CD23 site lies. Furthermore, comparison of the several sCD23–Fcε3-4 complexes in different crystal packing environments has shown that even in the complex, a range of Cε3–Cε4 interdomain angles (7°) can be accommodated (93), with concomitant, small differences in the number of van der Waals contacts and in some instances hydrogen bonds also. This conformational flexibility in the two components and plasticity at the interface are consistent with the low affinity of the interaction with a single head domain.

Reciprocal allosteric inhibition of receptor binding

The mutual inhibition of FcεRI and CD23 had earlier led to the suggestion that their binding sites in IgE-Fc might be overlapping (11, 84), but we now know that while both principally involve residues in Cε3, the two sites lie at opposite ends of the domain (82, 85) (*Fig.* 6). Communication between these two sites must therefore be through an allosteric mechanism, one component of which involves the relative disposition of the Cε3 domains. When IgE-Fc (or Fcε3-4) binds to sFcεRIα, the two Cε3 domains move apart and adopt a more open conformation compared with the uncomplexed IgE-Fc (or Fcε3-4). This affects the angle between the Cε3 and Cε4 domains in each chain, which increases. (It is important not to confuse here the bend in the IgE-Fc between the $(Cε2)_2$ domain pair and the Fcε3-4 region, which becomes more acute when IgE-Fc binds to sFcεRIα, as discussed earlier.) With an increased angle between the Cε3 and Cε4 domains, the binding site for sCD23 becomes in part inaccessible. Conversely, in the

complexes between Fcε3-4 and sCD23, the Cε3–Cε4 angle decreases, bringing the Cε3 domains into a more closed conformation, incompatible with binding to sFcεRIα. This is illustrated in *Fig. 6*.

Although these quaternary structural changes in the disposition of the Cε3 domains relative to Cε4 clearly contribute to the reciprocal inhibition of sFcεRIα and sCD23 binding, given the intrinsic flexibility of the Cε3 domains referred to earlier, there may be other aspects to this allosteric communication that additional crystal structures and NMR analyses in solution will subsequently reveal. The consequence of this mutual incompatibility for IgE binding to its two receptors is biologically essential, as trimeric sCD23 or soluble IgE–CD23 complexes (or mCD23 on a cell surface) could cross-link FcεRI-bound IgE on a mast cell or basophil, triggering an allergic reaction. It appears that the IgE molecule has evolved a structure that allows expression of functional activity independently through its two, very different receptors.

The existence of these two conformational states, open and closed, with respect to the Cε3 domains, corresponding to a greater and lesser Cε3–Cε4 angle, respectively, is consistent with intriguing observations reported much earlier concerning the relative binding affinities and binding capacities of IgE for CD23 and FcεRI at different temperatures (91). At lower temperature (4°C), the interaction of CD23 with IgE is more favorable (in terms of affinity and bindability, i.e. active fraction) than at higher temperature (37°C), whereas the converse is true for the FcεRI–IgE interaction. This observation was attributed by the authors, presciently, to a shift in equilibrium between conformational states in the IgE-Fc (91). Although the dependence of the equilibrium between open and closed states upon temperature has yet to be investigated, the association of each state with the binding of one receptor but not the other is now established. In fact, the IgE molecule in its Fc region likely adopts a range of different conformations in solution, incorporating also the bending and unbending of the $(C\varepsilon2)_2$ domain pair relative to the Fcε3-4 region and even extended conformations such as that seen in the anti-IgE Fab–IgE-Fc complex (58).

Summary and future perspective

Crystal structures have now revealed the precise locations of the binding sites in IgE-Fc for its two receptors FcεRI and CD23, while NMR, FRET, and other biophysical studies in solution have monitored the effects of receptor binding upon the conformation of the interacting partners. What has emerged is a remarkably dynamic picture. IgE-Fc has the ability to adopt a range of conformational states, involving movements of the $(C\varepsilon2)_2$ domain pair relative to the Fcε3-4 region, the Cε3 domains relative to each other, and also to the $(C\varepsilon4)_2$ domain pair. Changes in these relative domain orientations provide an explanation for the mutual exclusion of FcεRI and CD23 binding through an allosteric mechanism, although the discovery that IgE has evolved a uniquely flexible and responsive domain, Cε3, to accommodate both receptor-binding activities, suggests that the communication mechanism between the two sites may be even more intricate. Allostery may not only be considered in terms of two (or more) defined structural states but also rather the response of an ensemble of conformations to different binding events (94).

The interaction between IgE and FcεRI is clearly a target for therapeutic intervention, as demonstrated by the success of the anti-IgE monoclonal antibody omalizumab for many people with severe asthma and other allergic conditions. Other anti-IgE antibodies have been reported, e.g. the monoclonal antibody 8D6 that also blocks binding to FcεRI but, unlike omalizumab, can bind to IgE that is already bound to CD23, which might serve additionally to downregulate IgE synthesis (95). Blocking the binding of IgE to FcεRI clearly has efficacy, but the observation that IgE might be removed from its receptor – 'accelerated dissociation' – by molecules such as DARPin, or even anti-IgE antibodies such as omalizumab at very high concentration, is an exciting discovery. The explanation for such a phenomenon almost certainly lies in the dynamics and allosteric capacity of the IgE molecule, perhaps involving conformational changes such as the bending/unbending or opening/closing, or others as yet unidentified. The crystal structure of an anti-IgE antibody Fab complexed with IgE-Fc as described above and illustrated in *Fig. 4B* (58), shows that fully extended conformations can and do occur, if only transiently in the free antibody molecule. Such a conformation is incompatible with FcεRI binding as key amino acid residues in both Cε3 subsites are disrupted by the rearrangement of the Cε3–Cε2 linker region (58). The structure of this complex exemplifies a strategy for discovering, stabilizing – and subsequently targeting – transiently populated conformational states that are incapable of functional activity (96).

CD23 is also an attractive target for therapeutic intervention. An anti-CD23 antibody (lumiliximab) (97) was taken into early clinical trials for allergic asthma and did reduce serum IgE levels, but was not pursued. CD23's role in IgE regulation is complex, and the proposed mechanism

involving IgE–CD23–CD21 interactions at the B-cell surface requires further testing, but its emerging roles in allergen presentation and transcytosis both in the airways (asthma) and gut (food allergy) will again focus interest on CD23. The IgE–CD23 interaction involves flexibility at the interface and is subject to control not only by allostery in IgE but also modulation by the presence of Ca^{2+}. As trimerization is mediated by the stalk region, and functional activity depends upon its oligomeric state, there is also the prospect of exploiting allosteric effects within CD23. Finally, interest in IgE and the expression of its effector functions through both receptor interactions will undoubtedly develop further if the exciting potential of IgE monoclonal antibodies in cancer immunotherapy is realized (98, 99).

Despite this recently acquired wealth of structural data concerning IgE-Fc and its receptor interactions, it must be remembered that neither the three-dimensional structure of the FcεRI $\alpha\beta\gamma_2$ tetramer, nor that of the CD23 trimer, is known. And looking beyond the structure and function of these individual receptor molecules, it will be important to understand their behavior in the membrane: their associations with other cell surface molecules, their response to extracellular stimuli (IgE and allergen-dependent crosslinking), and interactions with intracellular signaling molecules. Fortunately, exciting new developments in super-resolution microscopy, fluorescent labeling chemistry, and techniques for single molecule tracking, offer real promise of answering these questions in the near future.

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