

Structure/function studies on IgE as a basis for the development of rational IgE antagonists

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Since the discovery of antibodies of the immunoglobulin (Ig)E isotype some 30 years ago, their pivotal role in mediating type I hypersensitivity responses has been extensively documented (1, 2). In normal adults, the plasma levels of IgE rarely exceed 100 µg/l, but are elevated in allergic and parasitic disease, and compelling evidence suggests that an IgE response protects against parasitic infestations (3–5). In industrialized societies and in developing countries, the incidence of allergic and asthmatic disorders has dramatically increased during this century, particularly over the past three decades. Epidemiologic surveys indicate that allergic manifestations now afflict more than a quarter of the population in industrialized countries, with 10% of children suffering from asthmatic airway inflammation (6, 7). Pollutants in the air such as diesel exhaust particles, and oxygen radicals produced by engine emissions or cigarette smoke have been implicated as adjuvants, enhancing IgE synthesis (8, 9). Other observations point to a connection between the decline of infectious diseases and the rise in allergic manifestations (10). Furthermore, repeated exposure to substances at the workplace can lead to the development of allergies. Agricultural workers show adverse responses to a widely ranging number of plant and animal allergens, baker's asthma is a well-documented occupational hazard, latex hypersensitivity in health-care workers has become a serious problem in recent years, and employees in the detergent industry report a high incidence of allergic reactions to biologic detergents (11). The socio-

economic impact of allergic diseases places a severe and increasing burden on public health care by reducing the quality of life and work performance of allergy and asthma victims. This is further aggravated by the fact that currently available medications for the management of these diseases are frequently associated with debilitating side-effects and are far from effective (6, 7, 9).

The central role of IgE antibodies and the structural basis of their interaction with class-specific high- (Fc_εRI) and low- (Fc_εR2) affinity receptors in immediate and delayed hypersensitivity reactions have been extensively studied over the last 30 years. Early studies on human (h) myeloma E protein established that IgE, like antibodies of other classes, comprises two heavy ε-chains combined with two light chains that are either of the κ or λ isotype (12–14). Both chain types are organized into variable (Vε) and constant (Cε) regions involved in antigen and Fc-receptor recognition, respectively (10, 11). After secretion by B cells, the IgE molecule binds with high affinity to Fc_εRI receptors predominantly expressed on mast cells and basophils, and with low affinity to Fc_εR2-receptors found on various inflammatory cells including macrophages and platelets. Both receptor classes have been identified on eosinophils, monocytes, and antigen-presenting cells (15). As a consequence, most IgE molecules are cell bound and extensively distributed on the surface of white blood cells and mast cells found in the mucosal lining of the eyes, lungs, skin, and intestines. The binding of IgE to both classes of receptors is a

reversible process. It sensitizes, but does not induce, mediator secretion from target cells expressing the receptors, unless the ligand is aggregated. Degranulation of the mediators of the allergic response occurs as a result of the interaction of receptor-bound IgE with cognate antigen, but most anti-IgE antibodies or lectins interacting with carbohydrate residues in IgE or the receptor can also induce secretory responses (16–18). Interestingly, many proteins from allergenic sources induce mediator secretion from mast cells and basophils, and we have suggested that there is a potential link between allergenicity and the mast cell/basophil secretagogue activity of antigens (19). The wide spectrum of diverse proinflammatory mediators which are released from IgE-activated mast cells and basophils (reviewed by Helm et al. [15]) causes the symptoms associated with immediate hypersensitivity responses. In addition, they play an important role in the selective activation of inflammatory cells, including eosinophils, the upregulation of MHC class II and Fc ϵ RII molecules, and the potential enhancement of IgE synthesis by a feedback mechanism, which amplifies the response to allergen (reviewed by Helm et al. [15]). These observations suggest that inhibition of the IgE-mediated degranulation should be associated with wide-ranging short- and long-term anti-inflammatory benefits.

IgE- and Fc ϵ RI-derived peptides and peptidomimetics as potential antiallergic drugs

In 1968, Stanworth et al. (12) demonstrated that a proteolytic fragment prepared from hIgE competitively inhibited the sensitization of skin mast cells by allergen-specific IgE. This marked the beginning of a quest for progressively smaller IgE- and receptor-derived peptides as competitive inhibitors, in the hope that these might act as antiallergic drugs by inhibiting the interaction between the ligand and its receptors (15). The therapeutic usefulness of these small peptides must be considered doubtful in view of the fact that, *in vivo*, peptides generally lack the bioavailability requirements to make them suitable for oral administration and penetration into tissue fluids. Furthermore, all IgE- and Fc ϵ RI-based peptides examined so far have at least four orders of magnitude lower IC₅₀ values than their native counterparts (15). As a consequence of the high-affinity and reversible nature of IgE/receptor interaction, peptides bound to IgE or to the receptor will eventually become represented on the surface of target cells as a function of their relative affinities and concentration ratios in the body fluids in the vicinity of the receptor-bearing

cells (20). It follows that, unless peptides having substantially higher binding affinities and *in vivo* stability can be identified, it is improbable that peptide-blocking agents can prevent the eventual sensitization of receptor-bearing cells by antigen-specific IgE *in vivo* (15, 20). The requirement that only a small proportion of the total receptor-bound IgE need be cross-linked to initiate exocytosis of mediators compounds what is already a challenging problem. In addition, it is worth noting that Fc ϵ RI α chain-derived peptides proposed by others (21) as potential antiallergic drugs may stimulate the formation of antireceptor antibodies. We have already demonstrated that antibodies raised against an α -chain peptide proposed as an inhibitor of the allergic response (21) induce mast-cell degranulation (15). It is therefore probable that the *in vivo* administration of such antiallergic drugs may precipitate a potentially lethal systemic anaphylactic shock.

A number of approaches to the problem of modifying polypeptide epitopes for optimal binding, bioavailability, and biodistribution have been reported for a wide array of protein–protein interactions (22). Early approaches focused on rational, structure-based optimization using established tenets of medicinal chemistry. Classical examples are Genentech's benzodiazepinedione-based inhibitors of fibrinogen-glycoprotein IIbIIIa binding, prepared *de novo* from consensus conformations of the native arginine-glycine-aspartic acid (RGD) recognition epitope (23, 24), and Bio-Mega's peptidomimetic subunit association inhibitor of HSV ribonucleotide reductase from a hexapeptide (AVVNDL) recognition epitope. Increasingly, however, structurally diverse oligopeptide and peptidomimetic libraries generated by combinatorial “chemical” or biologic methods have been utilized for ligand discovery, particularly where very limited structural information is available on the partners of the protein–protein interaction. Rapid advances in synthetic methods, analytic methods, computational capability (modeling and data handling), and assay procedures have facilitated these approaches. The ability to generate millions of potential “lead” structures in a simple, yet defined manner can greatly reduce the time and effort required to determine the same information via classical chemical syntheses. The development of combinatorial library techniques has its roots in the solid-phase peptide and oligonucleotide synthesis introduced three decades ago by Merrifield (25). Peptide combinatorial libraries (26, 27), consisting of thousands of individual peptides, are generated by extensions of this methodology involving either divide, couple, and recombine (28, 29); portion mixing (30, 31); or split synthesis techniques (32).

Solid-phase peptide libraries have been developed for a number of important biologic targets including opioid-, viral-, and hormone receptors (reviewed by Kieber-Emmons et al. [33]). For example, utilizing an on-bead binding assay with an anti- β -endorphin monoclonal antibody recognizing the continuous tetrapeptide epitope YGGF, Lam et al. were able to identify high-affinity ligands from an all-L-amino-acid library generated by split synthesis (34, 35).

Another method for generating peptide libraries was first described by Smith (36) in 1985. Peptide libraries are generated by cloning random nucleotide sequences in gene III or IV of the phage genome, and are presented on the surface of the filamentous phage. The technique allows up to 200 copies of the peptide to be displayed on the phage surface, presented in a loop formed by disulfide formation between the two flanking Cys residues. A number of protein-protein and protein-ligand interactions have been studied by this technique. Livnah et al. were able to identify a 20-residue peptide which mimics the function of the larger protein erythropoietin (160 AA). The mimic shows no sequence similarity, yet it can bind to and activate the erythropoietin receptor (37). Yanofsky et al. were able to develop a 15-residue peptide which binds to the type 1 interleukin-1 receptor with a dissociation constant of 2 nM, similar to that of the natural antagonist, a 20-kDa peptide which has a dissociation constant of 1.6 nM (38).

Synthetic oligonucleotide libraries have been applied to IgE/ $Fc\epsilon$ RI inhibition. Wiegand et al. reported the use of the SELEX method of library generation to generate oligonucleotides that bound to hIgE with high affinity and selectivity (39). Single-stranded DNA or RNA oligomers of 25–40 variable residues were produced which had dissociation constants in the range of 10–30 nM, but no information on exact binding sites was detailed. As yet, we have no knowledge of the immunogenicity of such compounds, an issue that should be resolved in view of the damaging effect of anti-DNA antibodies in systemic lupus. The therapeutic application of this class of inhibitor therefore requires careful assessment of *in vivo* responses in suitable animal models such as the human SCID mouse system.

Structure-based design of antiallergic vaccines

Acquiring information on the structure of the complementary site of interaction between IgE and its receptors holds tremendous promise for the rational design of antiallergic drugs. Unfortunately, despite intensive efforts worldwide, no

high-resolution structural information is currently available for either the ligand or its receptors. In the absence of such information, data obtained from genetic, protein-, and cell-engineering studies have been complemented by molecular modeling techniques in an attempt to gain information on the nature of the complementary site of interaction (reviewed by Helm et al. [15]). The primary sequence of hIgE has been available since 1974 (14), and, from the high sequence homology with IgG-Fc (40), two molecular models were proposed independently for IgE-Fc in 1986 (41, 42). These projected an arrangement whereby Cys241 in one ϵ -chain pairs with Cys328 in the opposite chain (41, 42). The "crossed" inter- ϵ -chain model, however, did not satisfy a number of biochemical observations (43), and we proposed a revised model when we demonstrated that the interchain disulfide bonds in $C\epsilon$ 2, like those in IgG, are parallel (43). The most pronounced structural feature of this model is the presence of an exposed inter- $C\epsilon$ 2/3 hinge link, computed to connect the globular portions of the $C\epsilon$ 2 and $C\epsilon$ 3 domains. Currently, most publications concerned with a structure/function relationship in IgE utilize this model as a framework for the analysis of structural and functional consequences of site-specific mutagenesis (44–46, 48, 49).

Based on an analogy with the IgG/ $Fc\gamma$ RI interaction (47), we suggested that the $Fc\epsilon$ RI binding region in hIgE might be found in this interdomain segment (43). This proposal was subsequently reiterated by Gould and her collaborators (44, 45, 48), who proposed that residues 329–340 are essential for the binding of IgE to $Fc\epsilon$ RI (44, 45) and that Arg334 played an important role in binding IgE to $Fc\epsilon$ RI (48). Our studies have, however, shown unequivocally that deletion of residues in IgE to Leu340 has no influence on the association of the ligand with either receptor, and causes only a modest increase (~30-fold) in the rate of dissociation from $Fc\epsilon$ RI (49). Furthermore, we demonstrated that the essential determinant for the interaction of IgE with both receptors depends on a consecutive sequence of 11 amino acids spanning residues 343–353 (49). This sequence has been computed to form a loop (A–B), constrained by Pro residues (41, 43, 49). C-terminal deletion of residues in IgE to Pro354 causes a >3000-fold increase in the rate of dissociation (49). This points to the importance of additional residues in the C-terminal $C\epsilon$ 3 domain in the maintenance of the high affinity of interaction, as proposed by Presta et al. (46). They concluded that six residues located in the C–D, E–F, and F–G loops, computed to form the outer ridge on the convex face of the $C\epsilon$ 3 domain, have an important function in the interac-

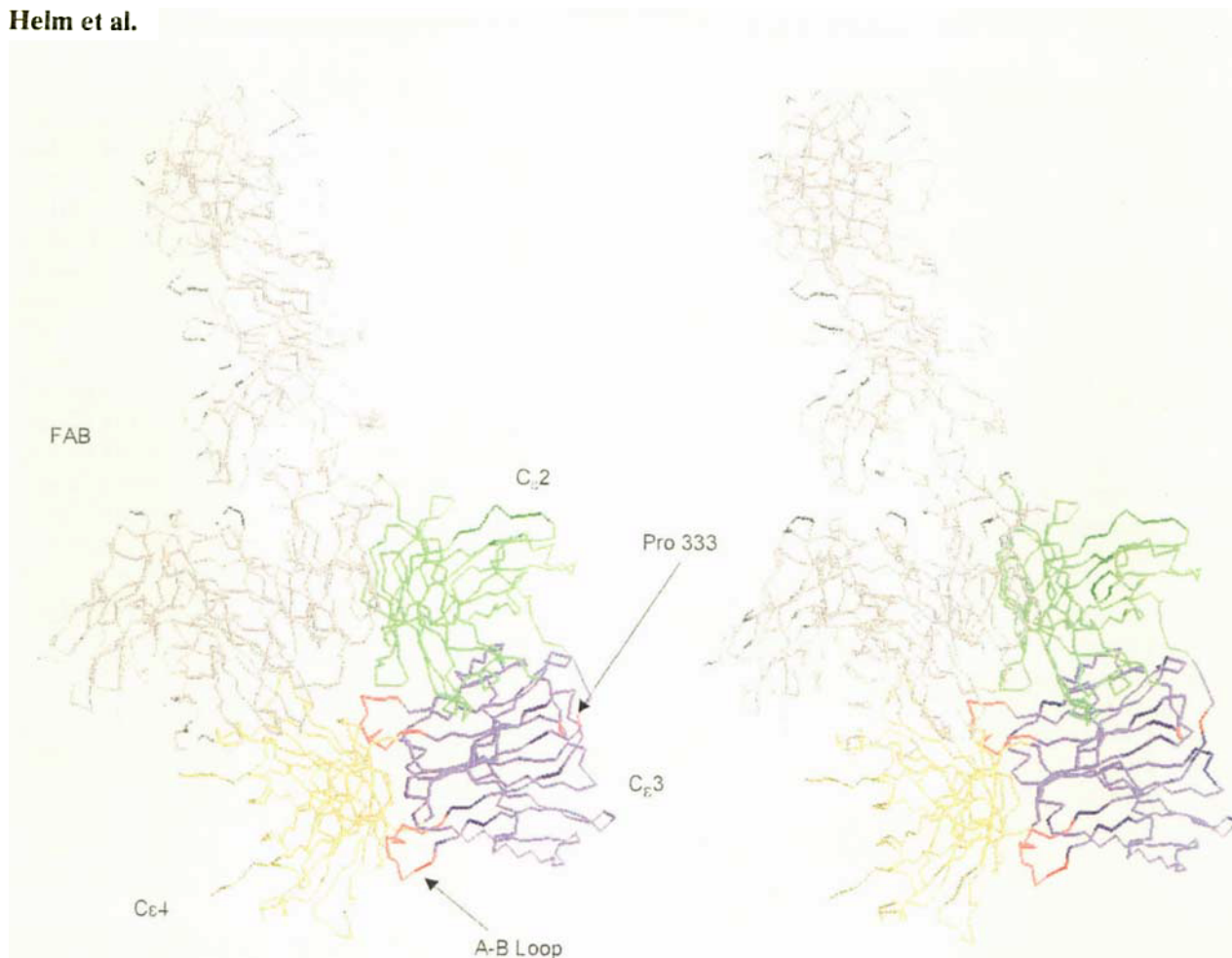


Fig. 1. Stereodrawing of the α -carbon trace of model of human IgE in bent conformation. Antigen-binding sites are shown in gray. $C_{\epsilon}2$ domains are green, $C_{\epsilon}4$ domains are yellow. $C_{\epsilon}3$ domains, which have been shown to comprise effector determinant residues in IgE, are depicted in blue. Pro333 residues in inter- $C_{\epsilon}2/3$ hinge-link region and residues in A-B loop in $C_{\epsilon}3$ are highlighted in red.

tion of IgE with $Fc_{\epsilon}RI$. However, we have questioned the interpretation of their experiments (15, 49). Their claim that the grafting of these IgE loops into IgG confers $Fc_{\epsilon}RI$ binding on the chimeric Ig is not validated by their own data (reviewed by Helm et al. [15, 49]).

Although the function of the $C_{\epsilon}2$ domains is unknown, we have demonstrated that the disposition of the $C_{\epsilon}2$ domains relative to the $C_{\epsilon}3$ domains has a crucial influence on the interaction of IgE with its receptors, even though the $C_{\epsilon}2$ domains do not participate in the interaction of IgE with either receptor (15). Fluorescence energy-transfer studies on rat IgE suggested that the molecule bends out of plane in this interdomain region (50), and our recent observations on the functional consequences of site-specific mutagenesis on hIgE provide strong support for the notion that the Pro333 residues are crucial in maintaining a relatively rigid bend in this interdomain region (51). We incorporated these observations into our latest model structure for hIgE shown in Fig. 1. In this model, the $C_{\epsilon}2$ domains and $(Fab)_2$ twist over one face of the $C_{\epsilon}3$

domains, breaking the $C2$ symmetry of the complex such that the $C_{\epsilon}2$ domains may restrict, by steric hindrance, the docking of IgE to a single $Fc_{\epsilon}RI\alpha$ receptor. This model could explain the apparently paradoxical observation that IgE molecules bind to $Fc_{\epsilon}RI\alpha$ and nonanaphylactogenic antibodies (which block the binding of IgE to $Fc_{\epsilon}RI$, but fail to cross-link receptor-bound IgE and initiate degranulation) with a 1:1 stoichiometry (reviewed by Helm et al. [15]). In contrast, one IgE molecule binds two anaphylactogenic antibodies.

Others, however, disagree with this proposal since their observations suggest that a human IgE fragment comprising residues 328–547 engages only a single copy of soluble $Fc_{\epsilon}RI\alpha$ (53). Since IgE and most IgGs engage the ligand-binding domain of the cognate receptor complex with a 1:1 stoichiometry (unlike the interaction of IgG with placental $Fc\gamma Rn$, where the antibody engages two copies of the receptor prior to transfer to the embryo [54]), identification of the structural basis underlying the 1:1 binding ratio can be expected to provide important information for the development of rational

therapeutic interventions in many Ig-mediated immunologic disorders.

We have already demonstrated that a disulfide bond-constrained peptide based on the sequence of the A–B loop in Cε3, which is essential for the docking of IgE into the receptor, can block the binding of the ligand to the receptor with an IC₅₀ in the micromolar range (15). Appropriate additional constraints imposed on this peptide should result in a molecule that will emulate the tertiary structure of the ligand more closely and therefore have improved blocking activity, but whether such constrained peptides will be able to match or even exceed the binding strength of the native ligand remains to be established. Since multiple sites across the Cε3 have been implicated as contributing to IgE/FcεRI binding, the success of high-affinity peptidomimetics of the A–B loop will ultimately depend on the detailed mode of interaction of the two protein partners. However, we have demonstrated that an antiserum raised in rabbits against the disulfide bond-constrained, but not the linear, peptide, binds to IgE in solution and inhibits the binding of the ligand to its receptor, but does not cross-link receptor-bound IgE (15, 51). This suggests that the design of peptides based on structural epitopes in IgE, which become concealed after the docking of the ligand into the receptor, has an important application for the development of active immunization schedules in allergic disease. Unfortunately, the Cε3 A–B loop peptide was only poorly immunogenic, and future immunization strategies with IgE-derived peptides must take into account the presence of epitopes recognized by both B cells and T cells (52). The bent model structure should provide a useful template for the design of IgE-derived peptide vaccines. Such peptides should have an application in the treatment of all IgE-mediated allergies through active immunization irrespective of the nature of the allergen (15).

Acknowledgments

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