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Peptide blocking of IgE/receptor interaction: possibilities and pitfalls

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The immunoglobulin (Ig)E receptor/effector system is best known for its role in allergy and asthma (1, 2), although compelling evidence suggests that an IgE response protects against parasitic infestations, which affect at least 1000 million people worldwide (3, 4). This beneficial effect is, however, considered of limited value in populations that live in an environment where parasitic infestations are rare and where the synthesis of an antibody of the IgE isotype is usually regarded as an unwanted immune response linked to the development of type I hypersensitivity (5, 6).

Epidemiologic studies indicate that allergic manifestations afflict more than a quarter of the population in industrialized countries, and a dramatic increase has been reported worldwide during the last three decades (5–8). There is evidence that pollutants in the air, such as diesel exhaust particles, oxygen radicals produced by engine emissions, or cigarette smoke, act as adjuvants to enhance IgE synthesis (7, 8). Other observations indicate a connection between the decline of infectious diseases and the rise in type I hypersensitivity responses (9). Delineating the multifactorial environmental causes of this allergy pandemic is likely to take many years.

The central role of IgE antibodies in immediate hypersensitivity responses has been extensively documented since their identification as the mediator of the allergic response (1, 2). Like antibodies of other classes, IgE is a Y-shaped molecule in which heavy ϵ -chains are associated with light (L) chains that are either of the κ or λ isotype. Both chain types are organized into variable ($V\epsilon$) and constant ($C\epsilon$) domains (10, 11). Limited proteolysis of human (h) IgE with papain produces two identical Fab fragments (L-chain and $V\epsilon+C\epsilon1$ of the IgE heavy chains) involved in antigen recognition, and an Fc fragment (disulfide-linked $C\epsilon 2-4$ homodimer). IgE antibodies bind via residues located in the Fc portion of the IgE molecule to the α -chain of the high-affinity receptor complex ($Fc\epsilon RI$), which is expressed predominantly on the surface of tissue mast cells and blood basophils, and also bind to low-affinity receptors ($Fc\epsilon RII$) found on various inflammatory cells, including macrophages and platelets (12–15). Both receptor classes have been identified on eosinophils, monocytes, and antigen-presenting cells (12, 15). In normal adults, the plasma levels of total IgE are $\sim 50 \mu g/l$ and even in severely allergic patients rarely exceed 1 mg/l. In contrast, the IgG concentration is

~5–15 g/l. Most IgE molecules are cell-bound and extensively distributed on cells of the mucosal lining of the eyes, nose, lungs, intestine, and skin. These tissues are the major target organs in immediate hypersensitivity reactions. The binding of multivalent antigen to the V-region of receptor-occupied IgE causes the aggregation of adjacent receptors. Mast cells and basophils respond by secretion of a wide spectrum of diverse proinflammatory mediators, including histamine, prostaglandins, leukotrienes, cytokines (including interleukins [IL]-3, -4, -5, -6, -10, -13, tumor necrosis factor- α (TNF- α), granulocyte/macrophage colony-stimulating factor (GM-CSF), and several serine proteases, the function of which is uncertain (16–18). These mediators not only cause the acute phase of the allergic response but also play an important role in the selective activation of inflammatory cell subpopulations. Via the secretion of IL-4, IgE-activated mast cells/basophils induce upregulation of MHC class II and Fc ϵ RII molecules and stimulate antibody class-switching in B cells to the IgE isotype (18–21). Therefore, an upregulatory feedback occurs which amplifies the response to allergen. IL-5 plays a key role in the activation of eosinophils (reviewed earlier in this series). Eosinophil-mediated cytotoxicity requires accessory mast cells or their mediators, and in tissues affected by immediate hypersensitivity, eosinophils are commonly found in close association with mast cells (22). Similarly, oxygen metabolites, which are released from IgE-activated eosinophils, macrophages, and monocytes, can induce mast-cell secretion through an IgE-independent stimulus (22, 23). Furthermore, the chemotactic effect of TNF- α on the recruitment of eosinophils, monocytes, and macrophages is well known, and infiltration of lung tissue by these cells has been observed in asthmatic patients in the late phase of the allergic response (24). The consequences of IgE-mediated target-cell activation are summarized in Fig. 1.

Collectively, these observations show that the prevention of the release of mediators from mast cells and basophils is highly desirable in order to inhibit/attenuate the clinical manifestations associated with both immediate- and delayed-hypersensitivity reactions.

Currently available medications can partially inhibit IgE-mediated target-cell exocytosis in certain populations of mast cells, while other therapeutic interventions are aimed at attenuating the physiologic effects of mediator release. Unfortunately, the mode of action of most IgE antagonists is ill-defined, and undesirable side-effects are common. The development of more rational inhibitors is therefore of the utmost importance in view of the socioeconomic impact of the disease (5, 6).

The binding of IgE to both types of receptors is a reversible process. It sensitizes, but does not activate, target-cell degranulation until the ligand is aggregated, usually by antigen, less frequently by anti-IgE antibodies and lectins (25–28). Methods that block the initial sensitization of target cells with antigen-specific IgE have been explored since the demonstration that human (h) myeloma IgE-derived Fc ϵ fragments, generated by proteolytic cleavage with papain (which produces peptides comprising h ϵ -chain amino acid [a.a.] residues 1–226 and 227–547 [29]), can competitively inhibit passive cutaneous anaphylaxis (PCA) (10), whereas cleavage products of pepsin digestion (which generates ϵ -chain fragments spanning residues 1–338, 339–349, and 350–547 [29]) do not block. The demonstration that an hIgE-derived Fc-fragment can competitively inhibit the binding of antigen-specific IgE to mast cells and basophils (10, 11) initiated the quest for progressively smaller Fc ϵ -derived peptides as potential blocking agents.

Mapping of the complementary binding site between human IgE and Fc ϵ RI α

Since IgE/Fc ϵ RI interaction is highly specific, the rational design of peptides as blocking agents has focused on the identification of the complementary site of interaction. Despite intensive efforts worldwide, there are currently no high-resolution structures available for any part of the IgE molecule or its receptors. In the absence of such information, model structures have been proposed for the ligand and its receptors, which are based on sequence similarity with known structures. These have been complemented and refined by low-resolution data obtained from protein-engineering studies to obtain information on the conformation of those regions of the molecules that contribute to the complementary interaction (30–32).

Over the years, data have been collected by screening digestion fragments, recombinant IgE-derived peptides, ϵ/γ and human/rodent chimeras, mutant forms of hIgE, and chemically synthesized Fc ϵ - and Fc ϵ RI α -derived peptides as inhibitors of IgE binding (33–40). There is considerable controversy about the exact location of the Fc ϵ RI-binding region in hIgE, and the literature is replete with inconsistencies (34–38). This can be attributed largely to the fact that almost all data reported before the 1990s relied on the inhibition of the PCA reaction. No assay was available to assess the direct binding of hIgE to Fc ϵ RI α , since the ligand recognizes only human and other primate Fc ϵ RI. Peptide inhibition of the PCA reaction was commonly considered an indication that the peptide blocked IgE-receptor interaction, and conflicting

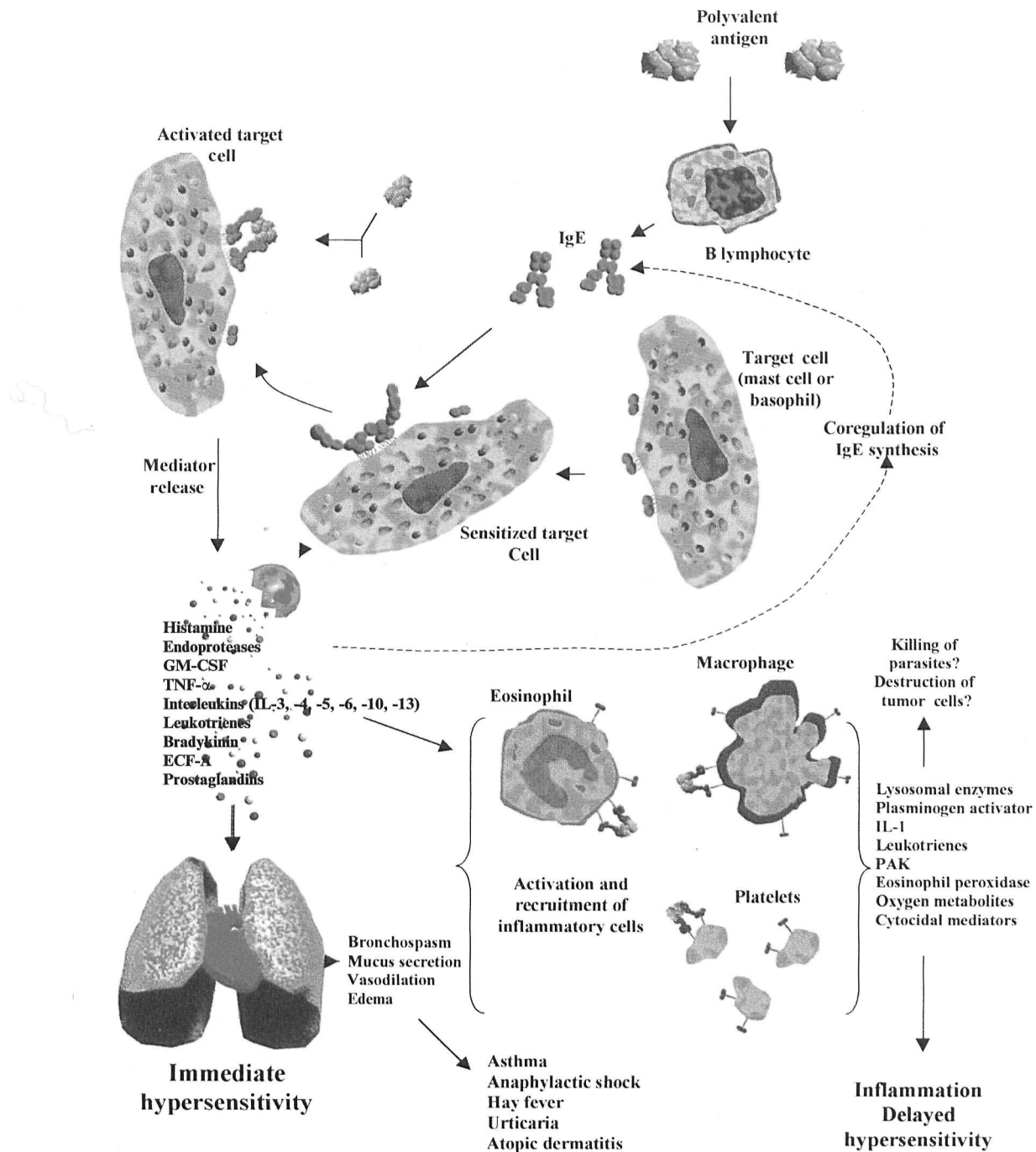


Fig. 1. IgE-mediated target-cell activation in immediate and delayed hypersensitivity. IgE binds with high affinity to class-specific Fc receptors expressed predominantly on mast cells and basophils. Initial ligand/receptor interaction does not activate discernible postreceptor responses. Cross-linking of receptor-bound IgE by allergen or parasite antigens initiates target-cell degranulation, resulting in release of pharmacologically active substances that cause clinical manifestations associated with type I hypersensitivity. Chemokines secreted during immediate hypersensitivity reaction upregulate IgE synthesis by B cells and contribute to late response through priming, activation, and recruitment of inflammatory cells into tissues affected by immediate hypersensitivity responses (diagrammatic representation, not to scale).

results were reported even by the same group of investigators (35, 36). Some of these observations still require re-evaluation and/or reinterpretation in view of the establishment of well-defined *in vitro* and cellular assay systems as a result of the cloning

and expression of the IgE-binding domain of hFc ϵ RI (41, 42).

An interesting example of such inconsistencies is the observation by Hamburger, who reported in 1975 that a small hIgE-derived pentapeptide

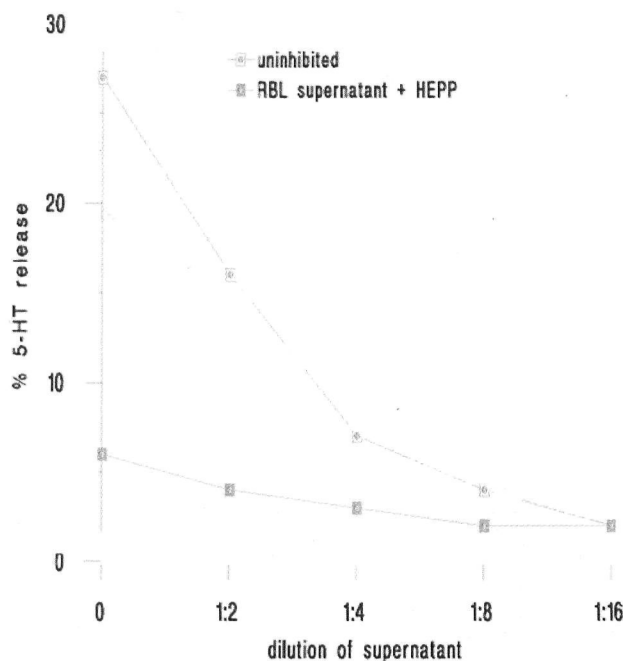


Fig. 2. ^3H -5-hydroxytryptamine (5-HT) release from RBL cells induced by supernatant from IgE-activated cells. RBL2/2/C cells were incubated overnight (o/n) with IgE (JW8 [102]) before challenge with specific antigen. Supernatant was harvested after 60 min and diluted as indicated with or without 50 μM HEPP (43–45) before addition to RBL2/2/C cells prelabeled with ^3H -5-HT. Other substrates for proteases, such as TAME, also attenuate mediator release. Cell degranulation was assessed in tissue-culture supernatant, as described in Refs. 28, 42, and 52. Data shown represent mean of two determinations carried out in duplicate.

(HEPP) comprising a.a. residues 330–334 in the N-terminal C ϵ 3 domain reduced the wheal and flare reaction induced by IgE and specific antigen (43). The findings were initially attributed to competitive inhibition of IgE/Fc ϵ RI interaction (43), but this was later held to be untenable (44). However, patients suffering from allergic rhinitis who were treated with HEPP reported relief of symptoms (45). We reassessed this observation when we found that proteases, including those associated with house-dust mite emanation, cause degranulation of cells of mast-cell lineage, because IgE-activated mast cells also secrete a number of proteases which induce mediator release (28). As shown in Fig. 2, this secondary burst of the release of inflammatory mediators can be attenuated by the inclusion of substrates for serine proteases such as the synthetic substrate *p*-toluenesulfonyl L-arginine methyl ester (TAME) or HEPP in the cell culture medium. This provides an unexpected, although self-evident explanation for the observed therapeutic effect of HEPP and points to the usefulness of developing inhibitors specific for mast cell/basophil proteases in the treatment of allergic responses.

A summary of the sequences of recombinant and synthetic peptides considered of interest in human and rodent IgE/Fc ϵ RI interaction in the context of their putative location is depicted in our model structure of hIgE (30) (Fig. 3). Although there is now a broad consensus that the principal determinants of IgE/Fc ϵ RI interaction are found in the C ϵ 3 domain (residues 329–440) (40, 46–52), there is still no agreement on the actual binding region. On the basis of our demonstration of the parallel nature of the inter- ϵ -chain disulfide bonds in hIgE, we developed a model structure that predicts that an exposed and probably flexible segment connects the globular portions of the C ϵ 2 and C ϵ 3 domains (30). Since there is evidence that rat IgE bends out of plane in this region of the molecule, we suggested that residues such as Pro333 in this potentially flexible interdomain region can facilitate the bending of hIgE (30). By analogy with the IgG/Fc γ RI binding site, we proposed that the receptor-binding region in IgE might be found in the structural equivalent of the flexible hinge region of other antibodies (30). Subsequently, Sutton, Gould, and their collaborators have claimed (50, 51), on the basis of the outcome of earlier PCA tests (34), that the N-terminal 11 a.a. residues (329–340) in C ϵ 3, which are included in this segment, are essential for Fc ϵ RI binding (50, 51). When the unreliability of PCA tests became apparent (35, 36), we re-evaluated previous observations and measured both inhibition and direct binding of hIgE-derived peptides to RBL2/2/C cells (which are transfected with the gene encoding hFc ϵ R α [41, 52]). We demonstrated that N-terminal IgE sequences can be deleted beyond residues 340 without any significant effect on the kinetics of IgE/Fc ϵ RI interaction (52). This indicates that the essential determinant for hIgE/Fc ϵ RI recognition depends on a consecutive sequence of 11 a.a. computed to form a loop (A–B) at the interface between the C ϵ 3 and C ϵ 4 domains (52) (summarized in Fig. 4). N- or C-terminal truncation of the IgE molecule has a negligible effect on the rate of association of biologically active ϵ -chain fragments with Fc ϵ R α . In contrast, the rate of dissociation increases several hundredfold when residues are deleted beyond a.a. 353 from the C-terminal end, indicating the importance of residues in the C-terminal C ϵ 3 domain in the maintenance of high-affinity interaction (52). In accord with others (39, 46, 47), our observations exclude any direct contribution of C ϵ 4-specific residues, as proposed by Stanworth (37) and Burt & Stanworth (38), and confirm and extend those made by Nissim et al. (46, 47), who were the first to demonstrate that the receptor-binding site in IgE is located in the C ϵ 3 domain.

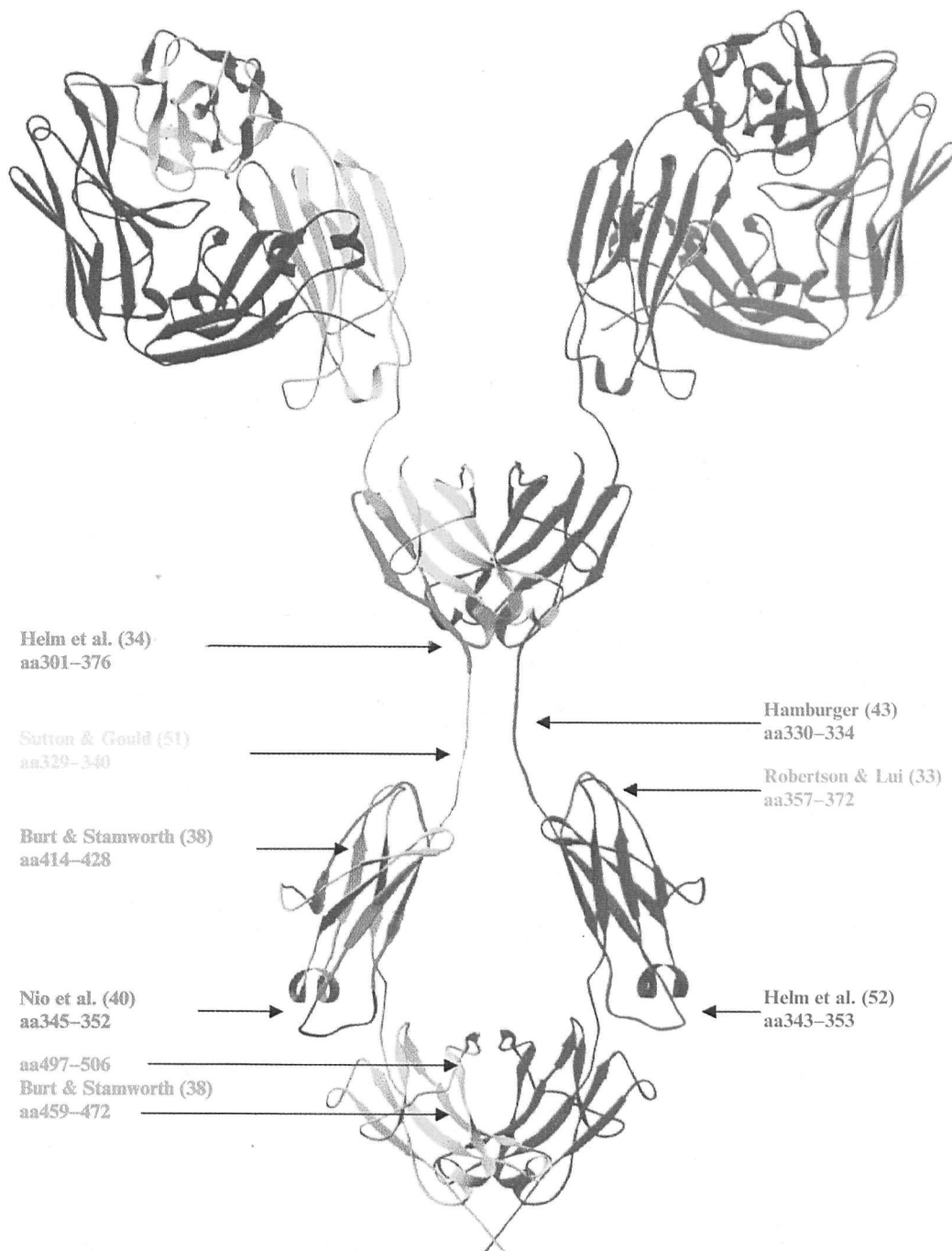


Fig. 3. Location and conformation of recombinant and synthetic human and rodent IgE-derived peptides in context of model structure for hIgE (30, 32). IgE-Fc-derived peptides assessed as potential blocking agents are color-coded.

Although our data might appear to be at variance with the conclusions of Presta et al. (49) (who proposed that six a.a. 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 ϵ 3 domain, are involved in receptor binding), analysis of their data shows that their results provide compelling support for our observations. Their claim that the grafting of loops C-D, E-F, and F-G and the

inter-C ϵ 2/3 switch region into IgG (which they refer to as IgGEL) conferred Fc ϵ RI binding on IgG is not supported by their own data. It is important to note in this context that the chimeric IgGEL construct still retains the endogenous IgG A-B loop, which, when exchanged for the A-B loop of IgE, effects an almost complete loss of receptor recognition (Ref. 49, Table 1, variant 1). They show that IgGEL and variant 1 have iden-

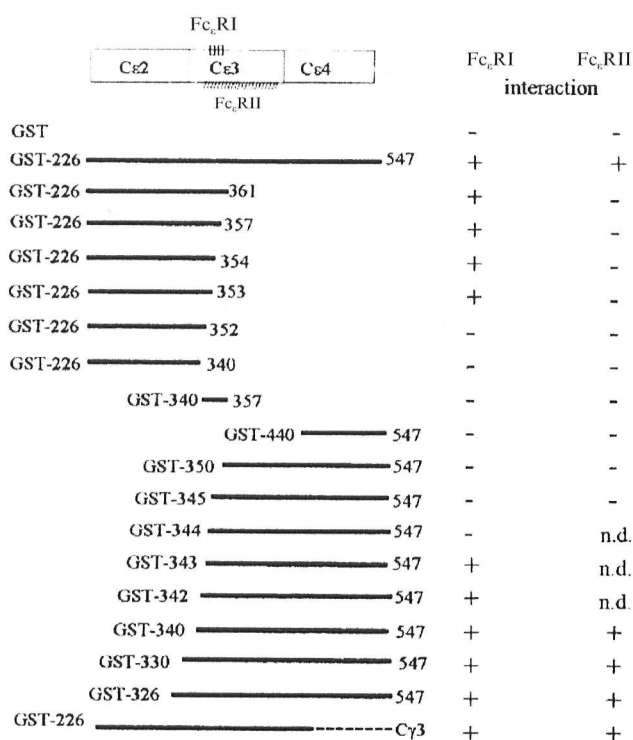


Fig. 4. Recombinant hIgE fragments employed in identification of receptor-binding regions. For experimental details, see Helm et al. (52).

tical binding characteristics; i.e., both constructs display a >97% decrease in receptor-binding activity when compared to native hIgE (Ref. 49, Table 1 and Fig. 3). Thus, their study and ours indicate that the A-B loop in IgE contributes the essential structural determinants for FcεRI recognition.

In complementary studies, the ligand-binding region of FcεRI has been located on a 180-a.a. region comprising the extracellular domain of the α-subunit (53), which is made up of two small Ig-like domains belonging to a subclass known as C2. Based on inhibition studies with monoclonal antibodies (54), construction of chimeric receptors (55, 57), phage display (56), homolog-scanning mutagenesis (58), and peptide inhibition (59), there is general agreement that the membrane proximal α2 domain comprises the IgE-binding site. The α1 domain of hFcεRI does not recognize IgE but confers high affinity on the IgE/α2 interaction. In addition, data suggest that binding is not localized in a single group of residues, but resides in several regions of the α2 domain which have been computed to form loop structures, and it has been suggested that together they may form a binding pocket (55, 57). Regions thought to contribute to the interaction are shown in Fig. 5.

Is there a potential therapeutic application for IgE or FcεRIα-chain-derived peptides?

Peptides generally lack the bioavailability requirements important for cellular activity and oral administration, and L-amino acid-containing peptides are quickly degraded *in vivo*. On the basis of delineation of residues involved in the complementary interaction, both ligand and receptor-derived peptides have been assessed as blocking agents (10, 11, 37, 38, 40, 43, 47–50, 52, 55, 59) by the *in vitro* inhibition of binding of IgE- or FcεRIα-derived peptides. However, all peptides so far examined have four to seven orders of magnitude lower IC₅₀ values when compared with their native counterparts. For example, the IgE-derived linear peptide identified by Nio et al. (40), which is almost identical to the sequence we found to be common to all IgE-derived peptides that engage hFcεRIα, has an IC₅₀ in the mmol range. Low affinity is mainly due to the ability of linear peptides to adopt a large number of conformations in aqueous solution. Several investigations of biologically important interactions in which linear peptides display low binding affinity for a given receptor have demonstrated that appropriate conformationally restrained analogs can exhibit enhanced specificity and affinity. The Pro343–Ser353 sequence has been computed to form an exposed loop, and this provided the basis of the disulfide bond-constrained peptide shown in Fig. 6 which blocks IgE/FcεRI interaction in a competitive manner with an IC₅₀ in the μmol range (Fig. 7). Similar inhibitory potencies have been observed by others for hFcεRIα-derived peptides (59, 60). These observations suggest that a substantial proportion of the hIgE/FcεRI-binding energy is contributed by limited regions in IgE-Fc and FcεRIα, an idea which is supported by observations made by Clackson & Wells, who show that a small subset of residues within a binding “surface” can contribute 75% of the total binding energy (61).

To extrapolate potential therapeutic utility from these observations is premature. In one reported study, mouse IgE- and hFcεRI-derived peptides (a.a. 119–129, D- and L-enantiomers) were incubated together overnight before addition to rat basophilic leukemia (RBL) cells and challenge with specific antigen (59). Complete inhibition of mediator release was observed at peptide concentration ~100 μM (59). *In vivo*, however, it will not be possible to preblock IgE/receptor interaction. Although of high affinity, IgE/receptor interaction is a reversible process, and peptides bound to the receptor or IgE will be in dynamic equilibrium with cell-bound IgE and IgE in the body fluids. As a result of this

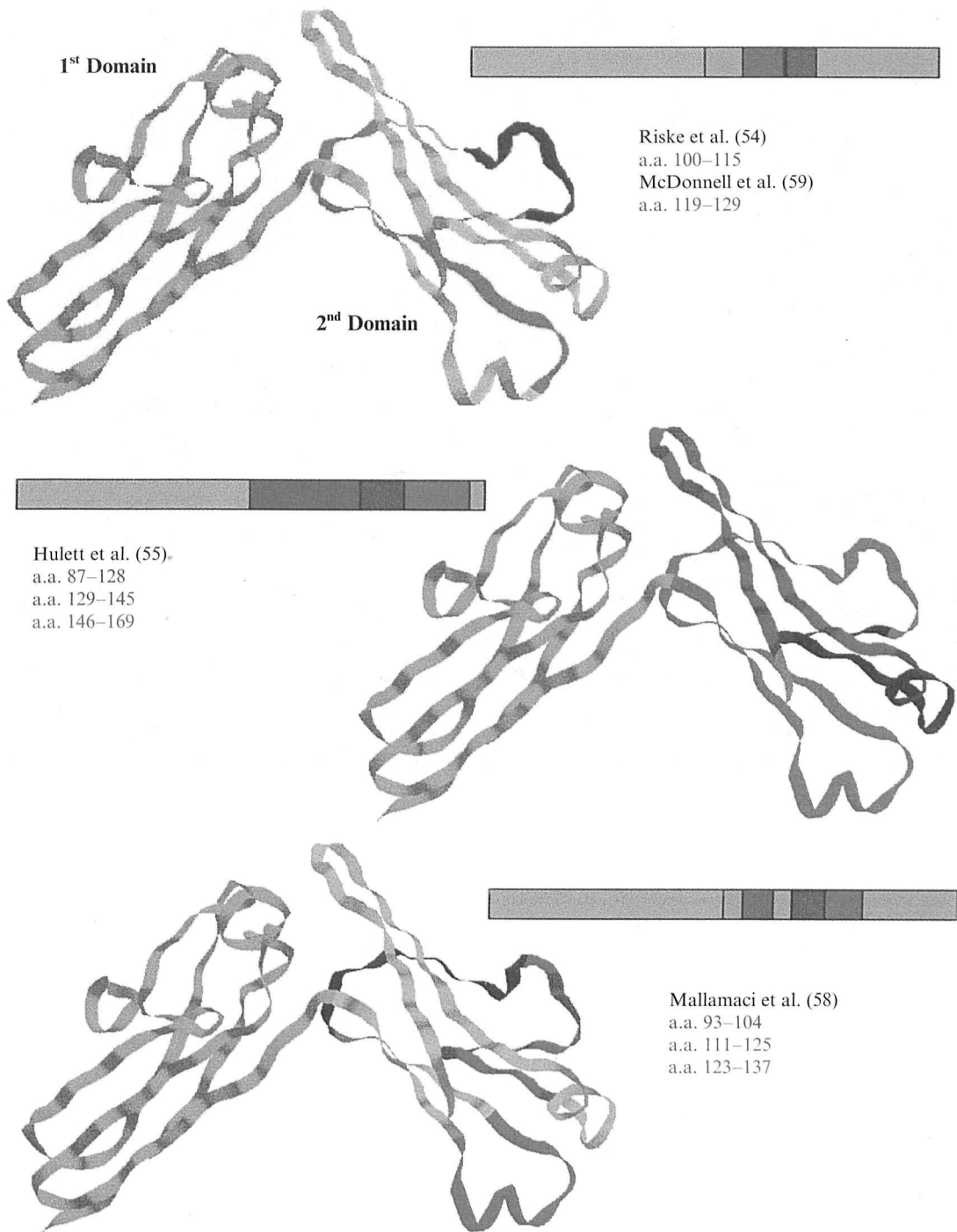


Fig. 5. Model structure of extracellular domain of α -chain of hFc ϵ RI (31, 32). Peptide sequences reported from different studies as being implicated in IgE binding are highlighted.

continuous exchange, peptides will become part of a pool and eventually become represented on the surface of target cells as a function of their relative concentration ratio in the vicinity of the receptor-bearing cells and of their binding affinities (63). When we assessed the inhibitory activity of Ce3 A–B loop- or α -chain-derived peptides, we found that only a small proportion of the total receptor-

bound IgE on mast cells and basophils has to be cross-linked to initiate exocytosis (shown in Fig. 8). Therefore, peptides, even when administered at high molar excess, cannot prevent the eventual sensitization and degranulation of the mediators of the allergic response from target cells (60, 63).

In addition, it is worth noting that the use of α -chain-derived peptides in therapy carries the

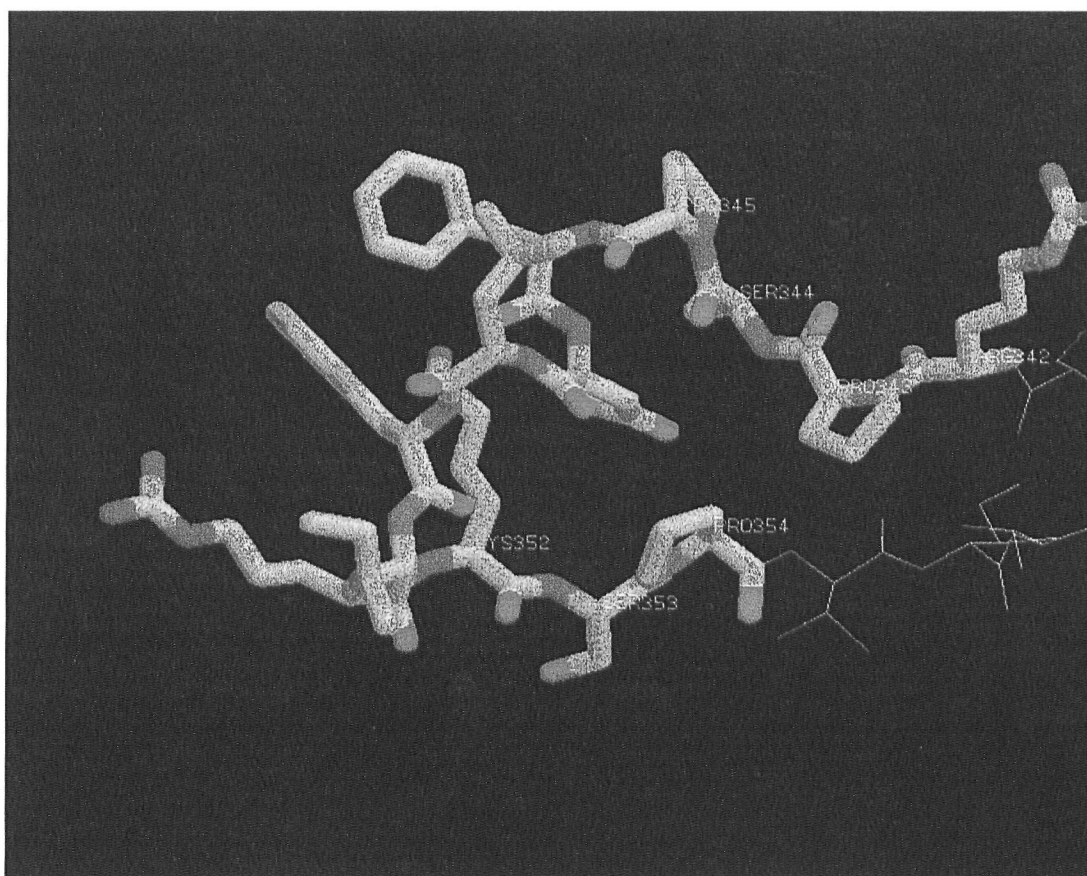


Fig. 6. Model structure of Arg342-Pro354 [C'-Arg342-N'Pro354 separation marked].

inherent danger of inducing an anti-Fc ϵ RI antibody response, resulting in a potentially lethal anaphylactic shock. Fig. 10B shows that an antiserum from mice immunized with the disulfide bond-constrained α -chain peptide comprising residues 119-129 induces degranulation, and some animals which were immunized with this peptide died (Helm, unpublished observations). In contrast, immunization with the IgE-derived A-B loop peptide induced the production of antibodies which inhibited IgE binding to the Fc ϵ RI α but failed to induce mediator release from cells sensitized with IgE (Helm & Sayers, paper in preparation [62]). These findings are summarized in Figs. 10A and B. Interestingly, several monoclonal and naturally occurring anti-hIgE antibodies have been described which block the binding of IgE to Fc ϵ RI, but fail to cross-link receptor-bound IgE and initiate degranulation (25, 26, 64). As reviewed earlier in this series, some of these anti-hIgE antibodies have shown promise in clinical trials after humanization, where they were used for the passive immunization of asthmatic patients. The structural basis of this phenomenon is unknown. It appears paradoxical in view of the fact that IgE is a

homodimer and antibody is divalent. However, unlike IgG/Fc γ Rn interaction, where the ligand can engage two receptors, IgE molecules bind to Fc ϵ RI in 1:1 stoichiometry (52, 65). A possible explanation arising from studies on the interaction of rat IgE with its receptor on RBL cells is that IgE adopts a bent conformation which then renders the ϵ -chain inaccessible to a second receptor molecule or to antibodies directed against epitopes in IgE that become masked after receptor engagement. The distance between the V-region and the C ϵ 4 domain of IgE in solution or complexed to Fc ϵ RI, which would be 18 nm if the molecule had an extended conformation, is only 7.1 nm (66). In the absence of high-resolution structural data, we incorporated these predictions into a model structure for the interaction between a bent form of IgE and Fc ϵ RI α . This model (shown in Fig. 9) is supported by the observation that IgE binds to Fc ϵ RI α and nonanaphylactogenic antibodies in 1:1 stoichiometry (25, 26, 64, 65). In contrast, one IgE molecule binds two anaphylactic antibodies (26).

These considerations suggest that IgE- but not α -chain-derived peptides, have an application as

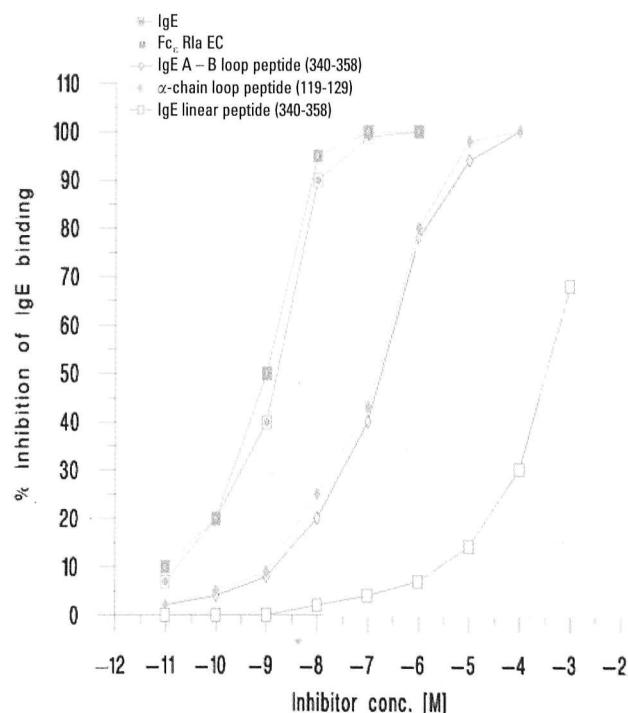


Fig. 7. Inhibition of IgE binding to RBL2/2/C cells by IgE- and α -chain-derived peptides. Extracellular domain of hFc_ε RI α (60, 62) and linear and disulfide bond-constrained peptides were preincubated for 1 h with ¹²⁵I-labeled IgE before addition to RBL2/2/C cells (52). To assess blocking of IgE binding to cellular receptor, cell pellet-associated radioactivity was counted. Mean values of two experiments carried out in duplicate are shown. For experimental details, see Refs. 30, 52, 60, 62.

immunogens by stimulating the synthesis of antibodies capable of preventing cell sensitization with IgE. The identification of the epitopes recognized by nonanaphylactogenic antibodies or, preferably, the identification of the sites in hIgE which become concealed after receptor recognition is the starting point for the design of inhibitors of mast cell/basophil blocking agents. Used as vaccines, such peptides will have a direct application in the treatment of IgE-mediated allergies and should actively protect subjects at risk of anaphylactic shock from insect stings or food hypersensitivity where passive immunization may not be effective.

Since there is little evidence of a beneficial role of IgE antibodies except in parasitic disease, active immunization against allergies appears to be an attractive prospect. However, other important functions might unexpectedly emerge. In the long term, the determination of the structure of the complex of hIgE/Fc with the soluble extracellular domain of the α -chain holds tremendous promise for the rational design of antiallergic drugs based on the coordinates of the complementary site of interaction.

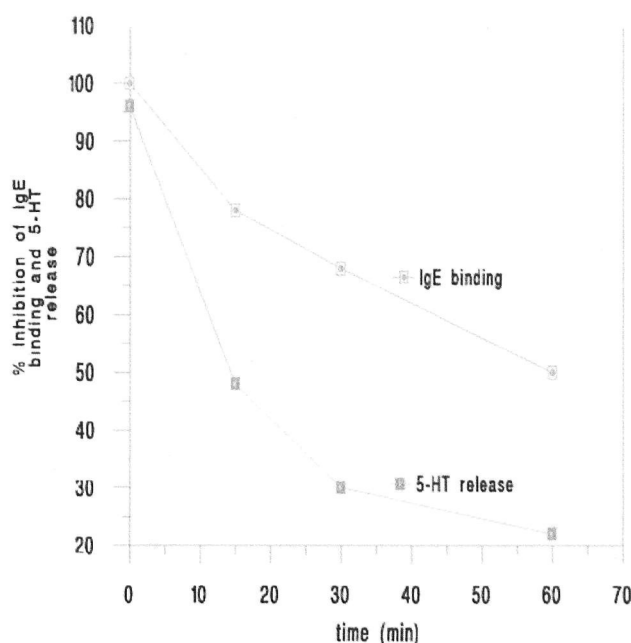


Fig. 8. Time-dependent changes in inhibition of IgE binding and mediator release. ¹²⁵I-labeled IgE (2 μ g/ml) and 200 000-fold molar excess of IgE A-B loop peptide (a.a. 340–358) were added to RBL2/2/C cells, and cell pellet-associated radioactivity was monitored for 60 min. To measure inhibition of 5-HT release, IgE (JW8, 2 μ g/ml) was incubated with 200 000-fold molar excess of IgE A-B loop peptide and 10 ng/ml specific antigen (NIP linked to HSA [101]), and 5-HT released into supernatant was monitored for 60 min. During this time, 40% of IgE became associated with receptor, while inhibition of mediator release decreased by nearly 80%. Mean values of two experiments carried out in duplicate are shown. For precise experimental details, see Refs. 30, 52, 60, 62. Almost identical results were obtained when h α -chain-derived peptide (a.a. 119–129 [59]) was assayed under equivalent conditions (data not shown).

Structure-based design of antiallergic drugs

Structure-based drug design has benefited tremendously from improvements in protein overexpression, purification techniques, and methods employed for the determination of protein structure. The discovery of low-molecular-weight compounds capable of selectively binding and modulating the activity of receptor motifs on the surfaces of proteins by related methods is a challenging proposition, and is now emerging as an increasingly important goal of medicinal chemistry programs. Structure determination, or, in its absence, homology modeling, of both partners involved in such interactions is the starting point. This is followed by the identification of "lead" structures. Leads will typically have dissociation constants of ~ 10 μ M or better and are invariably peptides or simple peptidomimetics. These may be generated by a number of methods. Endogenous peptide or

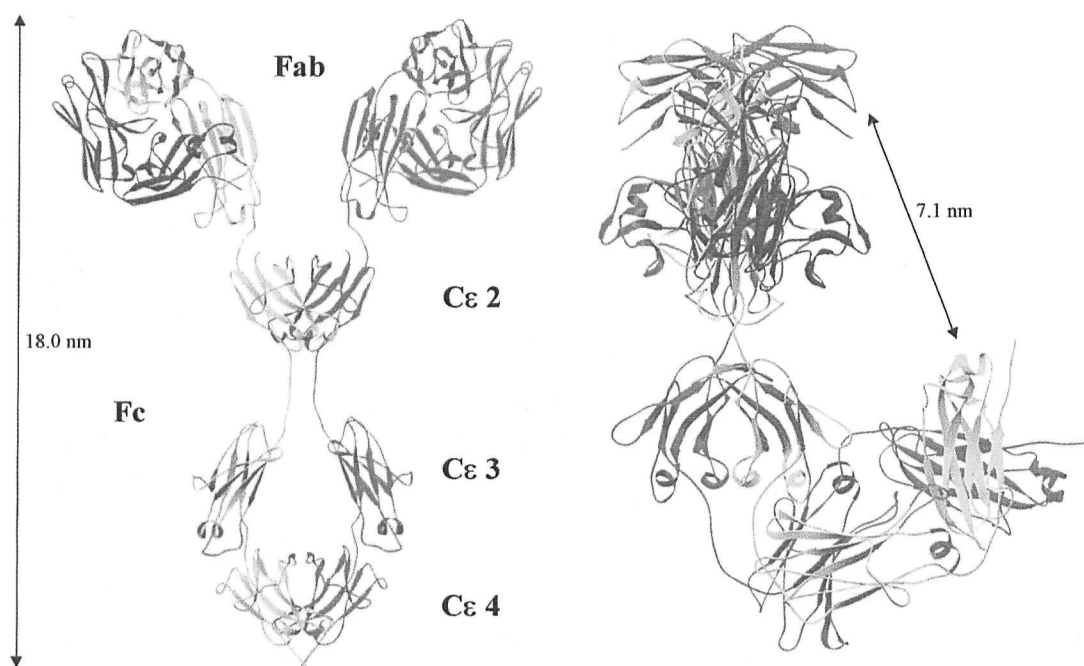


Fig. 9. Structural models of IgE in coplanar (left) and bent conformation (right). Structure projections are based on data obtained for conformation of rat IgE in solution and bound to Fc ϵ RI (66).

peptidic ligands for the receptor motif in question may provide a starting point (67). High-throughput screening of large numbers of individual compounds or affinity screening of pools of compounds assembled by combinatorial techniques is another rich source of leads (68, 69). Vast libraries of peptides can be created by a number of techniques, including cloning of complex mixtures of combinatorially synthesized oligonucleotides into specialized expression "display" vectors (84), or by solid-phase peptide-synthesis techniques (68, 69, 74). An oligonucleotide identified through this approach that inhibits IgE/Fc ϵ RI interaction has already been described (73). A lead may also be generated "rationally" as the direct result of analysis of the molecular interactions present at the docking surfaces. Many algorithms such as Monte Carlo, GRID, MCSS, AUTODOCK, and LEGEND have been developed to aid analysis of such molecular interactions (70). Resulting compounds will typically be linear peptides or template-constrained peptide loops. Various programs can screen databases containing libraries of atomic coordinates for vectorial, spatial, and electronic relationships between atoms, bonds, and functional groups as potential molecular templates in such a rational lead design process (71).

Although conformationally restrained peptides frequently exhibit enhanced specificity, affinity, and activity after oral administration relative to similar linear peptides (72), the next step in the drug

discovery process typically involves either systematic or focused combinatorial modification of such lead compounds. In the first instance, this optimization is likely to revolve around gaining at least three to four orders of magnitude in binding affinity while avoiding constellations of functionality known to be metabolically unstable. The crucial issues of bioavailability and selectivity will usually only assume overriding importance once acceptable binding affinity has been demonstrated *in vitro*. It is generally accepted that significant enhancement in binding affinity can be attained by reducing the conformational mobility of a lead so as to "freeze out" its active conformation as closely as possible. To this end, residue replacement within both the peptidic and template portions of the lead is undertaken. This could involve *N*-methylation, isosteric and isoelectronic functional group replacements, whole residue replacements (e.g., conformationally restricted amino acids), or incorporation of known secondary structural fragments (e.g., β -turn, Ω -loop mimetics, etc.), depending on the conformational hypothesis being pursued (78). This process of optimization has generally been performed in a stepwise, iterative manner, but the potential advantages of harnessing a combinatorial dimension to the process are starting to be appreciated and are likely to be considerable (68, 69).

Whichever method of optimization and refinement of a lead structure is pursued, the "minimum" structural requirement for effective binding should

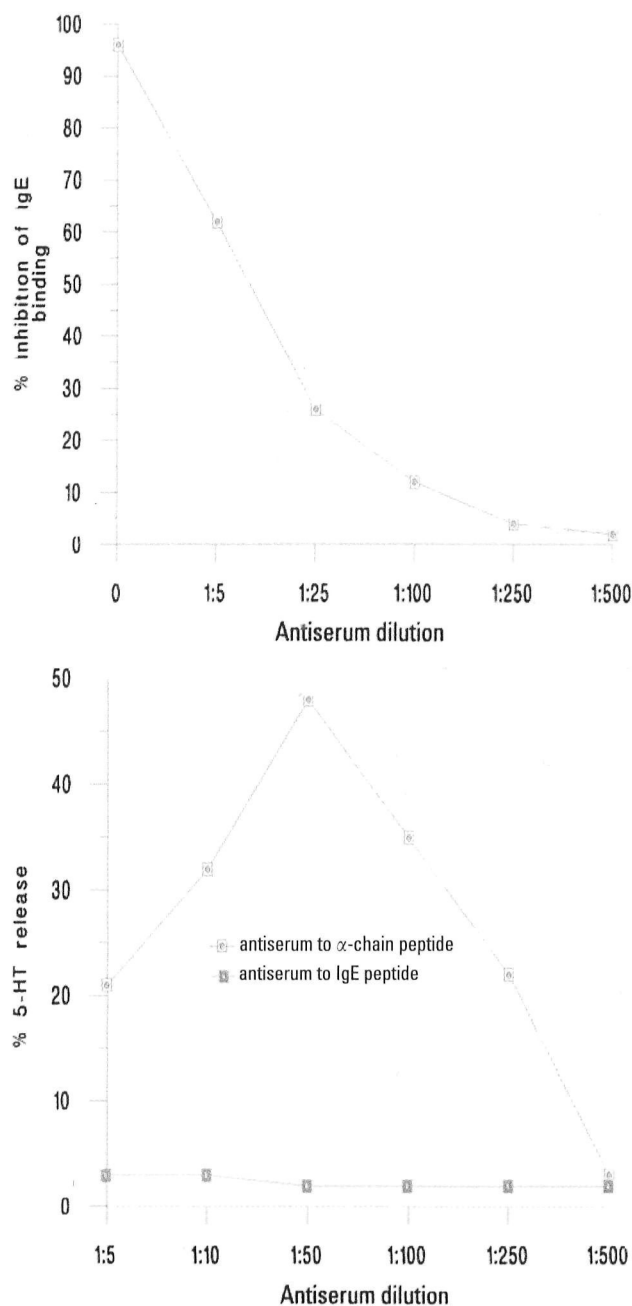


Fig. 10A. Blocking of IgE to RBL2/2/C cells by rabbit antiserum raised against IgE-derived A-B disulfide bond-constrained loop peptide (340–358) (62). 150 μ l of 2 μ g/ml ^{125} I-labeled IgE was incubated for 1 h with increasing dilutions of antiserum raised in rabbits against disulfide bond-constrained A-B loop peptide before incubation with RBL2/2/C cells for 30 min. Receptor-associated radioactivity was assayed as before. Mean values of two experiments carried out in duplicate are shown.

Fig. 10B. Anaphylactogenicity of IgE- and α -chain-derived peptides. Antibodies were raised in rabbits against disulfide bond-constrained IgE A-B loop peptide and in mice against Fc ϵ RI α -derived peptides (a.a. 119–129). RBL2/2/C cells were sensitized o/n with IgE (JW8) and challenged with dilutions of antiserum as indicated. 5-HT release was assessed as before.

eventually become apparent. This will ideally be a totally nonpeptidic compound that mimics the key structural properties of the complementary partner of the interaction by virtue of having appropriate functional groups oriented for interaction with the receptor (75–78). Examples of published drug discovery programs which have embraced hierarchic, structure-based approaches broadly similar to those indicated above include studies on the somatostatin-opioid (79) and cyclophilin-cyclosporin (80) interactions and mimics of streptavidin (81), RGD (72, 82, 83), and EPO (84, 85) binding motifs. However, a successful example of complete progression from a peptidic lead compound to a low-molecular-weight, nonpeptidic drug has yet to be disclosed for any receptor involved in cell–cell or cell–matrix interactions. Indeed, to what extent it will be possible to “reduce” peptidomimetic leads to very small nonpeptidic molecules remains an open question (86). Interesting in this regard are the studies of Huang (87) and a group at Hoffmann-LaRoche (89) who have recently disclosed small nonpeptidic inhibitors of the CD4-MHC and IL-2/IL-2R α -receptor interactions, respectively. The binding affinities in both cases are low, but whether this can be increased remains to be determined. Given our present limited understanding of the fundamental processes underlying static, let alone dynamic, molecular recognition phenomena in general, this is likely to be resolved only by further experimentation.

Application of such a hierarchic, structure-based optimization program to the disulfide bond-constrained peptide leads described by us and others for the IgE/Fc ϵ RI interaction is particularly difficult because of the inadequate structural information presently available for this protein–protein interaction. As detailed above, the homology model structures of IgE suggest that its binding domain is contained within a complex turn connecting antiparallel β -strands. This is properly classified as a Ω -loop (90). The Pro343–Ser353 motif comprises a sector within this loop, shown in Fig. 6, which spans about 9.5 Å.

Consequently, the design of a more rigid template for this motif is not trivial. Many template molecules have been reported, but the vast majority of these are so-called β -turn mimetics. They fall into two categories: those which mimic the four residues within specific β -turns (91) and those which simply position correctly two chains to enter and exit a reverse turn antiparallel, with a separation of $\sim$ 5 Å. Longer templates and particularly templates which specifically address 3-D-vectorial positioning of termini are much less common. Notable examples before 1994 are cited in an excellent review by Olsen et al. (92), and more

recent examples are due to Robinson (82, 88, 93), Schreiber (94), and Chmielewski (95). It is of course important also to consider the degree of flexibility afforded by a potential template. Nuclear magnetic resonance studies indicate that in aqueous solution the disulfide bond-constrained peptide loops are conformationally labile. This may indicate stringent conformational binding dependence (52). However, until higher resolution structural data for the binding domain become available, it would be prudent to maintain some degree of flexibility in the system.

Conclusion

IgE/Fc ϵ RI interaction is of high affinity, mainly because of the very slow dissociation of the ligand from the receptor once it is bound ($k_{-1}=1.3\times 10^{-5}\text{ s}^{-1}$). Ideally, a clinically effective inhibitor should be long-lived (the half-life of receptor-complexed antigen-specific IgE is ~21 days [96]), and should displace cell-bound IgE. Short IgE- or receptor-derived fragments described so far do not meet these requirements. They have short half-lives, although D-enantiomers appear to be more stable, and all IgE- or receptor-derived peptides have at least four orders of magnitude lower binding affinities (e.g., Fig. 7) than the native molecules. As a consequence of the reversible nature of IgE/Fc ϵ RI interaction, it will not be possible to prevent eventual sensitization with antigen-specific IgE. Since only a small proportion of the total receptor-bound IgE has to be cross-linked, it appears improbable that small peptides, even when administered at high molar excess, can prevent activation of the mediator release which causes the symptoms of allergy.

The future development of rational antiallergic drugs should be greatly assisted by structural information on the molecular forces involved in the complementary interaction between the ligand and its receptor. Detailed structural information can be expected to assist the rational design of small-molecular-weight inhibitors of the allergic response. Precise identification of the site(s) in IgE which are accessible to anti-IgE antibodies in solution but become masked once IgE engages the receptor should assist the development of IgE-derived peptides as immunogens to stimulate the formation of antibodies binding specifically to receptor-binding site(s) of IgE in the circulation and to membrane IgE on B cells. In the absence of high-resolution structure determination of the complementary ligand/receptor binding site, the identification of epitopes recognized by nonanaphylactogenic antibodies might assist the design of IgE-derived peptides as antiallergic vaccines (97). Active immunization could be particularly benefi-

cial to asthmatics and patients at risk of anaphylactic shock.

The rationale behind these potential therapeutic interventions is based on the expectation that inhibition of IgE/Fc ϵ RI interaction will prevent allergic responses. A recent report, however, shows that IgE knockout mice still suffer allergic reactions (98). This can be attributed to the fact that the receptor for IgG shares the signal-transducing γ -chain of the Fc ϵ RI complex and that IgG receptors are expressed in some mast-cell subpopulations. Attention should therefore be focused on identifying mechanisms of signal transduction common to all Fc receptors which share γ -subunits. A study of contact sites between the transmembrane domains of the ligand-binding and signal-transducing domains of the receptor complexes may assist the design of molecules which could act as "intermolecular wedges" capable of inhibiting receptor aggregation. This approach has already been applied to the development of a "molecular wedge" anticancer drug which inhibits *neu* oncogene receptor activation and to the inhibition of signaling via the T-cell receptor (99, 100). This kind of inhibitor may therefore have an application in combination therapy with drugs interfering with the IgE/Fc ϵ RI interaction, although recent observations in a mouse model system, which demonstrate that IgE enhances Fc ϵ RI expression and mast-cell degranulation (101), suggest that inhibition of mast-cell sensitization could be associated with wide-ranging anti-inflammatory benefits.

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