

Immunoglobulin E Receptors

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IgE mediates the allergic reaction known as type I immediate hypersensitivity. It also plays a role in late phase allergic reactions. This activity depends on the interaction of IgE with specific receptors on effector cells. The receptors are of two types, designated $\text{Fc}_\epsilon\text{R1}$ and $\text{Fc}_\epsilon\text{R2}$, and they differ in their affinity for IgE, their distribution on different cell types, and structure. $\text{Fc}_\epsilon\text{R1}$ has an affinity ($K_a = 10^9 \text{ M}^{-1}$), some two orders of magnitude higher than that of $\text{Fc}_\epsilon\text{R2}$ ($K_a = 10^7 \text{ M}^{-1}$) for monomeric IgE. $\text{Fc}_\epsilon\text{R1}$ is present on mast cells and basophils which are responsible for type I immediate hypersensitivity. $\text{Fc}_\epsilon\text{R2}$ is expressed by several types of inflammatory cells, including eosinophils, platelets and macrophages that may participate in late phase reactions. The amino acid sequences of $\text{Fc}_\epsilon\text{R1}$ and $\text{Fc}_\epsilon\text{R2}$ reveal that they are unrelated proteins. In common with other immunoglobulin receptors, $\text{Fc}_\epsilon\text{R1}$ is a member of the immunoglobulin superfamily. By contrast, $\text{Fc}_\epsilon\text{R2}$ is a member of the superfamily of hepatic lectins.

Knowledge of the three-dimensional structures of IgE and its receptors, and of the two IgE-receptor complexes, can be used in the design of novel drugs for the treatment of allergy. An allergic reaction begins when an allergen stimulates a B-cell committed to the synthesis of an IgE antibody to divide and differentiate to produce large amounts of the antibody. The secreted product binds to the receptors on effector cells and then cross-linking by the allergen triggers cell activity. Clearly, allergic reactions could be prevented by the use of competitive inhibitors of the IgE-receptor interactions. The primary requirement for such a competitor is that it should bind with high affinity to IgE or to the receptor, but be unable to mediate cross-linking of the cell-bound receptors.

These two requirements may be met by the four types of inhibitors illustrated in Fig. 1. Panels (a) and (b) represent inhibitors that mimic IgE or $\text{Fc}_\epsilon\text{R1}$. In (a) the inhibitor is the Fc fragment of IgE. In (b) it is a soluble fragment of $\text{Fc}_\epsilon\text{R1}$. Panels (c) and (d) show other types of proteins which bind to the same molecules, but not necessarily to the same site, interposing themselves between IgE and the cell-bound receptor. Monoclonal antibodies represent a versatile class of proteins which may be easily adapted for this purpose. The competitors shown in panels (c) and (d) are the Fab fragments of monoclonal antibodies. The intact antibodies may have undesirable effects on the modulation of B-cell activities (c) or may themselves act to cross-link the receptors on effector cells (d).

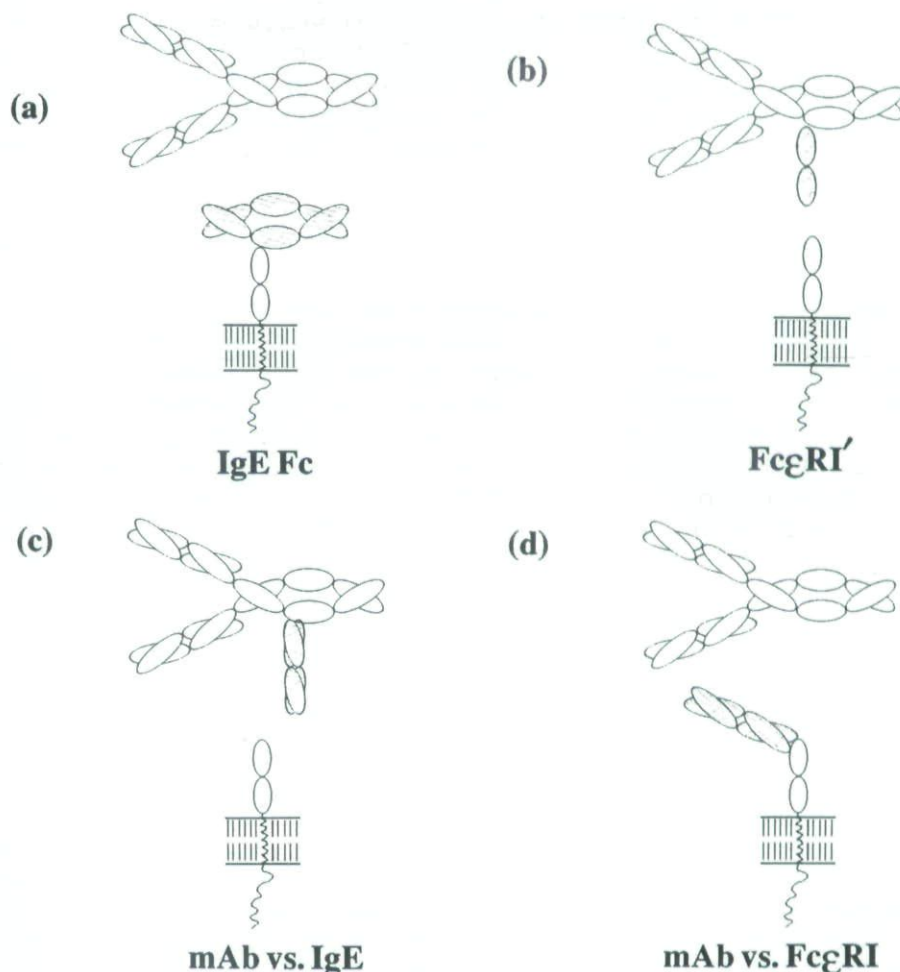


Fig. 1 Strategies for Competitive Inhibition of IgE binding to Fc ϵ R1 on Mast Cells. IgE is shown at the top, the mast cell Fc ϵ R1 at the bottom, and the competitor is interposed between them. Immunoglobulin or immunoglobulin-like domains are represented by ellipsoids, which are unfilled in the case of the native molecules and hatched in the case of the competitors. The competitors, named below each panel, are (a) the Fc fragment of IgE; (b) a soluble fragment of the receptor containing the two extra-cellular, immunoglobulin-like domains (Fc ϵ R1'); (c) the Fab fragment of a monoclonal antibody that recognises an epitope in C ϵ 3 of IgE; (d) the Fab fragment of a monoclonal antibody that recognises the receptor.

An intriguing example of a protein which fits the strategy (c) is the soluble fragment of Fc ϵ R2, which has been shown to interfere with the binding of IgE to Fc ϵ R1 on mast cells (1). The mutually exclusive binding of Fc ϵ R1 and Fc ϵ R2 to IgE may be rationalised by the observation that the two binding sites are near to one another in the three-dimensional structure of IgE (2). The competitor of the IgE-Fc ϵ R1 interaction need not be limited to IgE, IgE receptors and antibodies. They may also encompass non-peptide agents with affinity

and specificity for this system. The competitors shown in Fig. 1 are protein fragments, but they are still fairly large peptides, and as such could act as antigens (and combine with auto-antibodies), or even act as immunogens and elicit an antibody response. The ideal competitor would be a much smaller one. The design of such peptides, or indeed non-peptide competitors, will require structural data.

IgE Structure and the Location of Binding Sites for $\text{Fc}_\epsilon\text{R1}$ and $\text{Fc}_\epsilon\text{R2}$

Like other immunoglobulins IgE has two heavy (H) and two light (L) chains, each with variable (V) and constant (C) regions, associated into LH and HH dimers through parts of each chain. IgE is distinguished from other immunoglobulins in having ϵ -heavy chains, which have four constant region domains, termed $\text{C}_{\epsilon 1}$ - $\text{C}_{\epsilon 4}$. The two ϵ -chains are covalently linked by two disulphide bonds in $\text{C}_{\epsilon 2}$. Thus, this domain serves the same function as the hinge region in IgG, which is too short to form a globular domain. Protease digestion cleaves IgE into Fab and Fc fragments between $\text{C}_{\epsilon 1}$ and $\text{C}_{\epsilon 2}$. The Fc fragment of IgE is a dimer of the three carboxy-terminal domains of the ϵ -chain $\text{C}_{\epsilon 2}$ - $\text{C}_{\epsilon 4}$; the two chains are covalently linked by the two inter-chain disulphide bonds between the pair of $\text{C}_{\epsilon 2}$ domains and non-covalently associated in the $\text{C}_{\epsilon 4}$ domains. Since the allergen combining sites of IgE are in the Fab arms, the Fc fragment can bind to the receptor, without being cross-linked by allergen (Fig. 1a).

Smaller fragments of the ϵ -chain which can bind with high affinity to both receptors have been identified, by expression of ϵ -chain cDNA in *E. coli* and binding assays (2,3). To summarise these results, all fragments containing the 68 amino acid sequence of the ϵ -chain between Gln 301 and Lys 368, spanning the junction between $\text{C}_{\epsilon 2}$ and $\text{C}_{\epsilon 3}$, are active in binding to $\text{Fc}_\epsilon\text{R1}$. The $\text{Fc}_\epsilon\text{R2}$ binding site is contained in $\text{C}_{\epsilon 3}$. Interestingly, monomeric peptides containing $\text{C}_{\epsilon 2}/\text{C}_{\epsilon 3}$ junction sequence were recognised by $\text{Fc}_\epsilon\text{R1}$, but $\text{Fc}_\epsilon\text{R2}$ required dimeric $\text{C}_{\epsilon 3}$.

Two different three-dimensional structures have been proposed for the IgE Fc. They differ in the arrangement of the two inter-chain disulphide bonds in $\text{C}_{\epsilon 2}$, the original models having been made on the assumption of the crossed arrangement (4,5), and a more recent model on the assumption of a parallel arrangement of these bonds (6). The structure of the $\text{C}_{\epsilon 2}/\text{C}_{\epsilon 3}$ junction region differs quite dramatically in the two models, and hence it is not clear whether the part of the ϵ -sequence recognised by the receptor forms a deep groove or a relatively flat surface on the IgE molecule. In both models, however, the sequence Gln 301-Lys 368 forms two β -hairpins (hydrogen-bonded β -strands connected by a β -turn), one each in $\text{C}_{\epsilon 2}$ and $\text{C}_{\epsilon 3}$; the former appears to be more accessible to interaction with a receptor.

The Structure of $\text{Fc}_\epsilon\text{R1}$

$\text{Fc}_\epsilon\text{R1}$ is a multi-subunit protein, consisting of an α -chain which binds to IgE, a β -chain and two gamma-chains. The sequence of the α -chain shows it to be closely homologous to the IgG receptors that

have been characterised, and correspondingly it has two immunoglobulin-like domains in the N-terminal, extra-cellular region of its sequence (7).

The homology with immunoglobulins has been used to build a model of this region of the α -chain (B.J. Sutton and D. Martin, unpublished results). This clearly consists of two immunoglobulin-like domains. Neither domain, however, fits the pattern of the variable and constant immunoglobulin domains for which crystal structures are known, but rather they incorporate features of both. After careful alignment of the sequence of the receptor with the sequences of the V and C domains, models of the "core" regions of the two domains were built. This structure consists of two β -sheets, packed together and bridged by an internal disulphide bond. The "loop" regions which link the strands of β -sheet were then modelled by homology with loops of similar length from the extensive database of known immunoglobulin structures.

The completed model reveals a number of interesting features such as the close interaction between the two domains and the hydrophobic nature of the short peptide which links them. The model also places all of the glycosylation sites and also a proposed site of interaction with IgE at the surface; this information was not incorporated into the model building procedure.

The Structure of $\text{Fc}_\epsilon\text{R2}$

$\text{Fc}_\epsilon\text{R2}$ is unique amongst immunoglobulin receptors, in not being a member of the immunoglobulin superfamily. It is related instead to the family of hepatic (or C-type) animal lectins. Lectins are defined by their function of binding to carbohydrates in glycoproteins (11); but $\text{Fc}_\epsilon\text{R2}$ does not recognise the carbohydrate chains in IgE (2). No crystal structure for an animal lectin is currently available as the starting point for a model. Hence little can be said about the structure of the part of the molecule that binds IgE.

The primary structure of the receptor consists of several functional domains (12). As with other C-type lectins, $\text{Fc}_\epsilon\text{R2}$ is oriented in the cell membrane with its carboxy-terminus projecting out of the cell and its amino-terminus in the cytoplasm. The "domains" of the protein then, reading from the C-terminus to the N-terminus, include the lectin domain which binds to IgE, a series of three repeating sequences (four in the mouse), the transmembrane segment and the cytoplasmic tail. The receptor contains glycosylation sites and sites of proteolytic cleavage, an auto-protease catalytic domain, and alternative cytoplasmic tails, all of which can be anticipated to have important biological functions.

$\text{Fc}_\epsilon\text{R2}$ has been represented as a monomeric chain, but protein-protein cross-linking studies suggest that it may exist as a homodimer (13). Furthermore, the presence of a heptad repeat of leucines between residues 95 and 116 suggests (14) that the extra-cellular region may form a coiled-coil structure. Coiled-coil structures consist of two

right-handed α -helices coiled around each other with a left-handed superhelical twist (15). This reduces the helical repeat of the α -helix from 3.6 to 3.5 residues, and generates an exactly integral repeat of seven residues every two turns. The two helices interact with each other between hydrophobic residues at positions 1,4,8,11,15 etc. on each of the two chains. There is therefore a 4-3 repeat within the heptad repeat of coiled-coil structures. Models exist for an idealised poly-alanine coiled-coil (16) and for tropomyosin (17).

To examine whether the sequence in this region of $\text{Fc}_\epsilon\text{R2}$ could form a coiled-coil we have used two independent procedures. In the first of these, amino acid sequence homology with tropomyosin was examined. The two sequences were scanned and compared with each other at each position with a window of 30 amino acid residues, and if 12 or more were identical or conservatively replaced, a dot was placed corresponding to the two central residues in a plot of one sequence against the other. This dot plot procedure is the best way to detect all of the segments, including repeated segments, in common between two sequences (18). When the sequence of the human $\text{Fc}_\epsilon\text{R2}$ was compared in this way to tropomyosin (Fig. 2), a remarkable pattern of homologous repeated segments was detected extending from residue 50 to 150, i.e. extending the whole way between the membrane and the lectin domain. This is clearly seen in the dot plot, and the boundaries are well-defined.

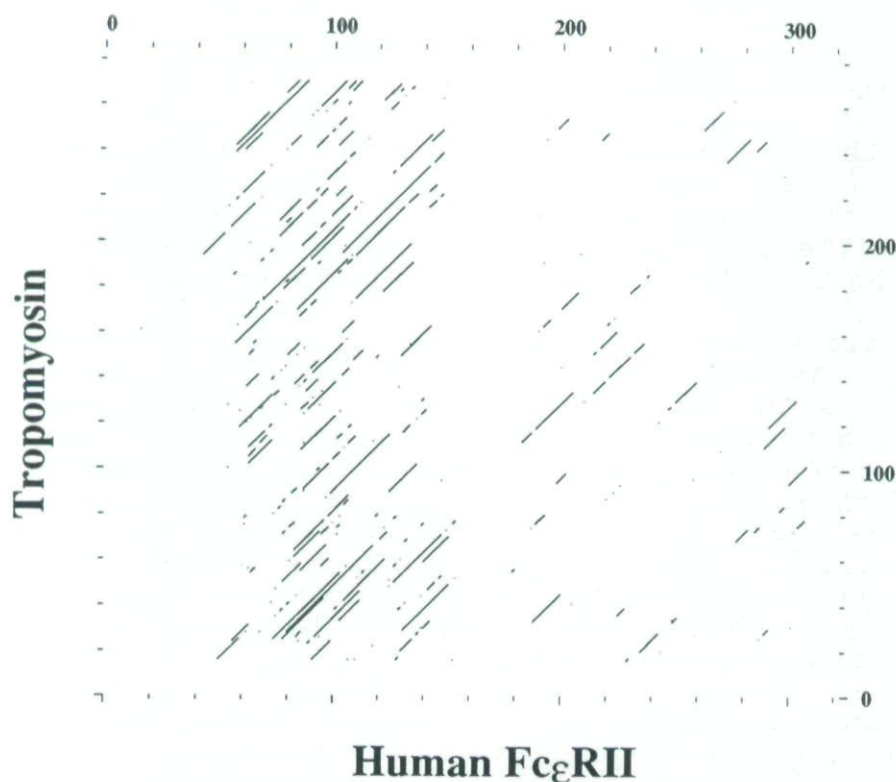


Fig. 2. Homology Dot Plot for Human $\text{Fc}_\epsilon\text{R2}$ against Tropomyosin. The amino acid sequence positions are numbered along each axis.

The homology with tropomyosin is indicative of a coiled-coil structure, but such a structure also requires the characteristic periodicity (the 4-3 repeat and exact 7 residue periodicity). In order to detect this periodicity within the Fc_εR2 sequence, the method of McLachlan (19) was applied. This method is particularly suited to the detection of "imperfect" repeats, i.e. patterns of sequence which repeat at exactly regular intervals, but which themselves are repeated imperfectly.

To illustrate the method, consider a segment of 56 residues from the sequence of tropomyosin. In order to discover whether a 7-residue long repeating pattern exists within this sequence, imagine the sequence set out in rows of seven as shown (Fig. 3). This sequence may then be tested for the presence of a pattern of (say) hydrophobic residues, by simply summing the number of residues in each column which are hydrophobic. In column one, all eight residues are hydrophobic, only two in each of the next two columns, all eight again in column four, etc. The more statistically significant the pattern, the more uneven the distribution of numbers in the column totals. A simple measure of the unevenness of the totals in the sum of their squares, which may then be compared with the sum of squares of the column totals calculated for a random sequence. The difference between these two numbers yields the "pattern strength" for a repeat of seven, $P_7 = 13.1$. The same procedure is repeated to search for repeats of 2,3,4 residues etc., and the pattern strength calculated for each of these may then be plotted against periodicity. This plot for tropomyosin reveals clearly the 7 residue repeat; the only other feature occurs at 14 and is a direct consequence of the heptad repeat. This method is completely independent of the exact nature of the pattern, which may itself be irregular and imperfectly repeated. The profile of the column totals reveals the nature of the pattern itself; in tropomyosin the 4-3 repeat (hydrophobic residues at 1,4, etc.) is clearly seen.

When this method is applied to Fc_εR2 residues 50-150, the same periodicity at 7 (and 14) is clearly detected, and the pattern of column totals reveals the same 4-3 pattern of tropomyosin, though it is less strong, as the smaller P_7 value of 4.8 indicates.

The combination of the two indicators, namely the homology with tropomyosin, and the existence of the seven residue repeat, together strongly suggest that the structure of the region of Fc_εR2 between 50 and 150 is a coiled-coil. The predicted length of this segment of the structure which extends from the membrane to the lectin domain is 15 nm (Fig. 4). Preliminary model building of the sequence of Fc_εR2, based upon the model structure for tropomyosin, clearly demonstrates that a coiled-coil structure may be adopted. Further, charged residues (Glu, Asp, Arg, Lys) are located in the model in positions that would allow the formation of salt bridges between the two polypeptide chains. Such bridges are important in stabilising the structure of tropomyosin.

**Search for a 7 residue repeat in a stretch of 56 residues in the
tropomyosin sequence**

L	K	G	T	E	D	E
L	D	K	Y	S	E	A
L	K	D	A	Q	E	K
L	E	L	A	E	K	K
A	T	D	A	E	A	D
V	A	S	L	N	R	R
I	Q	L	V	E	E	E
L	D	R	A	Q	E	R
8	2	2	8	0	1	1

Pattern strength $P_7=13.1$

Fig. 3. Method of Calculation of the "Pattern Strength" according to McLachlan (19). This method is illustrated using tropomyosin, as explained in the text.

In mouse $Fc\epsilon R2$, the stretch of sequence between the membrane and the lectin domain is longer, but the homology with tropomyosin and the seven residue repeat extends over the whole of this region. The predicted length of the coiled-coil stalk is 18 nm.

The two tests, namely homology with tropomyosin and the McLachlan pattern search, were applied to a wide range of receptor proteins. The Kupffer cell receptor sequence (20) was found to contain a stretch of 300 residues with homology to tropomyosin, and a significant 7 residue repeat. The asialoglycoprotein receptors similarly were found to contain a segment of about 50 residues. When modelled as coiled-coils, the lengths of these segments are 45 nm and 7.5 nm, respectively. Both of these receptors also have lectin domains that are homologous to that of $Fc\epsilon R2$. The region of homology can be seen in the dot plots. It appears that there is a sub-family of hepatic lectin receptor molecules which have coiled-coil stalks of different lengths.

If $\text{Fc}_\epsilon\text{R2}$ contains a coiled-coil stalk, as this model suggests, then it is perforce a dimer, and this structure constrains the mode of IgE binding. A possible model for IgE binding is shown in Fig. 4. This model is consistent with the following observations: (1) the existence of $\text{Fc}_\epsilon\text{R2}$ dimers (9); (2) the stoichiometry of $\text{Fc}_\epsilon\text{R2}$ binding to IgE, which is 2:1 on the assumption that $\text{Fc}_\epsilon\text{R2}$ is a monomeric chain (21), but 1:1 if it is a dimer; (3) the presence of two potential $\text{Fc}_\epsilon\text{R2}$ binding sites, on IgE (2). Alternatively, the two lectin domains in a single $\text{Fc}_\epsilon\text{R2}$ molecule may embrace the two $\text{C}_\epsilon 3$ domains on a single IgE molecule. The structure of this complex may have implications for the mechanism of antigen presentation to T-cells via $\text{Fc}_\epsilon\text{R2}$ on B-cells (22).

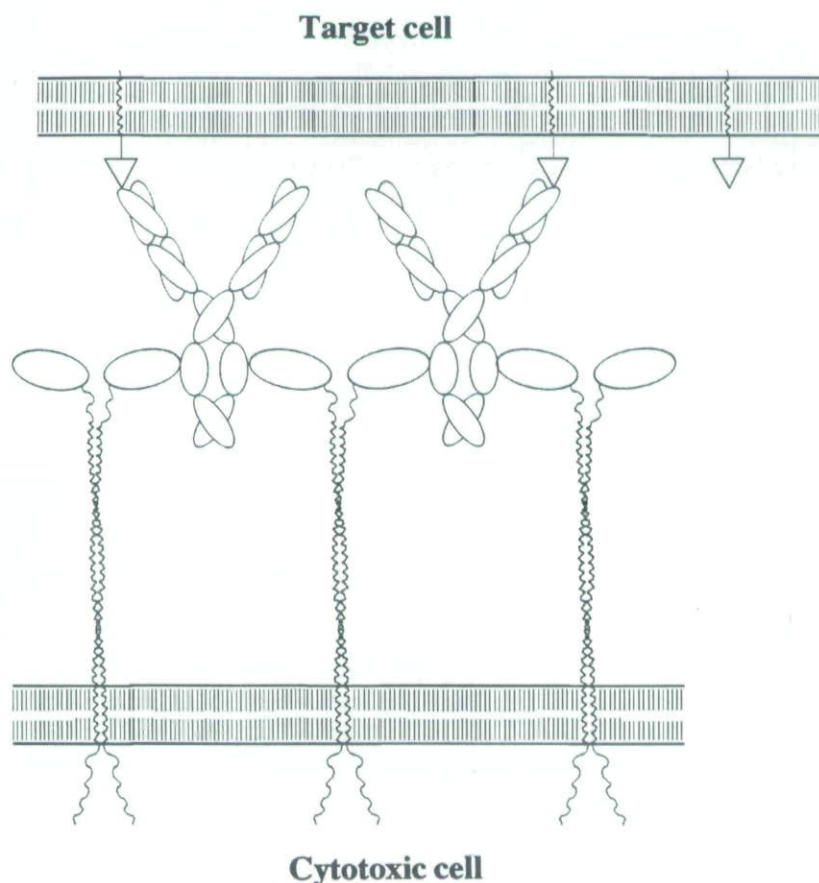


Fig. 4. Model of IgE Binding to $\text{Fc}_\epsilon\text{R2}$. This is illustrated by the interaction of an allergen-IgE complex with $\text{Fc}_\epsilon\text{R2}$ on a cytotoxic cell. The allergen (triangles), expressed at the surface of a target cell (e.g. a schistosome or an ovarian carcinoma cell), is attached to the Fab arms of two IgE molecules. IgE in turn bridges the allergen and $\text{Fc}_\epsilon\text{R2}$ attached to the cytotoxic cell (e.g. a platelet, an eosinophil or a macrophage). The IgE, $\text{Fc}_\epsilon\text{R2}$, and the cell membrane are drawn to scale, according to the models, described in the text. The ratio of IgE molecules: $\text{Fc}_\epsilon\text{R2}$ dimers in an infinite lattice would be 1:1 according to this model for the IgE- $\text{Fc}_\epsilon\text{R2}$ complex.

Future Direction

From this review, both the utility and limitations of the molecular models of IgE and its receptors must be apparent. In the case of IgE, the existing models leave considerable uncertainty about the three-dimensional structure of the receptor binding sites. There is no model yet for the lectin domain of Fc ϵ R2. If the aim of designing agents to block the allergic response at its inception--at the stage of the IgE-receptor interaction--is to be realised, it is clear that the exact determination of these structures by protein crystallography must claim a high priority.

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