The first event in the sequence of reactions that leads to an allergic response is the interaction of IgE with its high-affinity receptor, FceRI, on mast cells and basophils. Our aim is to define the structural details of the IgE–FceRI interaction, to provide a rational basis for the design of agents to interfere with this process and thus with the allergic response. Such studies will also contribute to our general understanding of molecular recognition between members of the immunoglobulin superfamily. We seek to identify the sequences involved in the interaction between the two proteins and to relate them to the three-dimensional structures of the proteins.

**IgE**

Like antibodies of other classes, IgE consists of two heavy chains and two (κ or λ) light chains organized into variable (V) and constant (C) immunoglobulin domains, stabilized by disulphide bonds. The class-specific ε-chain contains one V domain and four C domains, termed Ce1, Ce2, Ce3 and Ce4. Cleavage of human IgE with papain produces Fab and Fc fragments; the former is similar to the IgG-Fab fragment, whereas the latter has one more heavy chain domain pair (Ce2) than the prototypic IgG-Fc. The homology of the Ce3 and Ce4 sequences in IgE to those of Cy2 and Cy3 respectively in IgG1 is so close that the crystal structure of the IgG1-Fc serves as an excellent model for the relevant part of IgE [1].

Hamburger was first to speculate that the mast-cell-binding site on IgE might lie in the linker region between Ce2 and Ce3, on the basis of sequence differences between the ε- and γ-chains [2]. The first successful mapping experiment was carried out by Stanworth, Humphrey and coworkers, who separated the two fragments resulting from papain digestion of human IgE and showed that the mast-cell-binding activity was a property of the Fc fragment (Figure 1) [3]. The next advance was the demonstration by Perez-Montfort and Metzger that a trypsin cleavage site in the N-terminal sequence of Ce3 becomes protected when rat IgE is bound to FceRI on rat basophilic leukaemia cells [4].

More detailed mapping was attempted by cloning the human ε-chain cDNA and expressing DNA fragments in *Escherichia coli*; Figure 1 summarizes the results to date. Deletions were made from the N-terminus of the IgE Fc as far as CYS126 on the N-terminal side and Val401 on the C-terminal side, to define a binding sequence of 34 amino acids at the N-terminus of Ce3. Fragments containing this sequence inhibited the binding of radiolabelled IgE to basophils and/or the release of histamine from skin mast cells with almost the same IC_{50} as intact IgE [5–7]. The removal of 11 more residues on the N-terminal side, however, destroyed activity [5]. The effects of deleting additional amino acids from the C-terminus have not yet been tested.

Other workers, using either chimeric antibodies (Figure 1) [8, 9] or a deletion mutant starting at Ala126 [10], expressed in mammalian systems, also reached the conclusion that the sequences required for the recognition of IgE lie in Ce3. The importance of the N-terminal sequence, however, has been questioned recently by Eshhar's group, who reported that the replacement of the first 16 amino acids of human Ce3 with the homologous sequence from mouse Ce3 failed to allow the chimeric IgE to bind to the rodent receptor [11]. They concluded that the N-terminal sequence of Ce3 is not important for binding to the FceRI [11], but do not appear to have considered alternative explanations, such as the possibility that a sequence elsewhere in human Ce3 may interfere with binding to the rodent receptor. The N-terminal segments in human and in murine Ce3, which have 50% sequence identity, may be recognized by both receptors; this is consistent with the observation that rodent IgE binds to human FceRI, but not *vice versa*.

To resolve the ambiguity concerning the function of the N-terminal sequence in Ce3, we have carried out site-directed mutagenesis, targeting the 34 amino acid segment implicated in receptor binding (Figure 1). In the predicted three-dimensional structure, this sequence includes the linker region between Ce2 and Ce3 and a β-hairpin; only the...
Mapping of the FcERI-binding site in IgE

Above the domains in the ε-chain of IgE. The N-terminal domain borders of Cε2, Cε3 and Cε4 are at Val174, Asp137 and Gly138 respectively. Rows 1–13 represent three approaches to the mapping of the FcERI binding site. Rows 1 and 2 are the Fab and Fc fragments released by papain used by Stanworth, Humphrey and co-workers [3] to map the binding site to the Fc fragment. Rows 3–13 represent deletion mutants of the human ε-chain synthesized in E. coli. These have been named after the domains they contain, with primed numbers indicating truncated domains and letters indicating different lengths of deletions in the same domain; the N-terminal and C-terminal amino acids in each of these polypeptide chains are indicated at the right [5–7]. Rows 14–18 represent chimeric antibodies containing human (solid line) or murine (dashed line) ε-chain sequences. The names specify the domains containing murine ε-chain sequences. The interrupted lines in rows 17 and 18 represent sequences deleted from Cε3 to Val134 or to Cys138 respectively [9]. The vertical bar (both stripes) delineates the sequence from Cys138 to Tyr149 that destroys activity (see text for details).

<table>
<thead>
<tr>
<th>Row</th>
<th>Vε</th>
<th>Cε1</th>
<th>Cε2</th>
<th>Cε3</th>
<th>Cε4</th>
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<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Fab</td>
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<tr>
<td>2</td>
<td>Fab</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>3</td>
<td>fE2-4</td>
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<td></td>
<td>(Asp134-Lys140)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>fE2-3</td>
<td></td>
<td></td>
<td>(Asp138-Lys144)</td>
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<tr>
<td>5</td>
<td>fE2-2</td>
<td></td>
<td></td>
<td>(Asp138-Val144)</td>
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<tr>
<td>6</td>
<td>fE2-1</td>
<td></td>
<td></td>
<td>(Pro140-Lys146)</td>
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<td>(Asp144-Arg146)</td>
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<td>(Asp144-Lys146)</td>
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</table>

shows that the proline either is in the active site or is involved in determining the structure of this region of Cε3. Further mutants are now being studied.

**FcεRI**

FcεRI consists of four polypeptide chains, αβγ2. The IgE-binding site is in the extracellular part of the α-chain, and the β- and γ-chains are implicated in signal transduction. Examination of the extracellular region of the α-chain of FcεRI reveals that it consists of two domains, which we refer to as α(1) and α(2), that belong to the immunoglobulin superfamily. These, however, cannot be classified as either V-like or C-like, but belong rather to an intermediate type known as C2 [17]. Until recently, no three-dimensional structure of a C2-type domain had been determined, but the crystal structures of the extracellular domains of CD4 [18–20] and of CD2 [21], and in particular the second domain of CD2 [CD2(2)], which has been classified as C2-type, have proved to be extremely helpful for...
Table I

Characteristics of the binding of the Glu333 mutant to FceRI and FceRII

<table>
<thead>
<tr>
<th>Protein assayed</th>
<th>Protein assayed</th>
<th>FceRI</th>
<th>FceRII</th>
</tr>
</thead>
<tbody>
<tr>
<td>hlgE Fc</td>
<td></td>
<td>k_+ (M^{-1} s^{-1})</td>
<td>k_- (s^{-1})</td>
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<tr>
<td>hlgE Fc</td>
<td></td>
<td>3.0 ± 1.0 × 10^5 (3)</td>
<td>1.8 ± 0.5 × 10^{-1} (2)</td>
</tr>
<tr>
<td>hlgE Fc (Glu333)</td>
<td></td>
<td>3.3 ± 0.1 × 10^5 (2)</td>
<td>1.0 × 10^{-4} (2)</td>
</tr>
</tbody>
</table>

Figure 2

Schematic illustration of the modelled second extracellular domain of FceRI α-chain

The arrows represent β-strands, and the three N-linked glycosylation sites are represented by the large circles. Other features are referred to in the text.

modelling α(1) and α(2). The resulting model thus differs significantly from earlier modelling based on a more limited structure database of only C-type domains [22].

Firstly, a template structure was selected to model the core of each domain. For α(2) it was clear that CD2(2) provided the best model, because of common features, such as a disulphide bridge between strands B and F, and the absence of the tryptophan in strand C that is conserved in all V and C domains. (See Figure 2 for strand nomenclature.) The choice for α(1) was less explicit, but the B-F disulphide pattern, the conserved tryptophan in strand C, several V-like features and the identical length of the interdomain link region, led to the selection of the V-like CD2(1) as the better template. Once the strand regions of both domains had been assigned, the loop regions were modelled. Where homologous loops of equal length that fitted the geometrical constraints of the flanking strands could be found within immunoglobulin-like domains, these were selected [for example, CD4(2) was used for four loops]; for the remainder, the search was extended to the whole Brookhaven Protein Data Bank. Stereochemistry at the splice regions between strands and loops was regularized, and the side chains of some residues were manually adjusted, before dynamics simulation (for loop regions) and energy minimization routines were applied to the model. The modelling was performed using HOMOLOGY and DISCOVER (Biosyn Technologies Inc.); a detailed description will be published elsewhere (B. J. Sutton, A. J. Beavil and H. J. Gould, unpublished work). Coordinates were taken from the Brookhaven Protein Data Bank, except those for CD2, which were kindly provided by Drs. David Stuart and Yvonne Jones, Laboratory of Molecular Biophysics, Oxford.

The two-domain structure of CD2 affords a model for the relative positioning of α(1) and α(2), since the lengths of the linkers between the two domains are the same in both. However, there is no sequence similarity between the linker regions of the α-chain and of CD2; furthermore, there is a slight difference (7°) in the relative orientation of the two domains of CD2 in the two independent copies of the CD2 molecule in the crystal structure, and this variability is expected to be even greater in solution [21]. Modelling of the α(1)/α(2) interaction on CD2 must at best yield only a first approximation to the structure. Nevertheless, when
Immunoglobulin Superfamily Interactions

This is done, none of the loop regions conflict with each other, and a number of hydrophobic residues are found to be buried in the proposed interface.

It is known that the principal determinants of the IgE-binding site lie in \( \alpha(2) \) [23, 24]. A schematic representation of this domain is shown in Figure 2, which also indicates the locations of the three putative N-linked glycosylation sites in human \( \alpha(2) \). These three asparagine residues [as well as the four potential glycosylation sites in \( \alpha(1) \)] all lie in exposed surface regions of the model. It is known that glycosylation does not interfere with binding IgE, but the location of the IgE-binding site within \( \alpha(2) \) has yet to be determined. The interaction is blocked, however, by an antibody to an \( \alpha(2) \) peptide [25] that corresponds to the B-strand and BC loop in the model (coloured black in Figure 2). It may be that the antibody recognizes the loop region, since there is a potential glycosylation site adjacent to the B-strand segment. Also, it has been suggested that a stretch of seven residues conserved in the human and rat \( \alpha \)-chain sequences, but not in the homologous \( \gamma \)-receptors, forms part of the site [26]; these residues correspond to strand C (cross-hatched in Figure 2). Mapping studies of the binding site for IgG within the homologous second extracellular domain of FcεRII [24, 27] also indicate that a segment corresponding to strands F and G and to the intervening loop, constitutes a major part of the site (shaded grey in Figure 2). Finally, a polymorphism in FcεRII has been shown to affect binding IgG [27]; the corresponding position in \( \alpha(2) \) lies at the end of the C' strand (black circle in Figure 2).

With the further supposition that the binding sites on IgG and IgE occupy equivalent positions on their homologous receptors, all the available evidence points to the four-strand face (C'-C-F-G) as the determinant of the IgE-FcεRIα interaction. This face is free of potential glycosylation sites that might interfere with binding IgE. Site-specific mutagenesis experiments are in progress in our laboratory to test this proposition, and to identify key residues in the interaction.

A model of the complex with IgE may now be envisaged. Resonance-energy transfer experiments on IgE suggest that it is bent [28], and we have argued previously that the IgE-binding site is on the convex surface [29]. In outline, the argument rests on the observation that only one \( \varepsilon \)-chain is involved in binding [5], and that the stoichiometry of the IgE-FcεRIα complex is 1:1 [10, 23, 30]. If the receptor bound to the sides of IgE, the two equivalent sites on opposite sides of the molecule would generate a 2:1 complex, and the binding to one site would not interfere with the binding to the other. If, however, the receptor binds to the flat face of IgE, the observed 1:1 stoichiometry can be explained by postulating the accessibility of only one binding site—that on the convex surface. Figure 3 illustrates the suggested mode of interaction, and incorporates the distance between IgE and the cell membrane inferred from resonance-energy transfer [28]. This model also allows us to rationalize the influence of \( \alpha(1) \) on the binding of IgE to a site in the \( \alpha(2) \) domain [23]; we regard these models as a useful framework for the design of future experiments.

Figure 3
Possible mode of interaction between IgE and FcεRI at the cell surface

The model incorporates a number of features of the interaction that have been deduced from experimental evidence, as discussed in the text, in particular, the bent structure of IgE, the location of the FcεRI-binding site in IgE (in the linker region between Cε3 and Cε2 on the convex surface of IgE), the location of the IgE-binding site in the FcεRI (in the second domain of the \( \alpha \)-chain), the stoichiometry of the interaction, and the distance of IgE from the cell membrane. The experimentally determined distances [28] are also indicated.
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