Localization of the FcεRI Binding Site to the Third Constant Domain of IgE

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To map precisely the binding site of the high affinity receptor (FcεRI) on IgE we have constructed and expressed recombinant human and mouse IgE genes with anti-NIP specificity. Various mutated and chimeric molecules thus prepared were studied for their ability to bind to rat mast cells or transfected fibro-blasts expressing human FcεRI α chain. To avoid destruction of the FcεRI binding site due to conformational changes, we took advantage of the fact that human IgE does not bind to the rodent receptor, and by exon swapping between the human and mouse constant regions (Cε), generated chimeric human IgE molecules having either one or both of the murine Cε2 and Cε3. We found that replacement of the human Cε3 with the murine homologue was sufficient to confer on the human IgE the ability to bind to the rat FcεRI. Moreover, deletion of the Cε2 did not impair the receptor binding capacity of both human and murine molecules. We therefore conclude that all of the FcεRI binding site can be assigned to third constant region domain of IgE.

Results and Discussion

Many studies have been carried out in an attempt to map the site on the IgE molecule which accommodates the FcεRI. Several recent studies, utilizing different experimental approaches, implicated the Cε2, Cε3 and their interface as the sites that take part in the FcεRI binding [1, 2]. Because a large part of the data used was derived from manipulations which might have had deleterious effects on the overall conformation of IgE, and since the integrity of the native spatial structure of IgE is required for FcεRI-IgE binding [3], we decided to preserve the native IgE conformation as intact as possible. Because of the substantial homology in sequence and overall tertiary and quaternary structure between the mouse and the human IgE molecules [4], and the fact that human IgE does not bind to the rodent receptor, we reasoned it to be an excellent system to study the contribution of different murine Cε fragments to the FcεRI binding site. Thus, we constructed recombinant mouse and human ε heavy chain genes composed of the corresponding Cε exons and VH of an anti-NIP IgG. Following transfection into the λ chain producing J588L myeloma cells, functional recombinant IgE have been obtained which were identical to native IgE in their ability to bind to FcεRI and induce mast cell degranulation upon binding of NIP protein [5]. These basic constructs were used to generate chimeric human/mouse IgE molecules by replacing different human exons with their murine homologues [6]. Figure 1 describes schematically the different chimeric molecules and compares

their ability to bind to rat mast cell line (RBL-2H3) and CHO cells expressing the extracellular part of the human FcεRI α chain anchored to surface through the trans-membrane region of IL-2 receptor [7]. As can be seen in figure 1, both mouse and human rlgE and their chimeras could bind equally well to the human receptor. As anticipated, the human rlgE did not bind to the rat FcεRI, and the only murine domain that could confer on it the ability to bind to rodent receptor was Cε3 (CHM3 and CHM2M3, fig. 1). These data and additional results of direct binding measured by mast cell degranulation or rosette assay [6] clearly implicate the Cε3 as the domain containing the binding site of mouse IgE to the rodent FcεRI.

The data described above do not imply that the third constant region domain contains all the necessary elements and is sufficient by itself to account for all the high affinity interaction with the receptor. To

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Binding to FcεRI Rodent Human

Murine rlgE

MC,1

J MC.2 3/8 1 F~IV4Cr3 I I MC,λPT,ιωj

0.70

2.50

Human rlgE

HCj

I HCε2 ' , HCE3

HCε4

-//-

j 0.00

0.90

CHM3

HCι

HCε2

MCε3

HCε4 ---1 |

0.95

7.00

CHM2 I I HCι I 1

MCε2

HCε3

HCε4

0.00

1.50

CHM2M3

HCε1

MCε2

1_{Ji}
 MCε3
 ticpy
 0.60
 7.00
 16 a.a from MCr2
 CSPD
 I
 HCεl
 mc₂
 ¶U[^]-
 2.25
 5.00

 PCDD
 i L
 HCεl
 HI MCε3 --| | HCg
 1.50
 12.00

Fig. 1. Binding of the different recombinant and chimeric IgE molecules to human and rodent FcεRI. The composition of the constant region domains of the different rIgE studied is schematically displayed. Each block represents an exon. S represents the interchain disulfide bond in position 328 kept in the Cε2 deleted mutants. The degree of binding to either the human FcεRI α subunit expressing transfectant CHO-3D10 or the rat mast cell RBL-2H3 was evaluated by the competitive inhibition assay in which different amounts of the various IgEs was coincubated with radiolabeled IgE. For each preparation the concentration (μg/ml) that gave 50% inhibition was determined.

To examine the contribution of the Cε2 to the FcεRI binding, we have prepared two deletion mutants lacking either the entire human Cε2 (PCDD) or containing only 16 amino acid section (Val314-Pro329) of the carboxy terminus of Cε2 (CSPD). In both mutants we retained Cys328 which is involved in one of the two inter-heavy chain disulfide bonds of human IgE. When tested for their ability to bind to the human FcεRI, we found that both molecules inhibited the binding of human and mouse IgE in the competitive inhibition assay (fig. 1) and bound directly to RBL-2H3 cells or the human FcεRI α chain expressing CHO-3D10 cells in the rosette assay. Comparison of the different specific activities of the chimeric and mutated IgE molecules in the competitive inhibition assay reveals that PCDD was about 10-fold and CSPD about 4-fold less active than CHM3 or native IgE. This can be attributed to lower affinity of the mutated molecules. However, size analysis of the affinity purified Cε2 deleted molecules under nonreducing conditions revealed several molecular species, out of which the HL dimer was the major one. Using 125I molecules, we found that the main species that is actually bound to FcεRI is the H2L2 which comprises about 10–20% of the Cε2 deleted molecules [6].

Thus the apparent binding affinity of these mutated molecules should be corrected to be about 2- to 4-fold lower than that of intact rIgE. Taken together, our studies strongly suggest that the high affinity mast cell receptor binding site on human IgE can be mapped exclusively to the third

constant region domain and that the second domain does not participate in the actual binding but is necessary for the stabilization of the active conformation of the binding site.

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References

Perez-Monfort M, Metzger H: Proteolysis of soluble IgE receptor complexes: Localization of sites on IgE which interact with the Fc receptor. *Mol Immunol* 1982;19:1113–1125.

Helm B, Marsh P, Vercelli D, et al: The mast cell binding site on immunoglobulin E. *Nature* 1988;331:180–183.

Ishizaka T, Ishizaka K: Biology of immunoglobulin E. *Prog Allergy*. Basel, Karger, 1975, vol 19, pp 60–121.

Liu FT: Gene expression and structure of immunoglobulin epsilon chains. *CRC Crit Rev Immunol* 1986;6:47–68.

Schwarzbaum S, Nissim A, Alkalay I, et al: Mapping of murine IgE epitopes involved in IgE-Fcε receptor interactions. *Eur J Immunol* 1989;19:1015–1023.

Nissim A, Jouvin M-H, Eshhar Z: Mapping of the high affinity Fcε receptor binding site to the third constant region domain of IgE. *EMBOJ* 1991;10:101–107.

Hakimi J, Seals C, et al: A chimeric human FcεRI alpha subunit that binds IgE. *FASEB J* 1990;4:abstr 1407.

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