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Receptor phage

Display of functional domains of the human high affinity IgE receptor on the M13 phage surface

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In this paper we demonstrate that phage display technology is a suitable system for studying the interaction between the high-affinity receptor for IgE (FcεRI) and IgE. The α subunit extracellular domains of the human receptor were expressed on the surface of filamentous phage M13 fused to the carboxyl-terminal part of the gene III protein (pIII). Two constructs were made, the first with both the Ig-like domains of the receptor α chain and the second with only the C-terminal domain. The fusion genes were cloned in a phagemid vector to display monovalently the receptor on the phage surface. Our results indicate that the α receptor expressed on the phage is able to interact with IgE as demonstrated by an ELISA assay. In addition, by using the same system, we show that a single domain of the α receptor is sufficient for the interaction with IgE although with a binding affinity lower than that of the two-domain receptor.

FcεRI; Phage display; Receptor–immunoglobulin interaction

1. INTRODUCTION

The high-affinity receptor (FcεRI) for immunoglobulin E (IgE) present on mast cells and basophils plays a key role in the allergic reaction. Bridging of receptor bound IgE by a multivalent allergen triggers cellular degranulation and release of mediators that are responsible for the clinical symptoms of type I immediate hypersensitivity [1].

FcεRI has a tetrameric structure consisting of three different subunits: one α, one β and two γ chains [2]. The α subunit is a member of the immunoglobulin superfamily and contains two Ig-like domains (hereafter the N-terminal domain is called Dε1 and the C-terminal Dε2). Recently it has been clearly demonstrated that the extracellular part of the α chain is sufficient to produce interaction with IgE [3], and that it is fully functional in binding IgE with high affinity even after deglycosylation [4]. The analysis of functional domains by using monoclonal antibodies has suggested that the binding region is located within the second domain [5]. Furthermore chimeric receptors, engineered by exchanging homologous regions of human FcγRII and FcεRI, showed that the Dε2 provides the binding specificity for IgE but is not sufficient for high affinity binding [6].

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Displaying polypeptides on the M13 phage surface is an effective method for selecting and screening protein variants with the properties desired. Each phage particle carries the gene encoding the foreign protein, together with the polypeptide itself packaged on its surface [7]. The display of antibodies on the phage surface allowed protein mutants with higher affinity for the antigen to be selected [8]. Similar results were also obtained by engineering, as phage gene III fusions, proteins other than antibodies, such as human growth hormone [9,10] and bovine pancreatic trypsin inhibitor [11].

This paper describes the expression of a functional FcεRI on the phage surface. Our data indicate that the phage display is a suitable system to study FcεRI–IgE interaction. The displayed single domain (Dε2) is sufficient for the interaction with IgE but shows a lower affinity than the two-domain (Dε1+Dε2) receptor. The system described here is amenable to the construction of millions of different receptor mutants that can be affinity-selected using the cognate IgE as a ligand for the identification of residues involved in the binding.

2. MATERIALS AND METHODS

2.1. *Cloning of FcεRI α chain in the phagemid vector and rescuing of recombinant phage*

First strand cDNA was produced from RNA [12] of the KU8–12 cell line [13]. PCR was performed on first strand cDNA with the oligonucleotide primers FcεRI-1 (5'CGTGCTGCAGGTCCCTCAGAAACCTAAGGTCTCCT) and FcεRI-2 (5'GATCGCGGCCGCTCTACTTTTATTACAGTAATGTTGAGGG) to obtain the two-

domain construct (aa 1–172) . To clone the single domain, Dε2 (aa 86–172), the primer FcεRI-3 (5'GCAGCTGCAGGACTGGCTGC-TCCTTCAGGCCTCTG) was used instead of FcReI-1. The primers introduced two restriction sites (*PstI/NotI*) for directional cloning of the insert. After digestion with the restriction enzymes, the inserts were ligated with the phagemid vector pHENδ, kindly supplied by G. Ciliberto. This vector has been derived from pHEN1 [14] by deleting the N-terminal part of phage gene III. The inserts are produced as fusions with only the C-terminal part of pIII (from aa 249) as shown in Fig. 1.

Plasmids were transfected into the *E. coli* TG1 strain and selected on LB (Luria-Bertani) agar plates containing 100 μg/ml of ampicillin and 1% (w/v) glucose. A single transformant was grown in 2 × YT with 1% glucose and 100 μg/ml ampicillin. Superinfection was performed when the culture reached OD_{600}= 0.300 by adding 10^9 pfu (plaque forming units) per ml of culture of M13K07 wild type phage. Bacteria were kept at 37°C without shaking for 45 min, and then 1 h with shaking at 300 rpm. At this point the glucose was removed by spinning down the cells and aspirating the supernatant. Bacteria were grown for 20 h at 37°C in 2 × YT with 100 μg/ml of ampicillin and 20 μg/ml of kanamycin. Recombinant phage were purified and concentrated by two PEG precipitations [12b].

2.2. *ELISA assay*

Binding analysis of receptor phage to IgE was performed by ELISA. The hapten NIP-OSu (5-Iodo-4-hydroxy-3-nitrophenacetyl, succinimide ester) was coupled to ovalbumin following the manufacturers instructions (Cambridge Research Biochemicals). Nunc 96-well Maxi-Sorp plates were chosen and a volume of 100μl/well was used for all the incubations. Coating was performed with 10 μg/ml of NIP ovalbumin in 0.1 M carbonate buffer pH 8.6 at 4°C o.n. Blocking was done with 1% BSA (bovine serum albumin) in PBS 1 h at r.t. Culture supernatant of the cell line JW8/5/13 [15], which express the chimeric monoclonal antibody of IgE class, with human Fcε and mouse variable regions specific for the hapten NIP, were used to capture receptor phage. The culture supernatant of the cell line THG3-B5–13 [15] expressing IgG3 specific for the same hapten were used as control. The concentration of IgE and IgG3 in the culture supernatants was determined with the Bindazyme EIA Kits (The binding site); IgE and IgG3 were added to the ELISA plate at a concentration of 40 ng/ml and incubated o.n. at 4°C. Several concentrations of phage and different times of incubation were tried to set the reaction on saturation conditions, finally 5×10^{11} phage particles were added to each well and incubated for two hours at r.t.. After washing with PBS, 0.05% Tween-20, bound phage were detected with rabbit polyclonal antibody raised against M13, followed by alkaline phosphatase-conjugated anti rabbit antibody (Pierce). The reaction was developed with 4-nitrophenyl-phosphate in diethanolamine buffer and the OD_{405} was measured.

Competition of binding was performed. Receptor phage (10^{11}) were pre-incubated for 1 h at r.t. with dilutions of affinity-purified IgE from human myeloma (Scripps) non-reactive against the NIP hapten. After preincubation, receptor phage were added to the wells of an ELISA plate coated with NIP and anti-NIP immunoglobulins, as described in the ELISA assay.

3. RESULTS AND DISCUSSION

3.1. *Production of the FcεRI fusion phage*

Several strategies have been proposed for the identification of the binding site for IgE on the FcεRI. Inhibition of IgE interaction with FcεRI has been performed with monoclonal antibodies recognising different regions of the receptor molecule [5]. Chimeric IgG/IgE receptors have been produced in COS cells to characterise the minimum binding region [6]. The α chain of the receptor, a heavily glycosylated protein, is responsible for the interaction with IgE and both its immunoglobulin-like domains are needed for a high-affinity binding [6]. Sugar residues are not crucial for binding [16,17] and it has recently been demonstrated that soluble recombinant human a receptor can bind IgE with high affinity even after deglycosylation [4]. This observation opens up possibilities of producing a functional receptor in *E. coli*.

On the basis of these considerations we have cloned regions of the α chain of the high-affinity receptor for IgE as fusions with the phage gene III protein in the phagemid vector pHENδ (Fig.1). The expression unit of pHENδ is under the control of an inducible promoter (PlacZ) and the signal sequence pelB [14] drives the secretion of the recombinant protein into the periplasmic space. The vector contains a β lactamase gene, the ColE1 replication origin and the phage M13 intergenic region. It can be propagated as a common plasmid but, upon infection with helper phage, it is packaged into virion like particles. The phagemid system should allow the production of phage particles carrying on average one copy of recombinant pIII protein per phage virion [7]. This system was chosen since 'monovalent' phage display appears to enhance the discrimination between phage with different affinities [7,9]. The N-terminal part of pIII present in the original pHEN1 vector was deleted assuming that in a shorter fusion product the target protein would be more accessible for binding as described for the human growth hormone [7,8]. Moreover, we introduced an amber codon between the inserts and the pIII sequence to express the recombinant proteins in soluble form by using an *E. coli* non-suppressor strain.

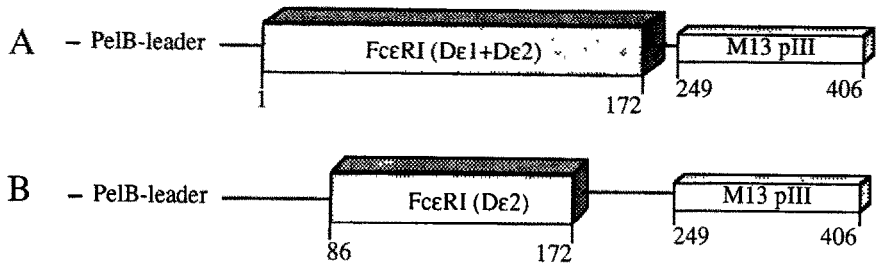

Fig. 1. Schematic representation of the fusion products: (A) two-domain receptor and (B) single-domain receptor. Numbers refer to the position of amino acids.

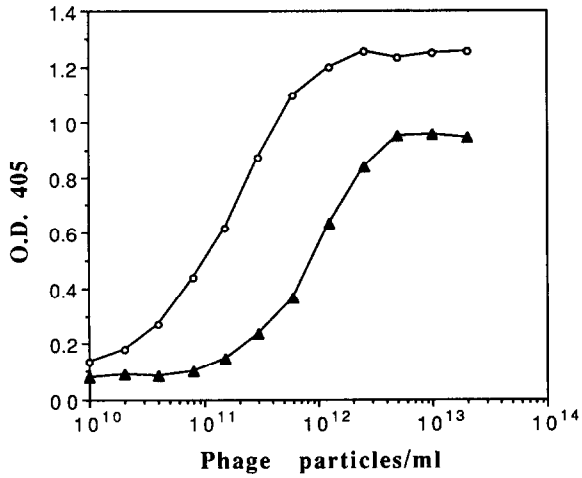

Fig. 2. Saturation curve of the binding to IgE of phage FcεRI Dε1+Dε2 (-○-) and FcεRI Dε2 (-▲-).

The extracellular part of the α chain of the IgE receptor, composed of two Ig-like domains, and a deletion mutant containing only the second domain (Dε2) were cloned in this system (Fig. 1). We obtained routinely 10^{11} fusion phages per ml of superinfected cells, titrated as ampicillin-transducing units.

3.2. *Binding of phage FcεRI to IgE*

In order to analyse the FcεRI–IgE interaction we have set up an ELISA assay. IgE reactive against a hapten, NIP, were captured on the ELISA plate coated with this hapten so that the immunoglobulins were oriented in such a way that the Fc would be free to interact with its receptor present on the phage surface. This experiment shows that both the two-domain receptor (Dε1+Dε2) and the single-domain (Dε2) bind IgE, but

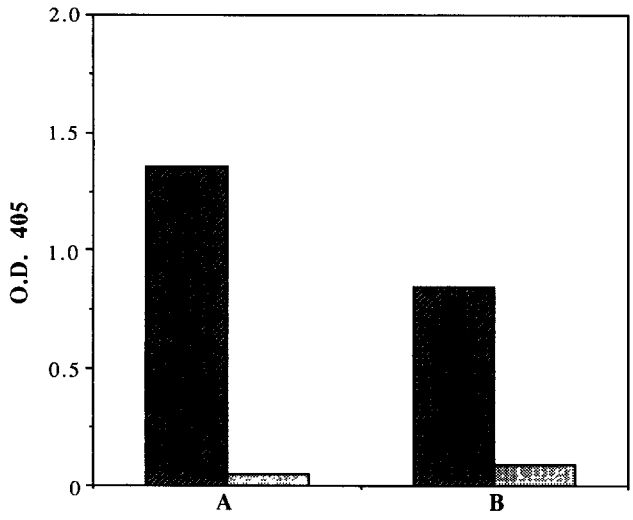

Fig. 3. Binding to IgE and to IgG3 of phage FcεRI Dε1+Dε2 (A) and FcεRI Dε2 (B) measured by ELISA. Symbols: IgE (▨), and IgG3 (□) The values of background reaction of the wild type phage have been subtracted from the $O.D._{405}$ values obtained with the recombinant phage.

there is a difference for the maximum binding achieved under saturation conditions (Fig. 2). The OD_{405} values are, in fact, lower for the Dε2 receptor. Preliminary experiments (data not shown) suggest that this difference in maximum binding obtained for the two receptors is a consequence of the partial loss of Dε2 bound phage during the two hours incubation with the revealing antibodies before the development of the binding reaction occurs. The specificity of the IgE–FcεRI reaction was controlled using immunoglobulins of a different isotype, IgG3, but reactive against the same hapten (Fig. 3). The specificity of the binding was further investigated with competition experiments using an excess of IgE non-reactive to the hapten NIP. The percentage of inhibition of the ELISA reaction was measured and the curve of inhibition for both receptor phages is shown in Fig. 4. The comparison of the two curves clearly indicate that the Dε2 receptor binds IgE with lower affinity. The production of soluble receptors is in progress for an accurate measurement of the dissociation constants.

Our data offer the first indication that a functional receptor can be produced on the phage surface. The receptor phage system is a feasible approach and could be very helpful in characterising the binding region within the receptor. A previous report has demonstrated, by using chimeric human FcγRII/FcεRI, that a single domain (Dε2) of the FcεRI is sufficient for IgE binding. Here we show that the Dε2 provides binding specificity for IgE working also in a structural context different from that of immunoglobulin receptors.

Functional proteins other than receptors, such as antibodies and hormones have been displayed as gene III fusions [8,10], showing that the phage display is a powerful system to select higher affinity binding mutants. The construction of a receptor mutant library could allow the identification, using a selection system similar to the one described here, of an improved form of the high-affinity receptor for IgE. Recently, several reports

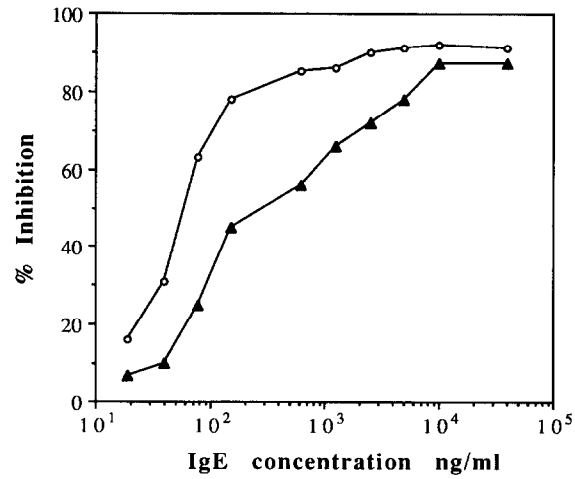

Fig. 4. Competiton of phage FcεRI Dε1+Dε2 (-○-) and FcεRI Dε2 (-▲-) binding obtained by preincubation with several dilutions of competitor.

describe the 'in vivo' use of soluble recombinant receptors as antagonist of the corresponding cytokines (i.e. IFN-γ receptor, [18,19]; IL4 receptor, [20]; IL1 receptor, [21]; TNF receptor, [22]) supporting the hypothesis that a soluble improved FcεRI, capable of efficiently competing the natural one, could be of great therapeutic interest.

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REFERENCES

[1] Vercelli, D. and Geha, R.S. (1989) Ann. Allergy 63, 4–11.
[2] Metzeger, H. (1992) Immun. Rev. 125, 37–48.
[3] Hakimi, J., Seals, C., Kondas, J.A., Pettine, L., Danho, W. and Kochan J. (1990) J. Biol. Chem. 265, 22079–22081.
[4] Blank, U., Ra, C. and Kinet, J.P. (1990) J. Biol. Chem. 266, 2639–2646.
[5] Riske, F., Hakimi, J., Mallamacci, M., Griffin, M., Pilson, B., Tobkes, N., Lin, P., Danho, W., Kochan, J. and Chizzonite, R. (1991) J. Biol. Chem. 266, 11245–11251.
[6] Hullet, M.D., McKenzie, I.F.C. and Hogarth, P.M. (1993) Eur. J. Immunol. 23, 640–645.
[7] Marks, J.D., Hoogenboom, H.R., Griffiths, A.D. and Winter, G. (1992) J. Biol. Chem. 267, 16007–1610.
[8] Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., McCafferty, J., Griffiths, A.D. and Winter, G. (1991) J. Mol. Biol. 222, 581–597.
[9] Bass, S., Greene, R. and Wells, J.A. (1990) Proteins: Structure, Function and Genetics 8, 309–314.
[10] Lowman, H.B., Bass, H.S., Simpson, N. and Wells, A.J. (1991) Biochemistry 30, 10832–10838.
[11] Roberts, B.L., Markland, W., Ley, A.C., Kent, R.B., Withe, D.W., Guterman, S.K. and Ladner, R.C. (1992) Proc. Natl. Acad. Sci. USA 89, 2429–2433.
[12] Maniatis, T., Fritsch E.F. and J. Sambrook. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edn., Cold Spring Habor Laboratory Press, Cold Spring Habor, N.Y.; (a) Vol. 2 chapter 14; (b) Vol. 1 chapter 4.
[13] Kochan, J., Pettine, L.F., Hakimi, J., Kishi, K. and Kinet, J.P. (1988) Nucleic Acids Res. 16, 3584–3584.
[14] Hoogenboom, H.R., Griffiths, A.D., Johnson, K.S., Chiswell, D.J., Hudson, P. and Winter, G. (1991) Nucleic Acids Res. 19, 4133–4137.
[15] Bruggermann, M., Williams, G.T., Bindon, C.L., Clark, R.M., Walker, R.M., Jeffers, R., Waldmann, H. and Neuberger, M. (1987) J. Exp. Med. 166, 1351–1361.
[16] Hempstead, B.L., Parker, C.W. and Kulczycki, Jr. (1981) J. Biol. Chem. 256, 10717–10723.
[17] Pecoud, A.R., Ruddy, S. and Conrad, H.D., (1981) J. Immunol. 126, 1624–1629.
[18] Ozmen, L., Gribaudo, G., Fountoulakis, M., Gentz, R., Landolfo, S. and Garotta, G. (1993) J. Immunol. 150, 2698–2795.
[19] Kurschiner, C., Ozmen, L., Garotta, G. and Dembic, Z., (1992) J. Immunol. 149, 4096–4100.
[20] Sato, A.T., Widmer, M.B., Finkelman, F.D., Madani, H., Jacobs, C.A., Grabstein, H.K. and Maliszewski, R.C., (1993) J. Immunol. 150, 2717–2713.
[21] Fanslow, W.C., Sims, J.E., Sassenfeld, H., Morrissey, J.P., Gillis, S., Dower, S.K. and Widmer, M.B. (1990) Science 248, 739–741.
[22] Lesselauer, W., Tabuchi, H., Gentz, R., Brockhaus, M., Schlaeger, E.J., Grau, G., Piguet, P.F., Pointaire, P., Vassalli, P. and Loetscher, H. (1991) Eur. J. Immunol 21, 2883–2886.

Note added in proof

After the submission of our manuscript we have been informed that the display of a functional human high affinity IgE receptor on phage surface has also been described by M. Robertson in J. Biol. Chem. 268 (1993) 12736–12743.