

The α Subunit of the Human IgE Receptor (FcERI) Is Sufficient for High Affinity IgE Binding*

(Received for publication, September 14, 1990)

John Hakimi†, Cary Seals‡, Jo Ann Kondas‡, Laura Pettine§, Waleed Danho¶, and Jarema Kochan§||

From the Departments of ‡Immunopharmacology, §Molecular Genetics, and ¶Peptide Research, Roche Research Center, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110

The α subunit of the FcERI binds IgE with high affinity. Previous studies have demonstrated that α subunit expression requires the presence of β and/or γ subunits, and it is not known how these two subunits contribute to the ability of the α subunit to bind IgE. In this report, we describe the expression and characterization of a human chimeric α subunit. The data demonstrate that high affinity IgE binding does not require the presence of the β and/or γ subunits and that this activity is localized to the extracellular domain (residues 26–201) of the human α subunit. Permanent cell lines expressing the chimeric receptor were used to characterize the binding parameters of the α subunit. These cell lines provide a means of identifying therapeutic agents which may be effective in the treatment/management of allergic diseases.

The high affinity receptor (FcERI) for immunoglobulin E (IgE) that is found on mast cells and basophils plays a central role in the allergic response (1). In the rat this receptor consists of three different subunits: an IgE binding α chain, one β chain, and two γ chains (1–5). Previous rat and mouse studies have demonstrated that high affinity cell surface IgE binding is dependent on the coexpression of the three subunits (5, 6). In the human system at least the α and γ subunits must be present for high affinity cell surface binding (7, 8). The functional contribution of the β and/or γ subunits at present is unknown. In this report we describe the construction and functional expression of a chimeric human α subunit. The data demonstrate that high affinity IgE binding is localized to the extracellular domain of the human α subunit and does not require the presence of β and/or γ . The availability of permanent cell lines expressing the high affinity FcERI α subunit provides a means of identifying therapeutic agents which may be effective in the treatment of allergic diseases.

MATERIALS AND METHODS

Proteins and Peptides—Human myeloma IgE was purified from the serum of a myeloma patient (PS) by precipitation with ammonium sulfate followed by DEAE-cellulose and Sephacryl S-300 chromatography as described previously (9). A second human myeloma IgE protein was provided by Dr. J. F. Greally of the University College,

Galway, Ireland (10). Cells secreting a recombinant human IgE protein were provided by Dr. Z. Eshhar of the Weizmann Institute, Israel, and IgE was purified on a anti-IgE monoclonal antibody (E48) affinity column provided by Dr. P. Haring of Roche, Switzerland. Purified polyclonal human IgE was provided by Dr. P. Goldsmith of NIH. Rat monoclonal IgE was purified from the ascites fluid of nu/nu mice bearing Immunocytoma IR 162 as described previously (11). IgE (PS) was biotinylated with biotinyl- ϵ -aminocaproic acid *N*-hydroxysuccinimide ester (Calbiochem) dissolved in dimethyl sulfoxide as previously described (12).

Peptides containing amino acids 81–103 and 160–197 of the IgE receptor were synthesized by solid-phase methods and purified by gel filtration and high pressure liquid chromatography using a μ Bondapak C₁₈ column (13). Antipeptide antibodies were prepared by immunizing rabbits subcutaneously with the keyhole limpet hemocyanin peptide conjugate (14) emulsified in complete Freund's adjuvant.

Vector Construction—The human FcERI α cDNA (15) was modified by site-directed mutagenesis (16) such that a *Bam*HI site was introduced at nucleotides 120–125 and an *Eco*RV site was introduced at nucleotides 879–884. The resulting *Bam*HI-*Eco*RV fragment of the modified cDNA was isolated and cloned into the *Bam*HI-*Sma*I sites of pBC12BI (17). This results in an in-frame fusion where the initiation codons and the first five amino acids of the preproinsulin gene are fused to amino acids 6–257 of the human α subunit. This particular construction pLJ1101 resulted in high level α expression when transfected into COS cells as judged by immunofluorescent staining of permeabilized cells using antipeptide antisera 81–103 and 160–197.

The vector containing the chimeric form of the α subunit was constructed by ligating the *Bam*HI-*Sca*I fragment of the modified α subunit (nucleotides 121–710) to an *Rsa*I-*Bam*HI fragment of the IL-2¹ receptor (nucleotides 876–1016) (18). This ligated fragment was then inserted into *Bam*HI-digested pBC12BI. The resulting vector, pLJ1275, directed the synthesis of a chimeric protein encoding amino acids 6–201 of the human α subunit (corresponding to the signal peptide and extracellular domain) and amino acids 240–271 (corresponding to the transmembrane and cytoplasmic domains) of the p55 IL-2 receptor.

Cell Culture and DNA Transfection—Cell lines COS-1 and CHO duk[−] were kindly provided by Dr. B. Cullen of the Howard Hughes Institute, Durham, NC. The conditions for cell growth, transient DNA transfection into COS cells, and permanent transfection into CHO duk[−] cells were as described (17). Amplification of chimeric α subunit expression was achieved by stepwise increasing concentrations of 0.1 and 1.0 μ M amethopterin. Enrichment of cells expressing high receptor numbers was achieved by two rounds of fluorescent activated cell sorting using IgE-biotin and streptavidin-FITC. Stable clones expressing high levels of cell surface IgE binding subunit were then obtained by limiting dilution cloning. Five independent clonal isolates were initially characterized which differed with respect to receptor expression but not with regard to binding affinity. The 3D10 clone was adapted to spinner cultures in Iscove's modified Dulbecco's media supplemented with 10% fetal calf serum and 80 μ g/ml gentamicin sulfate.

Immunofluorescent Analysis—Cells were incubated with biotinylated IgE (1–3 μ g/ml) or the two rabbit anti- α peptide antiserum (1:200 dilution) for 30 min in Iscove's modified Dulbecco's media supplemented with 10% fetal calf serum (complete medium) at 37 °C. The cells were washed 4 times with complete medium and incubated with streptavidin-FITC or donkey anti-rabbit IgG-FITC (Jackson ImmunoResearch) for 30 min at 37 °C in complete medium. The cells were then washed and either sorted on an Ortho cytofluorograph 50H or overlaid with 50% glycerol containing 0.25 M *N*-propylgallate and coverslip-mounted. The cells were observed on a Leitz Diaplan fluorescent microscope (25 $\times$ dry objective) and photographed with Kodak Tri-X film.

Binding Assays—IgE was radiolabeled with ¹²⁵I to a specific activity of 1–2 $\times$ 10⁶ cpm/ μ g protein using chloramine T (19). The bindability

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

|| To whom correspondence should be addressed.

¹ The abbreviations used are: IL-2, interleukin-2; FITC, fluorescein isothiocyanate.

of ^{125}I -IgE was assessed as previously described (20) and ranged between 50 and 75%. Binding assays were performed as described previously (21), and for competition assays, samples at various dilutions were preincubated with the cell suspensions (4×10^6 cells/ml) for 30 min prior to the addition of 250 ng/ml ^{125}I -IgE. Nonspecific binding was determined by adding a 50–100-fold excess of unlabeled IgE to the assay tubes, and these values were subtracted from experimental values to give specific binding. Kinetics of association and dissociation between IgE and the recombinant receptor were determined by the methods described previously (22–24). The forward rate constant (k_1) was calculated from the equation $k_1 = V_o / \text{IgE}_o R_o$, where V_o represents the initial rate of binding and IgE_o and R_o represent the initial molar concentrations of IgE and receptor, respectively. The dissociation rate constant (k_{-1}) was calculated by assuming that the dissociation of a ligand from receptors is a simple first-order decay process. The equilibrium constant (K_A) was calculated with the equation $K_A = k_1 / k_{-1}$.

RESULTS AND DISCUSSION

Cell Surface Expression of a Chimeric α Subunit—Previous studies have demonstrated that cell surface expression of the human FcERI α subunit is very inefficient in the absence of the γ subunit. In an effort to obtain cell surface expression of the α subunit to study in detail its binding properties, a chimeric receptor was engineered by substituting the transmembrane and cytoplasmic domains of the α subunit with those coding for the p55 IL-2 receptor. The p55 IL-2 receptor subunit is expressed efficiently on the cell surface independent of other subunits. Transfection of COS cells with the wild type FcERI α , pLJ1101, did not yield any cell surface binding of IgE or anti-peptide antisera (Fig. 1, B and D). Analysis of permeabilized cells using the anti-peptide antisera clearly demonstrated that the α subunit was indeed synthesized (results not shown). However, transfection of COS cells with a chimeric α subunit, pLJ1275, resulted in efficient cell surface expression of an α subunit which was able to bind IgE and was recognized by anti-peptide antisera (Fig. 1, A and C). The binding of biotinylated IgE was completely inhibited by a 50-fold excess of unlabeled IgE (results not shown, see below).

These results suggest that the α subunit transmembrane and/or cytoplasmic domains are involved in preventing the cell surface expression of the α subunit. The block exerted by these domains is obviously overcome by the presence of the γ subunit (5). It should now be possible to identify the regions which are exerting this block as well as the regions which are interacting with the γ subunit.

Binding Analysis—Permanent cell lines expressing the chimeric receptor were established in CHO cells using gene-

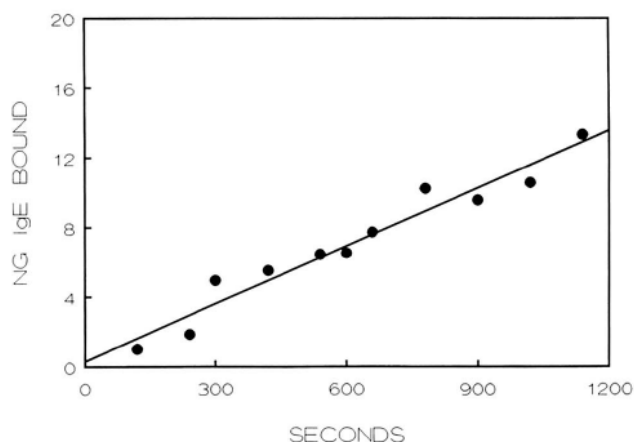


FIG. 2. Determination of the forward rate constant for IgE binding to 3D10 cells. Cells (2×10^6 cells/ml) were incubated with 1 $\mu\text{g}/\text{ml}$ ^{125}I -IgE (PS) at 25 °C. At indicated times, the reaction was stopped by the addition of a 100-fold excess of unlabeled IgE. Nonspecific IgE binding was subtracted from total binding at each time point. Data is expressed as nanograms of IgE bound per 4×10^5 cells.

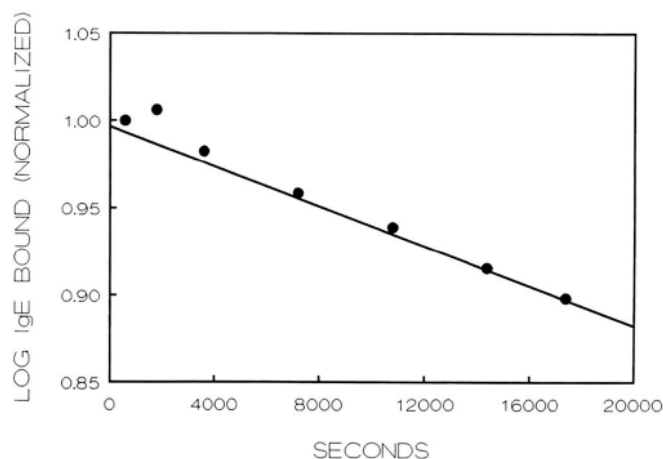


FIG. 3. Determination of the dissociation rate constant of IgE from 3D10 cells. Cells (2×10^6 cells/ml) were incubated with 0.5 $\mu\text{g}/\text{ml}$ ^{125}I -IgE (PS) for 60 min at 25 °C. Cells were washed and resuspended at the same density in media containing 25 $\mu\text{g}/\text{ml}$ unlabeled IgE. The amount of specific cell bound IgE was determined at the indicated times. The data were normalized to compensate for the difference in the concentration of cell-bound ^{125}I -IgE at time 0.

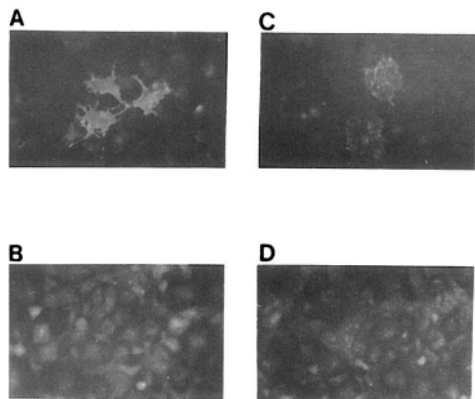


FIG. 1. Immunofluorescent analysis of FcERI α subunit expression on cells. COS cells were transfected for 40 h with pLJ1275 (panels A and C) or pLJ1101 (panels B and D). Cell surface FcERI α was examined by incubating monolayer cells with biotinylated IgE, followed by streptavidin-FITC (panels A and B) or with FcERI α anti-peptide antisera (panels C and D).

TABLE I
Inhibition of ^{125}I -IgE binding to 3D10 cells

Protein	IC ₅₀ $\mu\text{g}/\text{ml}$
Human IgE (PS)	2.3
Human IgE (10)	3.4
Human recombinant IgE	3.0
Human polyclonal IgE	15
Rat IgE (IR162)	34

linked co-amplification technology. To clearly demonstrate that the extracellular domain of the α subunit is sufficient for high affinity IgE binding, the equilibrium association constant was established by independent determinations of the association and dissociation rate constants as previously described for the human and rodent receptors. The receptor number on the CHO cells was determined by incubating the cells with 10 $\mu\text{g}/\text{ml}$ of ^{125}I -IgE for 2 h. Receptor density ranged between 4 and 10×10^5 molecules/cell.

The kinetics of association and dissociation between ^{125}I -

IgE and the chimeric IgE cell surface receptor were determined at 25 °C. A representative experiment is shown in Fig. 2 where the cells expressed 6.03×10^5 surface receptors, and the V_0 was determined during the first 100 s. The k_1 calculated from these results is $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. The dissociation rate of bound ^{125}I -IgE over a 5–6-h period was calculated from the slope of plot shown in Fig. 3, and the k_{-1} is $1.5 \times 10^{-5} \text{ s}^{-1}$. From three independent experiments the k_1 and k_{-1} calculated from our data are $1.1 \pm 0.26 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $1.3 \pm 0.18 \times 10^{-5} \text{ s}^{-1}$, respectively. The average K_A from our data is $0.85 \times 10^{10} \text{ M}^{-1}$. These data are similar to those described for the high affinity FcERI receptors on cultured cord blood basophils and COS cells transfected with the α and γ subunits (7, 24).

Specificity of receptor binding was evaluated in radioreceptor competition experiments (Table I). Dose-dependent inhibition of ^{125}I -IgE (PS) binding was observed with both human and rat IgE but not with human and rat IgG. Inhibition of 50% binding (IC_{50}) with two human myeloma IgE proteins and a chimeric recombinant human IgE was achieved with approximately 2–3 $\mu\text{g}/\text{ml}$ of protein.

Polyclonal human IgE (from hyper-IgE plasma) was 6-fold less effective in the competition assay. It is unknown whether this reflects a difference in the sample's intrinsic binding activity or partial inactivation during storage and purification. Rat IgE was at least 10-fold less active in the competition assay, yet the same rat IgE inhibited 50% binding in a rat radioreceptor binding assay at 2–3 $\mu\text{g}/\text{ml}$ (data not shown). By Scatchard analysis, the affinity of rat ^{125}I -IgE to the human FcERI was approximately 7-fold lower than that of human IgE (data not shown). Human IgE (PS) did not bind to the rat IgE receptor on RBL cells. These data confirm the cross-species specificity of IgE binding on rat and human FcERI. Early studies using human basophils indicated that approximately 15-fold more rat IgE was required for 50% inhibition of ^{125}I -IgE (PS) binding than unlabeled IgE (25). Subsequent studies using human cord blood basophils demonstrated no significant difference in equilibrium constants for rat and human IgE (24). More detailed binding analysis of rat IgE on the recombinant FcERI is required to characterize this species specificity.

It is clear from our data that the binding of human IgE to FcERI is localized to the extracytoplasmic region of the receptor and that the β and γ subunits of the receptor complex do not play a critical role in IgE binding. The chimeric receptor overexpressed on CHO cells provides us with the opportunity to study in detail the interaction of IgE and its receptor and to develop high flux receptor binding assays to identify antagonists of IgE/FcERI binding.

Acknowledgments—We thank R. Wright for cell sorting, E. Labriola-Tompkins for preparation of oligonucleotides, J. Swistok and

R. Makofske for preparation of synthetic peptides, and P. Lin, L. Anderson, and J. Wardwell-Swanson for excellent technical assistance. We are grateful to Drs. Z. Eshhar, P. Goldsmith, J. F. Greally, P. Haring, and T. Ishizaka for providing us valuable reagents.

REFERENCES

1. Metzger, H., Alcaraz, G., Hohman, R., Kinet, J. P., Publuda, V., and Quarto, R. (1986) *Annu. Rev. Immunol.* **4**, 419–470
2. Kinet, J. P., Metzger, H., Hakimi, J., and Kochan, J. (1987) *Biochemistry* **26**, 4605–4610
3. Shimizu, A., Tepler, I., Benfey, P. N., Berenstein, E. H., Siraganian, R. P., and Leder, P. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 1907–1911
4. Kinet, J. P., Blank, U., Ra, C., White, K., Metzger, H., and Kochan, J. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 6483–6487
5. Blank, U., Ra, C., Miller, L., White, K., Metzger, H., and Kinet, J. P. (1989) *Nature* **337**, 187–189
6. Ra, C., Jouvin, M.-H. E., and Kinet, J.-P. (1989) *J. Biol. Chem.* **264**, 15323–15327
7. Miller, L., Blank, U., Metzger, H., and Kinet, J. P. (1989) *Science* **244**, 334–337
8. Kuster, H., Thompson, H., and Kinet, J. P. (1990) *J. Biol. Chem.* **265**, 6448–6452
9. Ishizaka, K. (1985) *Methods Enzymol.* **116**, 76–94
10. Lawlor, E., Collins, W., and O'Conner, B. (1966) *Clin. Chem.* **32**, 697
11. Bazin, H., Querinjean, P., Beckers, A., Heremans, J. F., and Dessy, F. (1974) *Immunology* **26**, 713–723
12. Bayer, E. A., Skutelsky, E., and Wilchek, M. (1979) *Methods Enzymol.* **62**, 308–315
13. Barany, G., and Merrifield, R. B. (1980) *The Peptides: Analysis Synthesis, Biology* (Gross, E., and Meienhofer, J., eds) Vol. 2, pp. 1–255, Academic Press, New York
14. Lui, F. T., Sinnecker, M., Hamaoka, J., and Katz, D. (1979) *Biochemistry* **18**, 690–697
15. Kochan, J. P., Pettine, L. F., Hakimi, J., Kishi, K., and Kinet, J. P. (1988) *Nucleic Acids Res.* **16**, 3584
16. Morinaga, Y., Franceschiri, T., Inouye, S., and Inouye, M. (1984) *DNA Biotechnol.* **2**, 636–639
17. Cullen, B. (1987) *Methods Enzymol.* **152**, 684–702
18. Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J., and Honjo, T. (1984) *Nature* **311**, 631–635
19. McConahey, P., and Dixon, F. J. (1986) *Int. Arch. Allergy Appl. Immunol.* **29**, 185–189
20. Isersky, C., Kulczycki, A., Jr., and Metzger, A. (1974) *J. Immunol.* **112**, 1909–1919
21. Kulczycki, A., Jr., Isersky, C., and Metzger, H. (1974) *J. Exp. Med.* **139**, 600–616
22. Kulczycki, A., Jr., and Metzger, H. (1974) *J. Exp. Med.* **140**, 1676–1695
23. Ishizaka, T., Helm, B., Hakimi, J., Niebyl, J., Ishizaka, K., and Gould, H. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 8323–8327
24. Ishizaka, T., Dvorak, A. M., Conrad, D. H., Niebyl, J. R., Marquette, J. P., and Ishizaka, K. (1985) *J. Immunol.* **134**, 532–540
25. Conrad, D. H., Wingard, J. R., and Ishizaka, T. (1983) *J. Immunol.* **130**, 327–333