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

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The α subunit of the FcERI binds IgE with high affinity. Previous studies have demonstrated that a 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 means of identifying therapeutic agents which may be effective in the treatment of 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 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. Graily 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 IgG was purified from the ascites fluid of nu/nu mice bearing Immuno cytoma IR 162 as described previously (11).

IgE 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 µBondapak 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.

Cell culture and DNA transfection—Cell lines COS-1 and CHO duk—were kindly provided by Dr. B. Cullen of the Howard Hughes Medical 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 μM amethopterin. Enrichment of cells expressing high receptor numbers was achieved by two rounds of fluorescence 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 clones isolates were initially characterized which differed with respect to receptor expression but not with regard to binding affinity. The 3D10 clone was adapted to spinor cultures in Iscove’s modified Dulbecco’s media supplemented with 10% fetal calf serum and 80 μg/ml gentamicin as necessary.

Immunofluorescent analysis—Cells were incubated with biotinylated IgE (1–3 μg/ml) or the two rabbit anti-α peptide antisera (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 sucrose 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. Graily 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 IgG was purified from the ascites fluid of nu/nu mice bearing Immuno cytoma IR 162 as described previously (11).

The abbreviation 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 ($4 \times 10^6$ 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 ($k_+$) was calculated from the equation $k_+ = V_d/IGE \cdot R$, where $V_d$ represents the initial rate of binding and $IGE$ and $R$, represent the initial molar concentrations of IgE and receptor, respectively. The dissociation rate constant ($k_-$) 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 $K_a = k_+/k_-$. The dissociation rate constant of IgE from 3D10 cells. Cells ($2 \times 10^6$ cells/ml) were incubated with 0.5 µg/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 $4 \times 10^6$ cells.

RESULTS AND DISCUSSION

Cell Surface Expression of a Chimeric $\alpha$ Subunit—Previous studies have demonstrated that cell surface expression of the human FcERI $\alpha$ subunit is very inefficient in the absence of the $\gamma$ subunit. In an effort to obtain cell surface expression of the $\alpha$ subunit to study in detail its binding properties, a chimeric receptor was engineered by substituting the transmembrane and cytoplasmic domains of the $\alpha$ 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 $\alpha$, pLJ1101, did not yield any cell surface binding of IgE or antipeptide antisera (Fig. 1, B and D). Analysis of permeabilized cells using the antipeptide antisera clearly demonstrated that the $\alpha$ subunit was indeed synthesized (results not shown). However, transfection of COS cells with a chimeric $\alpha$ subunit, pLJ1275, resulted in efficient cell surface expression of an $\alpha$ subunit which was able to bind IgE and was recognized by antipeptide 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 $\alpha$ subunit transmembrane and/or cytoplasmic domains are involved in preventing the cell surface expression of the $\alpha$ subunit. The block exerted by these domains is obviously overcome by the presence of the $\gamma$ subunit. In an effort to obtain cell surface expression of the $\alpha$ subunit to study in detail its binding properties, the chimeric receptor was engineered by substituting the transmembrane and cytoplasmic domains of the $\alpha$ 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 $\alpha$, pLJ1101, did not yield any cell surface binding of IgE or antipeptide antisera (Fig. 1, B and D). Analysis of permeabilized cells using the antipeptide antisera clearly demonstrated that the $\alpha$ subunit was indeed synthesized (results not shown). However, after transfection of COS cells with a chimeric $\alpha$ subunit, pLJ1275, resulted in efficient cell surface expression of an $\alpha$ subunit which was able to bind IgE and was recognized by antipeptide 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).

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Binding Analysis—Permanent cell lines expressing the chimeric receptor were established in CHO cells using gene-linked co-amplification technology. To clearly demonstrate that the extracellular domain of the $\alpha$ 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 µg/ml $^{125}$I-IgE for 2 h. Receptor density ranged between 4 and $10 \times 10^6$ molecules/cell.

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

| Protein             | IC₅₀ (µg/ml) |
|---------------------|--------------|
| Human IgE (PS)      | 2.3          |
| Human IgE (10)      | 3.4          |
| Human recombinant IgE | 3.0         |
| Human polyclonal IgE | 15           |
| Rat IgE (IR162)     | 34           |

TABLE I

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

FIG. 1. Immunofluorescent analysis of FcERI $\alpha$ 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 $\alpha$ was examined by incubating monolayer cells with biotinylated IgE, followed by streptavidin-FITC (panels A and B) or with FcERI $\alpha$ antipeptide antisera (panels C and D).

FIG. 2. Determination of the forward rate constant for IgE binding to 3D10 cells. Cells ($2 \times 10^6$ cells/ml) were incubated with 1 µg/ml $^{125}$I-IgE (PS) for 30 min 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 $4 \times 10^6$ cells.

FIG. 3. Determination of the dissociation rate constant of IgE from 3D10 cells. Cells ($2 \times 10^6$ cells/ml) were incubated with 0.5 µg/ml $^{125}$I-IgE (PS) for 60 min at 25 °C. Cells were washed and resuspended at the same density in media containing 25 µg/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.
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 k_0 was determined during the first 100 s. The k_i calculated from these results is 1.4 × 10^6 M^-1 s^-1. The dissociation rate of bound ^125I-IgE over a 5–6-h period was calculated from the slope of the line shown in Fig. 3, and the k_-1 is 1.5 × 10^-5 s^-1. From three independent experiments the k_i and k_-1 calculated from our data are 1.1 ± 0.26 × 10^6 M^-1 s^-1 and 1.3 ± 0.18 × 10^-5 s^-1, respectively. The average k_S from our data is 0.85 × 10^-5 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 ^125I-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 μg/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 pg/ml (data not shown). By Scatchard analysis, the affinity of rat ^125I-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-reactivity 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 ^125I-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.

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