

Ligand binding properties of the basophilic IgE receptor of normal and allergic individuals

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Summary

The interaction of [^{125} I]-human myeloma IgE with the human basophilic IgE receptor of normal and allergic individuals was quantitated by direct ligand binding analysis after removal of endogenous IgE. Using a mixed leucocyte preparation containing 2 to 5% basophils, the [^{125} I]-IgE binding was specific for human IgE, paralleled the presence of basophils in the cell preparation, and was described by a time course similar to that reported for passive sensitization experiments. The K_d values were 4.0 to 13.2 nM for the normal individuals with 1.5 to 8.4×10^5 receptors per basophil, while the IgE receptor of allergic individuals was described by K_d values of 4.2 to 11.7 nM and 3.0 to 4.7×10^5 sites per basophil. Based upon the eleven individuals studied, these results suggest that the binding properties of the IgE receptor of normal and allergic individuals are similar.

Introduction

Cell surface receptors specific for Immunoglobulin E (IgE) on mast cells and basophilic leucocytes are believed to play a central role in immediate hypersensitivity disorders (Ishizaka & Ishizaka, 1978; Metzger & Bach, 1978). The cross-linking of two IgE-bound receptors by an allergen induces the explosive release of potent chemical mediators which presumably account for the clinical signs of asthma, hay fever, and hives (Ishizaka, Ishizaka, Conrad & Froese, 1978). Studies investigating the interaction of [^{125} I]-IgE and its receptor have for the most part been done in rat systems employing rat IgE and either rat peritoneal mast cells or rat basophilic leukaemia (RBL-1) cells (Conrad *et al.*, 1975; Kulczycki, Hempstead *et al.*, 1979; Rossi, Newman & Metzger, 1977). These studies have provided valuable information concerning the molecular structure of the receptor and the mechanism of its action. However, since it is questionable whether human IgE binds to the rat IgE receptor, we have investigated

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whether the interaction of [^{125} I]-human myeloma IgE with the human basophilic IgE receptor could be quantitated and characterized by direct ligand binding analysis. This provides a system more physiologically relevant for the study of the human allergic process. We were thus able to compare the receptor binding properties of normal and allergic individuals to ascertain whether differences between these two populations reside at the level of the receptor.

Materials and methods

Mixed leucocyte preparation

Blood, collected from normal and allergic donors in Vacutainer tubes containing EDTA (Becton-Dickinson, Rutherford, NJ), was treated with clinical dextran for 45 to 60 min at room temperature as described by Lichtenstein & Osler (1964). The supernatant layer was collected by aspiration and centrifugation and resuspended in autologous plasma. The cells were layered over Hypaque-Ficoll (Isolymp, Gallard-Schlesinger Corp., Carle Place, NY); the basophil enriched fraction was obtained according to the method of Böyum (1968). This mixed leucocyte preparation, containing 2 to 5% basophils as assessed by Alcian Blue (Gilbert & Ornstein, 1975), was washed twice with Tris buffer (14.5 mM Tris, 126 mM NaCl, 0.54 mM KCl, 0.1 mM $MgCl_2$, 0.005 mM $CaCl_2$, 0.56 mM Dextrose, 0.03% human serum albumin, pH 7.5). Where indicated, endogenous IgE was removed by twice washing the cells for ten minutes with 50 mM acetate buffer, pH 4.0 (Ishizaka & Ishizaka, 1974) followed by twice washing the cells with Tris. The cells were finally resuspended in Tris buffer at 3×10^5 to 1.5×10^6 basophils/ml.

[^{125} I]-IgE binding assay

In order to measure the total and non-specific [^{125} I]-IgE binding, 100 μ l aliquots of the mixed leucocyte preparation were incubated at 30° with 10 μ l [^{125} I]-IgE or 10 μ l heat inactivated (56°C—6 hr) [^{125} I]-IgE, respectively. To stop the binding reaction, 90 μ l of the cell [^{125} I]-IgE preparation was layered over 225 μ l 12% Ficoll in a 400 μ l microfuge tube. Receptor bound [^{125} I]-IgE and free [^{125} I]-IgE were separated by immediately centrifuging the cells for 2 min in a Beckman Spinco 152 microcentrifuge. The tip of the tube containing the cell pellet was cut and counted in a Nuclear Chicago gamma counter with an efficiency of 78% for [^{125} I]. Simultaneously, buffer was incubated with [^{125} I]-IgE to evaluate the background of the separation technique. Typically, this blank control represented less than 5% of the total binding. Specific [^{125} I]-IgE binding was calculated from the difference of the total and nonspecific binding after correcting each for their respective blank controls. Alternatively, identical results were obtained when the cell pellet was washed three times with cold Tris in order to remove free [^{125} I]-IgE. However, the centrifugation of the cells through 12% Ficoll was more rapid and applicable to the batch assay.

Materials

Reaginic human serum (idiopathic hyperimmunoglobulin E titre ~ 69 μ g/ml) and [^{125} I]-human myeloma IgE were obtained from Dr Roy Patterson, Northwestern University. The [^{125} I]-IgE (myeloma DB) had a specific activity of 16 to 18 μ Ci/ μ g. Human IgE (myeloma ND) serum was from a kit from Pharmacia Fine Chemicals, Piscataway, NJ; and the IgA, IgM, and IgG rich human sera were from Cappell

Laboratories, Cochranville, PA. The Ficoll, Tris and clinical dextran were from Sigma Chemical Co., St Louis, MO; the human serum albumin was from ICN Pharmaceuticals, Inc., Cleveland, Ohio; and the Alcian Blue was from Eastman Kodak Co., Rochester, NY.

Results

Removal of endogenous IgE

The presence of free IgE receptors in a mixed leucocyte preparation containing 2 to 5% basophils was detected by direct ligand analysis using [^{125} I]-human myeloma IgE. The quantitation and characterization of these receptors was hindered by the presence of significant levels of cell bound endogenous IgE. Since IgE dissociates rapidly from its receptor under acidic conditions (Ishizaka & Ishizaka, 1974), several stripping procedures were evaluated for removal of endogenous IgE. Washing the mixed leucocyte preparation twice for 10 min at 25°C with a pH 4 acetate buffer resulted in a significant increase in specific [^{125} I]-IgE binding without affecting the nonspecific [^{125} I]-IgE binding (Fig. 1). Inclusion of a third pH 4 wash resulted in an increase in nonspecific binding; no additional specific binding was uncovered. Buffers with different pHs were also tested for their ability to remove endogenous IgE from its binding site. However, pH 4 acetate buffer was optimal when compared to pH 3 glycine-HCl, pH 4.5 acetate, and pH 5 acetate buffers (data not shown). In addition, washing the cells with the pH 4 acetate buffer at 0°C was less efficient in terms of removal of endogenous IgE. These results suggested that two pH 4 washes at 25°C efficiently removed the endogenous bound IgE, and therefore, that binding to the twice washed cells reflected the total number of IgE binding sites.

Specificity of binding sites for [^{125} I]-IgE

The specificity of these binding sites for IgE and its physiological relevance were investigated using the twice pH 4 washed mixed leucocyte preparation. The binding

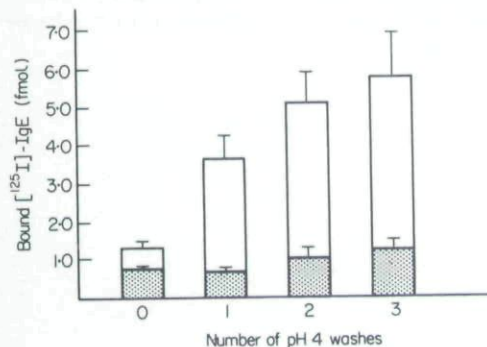


Fig. 1. Effect of washing cells with pH 4 buffer on subsequent [^{125} I]-IgE binding. A mixed leucocyte preparation was obtained after dextran sedimentation and Hypaque-Ficoll centrifugation as described in the 'Materials and methods'. Cells were washed either 0, 1, 2, or 3 times for 10 min at 25°C each with the pH 4 acetate buffer; then washed with Tris buffer such that each aliquot received five total washes (pH 4 + Tris). The cells in Tris buffer were incubated with [^{125} I]-IgE (total binding) or heat inactivated [^{125} I]-IgE (nonspecific binding) for 3 hr at 30°C. Plotted is a histogram of the specific ($\square$) and nonspecific ($\blacksquare$) [^{125} I]-binding using 75 000 basophils and 1.5 nM [^{125} I]-IgE. The data is expressed as the mean $\pm$ s.e. mean of three experiments. Identical histograms were observed under other conditions of [^{125} I]-IgE concentration and basophil number.

time course of [^{125} I]-IgE reached a plateau level by 120 min at 30° (Fig. 2). The binding remained stable for at least an additional 90 min. Interestingly, the time course of binding accurately reflects the time required to optimally passively sensitize basophils in terms of histamine release (Levy & Osler, 1967), demonstrating the physiological relevance of the binding time course. Routinely, a 3-hr incubation was adopted for the remaining experiments.

The binding of the [^{125} I]-IgE to the mixed leucocyte preparation was specific for only human IgE. Competitive binding experiments demonstrated that only unlabelled human myeloma IgE and hyperimmunoglobulin E sera inhibited the [^{125} I]-IgE binding (Fig. 3). Heat inactivation of the IgE at 56°C for 6 hr, a procedure which results in loss of IgE sensitizing activity due to alterations in the conformation of the Fc region (Dorrington & Bennich, 1973), significantly compromised its ability to compete with [^{125} I]-IgE (data not shown). Other classes of immunoglobulins, present at 1000-fold

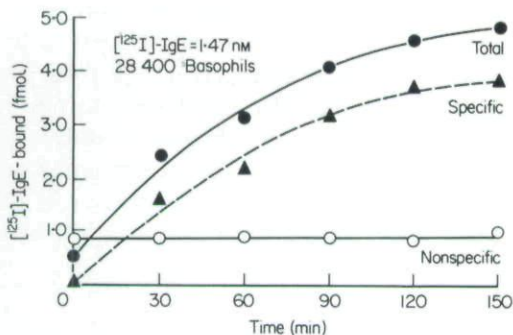


Fig. 2. Time course of [^{125} I]-IgE binding. At time zero, [^{125} I]-IgE (●) or heat inactivated [^{125} I]-IgE (○) was added to aliquots of a mixed leucocyte preparation containing 312 500 basophils per ml. After the indicated time of incubation at 30°C, receptor bound [^{125} I]-IgE was separated from free [^{125} I]-IgE by centrifugation of an aliquot of the cell preparation through 12% Ficoll in Tris. The specific [^{125} I]-IgE binding (▲) at each time point is the difference between the total binding and nonspecific binding.

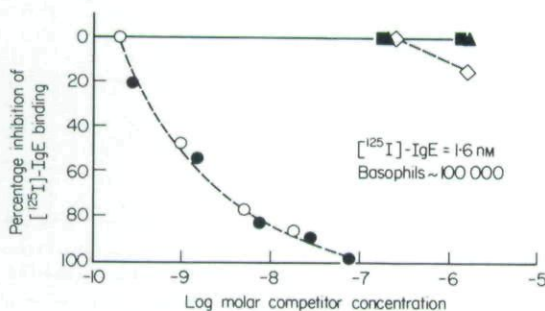


Fig. 3. Specificity of [^{125} I]-IgE binding. A mixed leucocyte preparation washed twice with pH 4 buffer and containing 100 000 basophils per sample was incubated with 1.6 nM [^{125} I]-IgE either in the absence or presence of dilutions of myeloma IgE serum (○), hyperimmunoglobulin E serum (●), IgG serum (▲), IgM serum (◇) or IgA serum (■) for 3 hr at 30°C. Plotted is the percentage inhibition of [^{125} I]-IgE binding by the indicated concentration of immunoglobulin.

excess, also failed to compete for the [^{125} I]-IgE binding site. Interestingly, the asymptotic approach of the IgE competition curve toward 100% inhibition suggested that heat inactivated [^{125} I]-IgE accurately estimated the nonspecific [^{125} I]-IgE binding. While nonspecific binding could also have been estimated by a 100-fold excess of unlabeled IgE, this procedure was not routinely employed because of the quantities of valuable IgE required.

Lymphocytes possess a low affinity IgE receptor whose physiological function remains to be elucidated (Spielgelberg & Melewicz, 1980; Yodoi & Ishizaka, 1980). In order to investigate the contribution of the lymphocyte receptor to the observed specific [^{125} I]-IgE binding in the mixed leukocyte preparation, the basophils were removed by filtration of the cells through two successive glass bead columns according to the method of Rabinowitz (1964). The resulting preparation was greater than 98% lymphocytes as assessed by differential cell counting. In addition, however, this lymphocyte preparation contained 0.4% basophils. The binding of [^{125} I]-IgE to this preparation was low, presumably reflecting the presence of the small percentage of basophils (Fig. 4). In contrast, the binding of [^{125} I]-IgE to the original mixed leukocyte preparation was linear with respect to the number of cells and significantly greater than to the 'lymphocyte' preparation. Similarly, a basophil-rich (8%) fraction recovered from the glass bead column in low yield showed high levels of binding comparable to the binding observed to the mixed leukocyte preparation. These results suggested that the lymphocyte IgE receptor did not contribute to the observed specific [^{125} I]-IgE binding in the mixed leukocyte preparation due to its low affinity relative to the basophilic IgE receptor; rather the observed binding reflected the interaction of [^{125} I]-IgE with the basophilic IgE receptor.

Equilibrium analysis of [^{125} I]-IgE binding

The IgE receptor plays a central role in the biochemical events leading to the release of the mediators of anaphylaxis, and hence, the allergic symptomatology. To investigate

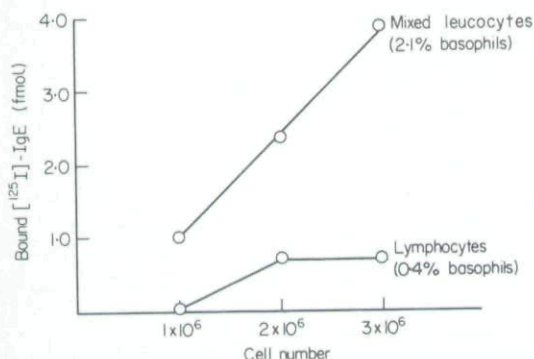


Fig. 4. Evaluation of the contribution of the low affinity lymphocyte receptor to the [^{125} I]-IgE binding. One aliquot of leucocytes obtained after dextran sedimentation and Hypaque-Ficoll centrifugation was filtered through two successive glass bead (0.25–0.32 mm diam.) columns yielding a cell preparation containing >98% lymphocytes as assessed by differential counting. This lymphocyte preparation (containing 0.4% basophils) and the non-glass bead filtered leucocyte preparation (containing 2.1% basophils) were washed twice with pH 4 acetate buffer, twice with Tris buffer, and finally resuspended in Tris at either 1×10^6 , 2×10^6 , or 3×10^6 cells per 0.1 ml. The cells were incubated with 1.8 nM [^{125} I]-IgE or heat inactivated [^{125} I]-IgE at 30° for 3 hr. Plotted is the specific [^{125} I]-IgE binding to these cell preparations.

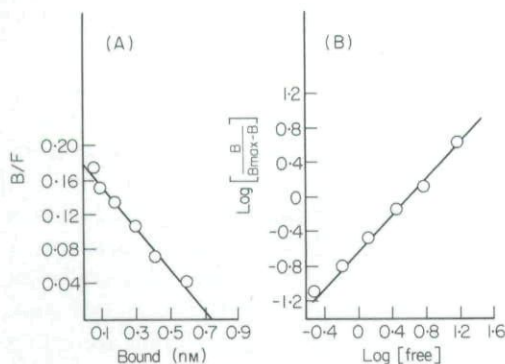


Fig. 5. Equilibrium analysis of [^{125} I]-IgE binding. The mixed leucocyte preparation was washed twice with pH 4 buffer as described in Fig. 1; then incubated for 3 hr at 30°C with varying concentrations of either [^{125} I]-IgE or heat inactivated [^{125} I]-IgE to measure total and non-specific binding, respectively. The specific binding was graphed on a Scatchard plot (A) and a Hill plot (B).

Table 1. Properties of the basophilic IgE receptor of normal and allergic individuals

Donor	Age/Sex	Serum IgE (ng/ml)	(n)	K_d (nM)	Receptors/basophil ($\times 10^{-5}$)
<i>Normal</i>					
JJ	46/F	402	4	5.9 ± 2.2	4.4 ± 0.6
BF	50/M	328	4	5.2 ± 0.9	6.4 ± 1.3
MB	28/F	108	3	5.2 ± 0.7	3.9 ± 0.4
KG	28/F	24	2	4.0	3.1
DF	49/M	~ 4	3	6.5 ± 1.1	1.5 ± 0.1
AM	47/F	<1	3	13.2 ± 2.4	8.4 ± 2.6
<i>Allergic</i>					
JS	29/F	10 000	3	4.2 ± 0.7	4.4 ± 0.5
KL	37/M	127	3	6.9 ± 2.0	4.5 ± 0.8
BE	33/F	71	3	6.0 ± 1.0	4.6 ± 0.7
EG	38/F	42	3	8.4 ± 2.8	4.7 ± 2.2
HH	48/M	20	2	11.7	3.0

Mixed leucocyte preparations from each donor were washed twice with pH 4 buffer to remove endogenous IgE; then equilibrium binding analyses using varying concentrations of [^{125} I]-IgE and heat inactivated [^{125} I]-IgE were performed. The K_d and total number of receptors per basophil were obtained from the Scatchard plot of the specific binding and are expressed as the mean $\pm$ s.e. mean of n individual experiments. Serum IgE was measured by radioimmunoassay (Kallestad Laboratories, Chaska, Mn).

whether differences between normal and allergic individuals were related to differences at the level of the IgE receptor, equilibrium analyses of the [^{125} I]-IgE-receptor interaction using leucocytes from five allergic and six normal donors were performed. Scatchard analysis of the binding data demonstrated the presence of a single class of high affinity binding sites; analysis on a Hill plot yielded a slope of 1.03 ± 0.01

suggesting no cooperative binding interaction between receptor sites (Fig. 5). The individual K_d values ranged from 4.0 to 13.2 nM for the normal individuals and from 4.2 to 11.7 nM for the allergic individuals (Table 1). This analysis of affinity was measured at equilibrium conditions because separation of bound and free [^{125} I]-IgE at 2.5 and 3.5 hr yielded identical K_d estimates as was expected from the time course of binding in Fig. 2. Interestingly, separation of bound and free [^{125} I]-IgE at 1 hr, at which time equilibrium had not been achieved, yielded a relatively flat Scatchard plot (data not shown). If the assumption that all the binding observed was to basophils is valid, then there were from 1.5 to 8.4×10^5 receptors/basophil for the normal individuals and from 3.0 to 4.7×10^5 receptors/basophil for the allergic individuals.

Discussion

The specific interaction of [^{125} I]-human myeloma IgE with the basophilic IgE receptor can be assayed by direct ligand binding analysis after removal of endogenous IgE by incubating the cells in pH 4 acetate buffer. This conclusion is based upon the following experimental observations: (1) Only human IgE-containing sera were capable of competing with [^{125} I]-IgE for binding to the mixed leukocyte preparation. Other immunoglobulin classes, heat inactivated IgE serum, and related proteins all failed to significantly inhibit the observed specific [^{125} I]-IgE binding. (2) The specific [^{125} I]-IgE binding paralleled the presence of basophils in the cell preparations studied. The lymphocyte-enriched fraction showed only low levels of binding, which were attributed to the presence of 0.4% basophils in the preparation. This is in agreement with previously reported autoradiographic procedures (Ishizaka, Tomioka & Ishizaka, 1970). Additionally, the linear Scatchard plot indicated little evidence for the inclusion of a second cell population to account for the observed [^{125} I]-IgE binding. (3) The time course of [^{125} I]-IgE binding plot showed a plateau by 2 hr at 30°C which accurately reflected the time reportedly required for optimal passive sensitization of basophils in terms of immunologic histamine release (Levy & Osler, 1967).

In equilibrium conditions, the affinity describing the interaction of [^{125} I]-IgE with the basophilic IgE receptor was on the order of 10^8 M $^{-1}$. This value is similar to that measured indirectly using the C1 fixation transfer technique (Ishizaka, Soto & Ishizaka, 1973) and the value estimated from the serum IgE level and the amount of IgE eluted from basophils by acidic buffer washes (Conroy, Adkinson & Lichtenstein, 1977). In addition, the total number of receptors per basophil measured directly in this study is in agreement with the maximum number of IgE molecules eluted from basophils of some of the allergic donors in the study by Conroy *et al.* (1977). However, the normal individuals used in this study showed significantly higher basophilic IgE receptor levels than the normal donors in the study by Conroy *et al.* (1977). This discrepancy may reflect the difference in the protocol used as this study measured the total IgE receptor levels directly by ligand binding analysis. Alternatively, the difference in the temperature of the acid washing procedure may account for the higher binding observed. We have continuously observed an increased efficiency of removal of endogenous IgE at 25°C *vis à vis* 0°C.

Comparison of the binding properties of the human and rat IgE receptors indicated that while the total number of binding sites per cell was similar, the affinity of the human receptor for human IgE was approximately an order of magnitude weaker than that of the rat receptor for rat IgE (Conrad *et al.*, 1975). This probably reflects an inherent difference in the size or conformation of the human IgE receptor *vis à vis* the

rat IgE receptor (Hempstead, Parker & Kulczycki, 1979), as well as differences between the Fc regions of IgE of the two species. However, several factors which could lead to an erroneously low estimate of the affinity should be considered. If there is heterogeneity among IgE molecules (Conroy, Adkinson & Lichtenstein, 1979), one can postulate that myeloma IgE differs from 'normal' IgE, and thus, may bind with a lower affinity. However, both myeloma (ND) serum and hyperimmunoglobulin E serum competed equally for the binding of the [^{125} I]-IgE, yielding 50% inhibition at a concentration equivalent to the [^{125} I]-IgE concentration. Secondly, the fraction of [^{125} I]-IgE capable of specifically binding the basophilic IgE receptor was estimated. At [^{125} I]-IgE concentrations in the nanomolar range, minimally 44% of the [^{125} I]-IgE was capable of binding as assessed by transferring of unbound [^{125} I]-IgE to fresh cell preparations. This would indicate that the question of the stability of the myeloma IgE could have only a relatively small effect on the affinity measurement. Thirdly, exposure of the cells to pH 4 buffer may irreversibly alter the receptor such that its affinity for [^{125} I]-IgE upon subsequent binding may be decreased. Unfortunately, the affinity of the receptor on cells not washed with pH 4 buffer cannot be directly assessed by these binding studies. Estimation of the affinity using the non-washed cells by correction for the dissociation of endogenous IgE during the assay suggests that the pH 4 wash did not have a deleterious effect. This observation was also supported by the similar time course of binding and stability of the two receptor preparations. Finally, non-equilibrium conditions could contribute to an underestimation of the affinity. Since identical K_d values and total binding site values were obtained at 2.5 and 3.5 hr of incubation, equilibrium conditions are indicated. The presence of equilibrium at every [^{125} I]-IgE concentration used was also indicated by the time course of binding and the linearity of the Scatchard plot.

The binding properties of the IgE receptor of normal and allergic donors were compared to ascertain if differences between these two populations reside at the level of the receptor. At least in terms of the eleven individuals studied the affinity and the total binding sites per basophil were identical for the two populations. Quantitation of the number of occupied receptors by the direct ligand binding method with [^{125} I]-IgE only yielded an approximate estimate of this parameter due to the dilution of the specific activity of the [^{125} I]-IgE by the endogenous IgE. However, it was apparent that a relationship between serum IgE level and receptor occupation was present, and thus, receptor occupation appeared independent of the allergic state of the individual (B. M. Weichman & R. M. Muccitelli, unpublished observation). Therefore, the inherent difference between normal and allergic individuals must reside within differences in the specificity of the IgE for the appropriate antigen, the 'releasability' of the cell when presented the antigenic challenge (Conroy *et al.*, 1977) or the sensitivity of the end organs to the released mediators.

Acknowledgments

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