Brief Definitive Report

THE MURINE LYMPHOCYTE RECEPTOR FOR IgE
II. Characterization of the Multivalent Nature of the B Lymphocyte Receptor for IgE

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Low affinity receptors for IgE ($Fc_\epsilon R$)[1] were initially discovered in the human system (1) and Spiegelberg and co-workers have studied this low affinity receptor on a variety of cell types (reviewed in reference 2). With regard to the lymphocyte $Fc_\epsilon R$, the murine B cell $Fc_\epsilon R$ has a relatively high affinity for its ligand, of the order of 10^8 M^{-1} (3). This observation stimulated investigation into the structure of this receptor and a recent study from this laboratory on the murine B lymphocyte $Fc_\epsilon R$ has shown that this receptor, when examined by SDS-PAGE, is a single polypeptide with a molecular mass of ~49,000 daltons (49K) (4). As part of this continuing study of the structure of the B cell receptor, the valency of the B cell $Fc_\epsilon R$ was investigated. IgE was found to co-isolate with the receptor when affinity chromatography on IgE-Affi-gel was used; in addition, when complexed to the B cell $Fc_\epsilon R$, nonhaptenated IgE would coprecipitate with haptenated IgE. In support of previously published work (5), the RBL high affinity receptor was clearly univalent in similar experiments.

Materials and Methods

Antibodies and Absorbents. Rat IgE from IR162 ascites was purified as previously described (6). Biotinylation of rat IgE was performed essentially as described by Berger et al. (7) for human C3. Briefly, a 30-fold molar excess of sulfo-NHS-biotin (a gift of Dr. E. Fujimoto, Pierce Chemical Co., Inc., Rockford, IL) was mixed with rat IgE overnight at 4°C and dialyzed for 24 h with borate-buffered saline (BBS), pH 8.0. Aliquots of stock biotinylated and normal rat IgE were radioiodinated with ^{125}I or ^{131}I (Amersham Searle Corp., Arlington Hts., IL) by the chloramine T procedure. After iodination, ~70% of the biotinylated rat IgE bound to avidin-agarose (Pierce Chemical Co., Rockford, IL); binding of non-modified IgE to the avidin was negligible. Adsorbents used were the avidin-agarose mentioned above and rat IgE-Affi-gel-10 (IgE-Affi) (Bio-Rad Laboratories, Richmond, CA) (4).

This work was supported by research grant AI 18697 from the U. S. Public Health Service with salary support to W. T. L. from National Institutes of Health Training Grant Number AI 07247. This article is publication No. 558 from the O'Neill Laboratories at the Good Samaritan Hospital, Baltimore, MD.

[1] *Abbreviations used in this paper:* BBS, borate-buffered saline; CHAPS, 3-[(3-Cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate; FBS, fetal bovine serum; $Fc_\epsilon R$, receptor for the Fc portion of IgE; IgE-Affi, IgE covalently coupled to Affi-Gel-10; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; RBL, rat basophilic leukemia cells; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Cells. The maintenance of the RBL cells and the isolation of murine B cells from *Nippostrongylus brasiliensis* infected mice (3) was performed as described elsewhere (4).

IgE-Affi Experiments. Purified murine B cells ($1-2 \times 10^8$) were surface radioiodinated as reported (4). Where indicated, the $Fc_\epsilon R$ was saturated with rat IgE (1 mg, 2 h at 4°C) before surface labeling. Subsequently, the cells were incubated overnight at 4°C, and the iodinated cells were washed 2 times by centrifugation and the $Fc_\epsilon R$ was solubilized in PBS containing 0.5% Nonidet P-40 (NP-40) plus protease inhibitors (4) (lysis buffer) and the clarified lysate (20 min, 3,000 *g*) was added to IgE-Affi. After mixing for 4 h, the IgE-Affi was washed and eluted with low pH (8) and the eluate was examined by SDS-PAGE. Detailed procedures are published elsewhere (4). In some experiments, purified B cells or RBL cells were incubated with various amounts of ^{125}I-rat IgE for 4 h at 4°C. The cells were then washed through a fetal bovine serum (FBS) gradient (9) and the amount of bound ^{125}I-IgE was determined with a gamma counter. After solubilization with lysis buffer, the clarified lysate was then rotated 4 h to overnight with 0.1 ml of IgE-Affi. The IgE-Affi was washed by centrifugation as indicated in results and the bound ^{125}I-IgE was determined as above.

Immobilized Avidin-Biotin Experiments. Purified B cell or nonadherent spleen cell preparations in 1–2 ml RPMI containing 10% FBS, were incubated with 5–6 μg ^{125}I-biotinylated rat IgE and 4–5 μg ^{131}I-normal rat IgE for 2 h (RBL) or 2 h to overnight (lymphocytes) at 4°C. To determine nonspecific binding, duplicate samples were prepared to which a 100-fold excess of unlabeled IgE was added for 20 min before the addition of radiolabeled IgE. After the incubation to allow binding, the cells were washed three times to remove unbound IgE and then solubilized using lysis buffer; in some experiments 3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate (CHAPS) (Pierce Chemical Co., Rockford, IL) was substituted for the NP-40 in the lysis buffer. The clarified lysate was added to 0.1 ml of avidin-agarose (in PBS containing the corresponding detergent) and rotated for 30 min in the cold. The avidin-agarose was centrifuged, collected, and washed five times by centrifugation with PBS-detergent buffer. Radioactivity in the sample was measured using a gamma counter at the whole cell, lysate, and after wash two and five. The counts in the ^{125}I channel were corrected for ^{131}I spillover and the corresponding duplicate sample containing excess cold IgE was subtracted to correct for nonspecific binding. The number of IgE bound molecules was then determined using the specific activities of the ^{131}I- and ^{125}I-IgE and by using 190,000 daltons as the mw of IgE.

Results and Discussion

As part of our continuing comparison study of the lymphocyte and mast cell $Fc_\epsilon R$, the valence of the murine B cell $Fc_\epsilon R$ was investigated. These studies were stimulated by an experiment in which the initial objective was to investigate the effect of ligand occupation on the capacity to radioiodinate the receptor. B cells were incubated with IgE, washed one time, and immediately surface radiolabeled with ^{125}I. As a control, an identical number of B cells were incubated with buffer only, iodinated in the same manner, and incubated with IgE after labeling. Subsequently, both groups of labeled cells were washed to remove free iodine and incubated overnight to allow partial dissociation of bound IgE. Next, the cells were washed, solubilized with lysis buffer, and the receptor was isolated using IgE-Affi (4) and examined by SDS-PAGE. In contrast to RBL cells (10), IgE did not influence the ability to iodinate the receptor; an essentially identical amount of 49K component, which has been identified as the murine B cell $Fc_\epsilon R$ (4), was isolated from both groups of cells. An unexpected observation was the appearance of two additional bands with mol wts of 70K and 23K, respectively, that were isolated from the sample that had IgE present at the time of labeling. The migration position of the two bands was identical with the heavy and light chains of rat IgE. Immunoprecipitation with monoclonal anti-IgE (11) confirmed

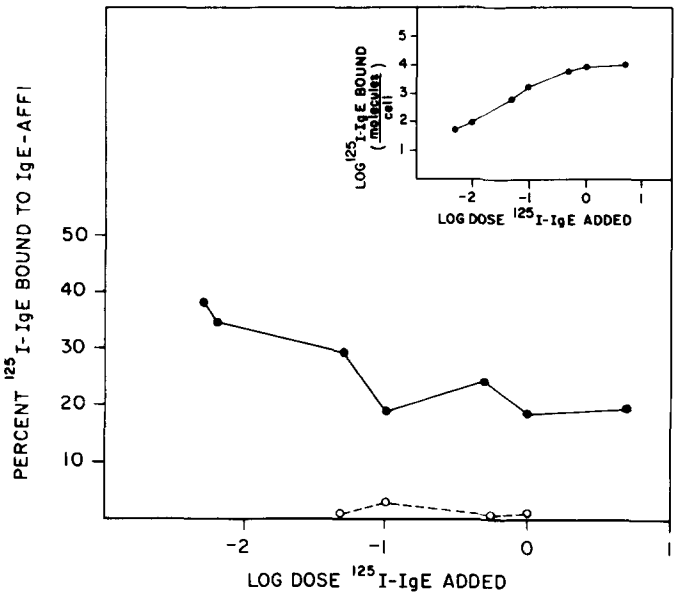

FIGURE 1. Binding of ^{125}I-IgE-receptor complexes by IgE-Affi. Purified murine (5.5×10^7) or RBL cells (3.25×10^6) were incubated with the indicated amount of ^{125}I-IgE. Subsequently, the cells, in a volume of 0.5 ml, were layered onto 1.5 ml of FCS and centrifuged. With B cells, the number of specifically bound molecules were determined and the inset shows the specific binding to the B cell plotted as a function of the amount of ^{125}I-IgE added to the sample. For both cell types, the cell pellet was solubilized with lysis buffer and mixed for 3 h with IgE-Affi. After two washes by centrifugation, the number of ^{125}I-IgE molecules bound to the IgE-Affi was determined and is expressed as the percent of the total ^{125}I-IgE initially added. The data is from a single experiment; two other experiments performed under similar conditions yielded comparable results. (●) IgE-receptor complexes from B cells; (○) IgE-receptor complexes from RBL cells.

that these additional bands were indeed from rat IgE. Thus, affinity chromatography on rat IgE-Affi was isolating both $Fc_\epsilon R$ and rat IgE. This experiment opened the possibility that the murine B cell is functionally multivalent.

The ability of the solubilized murine B cell $Fc_\epsilon R$ to cause co-isolation of labeled IgE when using an IgE affinity column is further illustrated in Fig. 1. Different amounts of radiolabeled IgE were incubated with murine B cells and the amount of specifically bound IgE was determined after washing the cells through FBS. The binding curve is shown in the inset of Fig. 1 and is similar to that reported by Vander-Mallie et al. (3). Increasing the amount of ^{125}I-IgE added resulted in an increase in the number of specifically bound receptors detected until saturation was achieved. Subsequently, the IgE-receptor complexes were solubilized with detergent and mixed with IgE-Affi. IgE-receptor complexes from RBL cells were prepared and mixed with the IgE-Affi in a similar manner. After 2 h the IgE-Affi was washed and the amount of bound IgE was determined. At all levels of saturation of the B cell, a significant percentage of the ^{125}I-IgE bound to the IgE-Affi; in contrast IgE-receptor complexes from RBL cells had no affinity for the IgE-Affi. Nonspecifically B cell bound ^{125}I-IgE, defined as ^{125}I-IgE bound in the presence of a 100-fold excess of cold IgE, when detergent solubilized, did not significantly bind to IgE-Affi. Thus, it can be concluded that the ^{125}I-IgE is

bound to the IgE-Affi via the B cell $Fc_\epsilon R$.

In order to further investigate this functional multivalency, we next determined whether the B cell $Fc_\epsilon R$ would allow co-precipitation of non-haptenated IgE along with haptenated IgE. Similar studies have been used with RBL cells, using mouse and rat IgE mixtures, to suggest univalency (5); thus, the RBL $Fc_\epsilon R$ continued to serve as a control in these experiments. Mixtures of ^{125}I-biotinylated-IgE and ^{131}I-IgE were incubated with RBL cells or murine B cells and subsequently washed to remove unbound IgE. Assuming that one IgE molecule corresponds to one $Fc_\epsilon R$, the amount of IgE bound per cell corresponded to a $Fc_\epsilon R$ number per cell of ~9,000 for B cells and 600,000 per RBL cells. The bound IgE was solubilized with essentially equal efficiency (85–90%) from both cell types. The solubilized IgE was then added to immobilized avidin-agarose, and after allowing the biotinylated IgE to bind, the avidin-agarose was washed with detergent buffer and the amount of IgE (normal and biotinylated) bound was determined after the second and fifth washes. The data in Fig. 2 is a composite of four experiments performed under different conditions (see below); in all cases a very similar ratio of the ^{131}I-IgE co-precipitated with the ^{125}I-biotinylated-IgE. Co-precipitation of non-haptenated IgE with the haptenated IgE was not seen with RBL cells and thus, this data is in agreement with the earlier study that used rat and mouse IgE. However, this phenomena clearly occurs with B cell $Fc_\epsilon R$ bound IgE. After two washes, about one in every five ^{131}I-IgE molecules was co-precipitated with the ^{125}I-biotinylated-IgE. Two different detergents, CHAPS and NP-40, were used for the co-precipitation experiments and gave identical results. The NP-40 solubilized extract was centrifuged at both 2,500 *g* and 25,000 *g* for 20 min; both procedures yielded essentially identical co-precipitation results and NP-40 concentration of 0.5% vs. 1% (vol/vol) also did not significantly alter the co-precipitation results. Thus, the possibility that membrane particles and/or receptor-detergent micelles are causing the observed multivalency are highly unlikely and multivalency of the $Fc_\epsilon R$ appears to be the explanation for the observed results.

The absolute valence (divalent vs. trivalent, etc.) cannot be stated with certainty. Assuming roughly equal proportions of biotinylated vs. normal IgE bound to the B cell, as was observed in these experiments, it would be expected that, as a percentage of total IgE molecules bound to the avidin, 25%, 30%, and 37.5% of the IgE would be non-biotinylated with divalent, trivalent, or tetravalent receptors, respectively. However, dissociation is constantly occurring, as is evidenced by the difference between the second and fifth washes (Fig. 2), and thus the amount of co-precipitated IgE is certainly underestimated. In spite of this, the 18.3% co-precipitation value obtained after two washes fits best with a divalent model. However, it should be noted that the data does not allow a distinction between two 49K $Fc_\epsilon R$ molecules being associated with each other and a single 49K molecule binding two ligands. Further work, perhaps with cross-linking reagents, may help to resolve this question.

What effect, if any, that multivalency plays in the function of the B cell $Fc_\epsilon R$ also remains to be determined. The multivalency clearly gives an advantage with respect to using affinity chromatography on IgE-coated adsorbents to isolate the receptor; multipoint binding greatly increases the strength of binding reactions

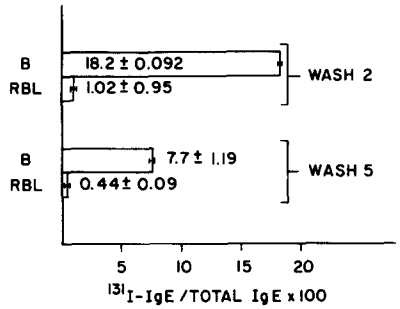

FIGURE 2. Coprecipitation of ^{131}I-IgE with ^{125}I-biotinylated-IgE when bound to the murine B cell Fc$_\epsilon$R or to the RBL cell Fc$_\epsilon$R. Murine B cells ($3–5 \times 10^7$) or RBL cells ($4–8 \times 10^5$) were incubated with 4 μg of ^{131}I-IgE plus 6 μg of ^{125}I-biotinylated IgE overnight (B cells) or for 2 h (RBL cells) at 4°C. Unbound IgE was removed by washing two times by centrifugation and the IgE-receptor complexes were solubilized with lysis buffer that contained either 0.5% (vol/vol) NP-40 or 0.8% (wt/vol) CHAPS as detergent. The clarified lysate was mixed for 30 min at 4°C with avidin-agarose before being washed the indicated number of times and counted. The data shown is a mean plus standard error of four experiments using the conditions indicated.

(12). Thus, it is interesting to speculate that a similar multivalency may exist with other IgE-binding components. The human lymphocyte Fc$_\epsilon$R from B lymphoblastoid cells has a relatively low affinity for IgE (K_a of $\sim 10^7$ M^{-1}) (13), however, isolation by human IgE-Sepharose is relatively efficient (4, 14). The RBL cell is known to have two IgE binding components, the high affinity Fc$_\epsilon$R already mentioned and a lower affinity Fc$_\epsilon$R, called H component, that can be isolated only by affinity chromatography on IgE-Sepharose (15). Although other low affinity Fc$_\epsilon$R such as the H component on RBL cells or 8866 B lymphoblastoid cells do not cause co-isolation of IgE by the procedures used in this study (L. Peterson and D. Conrad, unpublished observations), this may simply be a matter of affinity. The multivalency is no advantage in the coprecipitation experiments and the murine B cell "low affinity" Fc$_\epsilon$R is atypical with respect to affinity in the sense that the $\sim 10^8$ M^{-1} affinity (3) is higher than the IgE/Fc$_\epsilon$R affinity for the corresponding rat and human lymphocyte Fc$_\epsilon$R (13).

Summary

The murine B lymphocyte Fc$_\epsilon$R is functionally multivalent. Radiolabeled rat IgE, when bound to the B cell Fc$_\epsilon$R will co-isolate with the Fc$_\epsilon$R on a rat IgE affinity column; examination of the affinity column eluate by SDS-PAGE reveals the component previously identified as the Fc$_\epsilon$R as well as E and L chains from IgE. At low levels of Fc$_\epsilon$R saturation, up to 30% of the Fc$_\epsilon$R bound IgE becomes bound to IgE-Affi-Gel. By using a biotin-avidin system, the coprecipitation of non-haptenated IgE with haptenated IgE was examined and the results suggest (but do not prove) a divalent receptor.

The authors wish to thank Drs. Teruko and Kimishige Ishizaka for their generous assistance both in terms of reagents and advice. Thanks are also extended to Dr. Arnold Froese for helpful discussion and to Mr. Robert Knox for excellent technical assistance.

Received for publication 16 January 1984 and in revised form 13 March 1984.

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