The Cell Surface Receptor for Immunoglobulin E

I. THE USE OF REPEETITIVE AFFINITY CHROMATOGRAPHY FOR THE PURIFICATION OF A MAMMALIAN RECEPTOR

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(Printed in U.S.A.)

The IgE (immunoglobulin E) receptor of cultured rat basophilic leukemia (RBL-1) cells, shown previously to be an $M_w = 45,000$ to $50,000$ glycoprotein, was purified by repetitive affinity chromatography on IgE-Sepharose. RBL-1 cells were either labeled exogenously with $^{125}$I by the lactoperoxidase method or endogenously by growing cells in the presence of $[1^4]$glucosamine and $[3^H]$leucine. Receptor was solubilized with nonionic detergent and purified on Sepharose columns containing one of several conjugated proteins. Columns with non-IgE proteins were used to remove cell constituents adhering nonselectively to protein-conjugated Sepharose and columns with IgE were used to specifically bind the receptor. Initially, attempts to elute receptor from IgE columns resulted in major losses of binding activity. Analysis of $^3H$ radioactivity using polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) also showed that the receptor was still heavily contaminated. These contaminants were not labeled with $^{125}$I or $^3C$, indicating the desirability of utilizing an endogenous protein label when the purification of plasma membrane proteins is studied. Subsequent modifications of the procedure showed that when the receptor was eluted at $4°C$ with $0.5\%$ acetic acid, $1\%$ NP-40 (Nonidet P-40), followed by immediate neutralization, most of the binding activity was retained, which allowed further purification on a second IgE column. Receptor preparations then purified by repetitive affinity chromatography appeared to be at least $90\%$ pure based on the distribution of $^3H$, $^{125}$I, or $^3C$ radioactivity by SDS-PAGE in gels of differing porosities, and represented at least a $5000$-fold purification from the original cell extract with an overall yield of $10\%$ to $15\%$.

Mast cells and basophils are known to possess cell surface receptors which are highly specific for immunoglobulin E (IgE), the class of antibody of particular importance in allergic and parasitic diseases. After its synthesis by plasma cells, IgE becomes bound through its Fc region to mast cells and basophils (for reviews, see Refs. 1 and 2). Subsequent antigen binding to the Fab region of the receptor-bound IgE molecules causes the cells to release histamine and other mediators of immediate hypersensitivity apparently by the cross-linking of adjacent receptors (3).

Since studies of the IgE receptor on normal mast cells and basophils are hampered by limitations in cell number or purity, a rat basophilic leukemia cell line (RBL-1) (4) has been particularly useful in characterization of the receptor. RBL-1 cells bind $3 \times 10^7$ to $1.5 \times 10^8$ IgE molecules per cell with a binding specificity (4, 5) similar to normal rat mast cells. Kinetic studies of $^{125}$I-labeled rat IgE binding to RBL-1 cells are consistent with a reversible bimolecular reaction with a $K_d$ of $>6 \times 10^7$ M$^{-1}$ (6).

Receptor for IgE can be solubilized from RBL-1 cells with nonionic detergent without loss of specific receptor activity (6, 7). Solubilized material from $^{125}$I-labeled RBL-1 cells contains a single radiolabeled molecule which is co-precipitated with IgE and anti-IgE antibody immune complexes (8, 9). This receptor has been characterized as a glycoprotein with a molecular weight of $45,000$ to $50,000$ (9) when analyzed by sodium dodecyl sulfate gel electrophoresis (SDS-PAGE). Previous attempts at obtaining highly purified active receptor by binding and elution from insolubilized IgE (7, 10) have resulted in low recovery of activity, probably due to inactivation of the receptor upon elution from immunosorbents. Moreover, while the inactive receptor was apparently highly purified (8, 9), estimates of purity were made with a $^{125}$I-labeled receptor preparation from cells labeled on their surfaces by the lactoperoxidase method. Because the presence of contaminating unlabeled intracellular proteins could not be excluded by this method, receptor purity may have been seriously overestimated. In the present study, both endogenously and exogenously labeled RBL-1 cells have been used as a source of receptor. A reproducible repetitive affinity chromatography procedure for obtaining highly purified active receptor in reasonable yield is described.

MATERIALS AND METHODS

IgE and IgG—Rat monoclonal IgE was obtained from LOU/M/Ws1 rats bearing the IgE-secreting immunocytoma IR 102 in the ascites form (11). The IgE myeloma protein was purified from the serum and ascitic fluid using ammonium sulfate precipitation and gel chromatography and was radioiodinated as reported elsewhere (8, 12). A preparation of normal rat IgG was obtained by precipitating serum from 2- to 3-month-old normal rats at $40\%$ saturation with ammonium sulfate at $4°C$ and pH $8.0$, followed by dialysis and concentration by ultrafiltration.

RBL-1 Cells; Surface Radiosiodination and Biosynthetic Radiolabeling—RBL-1 cells (4) were cultured in spinner flasks using minimum Eagle’s medium containing (from Grand Island Biological Co.)
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15% heat-inactivated fetal calf serum, 2 mM L-glutamine and a 1:100 antibiotic-antimycotic mixture.

For surface labeling, proteins and glycoproteins of the RBL-1 cells were iodinated with 125I using a combination of lactoperoxidase and glucose oxidase as described previously (9). For biosynthetic labeling, RBL-1 cells entering the stationary phase of the growth curve 2 days after serum withdrawal were incubated in 50% fresh medium containing 1% fetal calf serum, and Pyronin Y. Electrophoresis was carried out on 1 ml of gel at 3 to 5 mA/gel thereafter. Radioactivity in 1- or 2-mm gel slices was either counted directly in a gamma counter (for 125I), or the gel slices were solubilized with trichloroacetic acid as described previously (17) and aliquots of the supernatant were counted in the appropriate manner.

Assays of IgE Receptor Activity—The number of binding sites for IgE per intact cell was evaluated as described previously (5). Binding activity of solubilized receptor was assessed by the following methods.

1. Ammonium sulfate precipitation of 125I-IgE complexed to receptor. An (NH4)2SO4 precipitation assay (7), based upon the decrease in 125I-IgE solubility in 40% or 44% saturated (NH4)2SO4, when active receptor is present, was used both at early and late stages of the purification procedure. Suitable dilutions of solubilized receptor were incubated for 2 h in 0.2 ml of buffer with 5 pmol of 125I-IgE at 4°C. Fifty microliters of this mixture and 50 μl of a solution of carrier protein were added to 0.25 ml of ammonium sulfate solution. After partial or extensive purification of the receptor, the radioactivity in the supernatant was either counted directly in a gamma counter (for 125I), or the gel slices were solubilized with trichloroacetic acid as described above (17), and aliquots of the supernatant were counted in the appropriate manner.

2. Binding of radiolabeled receptor to IgE-Sepharose or soluble IgE. After partial or extensive purification of the receptor, the radiolabeled receptor itself could be used to determine binding activity. Specific rebinding of receptor radioactivity to IgE-Sepharose and altered mobility of receptor radioactivity on gel exclusion chromatography after preincubation with soluble IgE were used to assay binding activity and are described in detail in the accompanying paper (14).

3. Immunoprecipitation Studies—The immunoprecipitation of labeled receptor complexed with IgE and antibody to IgE was carried out as previously described (9) and in the legend to Fig. 1. After washing, aliquots of the precipitates were analyzed for protein (18) and SDS-polyacrylamide gel electrophoresis was carried out in the usual manner.

RESULTS

Incorporation of L-[3H]Leucine and d-[14C]Glucosamine into RBL-1 Cells—In our analyses of receptor purification with limited quantities of receptor, the use of a radiolabeled receptor appeared highly desirable. Biosynthetic labeling of both proteins and carbohydrates in RBL-1 cells was evaluated and the optimal procedure in terms of cell recovery and labeling efficiency is described under "Materials and Methods." The use of leucine-free minimum Eagle's medium containing 15 to 20% undialyzed fetal calf serum was found to provide the minimal quantities of leucine needed for cell survival without undue dilution of the radiolabel and without significant reduction of the number of IgE receptors per cell. The final procedure resulted in greater than 40% incorporation of L-[3H]leucine radioactivity and about 10% of the d-[14C]glucosamine radioactivity into cells. After washing, the cells were solubilized in 1% NP-40 in NaCl/P04 buffer (9) and centrifuged at 30,000 x g, and the supernatant was used for further purification. More than 90% of the 3H radioactivity in this supernatant appeared to be in protein since it was precipitable with 5% trichloroacetic acid. Only 33% of the 14C radioactivity was precipitable with 5% trichloroacetic acid. Only 33% of the 14C radioactivity was precipitable with 5% trichloroacetic acid. Only 33% of the 14C radioactivity was precipitable with 5% trichloroacetic acid. Only 33% of the 14C radioactivity was precipitable with 5% trichloroacetic acid.

Purification of the IgE Receptor by Immunoprecipitation—We have previously reported that radiiodinated receptor and receptor biosynthetically labeled with d-[14C]glucosamine can be isolated with an apparently high degree of
radiochemical purity by co-precipitation with IgE-anti-IgE complexes (9). To determine if other cellular constituents had truly been eliminated from the precipitates, studies were carried out with receptor from intrinsically labeled cells using L-[3H]leucine in addition to L-[14C]glucosamine. The results of a typical experiment are shown in Fig. 1. Although only 0.13% of the 14C radioactivity was precipitated, as in the previous study, the major radioactive species in the precipitate had an apparent molecular weight, 65,000 in 5.6% gels (Fig. 1A), comparable with that of the IgE receptor described in gels of similar porosity (6, 9). This peak was not present in IgG-anti-IgG precipitates (Fig. 1B) so that by using the carbohydrate radiolabel, the receptor appeared to be markedly purified. However, while a larger fraction of the protein 3H radiolabel (0.71%) was precipitated with IgE-anti-IgE complexes, only a small amount was recovered in the receptor region. Since major bands in both specific and control precipitates were found in the smaller molecular weight region of the gels, it appears that proteins not heavily glycosylated (nor labeled with iodine during the lactoperoxidase iodination procedure) are present in both precipitates and only become observable in the 3H-labeled preparation.

**Affinity Chromatography Purification of the Radioiodinated IgE Receptor**—Initially, the behavior of the receptor on IgE columns was monitored primarily by radioactivity measurements using receptor preparations from 125I-labeled cells. IgE columns exposed to solubilized receptor and thoroughly washed contained 0.7 to 2.7% of the original added radioactivity (mean, 1.3% in 20 experiments). By comparison, control columns (albumin-Sepharose and rabbit IgG-Sepharose) or IgE columns, after precolumnation of the solubilized receptor with excess IgE, bound 0.2 to 0.7% (mean, 0.3% in six experiments). The IgE-Sepharose was in sufficient excess to bind most of all the solubilized receptor (14).

The elution of adsorbed radioactivity from IgE-Sepharose was studied using stepwise batch elutions. At least 0.1 n acetic acid or 1 M guanidine was needed for substantial recovery of radioactivity and inclusion of 1% NP-40 further improved yields to 0.8 to 1.4% of the applied radioactivity.

Most (60 to 68%) of the 125I radioactivity eluted with 0.1 to 0.5 n acetic acid was localized in a peak in the receptor area on SDS-PAGE (an apparent molecular weight of 60,000 in 5.9% gels) as illustrated in Fig. 2A. A similar pattern was obtained with radioactivity eluted with guanidine (Fig. 2B), suggesting that the IgE receptor had been considerably purified.

**Affinity Chromatography Purification of the Biosynthetically Labeled IgE Receptor**—Because of the possible presence of unlabeled contaminants in partially purified 125I-labeled receptor preparations, studies with biosynthetically labeled receptor were undertaken. After filtration of intrinsically labeled extracts through IgE-Sepharose, the columns were washed three times and eluted with either 4 ml of 0.1 n acetic acid (A) or 2 ml of guanidine (B). In each case, 4.45 × 10^3 cpm were eluted, the samples were dialyzed against water and lyophilized, and aliquots were analyzed in 5.9% gels.
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In order to obtain receptor of greater purity, more extensive washing was undertaken with the knowledge that the receptor affinity for IgE is high ($K_a \geq 10^{10}$ M$^{-1}$) and the rate of dissociation is exceptionally low ($k_{d} \leq 10^{-5}$ s$^{-1}$) (5, 20). Intermittent washes with a total of 120 ml of BBS buffer with 1% NP-40 at 4°C resulted in a marked reduction of $^3$H-labeled contaminants as evident by SDS-PAGE analysis (Fig. 3B). By using an IgG (rat or rabbit) and an IgE column in sequence and discarding the material adhering to the IgG column, substantial reduction in $^3$H labeled contaminants in acid eluates of the IgE eluates of the IgE column was also obtained (as determined by analysis using SDS-PAGE). Using the combination of prolonged washing and a nonspecific control column, the radiochemical purity of the recovered receptor was considerably improved, but more than 70% of the $^3$H radioactivity still migrated outside the receptor area on SDS-polyacrylamide gels.

Based on studies of nonspecific binding of other proteins to albumin-Sepharose (21), we utilized immunoadsorbents prepared by incubating the activated gel and IgE for only 2 h, by blocking possible unreacted iminocarbonate groups with 1 M ethanolamine, or by prewashing immunoadsorbents with 0.1 M sodium acetate buffer, pH 5.0, or 0.5 M acetic acid containing 1% NP-40, the usual elution medium. Although these alternative procedures produced variation in patterns of $^{125}$I contaminants noted, no overall improvement in purification was found. In fact, preincubation of the immunoadsorbent with acetic acid often reduced subsequent yields of receptor, presumably due to partial inactivation of the IgE.

Recovery of Active Receptor—The receptor was still impure even after extensive refinements of available methodology. Therefore, it was important to attempt to preserve the binding activity of the receptor during elution from the immunoadsorbent so that repetitive affinity chromatography might be possible. Initially, very little receptor activity could be recovered, as noted by others (7, 10). Since it seemed likely that the receptor was being irreversibly denatured during elution, it appeared desirable to keep the concentration of eluting agent and time and temperature of exposure of the receptor to a minimum. Subsequently, radioiodinated receptor which was 33% active (determined by specific rebinding to IgE-Sepharose) was obtained by rapidly passing 0.1 M acetic acid in 1% NP-40 through columns at 4°C and immediately diluting the effluent in 10 volumes of neutralizing buffer (initially 1% NP-40 in BBS buffer) (14). Higher concentrations of acetic acid (up to 0.5 M in 1% NP-40) when neutralized rapidly provided equally active receptor preparations in improved yield (14). When crude or partially purified receptor was exposed to comparable acidic conditions for as little as 5 s at 4°C (approximately the average period of exposure during elution from IgE columns), the binding activity measured by subsequent recovery of labeled receptor is significantly reduced. This emphasizes the need for very rapidly flowing columns and immediate neutralization. The procedure eventually adopted to obtain receptor for further purification involved elution with 0.5 M acetic acid, 1% NP-40 directly into 0.375 volumes of 2.0 M Tris, pH 8.5, 1% NP-40. When several additions of this eluting solution were made, approximately half of the receptor activity originally applied to the IgE-Sepharose column was recovered in the first eluted fraction (Table I, Experiments 1 and 2), which was uniformly the most purified as judged by $^{3}H:^{13}C$ ratios (e.g. Table II) or SDS-PAGE analyses. With a subsequent 0.5 M acetic acid, 1% NP-40 elution, the receptor yield was increased by 10 to 15%. Even when control columns and extensive washing were utilized (Table I, Experiment 3A), a total yield of 36.7% of the original receptor binding activity was obtained.

Further Purification of Partially Purified Receptor by Rebinding to IgE-Sepharose—The neutralized eluate from Fig. 3B (purified receptor obtained from a single IgE-Sepharose column) was incubated at 4°C with albumin-Sepharose and the unadsorbed material was incubated further with a
second IgE-Sepharose column. The eluate of the second IgE column revealed a single major peak for both isotopic labels (Fig. 3C), indicating considerable additional purification. If the albumin-Sepharose step was omitted, more contaminating \(^3\)H radioactivity remained (Fig. 3D). The major contaminant had an average molecular weight of 25,000 to 30,000 and could easily be separated from receptor by gel electrophoresis.

**Final Purification Scheme Adopted**—The purification method ultimately adopted is shown in Fig. 4. It involves four successive Sepharose columns containing rat or rabbit IgG, rat IgE, albumin, and rat IgE. In three experiments, eluates from the various columns used in purification were compared in 5.0 or 5.9% acrylamide gels. The IgG control column eluate did not contain a clearly identifiable receptor peak (Fig. 5A).

### Table I

**Yield of active IgE receptor during purification by single and repetitive affinity chromatography as determined by \(^125\)I-IgE binding**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Immunosorbent</th>
<th>Percentage of original binding activity which is recovered from IgE-Sepharose column*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgE-Sepharose</td>
<td>Flow-through: 3.5, Eluate 1: 48.8, Eluate 2: 5.8</td>
</tr>
<tr>
<td>2</td>
<td>IgE-Sepharose</td>
<td>~1.9, 49.2, n.t.</td>
</tr>
<tr>
<td>3A</td>
<td>Adsorbed with IgG-Sepharose, then IgE-Sepharose</td>
<td>8.4, 31.3, 5.4</td>
</tr>
<tr>
<td>3B</td>
<td>Eluate 1 from Experiment 3A on subsequent IgE-Sepharose</td>
<td>n.t., 48.9, n.t.</td>
</tr>
</tbody>
</table>

*In Experiments 1, 2, 3A, and 3B, the extracts applied to the column(s) bound 62.3, 25.8, 53.4, and 88 pmol of IgE, respectively.

**DISCUSSION**

In the present study, the IgE receptor of rat basophilic leukemia cells has been obtained in highly purified form in an overall yield of 10 to 15%. Using the repetitive affinity purification procedure shown in Fig. 4, the radiochemical purity of the receptor analyzed by SDSPAGE is usually 90 to 95% which represents at least a 5,000-fold purification from solubilized cell supernatants based on starting protein-bound \(^3\)H radioactivity. While a contaminant could potentially co-migrate with the receptor, the constancy of the \(^3\)H to \(^14\)C ratio...
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**FIG. 4.** Procedure adopted for purification of IgE receptor by repetitive affinity chromatography. The last column could be eluted either to yield active receptor (——) or inactive receptor (-----). Rab, rabbit; BSA, bovine serum albumin; HAc, acetic acid.

**FIG. 5.** Electrophoretic patterns of eluates from Columns 1, 2, and 4 of the purification procedure shown in Fig. 4. The biosynthetically labeled extract of Fig. 1 (2.8 ml) was used. Aliquots (1.1, 1.1, and 2.7 ml, respectively) of the column eluates were applied to 5.9% gels.

throughout the receptor peak area upon analysis both in 5 and 10% gels, which should have helped to detect any contaminating proteins, argues strongly against this possibility. Estimates of receptor purity based on specific binding activity for IgE are somewhat lower, maximally 68 to 78% (14), but this is almost certainly an underestimate mainly due to partial inactivation of receptor during the elution procedure. Also, receptor of similar isotopic purity has been obtained using a mixture of nine different 3H-labeled amino acids, making contamination by leucine-deficient proteins unlikely. Since incorporation times are long (15 to 21 h) relative to cell doubling times, contaminants with slow synthetic rates also appear unlikely.

The most essential step in the purification was the sequential use of IgE-Sepharose columns. The very extensive washing of affinity columns containing bound receptor and "control" columns are also useful in improving receptor purity. These features of our purification procedure and the use of intrinsically labeled receptor preparations may be advantageous both in the purification and evaluation of other cell surface receptors.

A. Kulczycki, Jr. and C. W. Parker, unpublished experiments.

In receptor preparations purified using immunoprecipitation or a single IgE affinity column, apparent co-purification of receptor and proteins labeled with L-[3H]leucine but not labeled with lactoperoxidase and 125I or D-[14C]glucosamine was noted (Figs. 1A, 3B, and 5B). Initially, the possibility was considered that some or all of these molecules might be receptor components migrating outside the M, = 45,000 to 50,000 range but not readily iodinated, as noted in studies of the la antigen (22) and acetylcholine receptor (23). However, these or very similar molecules bound to control precipitates (Fig. 1B) and to control Sepharose columns (Fig. 5A). Moreover, these substances were obviously not required for binding since very highly purified receptor without these molecules continued to bind specifically to IgE (Table I, Experiment 3B, and Ref. 14). Thus, it is clear that the M, = 45,000 to 50,000 glycoprotein itself binds to IgE even when other membrane components are absent. A more detailed analysis of this binding is presented in the accompanying paper (14). The 3H-labeled bands present in partially purified receptor preparations which did not co-migrate with the 14C-labeled band in all probability represent contaminants, and judging from the purification procedures used, a similar or greater level of contamination was almost certainly present in previously de-
scribed 125I-labeled preparations of IgE receptor obtained by immunoprecipitation (8, 9) or affinity chromatography (9, 10). It is apparent that by using 125I as the sole label of a surface receptor molecule, the actual degree of purification may be seriously overestimated.

The purity of our IgE receptor preparations compares quite favorably with other cell membrane receptors. Nonmammalian acetylcholine receptors have been highly purified from electric organs of the ray Torpedo and the eel Electrophorus by affinity chromatography using snake neurotoxins or acentylcholine analogues as ligands and these receptors appear to consist of multiple polypeptide chains (24-27). Since almost 1% of the electronig cell protein of Torpedo has acetylcholine receptor activity only a 100- to 300-fold purification is necessary to obtain pure receptor, whereas for the Electrophorus acentylcholine receptor protein somewhat more purification is required (28). Comparably rich sources of receptor have been unavailable for any mammalian receptor. However, an acetylcholine receptor containing 48,000-fold purification has been recently isolated from rat skeletal muscle by affinity chromatography (29), and like nonmammalian acetylcholine receptors, it consists of two major components and multiple minor ones. By contrast, the IgE receptor appears to be composed of only one type of glycoprotein unit.

Several groups have identified a receptor on mouse splenic lymphocytes and lymphoma cells for IgG which has also been considerably purified and appears to be a glycoprotein with an apparent molecular weight of 110,000 to 130,000 when analyzed by SDS-PAGE (30-32). On the other hand, other investigators studying the receptor for IgG on mouse splenic lymphocytes and leukemic cells have purified three different molecular species (33, 34), the largest of which is comparable in size to the IgE receptor. Whether these discordant findings result from proteolysis or the existence of different receptors for IgG remains to be established. The most highly purified surface peptide hormone receptor appears to be the gonadotrophin receptor from rat testis which also demonstrates one integral or peripheral proteins in the intact cell. It also may be that this contaminant is similar to a previously described nonglycosylated protein with binding affinity for immunoglobulin (36).

While the RBL-1 cells used as a source of IgE receptor in our studies are neoplastic and respond poorly to conventional stimuli for mediator release (37), their IgE receptors appear very similar or identical to those on normal rat mast cells (5, 38). With the availability of biologically active, purified IgE receptor, new insights into the nature of the IgE-receptor interaction should now be obtainable. In the accompanying paper (14), some of the characteristics of the purified active receptor in regard to stability, molecular size, and binding specificity are presented.

Acknowledgments—We would like to thank Drs. Susan E. Cullen for reviewing the final manuscript, Ms. Emily W. Wood for excellent technical assistance, and Ms. Mary Anne Blue and Ms. Nancy Grimshaw for skillful preparation of the manuscript.

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The cell surface receptor for immunoglobulin E. I. The use of repetitive affinity chromatography for the purification of a mammalian receptor.
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