IDENTIFICATION OF THE RAJI CELL MEMBRANE-DERIVED C1q INHIBITOR AS A RECEPTOR FOR HUMAN C1q

Purification and Immunochemical Characterization

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C1q is a 410,000 mol wt glycoprotein that circulates in plasma at a concentration of 65–70 μg/ml and constitutes a subunit of the first component of complement, C1 (1). In plasma, C1 circulates as a calcium ion-dependent pentamolecular complex consisting of one molecule of C1q, two molecules of C1r, and two molecules of C1s, to give a structural formula, $C1q_1C1r_2C1s_2$ (2). During activation of the classical pathway of complement, C1q, within the C1 macromolecule, functions as the recognition unit (3) by virtue of its ability to recognize and bind to the C_H2 domain of IgG (4, 5) and C_H3 or C_H4 domains of IgM (6, 7). It is a collagen-like molecule (1) with six flower-like globular heads that constitute the binding sites for Ig (4). Each globular head contains the carboxyl-terminal ends of three similar but distinct polypeptide chains (A, B, C) of ~20,000–22,000 mol wt that occur six times in the molecule, forming six structural and functional subunits (3, 8, 9). The amino-terminal regions of each chain are collagen-like and associate to form a helical configuration that represents the binding sites for $C1r_2C1s_2$ (9–10).

In addition to being a recognition unit of the classical pathway of complement, C1q functions as a ligand for a number of cellular receptors (recently reviewed in 11) such as B lymphocytes (12, 13), lymphoblastoid cells (14, 15), null cells, monocytes, polymorphonuclear leukocytes (16, 17), fibroblasts (18), and platelets (19, 20); and the binding of C1q to certain types of cells elicits some biological functions. For instance, we have shown previously (15) that lymphocytes or lymphoblastoid cells such as Raji cells are capable of lysing C1q-coated, chromium-labeled chicken erythrocyte (E_c)[1] target cells. That the pre-killing, effector cell–target cell contact is mediated by the effector cell C1q receptor (C1qR) was

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[1] *Abbreviations used in this paper:* C1qR, C1q receptor; CNBr, cyanogen bromide; DTT, dithiothreitol; EA, antibody-sensitized sheep erythrocytes; EACA, epsilon-amino-caproic acid; E_c, chicken erythrocytes; E_h, human erythrocytes; GVB, veronal-buffered saline containing 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 0.1% gelatin; HBSS, Hanks' balanced salt solution; NHS, normal human serum; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

demonstrated by pretreatment of the target cells with $F(ab')_2$ anti-C1q, which abrogated the cytotoxic effect (15). Furthermore, monomeric C1q was reported to inhibit the collagen-dependent aggregation of platelets (19, 20) and the release of serotonin (21). The importance of C1qR was not fully appreciated, partly because, in normal serum, C1q occurs as a subunit of the C1 complex. The amount of available free C1q was therefore thought to be very small. However, C1qR assumed new significance after it was observed that $C\bar{1}$-INA, the plasma inhibitor of activated C1, rapidly dissociates the activated C1r and C1s subunits from the C1 complex (22), thus leaving C1q bound to immune complexes.

In previous reports (23, 24), it was shown that a substance could be solubilized from membranes of lymphocytes or lymphoblastoid cells (Raji) that possesses the property of binding and precipitating free C1q, and hence was described as a C1q precipitin (23) or membrane-derived C1q inhibitor (M-C1qINH[3]) (24). In the present study, evidence is presented demonstrating that this membrane-derived inhibitor constitutes the cellular receptor for C1q.

Materials and Methods

Materials. The following materials were purchased: Nonidet P-40 (NP-40), lactoperoxidase, glutaraldehyde (Sigma Chemical Co., St. Louis, MO); $Na^{125}I$ and Bolton-Hunter reagent (New England Nuclear, Boston, MA); Ficoll-Hypaque (Pharmacia Fine Chemical, Piscataway, NJ), cyanogen bromide (CNBr) (Eastman Kodak Co., Rochester, NY), *N,N*-dimethylformamide (Aldrich Chemical Co., Milwaukee, WI), polyethylene glycol (PEG) (PEG 6000; Fisher Scientific Co., Pittsburgh, PA), and RPMI and other tissue culture reagents (Gibco Laboratories, Grand Island, NY).

Purified Proteins. Highly purified C1q was isolated according to a method previously described (25) and the protein concentration determined either by the method of Lowry et al. (26) or by measuring the optical density at 280 nM and using an extinction coefficient $E^{1\%}_{1cm}$ of 6.8. The homogeneity of a representative preparation of C1q used in these studies is shown in Fig. 1.

Radioiodination of Proteins. All radiolabeled proteins were labeled with ^{125}I by the Bolton-Hunter method as described (27) unless otherwise specified. Typically, 100 μg of isolated protein in 0.1 M sodium borate buffer (pH 8.5) were added to 100 μCi of Bolton-

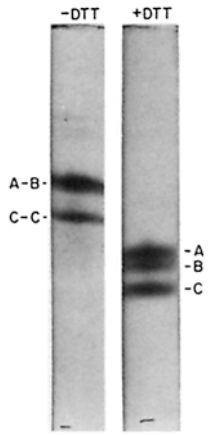

FIGURE 1. SDS-PAGE (7%) analysis of highly purified human C1q. Each gel contained 20 μg of protein. Unreduced human C1q consists of six A-B dimers and three C-C dimers that upon reduction yield equimolar amounts of three chains designated A, B, and C.

Hunter reagent and allowed to react for 30 min at 4°C. The reaction mixture was then dialyzed against phosphate-buffered saline (PBS), pH 7.0, at 4°C until the dialysate was free of ^{125}I.

Preparation of C1q-Sepharose 4B. Highly purified C1q was coupled to Sepharose 4B according to the method described by March et al. (28). Briefly, 50 ml of packed Sepharose 4B was washed extensively with deionized water and resuspended in 50 ml of 5 mM potassium phosphate tribasic buffer, pH 12.5 and stirred in an ice bath kept in a fume hood for 15 min. Then 7 g of CNBr dissolved in 3.5 ml of *N,N*-dimethylformamide were quickly added and the mixture was stirred for 5 min. The Sepharose beads were then washed in a Buchner filter funnel in the fume hood with 2 liters of water followed by 2 liters of coupling buffer consisting of 8 g $NaHCO_3$, 29.2 g NaCl/l, pH 9.0. The CNBr-activated Sepharose was mixed in a wide-mouth, 250-ml plastic bottle with 50 ml of human C1q (2 mg/ml) that had been dialyzed against coupling buffer, and the mixture was left for 16 h at 4°C with continuous stirring, after which it was placed in a Buchner funnel and evacuated to dryness. The amount of protein coupled to the Sepharose was determined by assaying the protein content in the filtrate. The C1q-Sepharose was then washed with 1 liter of PBS, pH 7.0 and resuspended in 50 ml of 1 M glycine, pH 8.0 for 2 h at 22°C to block the unreacted sites on the Sepharose beads. The slurry was washed by filtration with 1 liter of 5 mM sodium phosphate buffer, pH 7.5, containing 0.5 mM EDTA, 150 mM NaCl, and 0.02% NaN_3, and poured into a 1.5 × 20 cm column. Before each use, the C1q-Sepharose column was tested for its activity by taking 0.5 ml of packed beads and incubating them with 0.2 ml of monospecific, polyclonal antiserum to C1q for 1 h at 4°C. After incubation the mixture was centrifuged (500 rpm, 5 min) and the remaining anti-C1q activity of the supernatant tested by Ouchterlony analysis, as shown in Fig. 2.

Lymphoblastoid Cell Line. The cell line, Raji, used in this study was taken from a continuous culture line propagated in RPMI containing 10% fetal calf serum and 1% antibiotic-antimycotic mixture and was originally derived from a patient with Burkitt's lymphoma (29, 30).

Purification of C1q Receptor. The purification of C1qR was essentially the same as that described earlier (24) with only minor modifications. Briefly, 3×10^9 Raji cells were washed three times and resuspended in 100 ml of 5 mM sodium phosphate buffer, pH 7.5, containing 0.5 mM EDTA, 150 mM NaCl, 10 mM EACA, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). Cell membranes were prepared by freeze-thawing (five times) at −80°C and centrifugation for 1 h at 30,000 *g* at 4°C. The pelleted membranes were then solubilized by suspension in 50 ml of the above buffer containing 1% NP-40 and stirred for 20 h at 4°C. The solubilized membrane proteins were freed from insoluble material by centrifugation at 30,000 *g* for 60 min at 0°C and the total protein concentration was determined to be 30 mg. After dialysis against 5 mM $NaPO_4$ buffer, pH 7.5, containing 0.5 mM EDTA, 20 mM NaCl, 10 mM EACA, 0.5 mM PMSF, and 0.1% NP-40, 10 mg of the dissolved membrane solution was applied to a 1.5 × 20 cm C1q–Sepharose 4B column that had been equilibrated with the same buffer. The column was then washed with 600 ml of the starting buffer and the specifically bound proteins eluted with a linear NaCl concentration gradient with 300 ml of starting buffer and 300 ml of same buffer containing 1 M NaCl. Fractions containing C1q-binding activity were deter-

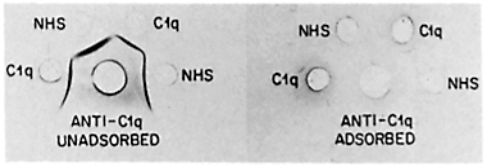

FIGURE 2. Depletion of anti-C1q activity on a C1q–Sepharose 4B column. A sample of a monospecific, polyvalent antibody to C1q was incubated with C1q-coated Sepharose. Depletion of anti-C1q was another means of showing that the Sepharose beads were coated with the C1q offered.

mined by single or double immunodiffusion techniques in a 0.8% agarose (sodium phosphate buffer, pH 7.2) containing 1.5% PEG 6000, using highly purified human C1q as described earlier (24, 31), and concentrated on an Amicon pressure filtration device with a Diaflow UM-10 ultrafiltration membrane (Amicon Corp., Danvers, MA). When the C1qR was used in a hemolytic assay, the detergent concentration was always reduced to ~0.01% by passage through a column of QAE-50 Sephadex as described (24).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis of C1qR. After radiolabeling with Bolton-Hunter reagent (27), the isolated C1qR was reduced and alkylated by boiling for 5 min in the presence of 0.1 M dithiothreitol (DTT) and 0.2 M iodoacetamide and was analyzed by application on a 1.5-mm thick, 5–10% acrylamide gradient containing slab gels, using the method of Laemmli (32). After electrophoresis, the gel was stained with Coomassie Blue, vacuum-dried, and analyzed by autoradiography.

Ultracentrifugation Analyses. Ultracentrifugation analysis of isolated C1qR was determined on a 5–40% sucrose-containing linear gradient in 5 mM $NaPO_4$ buffer containing 90 mM NaCl, 10 mM EACA, 0.5 mM EDTA, 0.5 mM PMSF, and 0.1% NP-40, pH 7.4. Centrifugation was carried out in a Beckman model L5-75B ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA) using an SW65 rotor for 20 h at 50,000 rpm. C1q (11S), C3 (9.5S), albumin (4.6S), and cytochrome *c* (1.7S) were used as markers.

In another set of experiments, an equilibrium density gradient ultracentrifugation was run in the following manner. First, the isolated C1qR was dialyzed overnight at 4°C against distilled water and then lyophilized. The dried residue was then dissolved in 8 ml of a solution comprised of 4 M guanidinium chloride, 0.05 M sodium acetate, pH 5.8, *N*-ethyl malimide, 0.1 M EDTA, 0.1 M EACA, and 0.005 M benzamidine, that contained cesium chloride to a density of 1.5 g/ml. The solution was centrifuged at 12°C for 72 h at 100,000 rpm in a Beckman ultracentrifuge using a swing-out rotor (SW60). The tubes were then frozen in liquid nitrogen and cut into two fractions: the bottom 2/5 and the bottom 3/5 by volume. The fractions were then dialyzed against 0.2 M sodium acetate, pH 5.8 and then against distilled water at 4°C. Finally, the material was lyophilized and then dissolved in 1.0 ml of distilled water. Protein and uronate were determined on an autoanalyzer (Techicon Instruments Corp., Tarrytown, NY).

Amino Acid Analysis. Amino acid analysis was performed with an amino acid analyzer (model 121M; Beckman Instruments, Spinco Division, Palo Alto, CA) equipped with an automatic sample injector and a model System AA automatic digital integrator. The sample was hydrolyzed in 6 N HCl for 20 h at 110°C in evacuated and sealed Pyrex tubes. ~25 nmol of hydrolyzed protein were applied to each column and the amino acid concentration was determined by comparison with a standard mixture of amino acids (100 nmol/ml).

Effect of Chondroitinase ABC on C1qR. To determine the effect of chondroitinase ABC, 0.1 μg purified ^{125}I-C1qR was dissolved in 0.1 μl of Tris-HCl, pH 8.0, and incubated with buffer alone or with 0.1 U of enzyme as described previously (31) for 12 h at 37°C. Then the sample was dialyzed against buffer containing 0.14 M Na_2SO_4, 0.01 cacodylic acid, and 0.1% NP-40. The chondroitinase-digested sample and the control sample were sequentially applied to a column of Sepharose CL-6B (0.5 × 120 cm; matrix, 108 cm) equilibrated with same buffer together with 250 μg of bovine nasal cartilage proteoglycan A1D1 (V_o marker, ≃2.2 × 10^6 mol wt) and 20 μg glucuronolactone (V_t marker, ≃176 mol wt) and eluted with the same buffer at a flow rate of 5 ml/h. Fractions were analyzed by Ouchterlony using isolated C1q and by the carbazole method of uronic acid determination (33).

Inhibition of EC1q Rosettes by C1qR or Monoclonal Antibody to C1qR. The ability of C1qR to inhibit C1q-dependent rosette formation was determined by a previously described rosette assay (34, 35) and consists of the following steps. First, human erythrocytes (E_h) were treated with 100 vol of 2% glutaraldehyde in 0.15 M PBS, pH 7.4, at 4°C for 16 h. The cells were washed with Hanks' balanced salt solution (HBSS) and further incubated with 10 vol of HBSS containing 10 mg/ml L-lysine for 30 min at 37°C, after which the cells were washed and resuspended in HBSS at 1 × 10^9/ml. Then 5 × 10^8 cells

in 1 ml HBSS were incubated with 200 μg C1q and a tracer amount (1 μg) of ^{125}I-C1q (8×10^5 cpm/μg). The excess unbound C1q was removed by centrifugation in HBSS. Rosette formation was then assayed by incubating 1.5×10^7 E_h C1q with 5×10^5 Raji cells in a total volume of 0.4 ml HBSS for 5 min at 37°C, centrifuging for 5 min at 600 rpm, and further incubation for 60 min at 4°C. To determine the inhibitory effect of isolated C1qR, the Raji cells were either pretreated with 5 μg/ml $F(ab')_2$ or the reaction mixture was incubated in the presence of 5 μg C1qR. The percent rosettes formed was determined by counting at least 100 lymphocytes in a microscope (Microstar; American Optical Scientific Instruments, Buffalo, NY).

C1q-dependent Cytotoxicity Assay. ^{51}Chromium-labeled, C1q-coated chicken erythrocyte target cells (^{51}Cr-E_c) were prepared as described earlier (15). The inhibitory effect of C1qR was tested by preincubating 10 μg C1qR with 8×10^4 ^{51}Cr-E_c and leaving the excess unbound C1qR in the reaction mixture, which contained, in addition, 2×10^5 Raji cells in a total volume of 0.4 ml of RPMI containing 0.4 human serum albumin, 2 mM L-glutamine, and 1% antibiotic-antimycotic mixture (penicillin 10,000 U/ml, fungizone 25 μg/ml, streptomycin 10,000 mcg/ml). Incubation was carried out at 37°C in a CO_2 incubator for 20 h. After incubation, the reaction mixture was centrifuged for 10 min at 1,000 rpm, the radioactivity of both pellet and supernatant determined, and cell damage expressed as the percentage of the total radioactivity released from the cell into the supernatant.

Effect of Isolated C1qR on C1q Hemolytic Function. There is evidence in the literature indicating that the C1q receptor reacts with the collagen-like region of the molecule (16, 24). Since this region also constitutes the site to which C1r and C1s bind, an experiment was performed to see if the isolated molecule could inhibit the hemolytic activity of C1 in serum. This was determined by incubating 5 μg/ml of purified C1qR (which had been repurified on a QAE-Sephadex column to reduce the detergent concentration) with or without 50 ng of C1q for 60 min at 37°C, followed by an additional 60 min incubation in the presence of 10 μl of C1q-depleted serum (containing 20 mM $CaCl_2$) and antibody-sensitized sheep erythrocytes (EA) (5×10^8/ml) in a total volume of 0.5 ml GVB (veronal-buffered saline containing 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 0.1% gelatin) (24). After incubation, the cells were centrifuged and the amount of hemoglobin released into the supernatants was determined spectrophotometrically at 700 nm. The inhibitory effect of the isolated substance was expressed as the percent inhibition of C1q hemolytic activity.

Results

Purification of C1qR by Affinity Chromatography. When a total of 10 mg of unlabeled and tracer amount (1×10^6 cpm/μg) of surface-labeled, NP-40-solubilized Raji cell membranes were chromatographed on a C1q–Sepharose 4B column and bound proteins eluted with a linear NaCl concentration gradient, two discrete, C1q-reactive peaks eluted at approximately 22 and 30 mmho/cm, respectively (Fig. 3). This seems to suggest that Raji cells possess two species of receptors with different affinities or charge heterogeneity. The two peaks were then separately pooled, concentrated, and designated arbitrarily as C1q receptor I and II (C1qRI and -II). From the tracer amount of radiolabeled protein incorporated, it was determined that the two C1q-reactive proteins represent ~7% of the total labeled Raji cell membrane proteins.

Immunodiffusion Analysis of Isolated C1qR. Equal concentrations of the pooled and concentrated peaks, C1qRI and C1qRII, were tested for their ability to bind and precipitate C1q using double (Fig. 4*A*) and single (Fig. 4*B*) immunodiffusion techniques. Both pools were found to be reactive, although the precipitation ring obtained by C1qRII (Fig. 4*B*) was apparently stronger than that for C1qRI, probably due to the disparity in concentration, since C1qRII was found to

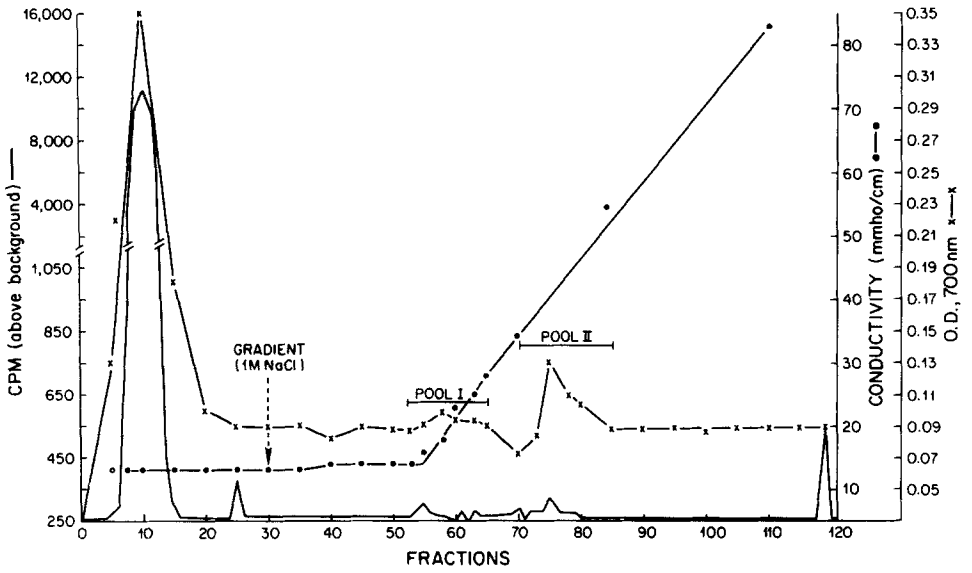

FIGURE 3. Affinity chromatography of solubilized Raji cell proteins on a C1q Sepharose 4B column. Pools I and II represent two peaks with C1q-binding activity. After concentration and repurification on QAE-Sephadex, the pools were arbitrarily designated as C1qRI and C1qRII.

contain at least two other protein bands (see below). Furthermore, C1qR was found to react with normal human serum (NHS) that contains EDTA but not NHS alone, indicating that under physiologic conditions where C1q is in complex with $C1r_2$ and $C1s_2$, the receptor does not bind to C1q in NHS.

SDS-PAGE Analysis of C1qR. Both C1qRI and C1qRII were radiolabeled with ^{125}I and analyzed on a 10% SDS gels. As shown in Fig. 5, both C1qR preparations contained a major band (80–90 kD) that, under reducing conditions, resulted in a single, 60–70 kD band. However, while C1qRII was relatively homogeneous, C1qRI was found to contain at least two distinctly visible bands that together represented ~50% of the preparation.

It is also of interest that the C1qR molecule has a much more pronounced ^{125}I band upon reduction than without reduction, indicating that the protein moiety might exist as a polymeric structure of disulfide-bonded 70,000 mol wt chains. Furthermore, some visible bands were observed at the top of the gel, and two or three protein bands were visible that did not label with ^{125}I.

Ultracentrifugation Analysis. Sucrose density ultracentrifugation analysis revealed that the C1qR sediments at ~4.2 S; the results of one such experiment is shown in Fig. 6. In addition, equilibrium density gradient ultracentrifugation showed that the isolated receptor could be separated into a protein-rich, low density fraction and a carbohydrate-rich, high density fraction (Table I). The large hydrodynamic size, coupled with the high buoyant density and uronic acid content, suggests that a proteoglycan is a constituent of the complex.

Amino Acid Analysis. The results of amino acid analysis data are represented in Table II. One of the striking features is that C1qR is rich in glycine, with glutamic acid and aspartic acid following closely behind, which explains its acidic

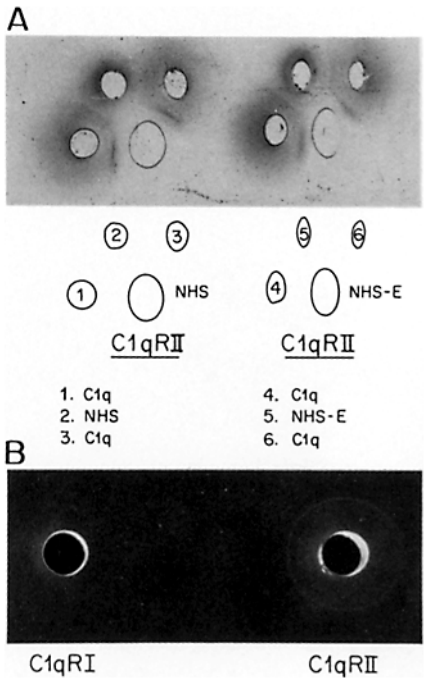

FIGURE 4. Immunodiffusion analyses of C1qR. Isolated receptor pools were analyzed by double (*A*) and single (*B*) immunodiffusion techniques. Both plates contained 0.8% agarose in PBS, pH 7.4, 1.5% PEG, and 0.02% NaN_3. The agarose in plate *B* contained, in addition, 25 μg/ml of purified C1q. C1qRII precipitated C1q in NHS containing EDTA (NHS-E) but not in NHS.

nature. The hexosamine ratio, or the ratio of galactosamine to glucosamine, was found to be 3.2. Furthermore, the uronic acid content of a typical C1qR preparation was found to be 23%, while galactosamine content was 21%.

Digestion of C1qR with Chondroitinase ABC. Previous studies postulated that the plasma-derived C1q inhibitor, which was identified as a chondroitin 4–sulfate proteoglycan (31), and the lymphocyte membrane–derived C1q inhibitor (C1qR), could be similar or identical. To assess this issue, an experiment was designed in which the C1qR was incubated with either buffer or chondroitinase ABC, as described in Materials and Methods. Both materials were then applied to a CL-6B column (0.5 × 120 cm; matrix height, 105 cm). After elution, it was found that the treated and untreated C1qR chromatographed in the same region, with a K_{av} of 0.45 (Fig. 7), indicating that C1qR is not digestable with chondroitinase. Furthermore, chondroitinase-digested C1qR did not lose its ability to precipitate C1q as assessed by a double radial immunodiffusion analysis, as described.

Inhibition of C1q-dependent Biological Functions by C1qR. The ability of isolated C1qR to inhibit C1q-dependent rosette formation was tested by preincubating glutaraldehyde-treated, C1q-coated E_h with or without 5 μg/ml of the receptor and then with either peripheral blood lymphocytes that had been purified by centrifugation on Ficoll-Hypaque (36) or Raji cells for 60 min at 4°C. As shown in Table III, 5 μg/ml of C1qR was able to inhibit rosette formation. Furthermore,

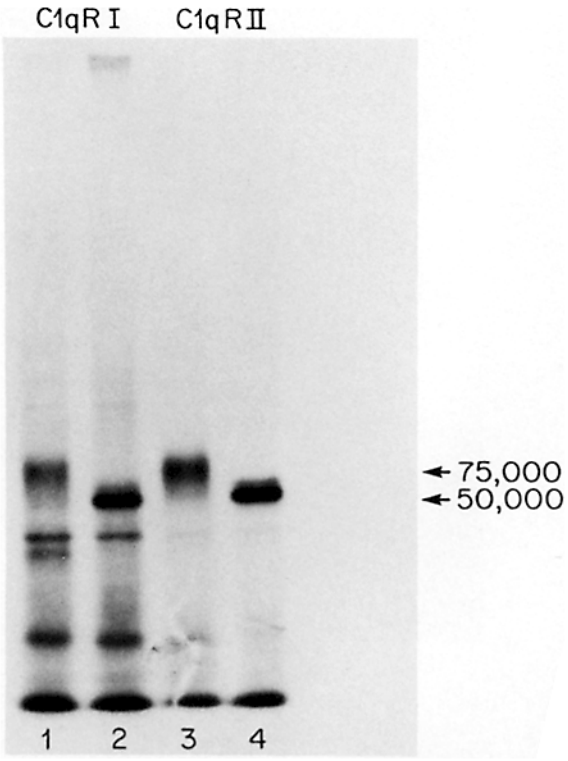

FIGURE 5. SDS-PAGE gel analysis of C1q-Sepharose pools. The pooled fractions in Fig. 3 were concentrated and radiolabeled before further purification. The specific activity was 7.7 $\times 10^3$ and 5.1 $\times 10^3$ cpm/μg for pool I (C1qRI) and pool II (C1qRII), respectively. ~5 $\times 10^4$ cpm (~7 μg) of each sample was applied to a Laemmli slab gel (10%) in the absence (*1* and *3*) or presence (*2* and *4*) of 0.1 M DTT. After electrophoresis the gel was stained with Coomassie Blue, vacuum-dried, and exposed in a Dupont Cronex cassette with Lightning Plus screens for 24 h at −80°C before autoradiographic analysis.

both the C1q-dependent cellular cytotoxicity (Table IV) and hemolytic functions (Table V) were found to be inhibited by C1qR.

Discussion

In previous reports (23, 24) we isolated a lymphocyte membrane-associated C1q precipitin (23) or M-C1q inhibitor (24) and demonstrated that it was capable of inhibiting some of the C1q-dependent biological functions. The questions that arose from these studies were: (*a*) What is the exact chemical nature of the membrane-derived C1q inhibitor? (*b*) What is its relationship to the plasma C1q inhibitor? and (*c*) Is the C1q inhibitor identical to the C1q receptor on lymphocytes? To answer these questions and further characterize the chemical, as well as the functional nature, the membrane-associated inhibitor was isolated to homogeneity using a C1q–Sepharose 4B affinity column as described earlier (23). The present results clearly demonstrate that the isolated C1q inhibitor constitutes the cellular receptor for C1q and that it may exist as two distinct populations of receptors possessing different affinities for the C1q molecule.

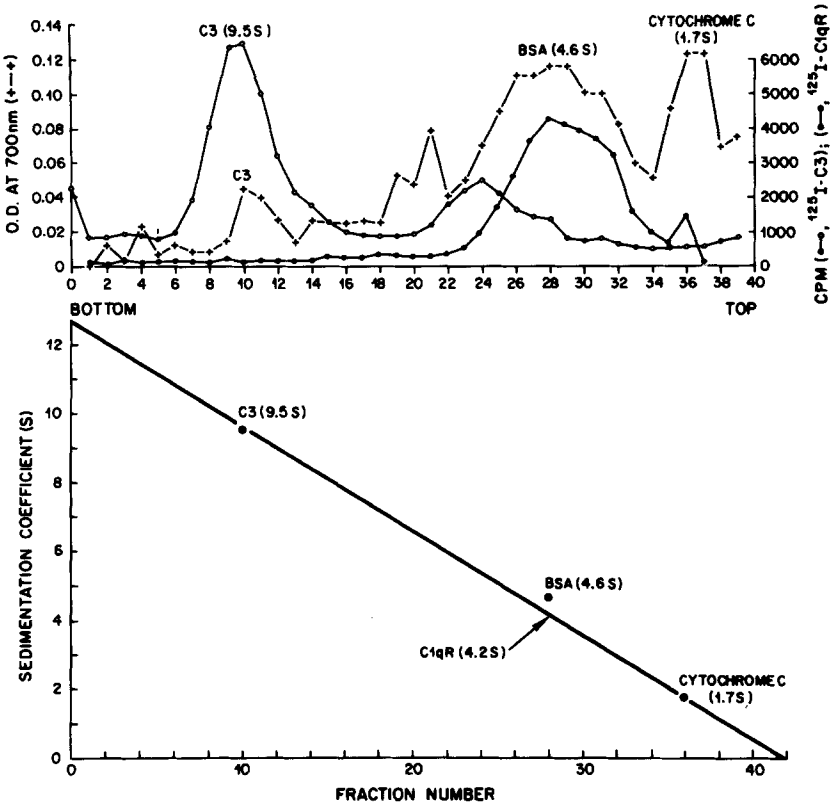

FIGURE 6. Sucrose density ultracentrifugation. Analysis of isolated C1qRII was determined in 5 mM $NaPO_4$ buffer containing 50 mM NaCl, 10 mM EACA, 0.5 mM EDTA, 0.5 mM PMSF, and 0.1% NP-40, pH 7.4. Centrifugation at 50,000 rpm was for 20 h at 4°C.

TABLE I

Distribution of Isolated ClqR in Cesium Chloride Density Gradient Ultracentrifugation

Fractions*	Density	Protein	Uronic acid
	g/ml	*μg*	*μg*
Bottom	1.55	24	6.25
Top	1.45	100	3.50

* A lyophylized preparation of ClqR that had been dissolved in 4 M guanidinum chloride, containing 0.05 M sodium acetate, pH 5.8, *N*-acetyl maleimide, 0.1 M EDTA, 0.1 M amino-caproic acid, 0.005 M benzamidine, and 1.5 g/ml cesium chloride, was centrifuged at 12°C for 72 h. The fractions were collected by freezing the tubes and cutting. Protein and uronate were determined on a Technicon autoanalyzer.

This conclusion is derived from the observations that when the detergent-solubilized membrane proteins were applied to the C1q–Sepharose 4B column, washed exhaustively, and the adsorbed materials eluted, two discrete peaks eluting at different ionic strengths (22 and 30 mmho/cm, respectively) were obtained. Both species had similar properties since both were capable of binding

TABLE II
Amino Acid Composition of ClqR

Amino acid	Total nanomoles	Res/1,000	Total micrograms
Aspartic acid	9.1	93.8	1.2
Threonine	4.9	50.5	0.6
Serine	8.0	82.5	0.8
Glutamic acid	10.0	103.1	1.5
Proline	7.9	81.4	0.9
Glycine	18.5	190.7	1.4
Alanine	7.3	75.3	0.6
Cysteine	0.0	0.0	0.0
Valine	4.1	42.3	0.5
Methionine	0.0	0.0	0.0
Isoleucine	2.5	25.8	0.3
Leucine	7.6	78.4	1.0
Tyrosine	3.0	30.9	0.5
Phenylalanine	3.4	35.1	0.6
Histidine	1.6	16.5	0.2
Lysine	5.3	54.6	0.8
Arginine	3.8	39.2	0.7
Glucosamine	2.3	23.7	0.4
Galactosamine	7.3	75.3	1.3

The percent total sample on the column is 67.28; total nanomoles of amino acids, 97; total micrograms of protein, 11.6. Hexosamine ratio: glucosamine/galactosamine, 0.3; galactosamine/glucosamine, 3.2.

and precipitating free C1q, although the concentration of C1qRI (after further purification) required to precipitate the same amount of C1q was one-half that of pool II. However, the conclusion that two distinct cell receptor populations might exist should await binding studies to determine their affinity constants. C1q possesses two binding sites for certain types of mucopolysaccharides such as low molecular weight heparin (37); the high affinity-binding site was shown to be the collagenous region of the molecule. That the C1q inhibitor is a cellular receptor for C1q (C1qR) is inferred from the following lines of evidences. (*a*) When the isolated material or C1qR was preincubated with C1q-bearing, glutaraldehyde-treated E_h, no rosette formation was obtained upon subsequent incubation with lymphocytes or Raji cells. (*b*) C1q-coated, ^{51}Cr-labeled chicken erythrocyte target cells were prevented from lysis by Raji cells when the target cells were first preincubated with the C1qR. That this complement-mediated cellular cytotoxicity (CDCC) is mediated by the effector cell–C1q receptor has been previously documented (15). (*c*) C1qR binds to free C1q and this C1qR-bound C1q was unable to reassemble and form a hemolytically active C1 in the presence of C1q-depleted serum and 20 mM Ca^{++}. (*d*) In addition, other experiments have shown that binding of ^{125}I-C1q to Raji cells was abrogated in the presence of C1qR, indicating that the isolated receptor could compete for

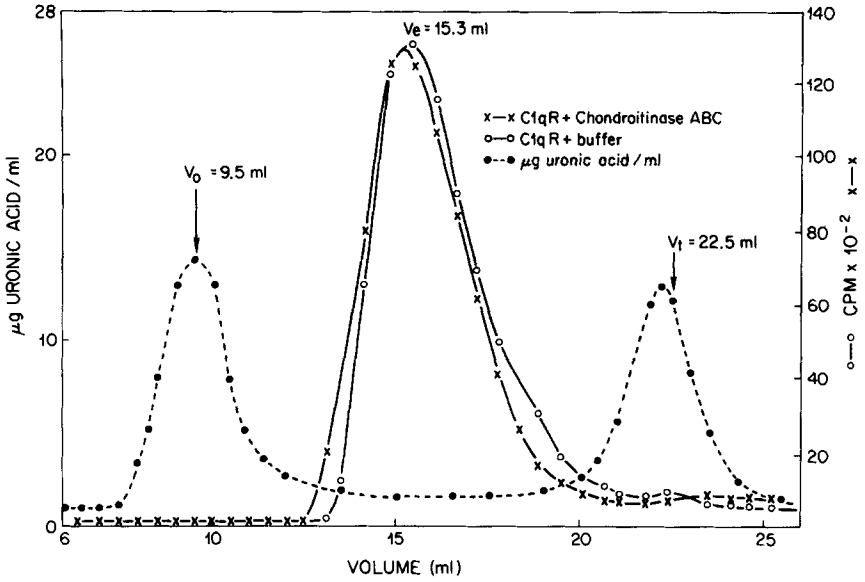

FIGURE 7. Chromatography of C1qR on Sepharose CL-6B. Radiolabeled C1qRII was first incubated with buffer or digested with chondroitinase ABC, as described in Materials and Methods, and then chromatographed in Sepharose CL-6B (0.5 × 120 cm).

TABLE III

Inhibition of Clq Rosettes by ClqR

Reaction mixtures	Percent rosettes*
Raji + EC1q	81
Raji + (EC1q + C1qR)‡	7
PBL + EC1q	39
PBL + (EC1q + C1qR)	10

* Rosettes were formed by incubating 0.2 ml peripheral blood lymphocytes (PBL) or Raji cells (2.5×10^6/ml) with 1.5×10^7 C1q-coated (15 μg) glutaraldehyde-treated human erythrocytes (EC1q) in a total volume of 0.4 ml HBSS for 5 min at 37°C, centrifuging 5 min at 600 rpm, and further incubating for 60 min at 4°C.

‡ Inhibition of EC1q rosettes by C1qR was determined by preincubating EC1q with 5 μg/ml C1qR for 15 min at 37°C before incubation with lymphocytes or lymphoblastoid cells.

the binding of C1q. (*e*) Furthermore, murine $F(ab')_2$ monoclonal antibody to C1qR was found to inhibit ^{125}I-C1q binding to Raji cells.[2]

The C1q molecule is collagen-like with six globular heads that are the binding sites for Ig. The collageneous tail of the molecule is considered to be the site where C1r and C1s bind in plasma. The collageneous region of the molecule constitutes the site through which C1q binds to receptors (14–17). Our finding that C1qR was unable to precipitate C1q in serum seems to confirm these findings.

The results obtained from equilibrium gradient ultracentrifugation indicate

[2] Ghebrehiwet, B. Murine monoclonal antibody that interacts with human C1q receptor. Manuscript submitted for publication.

TABLE IV
Inhibition of C1q-dependent Cytotoxicity by C1qR

Reaction mixture	Percent ^{51}Cr release*
Raji + ^{51}Cr-E_c	5
Raji + ^{51}Cr-E_c C1q	74
Raji + (C1qR + ^{51}Cr-E_c C1q)	10

* 1×10^5, ^{51}Cr-labeled, C1q-coated E_c target cells were incubated with 5×10^5 Raji cells in the presence or absence of 5 μg/ml C1qR in a total volume of 0.3 ml RPMI plus 5% FCS for 20 h at 37°C in an atmosphere of 95% air and 5% CO_2. After incubation, cells were centrifuged and the radioactivity of both pellet and supernatant determined. Cell damage is expressed as percent of total radioactivity released from the cells into the supernatant.

TABLE V
Reduction of C1q Hemolytic Activity by C1qR

Reaction mixture	Percent hemolysis	Percent inhibition
(C1q + GVB) + C1qD + EA*	89	—
(C1q + C1qR) + C1qD + EA	40	55‡

* Highly purified C1q was incubated either with GVB or 5 μg/ml C1qR before addition to C1q-depleted serum (C1qD) in the presence of 20 mM $CaCl_2$. Hemolysis was expressed as percent of NHS-induced hemoglobin release.
‡ The effect of C1qR is limited in part by the concentration of detergent (NP-40).

that a proteoglycan constitutes a part of the complex. However, amino acid analysis together with the glucosamine-to-galactosamine ratio and the insensitivity of the C1q receptor to digestion with chondroitinase seem to distinguish the C1q receptor from the serum C1q inhibitor, which has been previously characterized as chondroitin 4-sulfate proteoglycan (31). It is likely, however, that the C1qR and serum C1q inhibitor represent functional counterparts, in as much as both macromolecules are able to inhibit most of the C1q-dependent biologic reactions (23). Upon electrophoresis on SDS gels, the C1qR was shown to consist of a single protein band of 80–90,000 mol wt which, upon reduction, stains heavier, with a single band of ~60–70,000 mol wt. The ultracentrifugation data and the SDS-PAGE profile taken together suggest that the C1qR is a polymeric structure consisting of a protein portion noncovalently linked to a proteoglycan.

The biological significance of the receptor cannot be determined from these studies. It is clear, however, that the receptor does bind only to free C1q. Upon activation of the classical pathway of complement by immune complexes, $C\overline{1}$-INA, the plasma inhibitor of activated C1, binds to $\overline{C1r}$ and $\overline{C1s}$ to form an irreversible complex, and thus readily dissociates them from the C1q molecule. Under these conditions, the collageneous tail of the C1q molecule becomes exposed and possibly available to bind to cell surface receptors on monocytes, lymphocytes, or polymorphonuclear cells (PMN), though this type of C1q is believed to be conformationally changed (38). Whether binding of a C1q-

containing immune complex to a phagocytic cell C1q receptor represents one mechanism of immune clearance remains to be investigated. The binding of free C1q to cells possessing receptors for C1q promotes biological functions, as is apparent from studies which found that ^{51}Cr-labeled, C1q-E_c could be lysed by Raji cells as well as by lymphoblastoid cells in the absence of anti–target cell antibody (15). This C1q-mediated cellular cytotoxicity could be inhibited if the C1q-coated target cells were first pretreated with C1qR (15). In addition, C1q has been reported (18–20) to inhibit collagen-dependent platelet aggregation and serotonin release. Whether this inhibition is mediated by a specific platelet receptor for C1q or if the platelet recognizes the collagen portion of the C1q through a specific receptor for collagen is not clear. The C1q receptor isolated from Raji cells is similar if not identical to the receptor on PMN or platelets, as has been confirmed through immunoprecipitation studies using C1q-coated Sepharose beads and murine monoclonal antibody raised to the receptor (manuscript in preparation). More work is needed to determine the precise function of cellular C1q receptor.

Summary

We have shown previously that an activity which is capable of precipitating purified C1q and inhibiting some of the C1q-dependent biologic reactions could be solubilized from the membranes of both normal human peripheral B lymphocytes and a B cell–derived lymphoblastoid cell line (Raji), both of which are known to possess receptors for human C1q. In this report we present evidence that this membrane-associated C1q inhibitor is a chondroitinase-insensitive macromolecule and is the receptor for human C1q. The receptor was solubilized from membranes of Raji cells with Nonidet P-40 and purified to homogeneity using C1q–Sepharose 4B affinity chromatography. Equilibrium density gradient centrifugation analysis revealed that the complex could be resolved into a protein-rich, low density fraction and a carbohydrate-rich, high density fraction. The large hydrodynamic size, coupled with the high buoyant density, suggests that a proteoglycan is a constituent of the complex and indicates that the receptor might be a macromolecular complex of a proteoglycan portion noncovalently linked to a 60–70 kD glycoprotein. The glycoprotein moiety, in turn, consists of two or more identical (70,000 mol wt) polypeptide chains held together by disulfide bonds and constitutes the C1q receptor (C1qR). Sucrose density ultracentrifugation analysis showed that the isolated receptor sediments with an apparent rate of 4.2 S. Immunochemical analyses demonstrated that a typical preparation of the C1qR complex consists of ~23% uronic acid and ~21% galactosamine with a galactosamine-to-glucosamine ratio of 3.2. Binding of C1q to the receptor was found to be optimal at low ionic strength and neutral or near-neutral pH (7–7.4). The isolated receptor was found to inhibit C1q hemolytic function, abrogate C1q-dependent rosette formation, and block the C1q-dependent, cell-mediated cytotoxicity, all of which are activities mediated by the receptor.

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