The existence of a cell surface receptor for the complement protein C1q was first suggested in 1972 (1). The ligand C1q has a complex structure made up of six globular regions, each attached to a short collagen triple helix (2). C1q receptor (C1qR) binds to the collagenous region of C1q (3), but does not appear to be a general receptor for structural collagens (4). C1qR is an acidic glycoprotein with 15–20% carbohydrate (4). The detergent-solubilized protein is an elongated dimer of Mr 115,000 ± 7,000 and remains soluble in the absence of detergent (4). C1qR activity is found on most leukocytes, endothelial cells, fibroblasts, and platelets (5, 6), and binding of C1q to its receptor has been reported to mediate a range of phenomena, including phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), modulation of cytokine and immunoglobulin secretion, and polymorphoendothelium interaction (6, 7). Recently, three other proteins, mannan binding protein (MBP), lung surfactant protein (SP-A), and bovine conglutinin, which have similar primary structure to C1q, have been characterized. Each has collagenous and globular domains (reviewed in reference 8). Availability of the pure receptor (4, 9) prompted examination of its possible interaction with these three proteins, human MBP, human recombinant SP-A, and bovine conglutinin, which are known to be similar in ultrastructure to C1q (8) (MBP and SP-A: Fig. 1) or to have functional association with the complement system (10, 11) (MBP and conglutinin: Fig. 1).

Materials and Methods

Purification of Ligands. C1q used in this study was isolated from human serum as described by Reid (12). C1q was radioiodinated using a standard Iodogen reaction (13). Specific activity was 1–2 x 10^6 cpm/μg. MBP was also isolated from human serum (14, 15) by affinity purification on a mannan Sepharose column. Fractions containing MBP were further purified on a Superose 6 (Pharmacia Ltd., Milton Keynes, UK) gel filtration column, equilibrated and washed with 20 mM Tris/HCl buffer, pH 7.4, containing 1 M NaCl, 1 mM EDTA, 0.01% NaN₃, 0.01% Tween 20, followed by purification on a Mono Q (Pharmacia Ltd.) FPLC ion-exchange column.

SP-A (human, recombinant) was a generous gift from K.P. Schafer, Byk Gulden Pharmazeutica, Konstanz, FRG. This preparation contains a single polypeptide chain type (type α₅), although two highly homologous polypeptides (α₅, α₆) may occur naturally.

Conglutinin was purified from heat-inactivated (56°C, 30 min) bovine serum by adsorption to zymosan in the presence of 10 mM CaCl₂ (16). The zymosan was washed exhaustively with 5 mM sodium barbitone/HCl, 145 mM NaCl, 10 mM CaCl₂, pH 7.4, then conglutinin was eluted in the same buffer containing 10 mM EDTA in place of CaCl₂. Conglutinin was precipitated by dialysis against distilled water, dissolved in 10 mM Tris/HCl, 140 mM NaCl, 20 mM CaCl₂, pH 7.4 (TBS-Ca²⁺), centrifuged (10,000 g, 20 min), and applied to a mannan-Sepharose column (14). The column was washed in TBS-Ca²⁺, then bound protein was eluted with TBS containing 10 mM EDTA. Minor contaminants were removed by ion-exchange on a Mono-Q (Pharmacia) column equilibrated in 10 mM Tris/HCl, 50 mM NaCl, 1 mM EDTA, 0.05% NaN₃, pH 7.8. The column was developed with a 50–500 mM NaCl gradient.

Proteins used were >95% pure, as assessed by SDS-PAGE in reducing conditions (17). The polypeptide chain size of each of the four proteins is distinct by this method, and cross-contamination was not detected (Fig. 2).

Purification of C1q-Receptor. C1qR was purified from tonsil lymphocytes by the method of Malhotra and Sim (4).

Binding of C1qR to Ligands Immobilized on Microtiter Plates. Microtiter plate wells were coated with 100 μl of C1q or conglutinin at 10 μg/ml in 10 mM potassium phosphate, 5 mM EDTA, 100
Ultrastructure of C1q, SP-A, conglutinin, and MBP. C1q (a) is a hexameric structure made up of six bent collagen triple helices joined to a globular domain. SP-A is essentially identical in appearance in electron microscopy to C1q (8). MBP has a similar monomer structure, but when isolated consists of a range of oligomers mainly trimers and tetramers (c) and hexamers (13). Conglutinin (b) is a much larger protein, consisting of four subunits each made up of a collagenous and globular region.

mM KCl, pH 7.4, for 2 h at room temperature. Plates were washed in the same buffer and nonspecific binding sites were blocked with BSA (5 mg/ml in the same buffer). C1qR was isolated as described previously (4) and iodinated with 125I in a standard iodogen reaction (13). Specific activity was 0.5–4 x 10^6 cpm/ug. All binding assays were done in 10 mM potassium phosphate, pH 7.4.

**Binding to Immobilized C1q.** Serial twofold dilutions of 125I-labeled C1qR (50 μl; maximum concentration 2 μg/ml) were preincubated with 100 μl of potential binding competitors soluble collagen (type IV, human placental; Sigma Chemical Co., St. Louis, MO), MBP, SP-A, or C1q each at a constant initial concentration of 50 μg/ml, for 30 min at 37°C in the 10 mM potassium phosphate buffer. A control with no potential inhibitor was included.

Mixtures were then transferred to C1q-coated microtiter plate wells and left for 2 h at ambient temperature (18–22°C). After washing three times with 300 μl of 10 mM potassium phosphate buffer, pH 7.4, bound 125I-labeled C1qR was solubilized and removed with 400 μl of 4 M NaOH. Radioactivity obtained from each well was measured on an LKB 1275 minigamma counter.

**Binding to Immobilized Conglutinin.** A constant quantity of 125I-labeled C1qR (100 μl; concentration 2 μg/ml) was pre-incubated with serial twofold dilutions of the potential competitors MBP, C1q, and conglutinin (100 μl; maximum concentration 12.5 μg/ml) for 30 min at 37°C in 10 mM potassium phosphate buffer. A con-

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**Figure 1.** Ultrastructure of C1q, SP-A, conglutinin, and MBP. C1q (a) is a hexameric structure made up of six bent collagen triple helices joined to a globular domain. SP-A is essentially identical in appearance in electron microscopy to C1q (8). MBP has a similar monomer structure, but when isolated consists of a range of oligomers mainly trimers and tetramers (c) and hexamers (13). Conglutinin (b) is a much larger protein, consisting of four subunits each made up of a collagenous and globular region.

**Figure 2.** Purified ligands analyzed on 7.5% SDS-PAGE and detected by Coomassie Brilliant Blue staining. (Lane s) Reduced standards 1, α2-Macroglobulin (180 kD); 2, β-galactosidase (116 kD); 3, fructose-6-phosphate kinase (84 kD); 4, pyruvate kinase (58 kD); 5, fumarase (48.5 kD); 6, lactate dehydrogenase (36.5 kD). (Lane a) Mannan binding protein. (Lane b) Conglutinin. (Lane c) Lung surfactant protein. (Lane d) C1q.

**Figure 3.** (a) Binding of 125I-labeled C1qR to solid-phase bound C1q. Serial dilutions of 125I-labeled C1qR (50 μl; 2 μg/ml) were pre-incubated with constant concentrations (100 μl; 50 μg/ml) of collagen (●), C1q (○), conglutinin (□), MBP (▲), SP-A (▲), and loaded on to C1q-coated microtiter plates. Details of the assay are given in Materials and Methods. (b) Binding of 125I-labeled C1qR to solid-phase bound conglutinin. Serial dilutions (maximum quantity 1.25 μg) of conglutinin (□), MBP (▲), C1q (○), BSA (▲) were incubated with 125I-labeled C1qR (100 μl; 2 μg/ml) in 10 mM potassium phosphate buffer (pH 7.4) and loaded on to conglutinin-coated microtiter plates. After washing with loading buffer, bound cpm in the presence or absence of competing species were measured. Details are given in Materials and Methods.
of each C1gR ligand interaction by each of the other ligands collagen (not shown) and BSA (Fig. 3b) did not inhibit the inhibited over a similar concentration range. Again soluble receptor was not diminished by preincubation with soluble previously shown (4), this binding is saturable. Binding of the crotiter plates. Dose-dependent binding of radioiodinated C1q incubated with 100 μl of U937 cell suspension (10^6 cells) for 30 min at 37°C. Unbound ligand was separated by spinning the cell suspension through 100 μl of di-(n-butyl)-phthalate for 1 min at 10,000 g. Counts per minute bound to the cell pellet in the presence or absence of competitor were measured.

Binding of 125I-labeled C1qR to EAiC3b Cells. Sheep erythrocytes bearing bound iC3b (EAiC3b) cells were prepared by classical pathway activation followed by incubation with purified factors H and I (19). EAiC3b cells were suspended (10^6 cells/ml) in 5 mM sodium barbitone buffer (pH 7.4), containing 5% (wt/vol) glucose, 0.5 mM MgCl₂, and 0.15 mM CaCl₂ (buffer B). To prepare EAiC3b-conglutinin cells, EAiC3b (3 × 10^5 cells) were incubated with 100 μg of bovine conglutinin at 37°C for 30 min and then washed three times in buffer B. Different dilutions of 125I-labeled C1qR (100 μl; maximum concentration 2 μg/ml) were incubated with 100 μl of EAiC3b or EAiC3b-conglutinin cells for 30 min at 37°C. Unbound ligand was separated by spinning the cell suspension through 100 μl of di-(n-butyl)-phthalate at 10,000 g for 1 min. Counts per minute bound to the cell pellet were measured.

Results

Binding of 125I-labeled C1qR to Ligand Immobilized on Microtiter Plates. Dose-dependent binding of 125I-C1qR to solid-phase immobilized C1q was observed (Fig. 3a). As previously shown (4), this binding is saturable. Binding of the receptor was not diminished by preincubation with soluble type IV placental collagen, but was greatly reduced by constant concentrations of soluble C1q, conglutinin, MBP, and SP-A (Fig. 3a). Direct binding of the radioiodinated C1qR to immobilized conglutinin (Fig. 3b) was confirmed and it was shown that the C1qR-conglutinin interaction was inhibited in a concentration-dependent manner by preincubation of the soluble receptor with soluble unlabeled C1q, MBP, or conglutinin (Fig. 3b). Each of the three soluble proteins inhibited over a similar concentration range. Again soluble collagen (not shown) and BSA (Fig. 3b) did not inhibit the interaction.

The results shown in Fig. 3 indicate that C1q, MBP, SP-A, and conglutinin all bind to C1qR. Further studies (not shown) confirmed direct binding of radioiodinated C1qR to microtiter plate-bound MBP, and inhibition of C1qR-MBP interaction by C1q, conglutinin, and SP-A. The cross-inhibition of each C1qR-ligand interaction by each of the other ligands suggests C1q, MBP, SP-A, and conglutinin bind to the same site, or overlapping sites on C1qR. This is reinforced by the similarity in concentration dependence of inhibition (Fig. 3b).

Figure 4. Binding of 125I-labeled C1qR to U937 cells. Dilutions of MBP (Δ), conglutinin (□), C1q (○), C1qR (■), BSA (■) (maximum quantity 5 μg) were premixed with 125I-labeled C1qR and then incubated with U937 cell (10^5 cells) in 5 mM barbitone buffer (pH 7.4) containing 5% (wt/vol) glucose. Bound cpm in the presence and absence of competing species were measured, as described in Materials and Methods.
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