# Human Leukocyte C1q Receptor Binds Other Soluble Proteins with Collagen Domains

By R. Malhotra, S. Thiel, K. B. M. Reid, and R. B. Sim

*From the Medical Research Council Immunochemistry Unit, Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, United Kingdom*

## Summary

A receptor binding to the C1q subcomponent of complement has been reported by many workers. In this paper we report for the first time that C1q receptor binds not only to C1q, but also to three other structurally similar ligands, namely mannan binding protein (MBP), conglutinin, and lung surfactant protein (SP-A). All these ligands have been reported to enhance removal of species bound to their globular domain from blood (MBP, conglutinin, C1q) or lung (SP-A) through phagocytosis. One of the possible roles for ligand-receptor binding may be initiation of phagocytosis.

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 $M_r$ 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),[1] modulation of cytokine and immunoglobulin secretion, and polymorph-endothelium 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 × $10^6$ 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% $NaN_3$, 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 $\alpha_3$), although two highly homologous polypeptides ($\alpha_2$,$\alpha_3$) 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 $CaCl_2$ (16). The zymosan was washed exhaustively with 5 mM sodium barbitone/HCl, 145 mM NaCl, 10 mM $CaCl_2$, pH 7.4, then conglutinin was eluted in the same buffer containing 10 mM EDTA in place of $CaCl_2$. Conglutinin was precipitated by dialysis against distilled water, dissolved in 10 mM Tris/HCl, 140 mM NaCl, 20 mM $CaCl_2$, pH 7.4 (TBS-$Ca^{2+}$), centrifuged (10,000 *g*, 20 min), and applied to a mannan-Sepharose column (14). The column was washed in TBS-$Ca^{2+}$, 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% $NaN_3$, 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

[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cell-mediated cytotoxicity; MBP, mannan binding protein; SP-A, lung surfactant protein.

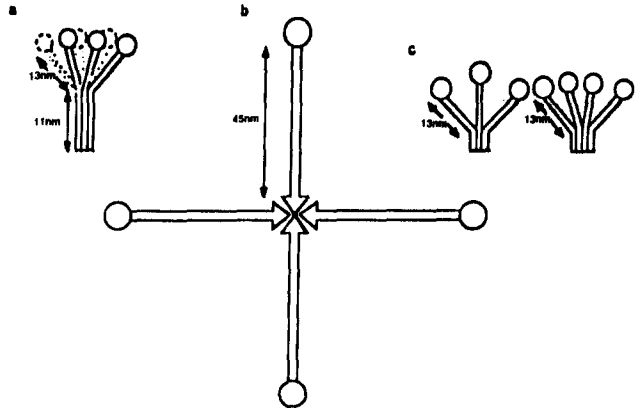


**Figure 1.** Ultrastructure of C1q, SP-A, conglutinin, and MBP. C1q (*a*) is a hexameric structure made up of six bent collagen triple helices joined to a globular domain. SP-A is essentially identical in appearance in electron microscopy to C1q (8). MBP has a similar monomer structure, but when isolated 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.

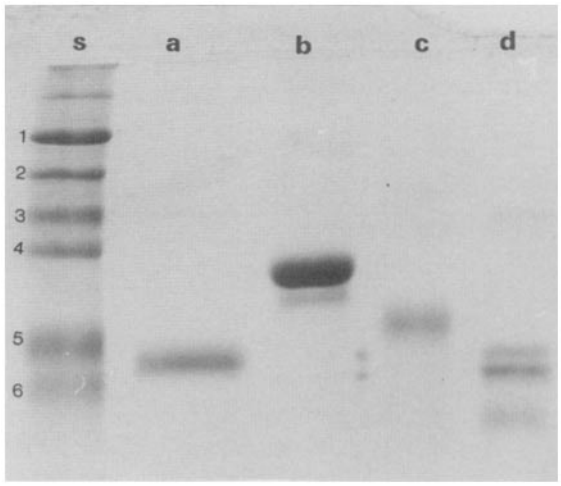


**Figure 2.** Purified ligands analyzed on 7.5% SDS-PAGE and detected by Coomassie Brilliant Blue staining. (Lane *s*) Reduced standards *1*, $\alpha_2$-Macroglobulin (180 kD); *2*, $\beta$-galactosidase (116 kD); *3*, fructose-6-phosphate kinase (84 kD); *4*, pyruvate kinase (58 kD); *5*, fumarase (48.5 kD); *6*, lactate dehydrogenase (36.5 kD). (Lane *a*) Mannan binding protein. (Lane *b*) Conglutinin. (Lane *c*) Lung surfactant protein. (Lane *d*) C1q.

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 $^{125}$I in a standard iodogen reaction (13). Specific activity was 0.5–4 × $10^6$ cpm/$\mu$g. All binding assays were done in 10 mM potassium phosphate, pH 7.4.

*Binding to Immobilized C1q.* Serial twofold dilutions of $^{125}$I-labeled C1qR (50 $\mu$l: maximum concentration 2 $\mu$g/ml) were preincubated with 100 $\mu$l 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 $\mu$g/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 $\mu$l of 10 mM potassium phosphate buffer, pH 7.4, bound $^{125}$I-labeled C1qR was solubilized and removed with 400 $\mu$l 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 $^{125}$I-labeled C1qR (100 $\mu$l: concentration 2 $\mu$g/ml) was pre-incubated with serial twofold dilutions of the potential competitors MBP, C1q, and conglutinin (100 $\mu$l; maximum concentration 12.5 $\mu$g/ml) for 30 min at 37°C in 10 mM potassium phosphate buffer. A con-

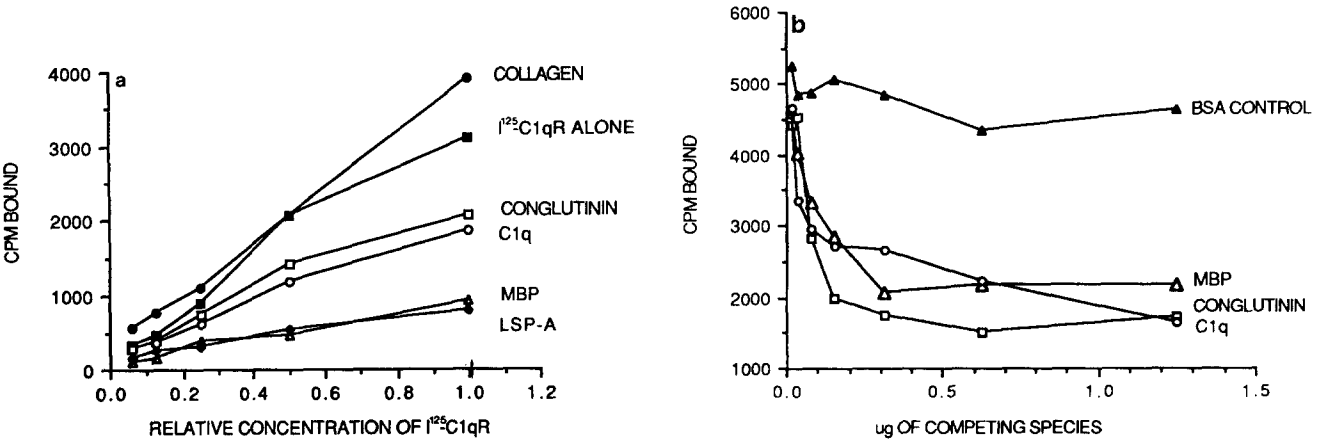


**Figure 3.** (*a*) Binding of $^{125}$I-labeled C1qR to solid-phase bound C1q. Serial dilutions of $^{125}$I-labeled C1qR (50 $\mu$l; 2 $\mu$g/ml) were preincubated with constant concentrations (100 $\mu$l; 50 $\mu$g/ml) of collagen (●), C1q (○), conglutinin (□), MBP (▲), SP-A (◆), and loaded on to C1q-coated microtiter plates. Details of the assay are given in Materials and Methods. (*b*) Binding of $^{125}$I-labeled C1qR to solid-phase bound conglutinin. Serial dilutions (maximum quantity 1.25 $\mu$g) of conglutinin (□), MBP (△), C1q (○), BSA (▲) were incubated with $^{125}$I-labeled C1qR (100 $\mu$l; 2 $\mu$g/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.

trol with no potential inhibitor was included. The mixture was transferred to conglutinin-coated microtiter plate wells. After incubation and washing (as above) the $^{125}$I-labeled C1qR bound in the presence or absence of competitor was measured, as above.

*Binding of Ligands to U937 Cells.* U937 cells were grown in 50-ml culture flasks in standard conditions (18). U937 ($10^7$ cells) were washed three times with PBS, followed by three washes with 5 mM sodium barbitone buffer containing 5% (wt/vol) glucose, pH 7.4 (buffer A). The cell pellet was suspended in 10 ml of buffer A. Different dilutions of potential competitors of C1q binding, namely MBP, C1q, C1qR, or conglutinin (100 μl; maximum concentration 50 μg/ml) were incubated with $^{125}$I-labeled C1q (100 μl; 1 μg/ml) for 30 min at 37°C in buffer A. The mixture was incubated with 100 μl of U937 cell suspension ($10^5$ 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 $^{125}$I-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 ($10^8$ cells/ml) in 5 mM sodium barbitone buffer (pH 7.4), containing 5% (wt/vol) glucose, 0.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$ (buffer B). To prepare EAiC3b-conglutinin cells, EAiC3b ($3 \times 10^8$ 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 $^{125}$I-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 10000 *g* for 1 min. Counts per minute bound to the cell pellet were measured.

## Results

*Binding of $^{125}$I-labeled C1qR to Ligand Immobilized on Microtiter Plates.* Dose-dependent binding of $^{125}$I-C1qR to solid-phase immobilized C1q was observed (Fig. 3 *a*). 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. 3 *a*). Direct binding of the radioiodinated C1qR to immobilized conglutinin (Fig. 3 *b*) 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. 3 *b*). Each of the three soluble proteins inhibited over a similar concentration range. Again soluble collagen (not shown) and BSA (Fig. 3 *b*) 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. 3 *b*).

*Binding of $^{125}$I-labeled-C1q to U937 Cells.* Binding assays based only on the use of proteins immobilized on microtiter plates serve as a useful indication of binding specificity, but represent a highly artificial system. Binding of potential ligands to intact cells bearing C1qR, a less artificial system, was therefore studied. Since the binding of monomeric C1q to C1qR is highly dependent on ionic strength (3), these tests were done at low ionic strength to maximize binding. Binding of radioiodinated C1q to U937 cells was inhibited in a concentration-dependent manner by co-incubation with soluble C1qR, MBP, or conglutinin (Fig. 4), indicating that binding of C1q to the cells does occur via C1qR, and that MBP and conglutinin compete for the same receptor. Binding of monomeric C1q to C1qR was considerably reduced (by ~74%) at physiological salt strength compared with the low salt conditions used here. This is consistent with the results of Arvieux et al. (3).

*Binding of $^{125}$I-labeled C1qR to EAiC3b.* A further intact cell system in which binding of C1qR to conglutinin could be examined was devised. Conglutinin is known to bind to the complement activation product iC3b (20). Sheep erythrocytes were sensitized with antibody (A) and coated with iC3b (EAiC3b) using purified complement classical pathway components. EAiC3b were then pre-incubated with and without conglutinin, washed, and the binding of $^{125}$I-labeled C1qR was assessed (Fig. 5). Cells treated with conglutinin bound more C1qR than did cells lacking conglutinin (Fig. 5). The moderate binding of radioiodinated C1qR in the absence of conglutinin reflects the presence of small amounts of C1q (used in the complement activation procedure) on these cells. This confirms the binding of C1qR to conglutinin attached in a physiologically relevant manner to iC3b.

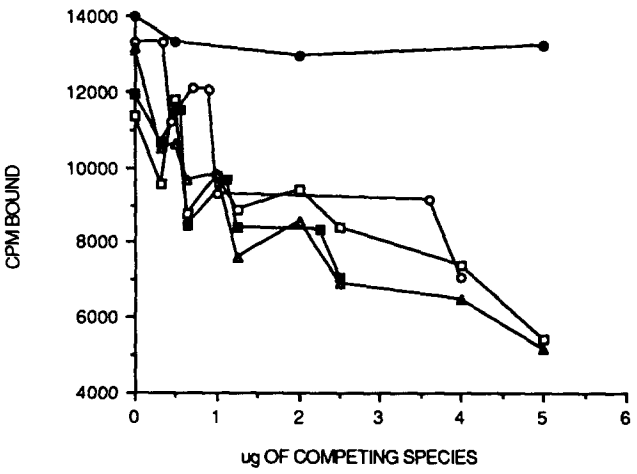


**Figure 4.** Binding of $^{125}$I-labeled C1qR to U937 cells. Dilutions of MBP (△), conglutinin (□), C1q (○), C1qR (■), BSA (●) (maximum quantity 5 μg) were premixed with $^{125}$I-labeled C1qR and then incubated with U937 cell ($10^5$ cells) in 5 mM barbitone buffer (pH 7.4) containing 5% (wt/vol)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

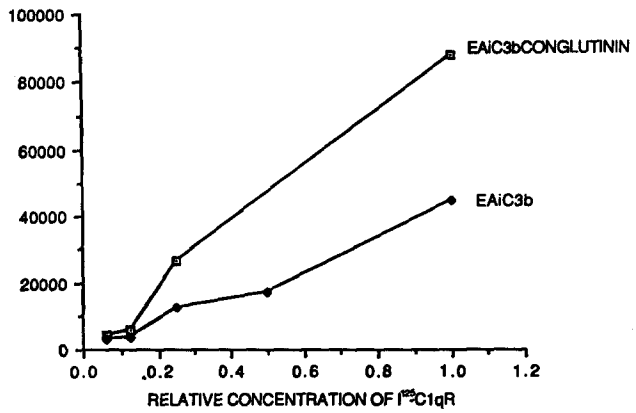


**Figure 5.** Binding of $^{125}$I-labeled C1qR to EAiC3b cells in the presence or absence of conglutinin. EAiC3b cells were preincubated with (⊡) or without (◆) conglutinin as described in Materials and Methods, then exposed to $^{125}$I-labeled C1qR in sodium barbitone buffer (pH 7.4) containing 5% (wt/vol) glucose. Unbound ligand was separated and cpm bound to cells were measured on an LKB gamma counter.

## Discussion

In this study we have demonstrated that binding to C1qR is a common function of four different proteins, which were not, with the exception of C1q and MBP, previously known to have strong functional similarities. Their structural similarity is, however, evident (Fig. 1). All of these proteins contain polypeptide chains made up of a short, $NH_2$-terminal noncollagen-like sequence, followed by a collagenous region of repeating Gly-Xaa-Yaa sequence, and a COOH-terminal end containing a recognition domain (8). Since C1qR is known to bind to the collagenous region of C1q (3), it is likely that it binds to the collagenous segments of MBP, SP-A, and conglutinin.

The established ligand, C1q, is a well-characterized protein that initiates activation of the classical complement pathway (2). It also functions as an opsonin, by interaction with C1qR on phagocytic cells (6). The soluble MBP used here occurs as a trace protein in serum (8, 14). It has a C-type lectin domain in the COOH-terminal region of the polypeptide, and binds to mannose/*N*-acetyl-D-glucosamine–containing structures, particularly yeast mannans, and also to mannose-rich surface polysaccharides on HIV and on virulent gram-negative bacteria (8, 21, 22). Its role as an opsonin in clearance of wild-type virulent *Salmonella montevideo* has been examined (21). Our results strongly suggest that the opsonic function of this protein is mediated by interaction with C1qR on phagocytes. Serum MBP when bound to, for example, yeast mannan, is also now known to mediate activation of the complement classical pathway (11, 14), and possibly also the alternative pathway (23), so in vivo its direct opsonic activity may be less significant than its role in stimulating deposition of another highly effective opsonin, C3.

SP-A is a major protein of pulmonary surfactant. Like MBP, it contains a C-type lectin domain within the primary sequence (8) and it has been indicated that it serves as an opsonin (24). It is thought to have a $Ca^{2+}$-dependent mannose/fucose specificity (8). The "target" for its lectin activity is unknown, but our results suggest that one of its functions may be to stimulate phagocytosis of material deposited in the lung, via interaction with C1qR on lung macrophage.

Conglutinin has until recently been considered as an obscure trace bovine serum protein. It has been detected in human plasma, again at very low levels (25). It has $Ca^{2+}$-dependent lectin activity of specificity similar to that of MBP, and binds directly to some yeasts (8, 10, 20, 25, 26). Its interaction with mannose-rich oligosaccharide on the complement activation fragment iC3b is well established (20). Opsonic (antibactericidal) activity has been demonstrated for conglutinin, although this effect appears to depend on prior deposition of iC3b on bacteria (10). Again our results suggest that the opsonic activity of conglutinin may be mediated by interaction with C1qR.

Demonstration of the interaction of C1q, MBP, SP-A, and conglutinin with C1qR strongly suggests a similar function for these structurally related proteins. The rodent protein RaRF (27) may be a further member of this family, both in terms of activity and structure. In addition to having roles as opsonins the newly identified C1qR ligands may be expected to mediate other phenomena associated with C1q-C1qR interaction.

We thank Miss B. Moffatt and Mrs. J. U. Newell for technical assistance, and K. P. Schafer (Byk Gulden Pharmaceutica, FDR.) for generously providing SP-A.

R. Malhotra was partially funded by an overseas research scholarship, and he thanks the Radhakrishnan Memorial and Christ Church, Oxford for additional support.

Address correspondence to Dr. R. B. Sim, Medical Research Council, Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, United Kingdom. S. Thiel's present address is Institut for Medicinsk Mikrobiologi, Bartholins Building, University of Aarhus, University Park, 8000 Aarhus, Denmark.

*Received for publication 2 April 1990 and in revised form 1 June 1990.*

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