# Complement Receptor 1/CD35 Is a Receptor for Mannan-binding Lectin

By Ionita Ghiran,* Sergi F. Barbashov,* Lloyd B. Klickstein,‡ Sander W. Tas,* Jens C. Jensenius,§ and Anne Nicholson-Weller*

*From the *Harvard-Thorndike Laboratory, the Division of Allergy and Inflammation, and the Division of Infectious Disease, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215; the ‡Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and the §Department of Medical Microbiology and the Department of Immunology, University of Aarhus, Aarhus 8000, Denmark*

## Abstract

Mannan-binding lectin (MBL), a member of the collectin family, is known to have opsonic function, although identification of its cellular receptor has been elusive. Complement C1q, which is homologous to MBL, binds to complement receptor 1 (CR1/CD35), and thus we investigated whether CR1 also functions as the MBL receptor. Radioiodinated MBL bound to recombinant soluble CR1 (sCR1) that had been immobilized on plastic with an apparent equilibrium dissociation constant of 5 nM. *N*-acetyl-D-glucosamine did not inhibit sCR1–MBL binding, indicating that the carbohydrate binding site of MBL is not involved in binding CR1. C1q inhibited MBL binding to immobilized sCR1, suggesting that MBL and C1q might bind to the same or adjacent sites on CR1. MBL binding to polymorphonuclear leukocytes (PMNs) was associated positively with changes in CR1 expression induced by phorbol myristate acetate. Finally, CR1 mediated the adhesion of human erythrocytes to immobilized MBL and functioned as a phagocytic receptor on PMNs for MBL–immunoglobulin G opsonized bacteria. Thus, MBL binds to both recombinant sCR1 and cellular CR1, which supports the role of CR1 as a cellular receptor for the collectin MBL.

Key words: C1q • opsonins • neutrophil • erythrocyte • innate immunity

## Introduction

Mannan-binding lectin (MBL)[1] is a C-type or $Ca^{2+}$-dependent lectin with primary specificity for fucose, mannose, and *N*-acetylglucosamine (1). MBL is composed of 32-kD polypeptides, each of which has an $NH_2$-terminal cysteine-rich region, a middle stretch of a collagen-like sequence, and a calcium-binding carbohydrate recognition domain (CRD) in the COOH terminus (2, 3). The collagen regions of three polypeptides form a triple helix, thus forming the 90+ kD trimeric subunit. In serum, large molecular mass complexes (200–700 kD) of MBL circulate, which are probably stabilized by interaction through the cysteine-rich, $NH_2$-terminal regions of adjacent trimeric subunits (4).

MBL, which is present constitutively in plasma at ~2 μg/ml, is part of the innate immune system because of its ability to recognize carbohydrate expressed by pathogens (for reviews, see references 5–7). Once the CRDs of MBL firmly bind to foreign carbohydrates, there are two pathways by which MBL may participate in a host defense response. The first is by activating MBL-associated serine protease (MASP)-1 and MASP-2 (8, 9). MASP-2 has the capacity to cleave and activate complements C4 and C2, in a manner analogous to C1, and thereby generates the opsonic fragments C4b and C3b (8–11). The second pathway MBL uses to effect host defense is by functioning as a pri-

This work was presented in part at the 18th International Complement Workshop, July 2000 and has appeared in abstract form (2000. *Immunopharmacology*. 49:3).

I. Ghiran and S.F. Barbashov contributed equally to this work.

S.F. Barbashov's present address is Protein Division, Ilex Oncology Corporation, Boston, MA 02215.

Address correspondence to Anne Nicholson-Weller, Beth Israel Deaconess Medical Center, Dana Bldg. 617, 330 Brookline Ave., Boston, MA 02215. Phone: 617-667-3307; Fax: 617-277-6061; E-mail: anichols@caregroup.harvard.edu

[1]*Abbreviations used in this paper:* CR1, complement receptor 1; CRD, carbohydrate recognition domain; C1qRp, C1q receptor of phagocytosis; LHR, long homologous repeat; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; MFC, mean fluorescent channel; sCR1, soluble CR1; TBS, Tris-buffered saline.

mary opsonin. MBL opsonizes *Salmonella* (12) and influenza A (13) for uptake by phagocytic cells. While the putative receptor for MBL has been called a "collectin receptor," identification of the receptor(s) has been elusive. Two different molecules termed C1q receptors, with reported activity also for MBL, do not fully explain the opsonic activity of MBL. The first molecule described to bind soluble C1q and MBL (14, 15) is now thought to be calreticulin, which is an intracellular protein (for a review, see reference 16). Although calreticulin is likely an important C1q-binding protein during tissue damage, it is not a cell surface receptor (17). The second C1q receptor was identified by screening mAbs (18) that reacted with monocytes and partially inhibited this cell's ability to be stimulated by either immobilized C1q (19) or MBL (20) for enhanced phagocytosis of erythrocyte targets coated with either IgG or C4b/C3b. The mAbs recognized a 126-kD transmembrane protein (21), termed C1q receptor for phagocytosis (C1qRp). While C1qRp has a definite role in this assay, there are no unequivocal data that C1qRp actually binds C1q or MBL. Thus, identification of receptor(s) that bind MBL-opsonized particles has remained an open question.

CR1 has recently been shown to act as a receptor for soluble C1q (22, 23). Because of the structural homology between C1q and MBL at the primary, secondary, and tertiary (by electron microscopy) levels (24), it was logical to assess if CR1 might also function as a receptor for MBL. Using in vitro assays with purified proteins, we have determined that MBL can bind to recombinant soluble CR1 (sCR1), which is comprised of the entire extracellular domain of the common form of CR1 but lacks the cytoplasmic and transmembrane domains (25). Although MBL–sCR1 binding was favored by the presence of calcium, it did not involve the CRD of MBL. MBL binding to cells paralleled CR1 expression on PMNs. Immobilized MBL also mediated E adhesion, which was specifically inhibited by polyclonal anti-CR1 Fab. MBL-opsonized *Salmonella* were not ingested by PMNs unless either the bacteria were coopsonized with suboptimal doses of anti–*Salmonella* IgG, or the PMNs were preactivated by incubation with soluble fibronectin. Under both of these phagocytic conditions, the opsonic effects of MBL could be blocked by the pretreatment of PMNs with polyclonal anti-CR1 Fab. These data provide evidence that cellular CR1 is a functional receptor for MBL.

## Materials and Methods

*Buffers and Reagents.* The following buffers were used: Tris-buffered saline (TBS: 150 mM NaCl, 50 mM Tris-HCl [pH 7.5]), TBST (TBS, 0.05% Tween 20), TBST-$Ca^{2+}$ (TBST, 10 mM $CaCl_2$), and TBST-EDTA (TBST, 10 mM $Na_2EDTA$); "binding buffer" was used in microtiter well-binding assays (140 mM NaCl, 0.05% Tween 20, 10 mM Tris-HCl [pH 7.5]); E adhesion buffer ($HBSS^=$ [HBSS without calcium and magnesium] diluted with an equal volume of 5% D-glucose, 0.1% gelatin); protein-coating buffer for the immobilization of protein to plastic (0.04 M $NaHCO_3$, 0.01 M $Na_2CO_3$, pH 9.6); borate buffer for FITC labeling (50 mM borate, pH 9.2, 200 mM NaCl, 20 mM $CaCl_2$, 100 mM mannose); and FACS® buffer ($HBSS^=$, 0.1% gelatin, 0.1% sodium azide). Trypan blue was purchased from Sigma-Aldrich.

SDS-PAGE (4 μg/lane) of reduced samples (5% 2-mercaptoethanol) was performed using 12% precast gels (Novex) with a Tris-glycine buffer system. The gel was stained with Coomassie R-250 (Sigma-Aldrich). Protein-binding assays and ELISAs were performed using Immulon 1 Removawell Strips (Dynatech).

*Human Proteins.* Plasma fibronectin (F2006) was purchased from Sigma-Aldrich. Human C1q was isolated from fresh human serum as described previously (22). Recombinant human sCR1 was a gift of Drs. U. Ryan and H. Marsh (Avant Immunotherapeutics, Needham, MA).

MBL was isolated as described previously (26) with minor modifications. In brief, 1 liter of previously frozen citrated human plasma (obtained from the Beth Israel Deaconess Medical Center blood bank) was precipitated with 7% (wt/vol) polyethylene glycol 3350 (Sigma-Aldrich) at 4°C. After 2 h, the precipitate was harvested by centrifugation, resuspended in 400 ml of TBST-$Ca^{2+}$, and stirred overnight at 4°C. The clotted material was discarded and the supernatant was mixed with a slurry of 30 ml of mannan-agarose (Sigma-Aldrich). After incubation, with stirring for 2 h at 4°C, the beads were collected, extensively washed with TBST-$Ca^{2+}$ in a Buchner funnel, packed into a column, and washed with TBST-$Ca^{2+}$. The column was eluted with TBST-EDTA. The resulting MBL-containing fraction was recalcified, adjusted to pH 7.5, and chromatographed on a 3-ml maltose-agarose (Sigma-Aldrich) affinity column equilibrated with TBST-$Ca^{2+}$. Material eluted with TBST-$Ca^{2+}$, 100 mM *N*-acetyl-D-glucosamine (Sigma-Aldrich) was dialyzed against TBST-EDTA and passed through a microcolumn ($V_t$ = 300 μl) containing a mixture of protein A–Sepharose (Pierce Chemical Co.) and rabbit anti–human IgM (IgG fraction; Sigma-Aldrich) that was immobilized on Sepharose CL-6B (Amersham Pharmacia Biotech) at 1 mg/ml packed gel using cyanogen bromide (Sigma-Aldrich). Isolated MBL was concentrated to 1–1.2 mg/ml using a Centriplus 30 device (Millipore) and the pH was adjusted to 4.5 with acetic acid. Replicate samples of 150 μl were applied to a TSK G4000 $SW_{XL}$ column (Supelco). The column was equilibrated with 50 mM KCl, 0.1 mM EDTA, and 20 mM $KH_2PO_4$ (pH 4.75) and run at 1 ml/min. MBL eluted with a retention time of 8.3 min ($M_r \approx$ 650 kD). The MBL peaks from several TSK runs were pooled, dialyzed against TBS, and concentrated using Centriplus 30 devices. Routinely, MBL preparations from the TSK column step were used in experiments. Recombinant MBL was provided by Dr. A. Ezekowitz (Massachusetts General Hospital, Boston, MA [20]).

FITC-labeled MBL was prepared by incubating MBL (0.8 mg) with FITC (25 μl of 1 mg/ml DMSO; Sigma-Aldrich) in 0.5-ml borate buffer for 16 h at 4°C. To stop the reaction, $NH_4Cl$ was added (final concentration, 50 mM), and the mixture was kept at room temperature for 2 h. FITC-MBL was separated from free FITC by passage over a PD-10 column (Amersham Pharmacia Biotech) that had been equilibrated in PBS. The ratio of absorbance measured at 495 nm to 280 nm was 1.08.

MBL was radioiodinated in mild conditions, which included a low concentration (5 μg/ml) of coating Iodogen (Pierce Chemical Co.), and incubated for only 2 min at 0°C with $^{125}I$-sodium (NEN Life Science Products). Radioiodinated MBL was separated from free $^{125}I$ by passage over a small column of maltose-agarose equilibrated in TBST-$Ca^{2+}$ and eluted with TBST-EDTA. The final specific activity was $1.2 \times 10^7$ cpm/μg, with 90% of cpm precipitable by 10% TCA.

*Antibodies.* Rabbit anti–*Salmonella* IgG was purchased from Fitzgerald Industries International. Anti-C1qRp IgM mAb (R3) was a gift of Dr. A. Tenner (University of California at Irvine, Irvine, CA). Control mouse IgM was purchased from BD PharMingen. Anti-MBL mAbs (131-1) and anti-CR1 mAb YZ-1 were prepared from hybridoma-conditioned media by protein A affinity chromatography. FITC-labeled goat anti–rabbit IgG was purchased from Jackson ImmunoResearch Laboratories. Rabbit anti–human CR1 Fab fragments were prepared from an IgG fraction using the ImmunoPure® Fab Preparation kit (Pierce Chemical Co. [27]). The papain digest was subsequently passed over a protein A column (Pierce Chemical Co.) and the nonretained fraction showed a 50-kD band, or a 25-kD band when analyzed by SDS-PAGE with Coomassie staining under nonreducing or reducing conditions, respectively. For the E adhesion experiment, an aliquot of the polyclonal Fab anti-CR1 preparation was passed through an affinity column of recombinant sCR1 immobilized on polyacrylamide beads (3M Emphaze; Pierce Chemical Co.). "Absorbed" Fabs were obtained from the drop-through fraction. Fab and CR1-absorbed Fab preparations were extensively dialyzed against PBS, and the protein concentrations were determined by the micro-BCA method (Pierce Chemical Co.), using BSA as a standard. For the phagocytic assay, polyclonal anti-CR1 Fabs were neutralized by the addition of sCR1 at a molar ratio of 3 sCR1/40 Fab (3 μg of sCR1/10 μg Fab).

0.6 mg unabsorbed anti-CR1 was mixed with 17 μl FITC (1 mg/ml in DMSO) in 0.3 ml borate buffer for 2 h at 4°C; the reaction was terminated and the FITC-Fab was isolated as described for FITC-MBL (see above). The ratio of absorbance measured at 495 nm to 280 nm was 0.6.

*Binding of Radiolabeled MBL to Immobilized sCR1.* Microtiter wells were coated with sCR1 at 5 μg/ml using coating buffer. The wells were blocked with SuperBlock (Pierce Chemical Co.). Then, $^{125}$I-MBL was added in binding buffer. After the 40-min binding incubation at 21°C, the wells were emptied by aspiration, washed twice with the binding buffer, separated, and individually counted in a γ counter. The binding data were analyzed using Prism v2.0 software (Graph Pad). A binding curve was fit to the data using nonlinear regression, and the $K_d$ was derived from the curve.

*ELISA for Bound sCR1 or Bound MBL.* The coating, blocking, and binding steps were performed as described above. After washing with binding buffer, purified anti-CR1 mAb (YZ-1) or anti-MBL mAb (131-1) was added. Finally, the assay was developed using goat anti–mouse IgG conjugated with horseradish peroxidase (Pierce Chemical Co.) and substrate tetramethylbenzidine as described above.

*Isolation of Cells.* For E, a normal donor was selected whose E expressed high levels of CR1 on the basis of $^{125}$I-$(C3b)_2$ binding and monospecific $^{125}$I–anti-CR1 $F(ab')_2$ binding (27), and as reconfirmed by FACS® (see below). 3 ml of venous blood was collected in a glass tube containing 68 μl of 7.5% EDTA solution ($K_3$, Vacutainer; Becton Dickinson). The blood was centrifuged for 5 min at 1,000 *g*. After centrifugation and removal of the plasma and buffy coat layers, the E were washed several times in E adhesion buffer.

Leukocytes from a healthy volunteer were isolated from 40 ml of venous blood drawn into a syringe containing 6 ml of sodium citrate/citric acid (0.15 M, pH 5.5) and 14 ml of 6% Dextran-70 (McGaw). To minimize the upregulation of CRs which can occur during cell isolation, all of the procedures were performed at room temperature and the leukocytes were not fractionated into subpopulations. After 50 min of sedimentation, the leukocyte-rich fraction was removed and the cells were pelleted by centrifugation. Contaminating E were removed by hypotonic lysis, and the leukocytes were resuspended in $HBSS^{=}$ and used within 30 min of isolation. 98% of the cells were alive as assessed with acridine orange/ethidium bromide staining.

*Flow Cytometry.* Replicate samples of PMA-treated or control leukocytes from the same donor were reacted with either FITC-labeled, rabbit anti-CR1 Fab (100 μg/ml) or an equivalent amount of FITC-MBL. FITC-nonimmune Fab and FITC-ovalbumin were used to set the respective background gates. All cells were fixed in 1.5% paraformaldehyde before analysis of 10,000 cells using a FACScan™ instrument with v1.0 CELLQuest™ software (Becton Dickinson).

*E Tip-Plate Adhesion Assay.* E ($10^9$) were quantified using a hemacytometer and suspended in 1 ml adhesion buffer containing anti-CR1 Fab (100 μg), or as a control, an equivalent concentration of anti-CR1 Fab that was specifically absorbed to remove anti-CR1 reactivity (see Antibodies, above). After 1 h at room temperature, each E suspension was diluted in adhesion buffer to $5 \times 10^7$ cells/ml for use in the tip-plate assay for cell adhesion. Tip-plate assays were performed as originally described (28) and modified (23). In brief, 20-μl drops of MBL at 5 or 20 μg/ml in adhesion buffer were applied to marked spots on Lab Tek petri dishes (100 × 25 mm; Nunc) and incubated at 37°C for 1 h. After appropriate washing of the individual MBL spots, the uncoated areas of the plate were blocked by flooding the entire plate with HBSS containing 0.5% gelatin (1 h at 37°C), followed by washes with adhesion buffer. Replicate plates were made for assay with each type of E: buffer treated, anti-CR1 treated, and control anti-CR1 (CR1-absorbed) treated. The E suspensions were added to the plates to allow adhesion over 1 h at 37°C. Nonadherent E were aspirated and the plate was gently washed. Adherent cells were fixed with 2% paraformaldehyde in PBS and the cells were quantified using phase–contrast microscopy. After fixation, the plates could be analyzed immediately, or stored at 4°C for later analysis.

The ligand-coated areas were scanned by low-power microscopy to determine the evenness of adherence. Within the ligand-coated areas, the E in three to five random fields were quantified at 290× power using an inverted microscope (Diaphot 300; Nikon) with a digital camera (RC 300 CCD; Dage-MTI Inc.) attached to a computer. Images were analyzed using IPlabSpectrum software v3.1a (Scanalytics).

*Phagocytic Assays with PMNs.* Purified PMNs were incubated with either buffer alone (RPMI 1640 plus 2% autologous serum), a rabbit anti-CR1 Fab (100 μg/ml final), the same amount of anti-CR1 that had been preabsorbed with sCR1 (3), or a rabbit nonimmune IgG fraction. *Salmonella montevideo* ($10^6$ cells; American Type Culture Collection) in 100-μl volume were opsonized with buffer, MBL at various concentrations, anti–*Salmonella* IgG (0.5 μg/ml), or the sequential addition of MBL and anti–S*almonella* IgG with a wash between additions. PMNs and opsonized bacteria, at a ratio of 1 effector to 10 targets, were incubated together for 30 min at 37°C with continuous rocking. The PMNs were washed in cold buffer, and then pelleted on a slide using a Cytospin centrifuge (Shandon Southern Instruments). The cells were stained with modified Wright-Giemsa (Hema 3 kit; Biochemical Sciences) and the percentage of PMNs that had ingested one or more organisms was determined. Random fields were chosen, and at least 300 PMNs were scanned. Alternatively, phagocytosis was evaluated using flow cytometry with *Salmonella* labeled with FITC before opsonization. At the conclusion of this assay, 0.4% Trypan blue was added for 5 min to

quench the extracellular fluorescence due to adherent, non-ingested bacteria. Subsequently, the PMNs were washed twice in FACS® buffer and analyzed within 0.5 h using a FACScan™ instrument and v1.0 CELLQuest™ software (Becton Dickinson).

## Results

*Characterization of MBL.* MBL was isolated as described in Materials and Methods, and its relative molecular mass of 650 kD was determined by gel filtration chromatography on a TSK column (Fig. 1 A, peak 1). The identity and purity of MBL were confirmed by ELISA (data not shown) and SDS-PAGE (Fig. 1 B), respectively. Under reducing conditions, MBL is seen as major band of ~32,000 mol wt as described. In the sample before the gel filtration step, the additional band of ~64,000, which has been described as a nonreducing dimer of MBL, is also seen (9). The lectin function of the isolated MBL from the TSK peak was confirmed by its ability to bind, when immobilized to plastic, biotinylated mannan in the presence of 0.3 mM $CaCl_2$, and this binding was completely inhibited by either 100 mM *N*-acetylglucosamine or 10 mM EDTA (data not shown).

*MBL Binds to sCR1.* Determination that CR1 was a receptor for C1q was based in part on the ability of immobilized CR1 to bind labeled C1q (22). Similar studies were designed to test whether labeled MBL would bind to immobilized sCR1. Furthermore, since MBL is a $Ca^{2+}$-dependent lectin it would be important to determine if any binding to CR1 were affected by calcium. $^{125}$I-MBL was added to wells containing immobilized sCR1 in binding buffer with different concentrations of $CaCl_2$ ranging from 0 to 10 mM. There was measurable MBL binding with no added $CaCl_2$, suggesting that the CRD of MBL was not involved in binding to CR1. However, the optimal calcium concentration was 0.3 mM, which was used throughout the remainder of the binding experiments that used isolated proteins (Fig. 2 A). To substantiate that the CRD was not involved, an experiment was performed with *N*-acetylglucosamine, which binds to the CRD of MBL with high affinity and can displace other carbohydrates from the CRD. The addition of 100 mM *N*-acetylglucosamine had no effect on MBL binding to sCR1 (Fig. 2 B), thus confirming that the CRD was not involved in MBL-CR1 binding.

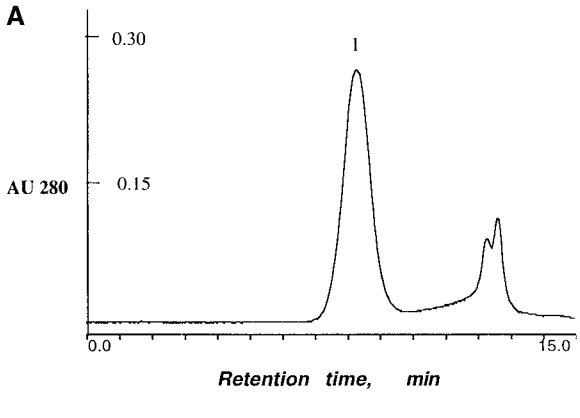


**Figure 1.** Purity of MBL. (A) Gel filtration chromatography on a TSK G4000 $SW_{XL}$ column (flow rate 1 ml/min) of an aliquot from the concentrated pool of MBL after absorption with anti–human IgM-Sepharose and protein A–Sepharose. (Peak 1 is the MBL and eluted at $M_r$ 650,000.) (B) Protein electrophoresis of isolated MBL. Lane 1, concentrated pool from peak 1 (see A). Lane 2, protein peak from anion exchange analysis of TSK pool. Reduced MBL has a mobility of corresponding to 32,000 mol wt.

Binding of C1q to CR1 is highly sensitive to ionic strength (22), prompting an examination of the influence of salt on MBL–sCR1 binding. In this experiment, MBL was immobilized on plastic and sCR1 was the soluble ligand. Bound sCR1 was detected by ELISA using the anti-CR1 mAb, YZ-1. sCR1 binding to MBL was also inhibited by increasing ionic strength (Fig. 2 C). Binding in high-salt buffers (2× and 5× TBST) was minimal. However, sCR1 bound in 1× TBST could not be completely removed by subsequent washings with high-salt buffer containing 750 mM NaCl. Thus, the initial interaction between MBL and sCR1 is salt sensitive, but once binding occurs the interaction becomes relatively salt insensitive. The ability of MBL-sCR1 binding to withstand the high salt is also a control that the high salt is not simply removing the coated sCR1 from the plate, which would be a trivial explanation for the lack of binding seen in 2× and 5× physiological salt.

Equilibrium binding assays were performed in 1× TBST, 0.3 mM $CaCl_2$, to determine the apparent dissociation constant of MBL binding to sCR1. $^{125}$I-MBL was added to microtiter wells containing immobilized sCR1. Control binding to wells with only blocking agent was subtracted as a background for all concentrations of labeled MBL. $^{125}$I-MBL bound to sCR1 in physiologic ionic strength, with one apparent binding site with a $K_d$ of 5.2 nM (Fig. 3 A). A Scatchard analysis of the data is shown in Fig. 3 B. This experiment was repeated with a different preparation of MBL, and analysis of the binding yielded a $K_d$ of 3.0 nM. $^{125}$I-sCR1 binding to immobilized MBL was also analyzed and the calculated $K_d$ was 45 nM. The apparent affinity of $^{125}$I-sCR1 binding to immobilized MBL increased to 28 nM if the MBL were immobilized through its CRD domain (data not shown).

The fact that C1q and MBL both serve as ligands for sCR1 raised the question whether C1q is able to compete with MBL for binding to sCR1. Because C1q binds sCR1 less avidly than MBL in our assay, it was necessary to design this experiment so that C1q binding would be maximized. A low ionic strength binding buffer (0.67× TBST, 0.3 mM $CaCl_2$) was used, and different concentrations of C1q were preincubated with immobilized sCR1 (2 μg/ml) be-

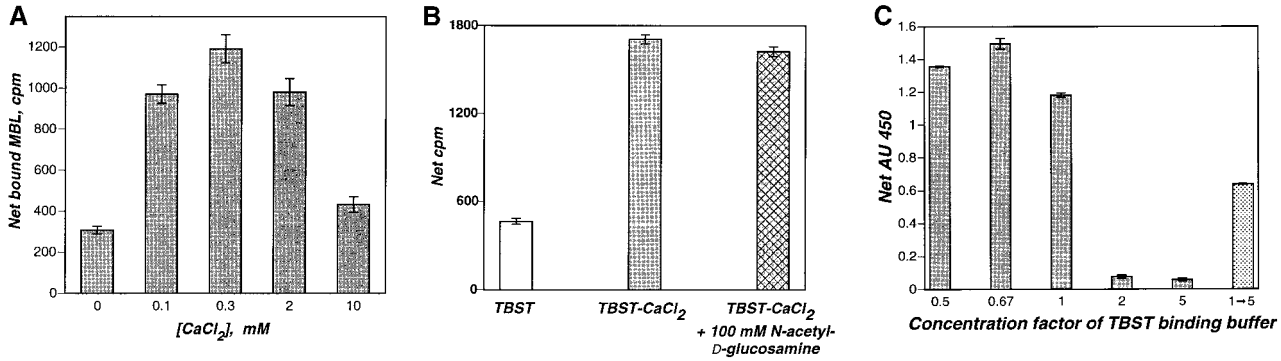


**Figure 2.** Binding conditions for MBL–sCR1. (A) Effect of $CaCl_2$ concentration. Microtiter wells were coated with recombinant sCR1 (8 μg/ml) in carbonate-bicarbonate buffer. After washing and blocking, $^{125}$I-MBL (70 ng/ml) was added for 40 min at 21°C in the presence of different $CaCl_2$ concentrations. (B) Effect of N-acetylglucosamine on MBL binding to sCR1. $^{125}$I-MBL (70 ng/ml) in TBST, or TBST, 0.3 mM $CaCl_2$ in the presence or absence of 100 mM N-acetyl-D-glucosamine was added to wells containing immobilized sCR1. Mean background values of gelatin-blocked wells, which did not receive sCR1, varied with the buffer condition and were specifically subtracted. Results are means ± SE, $n = 4$. This experiment was repeated with similar results. (C) sCR1 binding to immobilized MBL in different salt conditions. MBL was plated in microtiter wells at 1 μg/ml in carbonate-bicarbonate buffer. After blocking, different groups of wells were washed with Tris saline, 0.05% Tween 20, and 0.3 mM $CaCl_2$, having different concentrations of NaCl (0.15 M = 1×). sCR1 was added (1 μg/ml) in specific salt buffer. After incubation for 40 min at 21°C, each well was washed with the same buffer used for binding sCR1. Bound sCR1 was detected by ELISA using anti-CR1 mAb YZ-1 followed by a secondary anti–mouse peroxidase-conjugated antibody. The background values for each buffer condition were subtracted. The net means ± SE ($n = 4$) are plotted. The experiment was repeated with similar results.

fore the wells were washed and MBL (100 μl, 1 μg/ml) was added. Bound MBL was detected using the ELISA format. Preincubation of sCR1 with increasing doses of C1q provided a dose- dependent inhibition of MBL binding to sCR1 (Fig. 4). These results are compatible with the homologous proteins C1q and MBL sharing a common binding site on CR1, or binding to adjacent sites on CR1.

*E Adhesion to MBL Is Specifically Blocked by Anti-CR1 Fab.* Human E ($10^9$/ml) were pretreated with buffer or 100 μg/ml of rabbit anti-CR1 Fab, or as a control, 100 μg/ml of the same anti-CR1 Fab preparation that was specifically adsorbed with immobilized sCR1. E were able to adhere to immobilized MBL (application of 20 μl of 5 or 20 μg/ml to plastic) and antigen-absorbed Fab binding to E had no effect on the adhesion. Pretreatment of the E with anti-CR1 almost completely inhibited this interaction, which indicated the specificity of CR1 as the receptor for MBL on E (Fig. 5). There was almost no E binding (0–3 cells/field) to the background area of the plate, which was blocked with gelatin (0.5%) containing buffer.

*Positive Correlation of MBL Binding and CR1 Expression on PMNs.* PMNs have the ability to change their surface expression of CR1. PMA stimulation causes an initial increase in CR1 expression from a preformed intracellular pool (29–32), followed by a decrease in CR1 expression due to its ligand-independent endocytosis (33, 34). We assessed PMNs with and without the addition of 50 nM PMA to see the effects on FITC–MBL binding. After 10 min of PMA stimulation, the mean fluorescent channel (MFC) of CR1 expression had shifted from 3.2 to 30.7, or 9.6-fold. Correlated with the increase in CR1 expression was a 15.4-fold increase in FITC–MBL binding (Fig. 6, top). After 40 min of PMA stimulation, CR1 expression was downregulated on the PMNs with an associated downregulation of FITC–MBL binding (Fig. 6, bottom). PMA induced similar, but less pronounced, coordinate

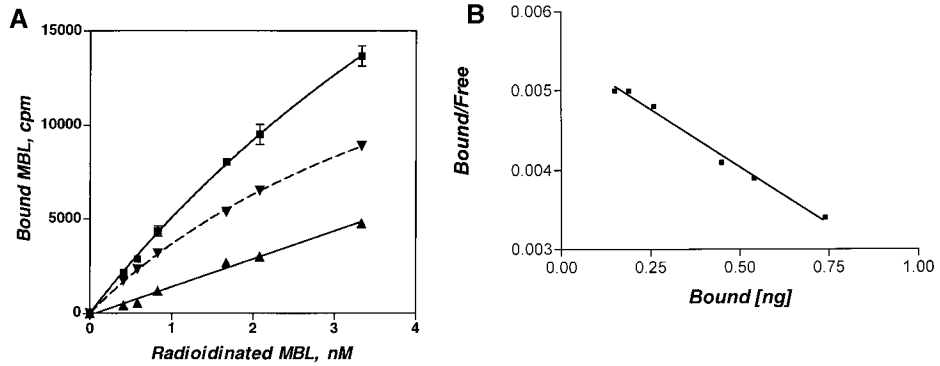


**Figure 3.** Derivation of binding constants. (A) Binding of $^{125}$I-MBL to immobilized sCR1. Microtiter wells were precoated with sCR1 (0.1 ml at 5 μg/ml) and washed before the addition of increasing amounts of $^{125}$I-MBL in TBST, 0.3 mM $CaCl_2$ for binding (40 min at room temperature). After washing the plate, the individual wells were separated and counted in a γ counter to determine the amount of bound $^{125}$I-MBL. $^{125}$I-MBL binding to blocked wells defined nonspecific counts; binding data: total (■), nonspecific (▲), and calculated specific binding (▼). Curves were fit by nonlinear regression analysis, and the $K_d$ of 5.2 nM was derived from the specific binding curve, as described in Materials and Methods. Data points are means ± SE, $n = 4$. The experiment was repeated with similar results. (B) Scatchard plot of binding data. The line was fitted with the method of least squares.

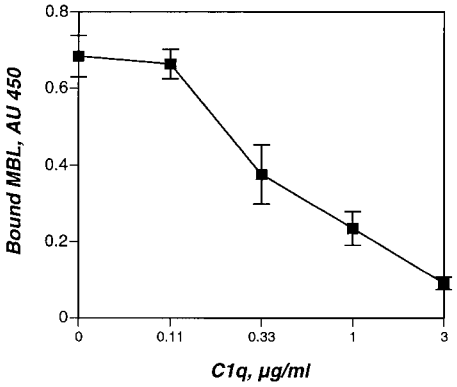


**Figure 4.** Competition of MBL with C1q for binding to plated sCR1. Plated sCR1 (2 μg/ml) was preincubated for 20 min at 21°C with 0.67× TBST, 0.3 mM $CaCl_2$ alone, or the same buffer containing different concentrations of C1q. After washing, MBL (0.1 μg in 100 μl) was added for 5 min at 21°C. After washing, bound MBL was detected by ELISA using anti-MBL mAb, second anti–mouse horseradish peroxidase-conjugated antibody, and tetramethylbenzidine substrate.

changes in CR1 expression and MBL binding in the gated monocyte population (data not shown).

*MBL Uses CR1 to Synergize with an FcR for the Phagocytosis of S. montevideo by PMNs.* *S. montevideo* avidly binds MBL (12). In a series of preliminary experiments, isolated serum MBL or recombinant MBL used in concentrations from 0.1 to 20.0 μg/ml were never opsonic alone (data not shown). We reasoned that if CR1 were indeed the MBL receptor that mediated phagocytosis, the PMNs might need a second signal, such as FcγR ligation, as is the case for opsonization with C3b and C4b. To determine a suboptimal dose of IgG, FITC-labeled bacteria were opsonized with 0, 0.5, 15, or 100 μg/ml of anti–*Salmonella* IgG and then used in the phagocytic assay, and the results were analyzed by flow cytometry. The MFC of the PMN population increased progressively with higher doses of opsonizing IgG, and 0.5 μg/ml was selected as a suboptimal concentration. When the bacteria were opsonized with 0.5 μg/ml of rabbit anti–*Salmonella* antibodies, the addition of MBL augmented phagocytosis (Fig. 7). The optimal concentration of MBL was 1 μg/ml, which is close to the normal concentration of MBL in plasma (1.8 μg/ml [35]). The MBL-augmented phagocytosis was inhibited by 75% if the PMNs were pretreated with 100 μg/ml of anti-CR1 Fab. As confirmation that the Fab preparation was blocking CR1, its inhibitory activity was completely removed by preabsorption with sCR1 (Fig. 8).

*Fibronectin-stimulated PMNs Can Utilize MBL Alone as an Opsonin.* Having evidence that CR1 might be the receptor for MBL, we wanted to determine if fibronectin-treated PMNs can ingest particles opsonized only with CR1 ligands, as has been shown for monocytes (36, 37). PMNs were pretreated with buffer or soluble fibronectin and then added to the phagocytic mixture containing MBL- or buffer-opsonized bacteria. Again, MBL-opsonized bacteria were not ingested by unstimulated PMNs. However, pretreatment of the PMNs with fibronectin made these cells competent to ingest MBL-opsonized bacteria. Pretreatment of the bacteria with fibronectin had no effect. The MBL opsonic effect was significantly inhibited when the PMNs

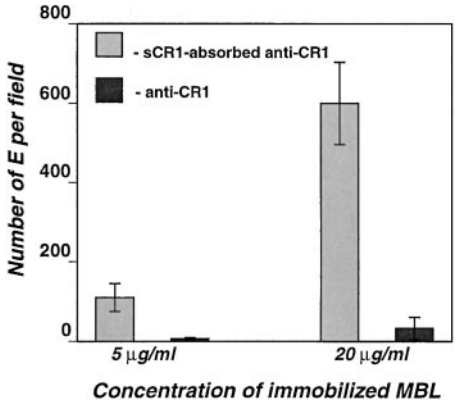


**Figure 5.** Adhesion of E to immobilized MBL can be blocked by pretreatment of the E with anti-CR1 Fab. 20-μl aliquots of MBL at 5 μg/ml or 20 μg/ml were each "spotted" three times on a petri dish. After washing the spots and then blocking the entire dish surface with 0.5% gelatin, the dish was incubated for 45 min at room temperature with E (15 ml; $5 \times 10^7$/ml). The E had been preincubated with 100 μg anti-CR1 Fab, or as a control, 100 μg anti-CR1 that had been preabsorbed with immobilized sCR1. The dishes were gently washed twice with adhesion buffer, fixed in paraformaldehyde (2% in PBS), and quantified by light microscopy. Results represent the mean number of adherent cells/field ± SD in nine representative fields (three fields × three spots). Each field was 0.065 $mm^2$. In the regions between the immobilized MBL, the background cell adhesion to 0.5% gelatin ranged from zero to three cells per field.

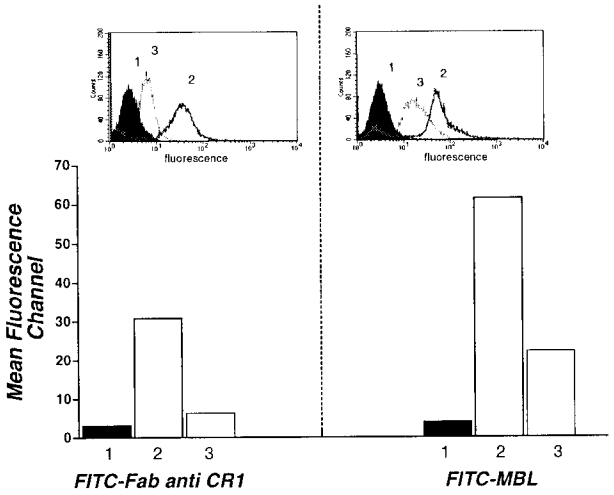


**Figure 6.** MBL binding parallels PMA-induced changes in CR1 expression on PMNs. Freshly isolated leukocytes were exposed to buffer (1), 50 nM PMA for 10 min (2), or 40 min (3) at room temperature. Samples were removed and assessed for CR1 expression by direct staining, analysis by flow cytometry, and FITC-MBL (50 μg/ml) binding, as described in Materials and Methods. FITC-labeled, nonimmune rabbit Fab binding defined the background (2.6–3.2 MFC) for CR1 expression; FITC-ovalbumin binding defined the background (3.2–4.0 MFC) for FITC–MBL binding. PMNs were selected for analysis with flow cytometry using forward and side scatter. The results of the histograms (top) are displayed as bar graphs (bottom). This experiment was repeated with leukocytes from a different donor with similar results.

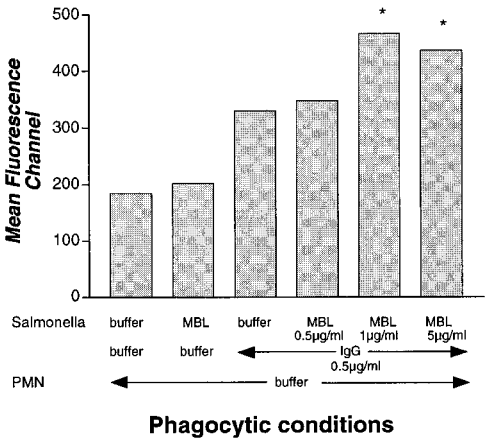


**Figure 7.** Role of MBL in opsonizing *S. montevideo* for ingestion by PMNs. FITC bacteria were opsonized with either buffer or 0.5 μg/ml anti-*Salmonella* IgG with or without different concentrations of MBL; at 37°C, the cells were incubated with Trypan blue to quench extracellular fluorescence. At least 10,000 PMNs for each reaction condition were analyzed by flow cytometry as described in Materials and Methods. The MFC was significantly increased for opsonization using MBL at 1.0 and 5.0 μg/ml in combination with IgG compared with opsonization with IgG alone. $*P < 0.05$.

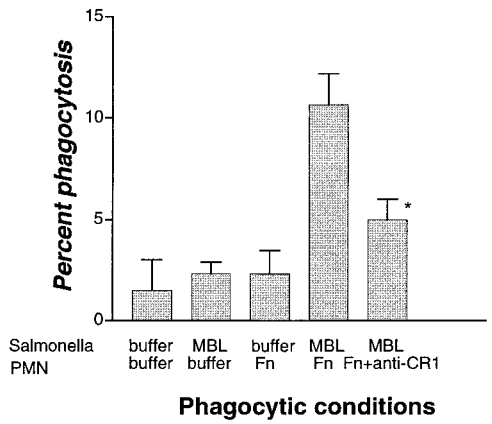


**Figure 9.** Effect of fibronectin pretreatment on the ability of PMNs to recognize MBL-opsonized bacteria. PMNs were pretreated with antibody fractions or buffer, and then incubated with buffer or 50 μg/ml fibronectin (Fn). Subsequently, the PMNs were added to bacteria that had been opsonized with buffer or MBL (1 μg/ml). After a 35-min incubation under routine conditions the cells were pelleted on a slide and stained to allow the determination of the percentage of PMNs that had ingested one or more bacteria. Results represent the mean ± SD values for at least 300 PMNs. $*P < 0.05$. This experiment was repeated with similar results.

were pretreated with anti-CR1 Fab (Fig. 9). These data confirm a role for CR1 as a receptor for MBL.

*Antibody to C1qRp Fails to Inhibit the Ingestion of MBL-opsonized Bacteria.* C1qRp is present on PMNs (18), and it has been described as having a role in MBL-mediated ingestion by monocytes of IgG-C3b/C4b–opsonized targets (38). To test for C1qRp involvement in our assay, PMNs were pretreated with either anti-C1qRp mAb R3, a control IgM mAb, anti-CR1 Fab, or control IgG. The target bacteria were opsonized with buffer, MBL alone, or MBL plus suboptimal amounts of anti–*Salmonella* IgG. Anti-C1qRp had a small and statistically insignificant effect on the ability of PMNs to ingest MBL-IgG–opsonized bacteria (Fig. 10). As in prior experiments, anti-CR1 pretreatment of PMNs did significantly inhibit ingestion compared with a control IgG.

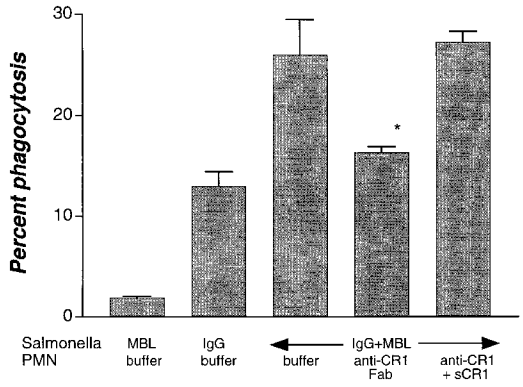


**Figure 8.** Effect of anti-CR1 Fab on the ability of PMNs to ingest MBL-opsonized bacteria. PMNs were incubated with buffer, anti-CR1 Fab (100 μg/ml), or sCR1-absorbed anti- CR1 Fab (100 μg/ml) and mixed with bacteria that had been opsonized under different conditions. After a 35-min incubation under routine conditions the cells were pelleted on a slide and stained to allow the determination of the percentage of PMNs that had ingested one or more bacterium. Results represent the mean ± SD values for at least 300 PMNs. Anti-CR1 pretreatment of the PMNs, compared with pretreatment with either buffer or sCR1-absorbed anti-CR1, significantly inhibited the phagocytosis of MBL-IgG–opsonized bacteria. $*P < 0.05$. This experiment was repeated three times with similar results.

## Discussion

The recognition that CR1 could act as a receptor for C1q suggested that CR1 might also be a receptor for MBL.

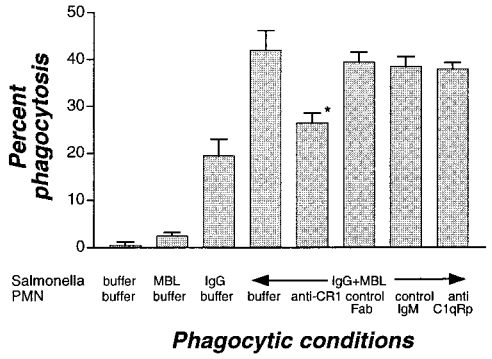


**Figure 10.** Effect of anti-C1qRp on the ingestion of MBL-IgG–opsonized bacteria by PMNs. PMNs were preincubated with either buffer, 100 μg/ml anti-CR1 Fab, 100 μg/ml rabbit nonimmune IgG, 10 μg/ml mAb anti-C1qRp (R3), or 10 μg/ml control IgM, and then mixed with bacteria that had been opsonized with MBL (1 μg/ml) with or without anti–*Salmonella* IgG (0.5 μg/ml). After a 35-min incubation under routine conditions the cells were pelleted on a slide and stained to allow the determination of the percentage of PMNs that had ingested one or more bacteria. Results represent the mean ± SD values for at least 300 PMNs. $*P < 0.05$. This experiment was repeated with similar results.

MBL that was purified from plasma (Fig. 1) retained its lectin activity. $^{125}$I-MBL was able to bind to immobilized sCR1 in the absence of added calcium ions, but the binding was favored by added calcium, with an optimal concentration of 0.3 mM $CaCl_2$ (Fig. 2 A). The fact that 100 mM *N*-acetyl-D-glucosamine had no deleterious effect on MBL-sCR1 binding is strong evidence that the CRD of MBL is not involved (Fig. 2 A). It is not obvious why calcium should have an effect on the MBL binding to sCR1 because the two tightly bound calcium ions are found in the CRD. It is possible that the calcium binding in the CRD affects the conformation of the more removed sCR1-binding site, which by analogy to C1q, is thought to be in the collagen domain of MBL. It is of interest that another collectin, surfactant protein D, displays calcium-dependent binding to its receptor, and that receptor binding is also not antagonized by surfactant protein D's specific sugar ligand, i.e., maltose (39, 40).

In our previous study, immobilized sCR1 provided good binding for labeled C1q, but labeled sCR1 binding to immobilized C1q was barely measurable (22). We interpreted this to mean that C1q had multiple binding sites (probably located in its six identical collagen stems), whereas sCR1 had only one binding site (22). In this study we found that radioiodinated MBL bound to immobilized sCR1 with much higher affinity ($K_d$ of 5.2 nM; Fig. 3, A and B) than radioiodinated sCR1 bound to immobilized MBL ($K_d$ of 45 nM). Again, the likely explanation is that polymeric MBL is a better soluble ligand because it has potentially six identical binding sites, whereas sCR1 has one apparent binding site. This model is consistent with the apparent single binding constant when either MBL or sCR1 was the soluble ligand in binding studies.

The adverse effects of salt on MBL–sCR1 binding were not unexpected; similar effects on C1q binding to sCR1 (22) and C1q binding to CR1-bearing cells have been noted (41–43). What was surprising was the ability of MBL to exhibit measurable binding to immobilized sCR1 under conditions of physiological salt (Fig. 2 B) which could not be demonstrated in equilibrium binding assays using C1q, although it was demonstrated in kinetic binding studies of C1q binding to immobilized sCR1 (22). The $K_d$ of 5.2 nM for MBL binding to sCR1 was in the same range as the $K_{eq}$ (3.9 nM) for C1q binding in physiological salt buffer that was calculated from kinetic binding studies using a BIAcore instrument (22). Thus, the affinity of MBL and C1q for sCR1 is similar, but MBL binding is apparently more stable. The ability of MBL to bind in physiological salt, and then remain bound even when the salt concentration was increased fivefold, is evidence for this (Fig. 2 B, right column). Thus, the association phase of binding is very salt sensitive, whereas the dissociation phase is much less salt sensitive. It will remain to be shown if the more avid MBL binding translates into MBL being a preferred ligand for CR1.

The fact that MBL and C1q compete for binding to sCR1 suggests that the binding site on CR1 is identical or adjacent such that the binding of one ligand provides steric hindrance for the binding of the other (see Fig. 4). We believe the C1q binding site on CR1 is long homologous repeat (LHR)-D (22). Consistent with our results, others have shown that MBL competes with the collagen domain of C1q for binding to PMNs and monocytes (20). When C1q binds to LHR-D, the C4b and C3b binding sites on CR1, namely LHR-A, -B, and -C, respectively (44–46), are still available. Functionally, this allows C1q to participate in additive binding with either C4b or C3b to CR1 (23). By analogy, MBL might recruit C4b or C3b for additive binding to CR1.

In contrast to our results, Bajtay et al. (47) have recently reported that MBL and C1q bound to different cell types: specifically, C1q and MBL both bound to human monocyte–derived macrophages and to monocytoid cell lines, but only C1q bound to B and T cells. Their results showed a small positive subpopulation of T cells, which is consistent with CR1 being expressed on only a subset of T cells (48, 49). We have no explanation for their inability to demonstrate MBL binding to B cells, which do express CR1. They showed that MBL and C1q did not compete for binding to monocytes or THP-1 cells in PBS, whereas we found it necessary to use low ionic strength buffers to demonstrate any significant C1q binding to either cells or purified sCR1 (22). C1q, or trace contaminants in the preparation, may have more promiscuous binding, as we have recently reported that high concentrations of immobilized C1q elicit a superoxide response from PMNs and this response does not involve CR1 (50). As we did, they found that MBL bound well in normal ionic strength and that MBL alone was a poor agonist. In sum, most of the differences in our findings relate to C1q binding and not to MBL binding.

Our experiments with cells provide evidence that MBL binding to recombinant sCR1 immobilized on plastic was relevant to MBL binding to CR1 on cells. MBL binding could be positively associated with CR1 expression on resting and PMA-stimulated PMNs (Fig. 6), although these data do not demonstrate the specificity of the binding. Others have reported a phorbol ester–induced reduction in C1q binding to PMNs, which is analogous to our results with MBL binding (51). The MBL binding was performed in normal ionic strength, emphasizing that cellular CR1 would function in vivo. However, the adhesion experiment with E required low ionic strength to demonstrate E binding to immobilized MBL, possibly due to the fact that the MBL immobilized on plastic may not be in the optimal conformation to interact with cellular CR1. The specificity of the MBL–CR1 binding was emphasized by the ability of anti-CR1 Fab to block E adhesion, whereas a similar concentration of the same Fab preparation that had been specifically absorbed with immobilized sCR1 did not block E adhesion (Fig. 5).

The lack of an effect of anti-ClqRp on PMN phagocytosis is perhaps not surprising (Fig. 10). The phagocytic assay in which C1qRp has been shown to be active requires MBL or C1q to be immobilized on plastic (38), whereas our assay is detecting the ingestion of MBL-opsonized bac-

teria. Furthermore, there are no data for PMNs that C1qRp is directly involved in binding MBL or C1q.

Two lines of evidence strongly support CR1 as the MBL receptor on PMNs. First, MBL behaves like C3b and C4b, the best-characterized CR1 ligands, in terms of not triggering phagocytosis unless either IgG is also bound to the target, or the phagocytic cell is preactivated (48–51). Second, polyclonal anti-CR1 inhibited the effects of MBL, and the inhibition was specifically blocked by absorption of the Fab preparation with soluble or immobilized recombinant sCR1. To date, there are limited examples of MBL alone serving as an opsonin: one involving the phagocytosis of *S. montevideo* by PMNs (12) and the second involving the phagocytosis of influenza virus by PMNs (13). Possible explanations for these findings include substantial activation of the PMNs during purification, or a small amount of contaminating IgG in the MBL sample. We have found it a challenge to remove all the IgG from our MBL preparations.

Our data emphasize that CR1 is a receptor for all the primary opsonins of complement, namely, MBL, C1q, C4b, and C3b. Deficiency of the CR1 ligands MBL, C1q, or C4b is associated with an autoimmune phenotype in humans; however, the mechanism is not understood. Deficiency of C1q is almost invariably associated with severe autoimmune pathology (for a review, see reference 52), whereas deficiency of MBL has a much less severe phenotype, manifest as a predisposition towards infectious and autoimmune complications (for a review, see reference 6). If ligand binding to CR1 were involved in the prevention of an autoimmune phenotype, then our data suggest that the putative autoimmune trigger might bind C1q well but MBL poorly. Alternatively, there may be receptor redundancy for some substances that bind MBL but not for those that bind C1q. For example, particles or antigens that would ordinarily bind MBL, in the presence of MBL deficiency might interact with mannose receptors to prevent autoimmunity (5).

We thank Drs. Henry Marsh and Una Ryan (Avant Immunotherapeutics) for sCR1, Dr. Alan Ezekowitz (Massachusetts General Hospital, Harvard Medical School) for recombinant MBL, and Dr. Andrea Tenner (University of California at Irvine) for mAb R3 (anti-C1qRp). We are grateful to Drs. Greg Stahl and Charles Collard (Brigham and Women's Hospital, Harvard Medical School) for their help with the purification protocol for MBL.

This work was supported by National Institutes of Health grant AI42987 to A. Nicholson-Weller. L.B. Klickstein was supported by grants from the S.L.E. Foundation and the Lupus Foundation of America. S.W. Tas was supported by the Dutch Kidney Foundation. J.C. Jensenius was supported by the Danish Medical Research Council.

*Submitted: 10 November 1999*
*Revised: 23 October 2000*
*Accepted: 30 October 2000*

## References

1. Kawasaki, N., T. Kawasaki, and I. Yamashina. 1983. Isolation and characterization of a mannan-binding protein from human serum. *J. Biochem.* 94:937–947.
2. Drickamer, K., M.S. Dordal, and L. Reynolds. 1986. Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to collagenous tails. *J. Biol. Chem.* 261:6878–6887.
3. Sheriff, S., C.Y. Chang, and R.A. Ezekowitz. 1994. Human mannose-binding protein carbohydrate recognition domain trimerizes through a triple α-helical coiled-coil [published erratum at 3:103]. *Nat. Struct. Biol.* 1:789–794.
4. Lipscombe, R.J., M. Sumiya, J.A. Summerfield, and M.W. Turner. 1995. Distinct physicochemical characteristics of human mannose binding protein expressed by individuals of differing genotype. *Immunology.* 85:660–667.
5. Fraser, I.P., H. Koziel, and R.A. Ezekowitz. 1998. The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity. *Semin. Immunol.* 10:363–372.
6. Turner, M.W. 1996. Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol. Today.* 17:532–540.
7. Weis, W.I., M.E. Taylor, and K. Drickamer. 1998. The C-type lectin superfamily in the immune system. *Immunol. Rev.* 163:19–34.
8. Matsushita, M., and T. Fujita. 1992. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like protease. *J. Exp. Med.* 176: 1497–1502.
9. Thiel, S., T. Vorup-Jensen, C.M. Stover, W. Schwaeble, S.B. Laursen, K. Poulsen, A.C. Willis, P. Eggleton, S. Hansen, U. Holmskov, et al. 1997. A second serine protease associated with mannan-binding lectin that activates complement. *Nature.* 386:506–510.
10. Ikeda, K., T. Sannoh, N. Kawasaki, T. Kawasaki, and I. Yamashina. 1987. Serum lectin with known structure activates complement through the classical pathway. *J. Biol. Chem.* 262:7451–7454.
11. Ohta, M., M. Okada, I. Yamashina, and T. Kawasaki. 1990. The mechanism of carbohydrate-mediated complement activation by the serum mannan-binding protein. *J. Biol. Chem.* 265:1980–1984.
12. Kuhlman, M., K. Joiner, and R.A.B. Ezekowitz. 1989. The human mannose-binding protein functions as an opsonin. *J. Exp. Med.* 169:1733–1745.
13. Hartshorn, K., K. Sastry, M.R. White, E.M. Anders, M. Super, R.A. Ezekowitz, and A.I. Tauber. 1993. Human mannose-binding protein functions as an opsonin for influenza A viruses. *J. Clin. Invest.* 91:1414–1420.
14. Malhotra, R., R.B. Sim, and K.B.M. Reid. 1990. Interaction of C1q, and other proteins containing collagen-like domains, with the C1q receptor. *Biochem. Soc. Trans.* 18:1145–1148.
15. Malhotra, R., S. Thiel, K.B.M. Reid, and R.B. Sim. 1990. Human leukocyte C1q receptor binds other soluble proteins with collagen domains. *J. Exp. Med.* 172:955–959.
16. Kishore, U., and K.B.M. Reid. 2000. C1q: structure, function, and receptors. *Immunopharmacology.* 49:159–170.
17. Nicholson-Weller, A., and L.B. Klickstein. 1999. C1q binding proteins and C1q receptors. *Curr. Opin. Immunol.* 11:42–46.
18. Guan, E., S.L. Robinson, E.B. Goodman, and A.J. Tenner. 1994. Cell-surface protein identified on phagocytic cells modulates the C1q-mediated enhancement of phagocytosis. *J. Immunol.* 152:4005–4016.
19. Bobak, D.A., T.G. Gaither, M.M. Frank, and A.J. Tenner.

1987. Modulation of FcR function by complement: subcomponent C1q enhances the phagocytosis of IgG-opsonized targets by human monocytes and culture-derived macrophages. *J. Immunol.* 138:1150–1158.
20. Tenner, A.J., S.L. Robinson, and R.A.B. Ezekowitz. 1995. Mannose binding protein (MBP) enhances mononuclear phagocyte function via a receptor that contains the 126 Mr component of the C1q receptor. *Immunity.* 3:485–493.
21. Nepomuceno, R.R., A.H. Henschen-Edman, W.H. Burgess, and A.J. Tenner. 1997. cDNA cloning and primary structure analysis of $C1qR_p$, the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis in vitro. *Immunity.* 6:119–129.
22. Klickstein, L.B., S. Barbashov, T. Liu, R.M. Jack, and A. Nicholson-Weller. 1997. Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity.* 7:345–355.
23. Tas, S.W., L.B. Klickstein, and A. Nicholson-Weller. 1999. C1q and C4b are additive ligands for CR1 and mediate erythrocyte adhesion. *J. Immunol.* 163:5056–5063.
24. Lu, J.H., S. Thiel, H. Wiedemann, R. Timpl, and K.B. Reid. 1990. Binding of the pentamer/hexamer forms of mannan-binding protein to zymosan activates the proenzyme C1r2C1s2 complex, of the classical pathway of complement, without involvement of C1q. *J. Immunol.* 144:2287–2294.
25. Klickstein, L.B., W.W. Wong, J.A. Smith, J.H. Weis, J.G. Wilson, and D.T. Fearon. 1987. Human C3b/C4b receptor (CR1). Demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristic of C3/C4 binding proteins. *J. Exp. Med.* 165:1095–1112.
26. Tan, S.M., M.C. Chung, O.L. Kon, S. Thiel, S.H. Lee, and J. Lu. 1996. Improvements on the purification of mannan-binding lectin and demonstration of its $Ca^{2+}$-independent association with a C1s-like serine protease. *Biochem. J.* 319: 329–332.
27. Wilson, J.G., W.W. Wong, P.H. Schur, and D.T. Fearon. 1982. Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus. *N. Engl. J. Med.* 307:981–986.
28. Diamond, M.S., D.E. Staunton, A.R. de Fourgerolles, S.A. Stacker, J. Garcia-Aguilar, M.L. Hibbs, and T.A. Springer. 1990. ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). *J. Cell Biol.* 111:3129–3139.
29. Fearon, D.T., and L.A. Collins. 1983. Increased expression of C3b receptors on polymorphonuclear leukocytes by chemotactic factors and by purification procedures. *J. Immunol.* 130: 370–375.
30. Berger, M., J. O'Shea, A.S. Cross, T.M. Folks, T.M. Chused, E.J. Brown, and M.M. Frank. 1984. Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. *J. Clin. Invest.* 74:1566–1571.
31. Sengelov, H., L. Kjeldsen, W. Kroeze, M. Berger, and N. Borregaard. 1994. Secretory vesicles are the intracellular reservoir of complement receptor 1 in human neutrophils. *J. Immunol.* 153:804–810.
32. Kumar, A., E. Wetzler, and M. Berger. 1997. Isolation and characterization of complement receptor type 1 (CR1) storage vesicles from human neutrophils using antibodies to the cytoplasmic tail of CR1. *Blood.* 89:4555–4565.
33. Changelian, P.S., R.M. Jack, L.A. Collins, and D.T. Fearon. 1985. PMA induces the ligand-independent internalization of CR1 on human neutrophils. *J. Immunol.* 134:1851–1858.
34. O'Shea, J.J., E.J. Brown, T.A. Gaither, T. Takahashi, and M.M. Frank. 1985. Tumor-promoting phorbol esters induce rapid internalization of the C3b receptor via a cytoskeleton-dependent mechanism. *J. Immunol.* 135:1325–1330.
35. Lau, Y.L., S.Y. Chan, M.W. Turner, J. Fong, and J. Karlberg. 1995. Mannose-binding protein in preterm infants: developmental profile and clinical significance. *Clin. Exp. Immunol.* 102:649–654.
36. Pommier, C.G., S. Inada, L.F. Fries, T. Takahashi, M.M. Frank, and E.J. Brown. 1983. Plasma fibronectin enhances phagocytosis of opsonized particles by human peripheral blood monocytes. *J. Exp. Med.* 157:1844–1854.
37. Wright, S.D., L.S. Craigmyle, and S.C. Silverstein. 1983. Fibronectin and serum amyloid P component stimulate C3b- and C3bi-mediated phagocytosis in cultured human monocytes. *J. Exp. Med.* 158:1338–1343.
38. Bobak, D.A., M.M. Frank, and A.J. Tenner. 1988. C1q acts synergistically with phorbol dibutyrate to activate CR1-mediated phagocytosis by human mononuclear phagocytes. *Eur. J. Immunol.* 18:2001–2007.
39. Holmskov, U., P. Lawson, B. Teisner, I. Tornoe, A.C. Willis, C. Morgan, C. Koch, and K.B. Reid. 1997. Isolation and characterization of a new member of the scavenger receptor superfamily, glycoprotein-340 (gp-340), as a lung surfactant protein-D binding molecule. *J. Biol. Chem.* 272:13743–13749.
40. Holmskov, U., J. Mollenhauer, J. Madsen, L. Vitved, J. Gronlund, I. Tornoe, A. Kliem, K.B. Reid, A. Poustka, and K. Skjodt. 1999. Cloning of gp-340, a putative opsonin receptor for lung surfactant protein D. *Proc. Natl. Acad. Sci. USA.* 96:10794–10799.
41. Tenner, A.J., and N.R. Cooper. 1980. Analysis of receptor-mediated C1q binding to human peripheral blood mononuclear cells. *J. Immunol.* 125:1658–1664.
42. Tenner, A.J., and N.R. Cooper. 1981. Identification of types of cells in human peripheral blood that bind C1q. *J. Immunol.* 126:1174–1179.
43. Jack, R.M., B.A. Lowenstein, and A. Nicholson-Weller. 1994. Regulation of C1q receptor expression on human polymorphonuclear leukocytes. *J. Immunol.* 153:262–269.
44. Klickstein, L.B., T.J. Bartow, B. Miletic, L.D. Rabson, J.A. Smith, and D.T. Fearon. 1988. Identification of distinct C3b and C4b recognition sites in the human C3b/C4b receptor (CR1, CD35) by deletion mutagenesis. *J. Exp. Med.* 168: 1699–1717.
45. Krych, M., D. Hourcade, and J.P. Atkinson. 1991. Sites within the complement C3b/C4b receptor important for the specificity of ligand binding. *Proc. Natl. Acad. Sci. USA.* 88: 4353–4357.
46. Makrides, S.C., S.M. Scesney, P.J. Ford, K.S. Evans, G.R. Carson, and H.C.J. Marsh. 1992. Cell surface expression of the C3b/C4b receptor (CR1) protects Chinese hamster ovary cells from lysis by human complement. *J. Biol. Chem.* 267:24754–24761.
47. Bajtay, Z., M. Jozsi, Z. Banki, S. Thiel, N. Theilens, and A. Erdei. 2000. Mannan-binding lectin and C1q bind to distinct structures and exert differential effects on macrophages. *Eur. J. Immunol.* 30:1706–1713.
48. Wilson, J.G., T.F. Tedder, and D.T. Fearon. 1983. Characterization of human T lymphocytes that express the C3b receptor. *J. Immunol.* 131:684–689.
49. Cohen, J.H., J.P. Aubry, J.P. Revillard, J. Banchereau, and M.D. Kazatchkine. 1989. Human T lymphocytes expressing the C3b/C4b complement receptor type one (CR1, CD35)

belong to Fc γ receptor-positive CD4-positive T cells. *Cell. Immunol.* 121:383–390.
50. Tyagi, S.R., A. Nicholson-Weller, S.F. Barbashov, S.W. Tas, and L.B. Klickstein. 2000. Intracellular adhesion molecule 1 and $\beta_2$ integrins in C1q-stimulated superoxide production by human neutrophils. An example of a general regulatory mechanism governing acute inflammation. *Arthritis Rheum.* 43:2248–2259.
51. Bobak, D.A., M.M. Frank, and A.J. Tenner. 1986. Characterization of C1q receptor expression of human phagocytic cells: effects of PDBu and fmlp. *J. Immunol.* 136:4604–4610.
52. Bowness, P., K.A. Davies, P.J. Norsworthy, P. Athanassiou, J. Taylor-Wiedeman, L.K. Borysiewicz, P.A. Meyer, and M.J. Walport. 1994. Hereditary C1q deficiency and systemic lupus erythematosus. *Q. J. Med.* 87:455–464.