Mannan-binding lectin (MBL) constitutes an important part of the human innate immune defense system. It has been shown to mediate the activation of complement upon binding to specific microbial carbohydrate motifs, to directly opsonize organisms, and to enhance the phagocytosis of targets suboptimally opsonized with IgG or complement components C3b or C4b. This enhancement of phagocytic activity induced by MBL and other molecules that contain a collagen-like region contiguous with a pattern recognition domain is mediated by a 126,000 M_r surface glycoprotein, designated C1qR_p. Although it has been known that the collagen-like domain of these “defense collagens” contains the interaction site(s) that triggers this enhancement of uptake, the specific interaction site has not been identified. To address this issue, wild type and mutant MBL constructs were generated, inserted into baculovirus, expressed in SF9 cells, and the recombinant MBL (rMBL) proteins purified by mannan affinity chromatography. The effect of wild type and mutant rMBL on the phagocytosis of targets suboptimally opsonized with IgG or with IgM and C4b by human peripheral blood monocytes was then assessed. Two mutants, one of which has five GXY triplet deletions below the kink region of MBL and the other one having only two of the GXY triplets deleted below the kink, failed to enhance phagocytosis, suggesting the importance of the specific sequence GEKGP in stimulating phagocytic activity. Similar sequences were detected in other defense collagens, implicating the consensus motif GE(K/Q/R)GEP as critical in mediating the enhancement of phagocytosis through C1qR_p. Clarification of specific ligand-C1qR_p interactions should facilitate the investigation of the signal transduction processes involved in the cell activation, as well as provide the basis for the design of specific modulators of the functions mediated by this receptor.

Vertebrates have developed very complicated defense mechanisms, including innate and adaptive immune systems to prevent infection by pathogenic microorganisms. Although effective individually, the complement system and phagocytic cells also interact with each other synergistically to facilitate the innate (first-line) immune defense system. Mannan-binding lectin (MBL), a serum protein of hepatic origin belonging to a family of Ca^2+-dependent collagenous lectins, is an important component of the innate immune system (1, 2). MBL is a multichain molecule of up to six subunits; each subunit consists of three identical 32-kDa polypeptide chains that contain a cysteine rich NH_2-terminal domain, which stabilizes the collagen α-helix of the second domain and a third COOH-terminal carbohydrate-binding domain (3). The collagen-like domain consists of 18–20 repeats of the triplet sequence GXY (where Y is often Pro or Hyp) (3). Human MBL is present in blood as a mixture of oligomers of its subunit with trimer/tetramers and pentamers/hexamers, constituting ~80 and 15% of the pool, respectively. MBL activates the complement system via two serum proteases, MASp-1 and MASp-2 (4–6). This lectin pathway of complement activation leads to complement-dependent lysis by a process that requires C4, C2, C3, and the macro-molecular attack complex (7, 8).

In addition to providing protection to the host through triggering complement activation, MBL is a member of a family of proteins called the defense collagens (9). This group of proteins contains a characteristic NH_2-terminal collagen-like domain as well as a globular carboxyl-terminal domain that includes recognition sites for what Medzhitov and Janeway (10) designated as pathogen-associated molecular patterns. All members of this family, which includes Clq, pulmonary surfactant proteins A and D (SP-A and SP-D), conglutinin, and ficolin (11), have been shown to play a role in defense against potential pathogens (1, 12–14). Previous work has shown that MBL, Clq, and SP-A enhance FcR-mediated phagocytosis by both monocytes and macrophages in vitro and stimulate CR1-mediated phagocytosis in human culture-derived macrophages and in phorbol ester-activated monocytes (15, 16); this enhancement is mediated via a 126,000 M_r surface glycoprotein designated C1qR_p (16, 17).

It has been shown earlier that the enhancement of phagocytic activity is mediated via the collagen-like fragment of Clq, as the isolated pepsin-resistant fragments, but not the pepsin-sensitive collagenase-resistant fragments, retain full activity (18). Thus, it has been hypothesized that common structural features of the collagen-like domains may provide a basis for this biological function of MBL, Clq, and SP-A. In addition, it was known that Clq in complex with C1r and C1s had no ability to enhance phagocytosis, suggesting that the regions above or below the kink region (where C1r and C1s associate above or below the kink region (where C1r and C1s associate...
with C1q) would be likely candidates for the receptor interaction domain.

This study was undertaken to determine the exact interaction site in the collagen-like domain of these defense collagens that mediates the enhancement of phagocytosis. A region containing a common amino acid sequence was noted when sequences of MBL and C1q near the kink region were examined. We used the baculovirus expression system for generating recombinant MBL and various mutants of MBL. The effect of the purified rMBL and mutant MBL on the FcR and CR1-mediated phagocytosis by monocytes was then assessed. Two of the mutants constructed with a common deletion of two GXY triplets below the kink did not enhance phagocytosis, whereas wild type rMBL and other mutants retained activity. These data provide evidence that this sequence is necessary for the C1qRp-mediated enhancement of phagocytosis.

EXPERIMENTAL PROCEDURES

Media, Reagents, and Antibodies—Pfu polymerase was purchased from Stratagene (La Jolla, CA). Restriction enzymes and RPMI were purchased from Life Technologies, Inc. Ex-Cell™ X401 was purchased from Stratagene (La Jolla, CA). Restriction enzymes and RPMI were provided evidence that this sequence is necessary for the C1qRp-phagocytosis by monocytes was then assessed. Two of the mutants constructed with a common deletion of two GXY triplets below the kink did not enhance phagocytosis, whereas wild type rMBL and other mutants retained activity. These data provide evidence that this sequence is necessary for the C1qRp-mediated enhancement of phagocytosis.

Experimental Procedures

FIG. 1. Schematic flow sheet for generation of mutant human recombinant MBL plasmids. PCR products were engineered such that the desired mutant MBL would be created upon incorporation of the sequences into Bluescript KS+. pBS was used to construct the mutant sequences, which were then cloned into the baculovirus vector pVL1393 for subsequent expression in Sf9 cells (see “Experimental Procedures”).

The PCR B product for each mutant was first cloned into Bluescript KS+. The PCR A fragment and the pBS-PCRB clones were then digested by SmaI and ligated to form each mutant construct. For all mutant constructs the orientation of the PCR A insert was determined by restriction mapping and the full-length recombinant human MBL (rMBL) cDNA was sequenced and then introduced into the baculovirus transfer vector, pVL1393 (PharMingen, San Diego, CA) according to the manufacturer’s instructions.

Expression and Purification of the Recombinants MBL Proteins—The recombinant vectors were purified and then co-transfected with linear AcNPV DNA into Sf9 cells. Transfection was performed as described in the Baculovirus Expression System Manual from PharMingen. Recombinant baculovirus were amplified into high titer stocks. For protein expression, the recombinant virus stock was used to infect Sf9 cells, and the cells were cultured at 27 °C for 5 days.

Recombinant proteins were secreted from infected Sf9 cells in serum-free Ex-Cell™ 401 medium in the presence of 0.3 mM L-ascorbic acid to enhance the hydroxylation of lysine and proline residues (25). The supernatants were extensively dialyzed against TBS-T Ca2+ (50 mM Tris, 1 mM NaCl, 0.05% Tween 20, 20 mM CaCl2, pH 7.8) and then incubated with agarose-mannan beads (Sigma) for 2 h at 4 °C. After washing, proteins were eluted with TBS-T EDTA (50 mM Tris, 1 mM NaCl, 0.05% Tween 20, 10 mM EDTA, pH 7.8). The eluted fractions were recalcified to 40 mM CaCl2 and assayed by ELISA (see below) for MBL, and the positive fractions were pooled. The procedure was repeated on a second mannann affinity column to enhance purity (26). The eluted recombinant proteins were concentrated using Centricon 30 (Millipore, Bedford, MA). Proteins were then subjected to SDS-polyacrylamide gel electrophoresis (PAGE) under reducing (50 mM dithiothreitol) and nonreducing conditions and the silver-stained gel compared with Western blot patterns to determine purity.

ELISA for MBL—MBL concentrations were assessed using a sandwich ELISA. Briefly, microtiter plates were coated with polyclonal anti-MBL antibody at a concentration of 10 μg/ml (100 μl/well) in
coating buffer (0.1 x carbonate buffer, pH 9.6) and incubated overnight at 4 °C. The microtiter plates were washed three times with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) and blocked for 1 h at 37 °C by adding 200 µl of PBS containing 3% milk to each well. Samples and MBL standards were loaded into wells in duplicate, incubated at room temperature for 2 h and washed as described before. Monoclonal anti-MBL (2 µg/ml) diluted in PBST-1% milk (100 µl/well) was added to each well and incubated at room temperature for 1 h. After washing, plates were incubated with horseradish-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 min at room temperature. Color was developed using OPD as the substrate and the absorbance at 405 nm read on a microplate reader ( Molecular Devices, Menlo Park, CA). Serum MBL standard, kindly provided by Prof. Kawasaki (Kyoto University, Kyoto, Japan), was used to calibrate our plasma standard.

Circular Dichroism (CD)—Circular Dichroism was recorded using a Jasco J720 spectropolarimeter. Data were collected at 0.5-nm intervals, and four scans were averaged but not smoothed. A cell of 1-mm path length was used. Protein concentration used was 1 mg/ml in TBS-T 0.1 M carbonate buffer (pH 9.6) and incubated overnight at 37 °C, 5% CO2. The microtiter plates were washed three times with PBS containing 3% milk to each well. Samples and MBL standards were loaded into wells in duplicate, incubated at room temperature for 2 h, and washed as described before. At 37 °C, 5% CO2, targets were then added (105/100 µl) and incubated for 30 min at 37 °C, 5% CO2 to allow phagocytosis to occur. Although monoclonal readily bind and ingest IgG-opsonized particles and avidly bind C4b-coated particles, these resting phagocytes do not ingest particles bound via CR1 unless activated with phorbol esters (28). Therefore, in the experiments in which monoclonal were assessed for CR1-mediated phagocytosis, 10 ng/ml of phorbol dibutyrate was added with the opsonized targets. After removing targeting sequence deleted that were not cell-associated, nonengaged Ea, were lysed in hypotonic buffer. The cells were then fixed and stained, and phagocytosis was quantitated using light microscopy. The number of opsonized targets ingested/100 effector cells is defined as the phagocytic index (PI), whereas the percentage of effector cells (monocytes) ingesting at least one E target is defined as the percent of phagocytosis. In each experiment, duplicate sample wells per condition were used, and ~200 cells were scored per well. All experiments were reproducible with experiments performed on separate days with different donors.

In some experiments, monoclonal (5 x 107/ml) were pretreated for 20 min at room temperature with 20 µg/ml R3 or control mouse monoclonal IgM (Sigma) prior to a 2-fold dilution and the addition of the cells to chambers as described above. Statistical analysis was performed using Sigma Stat, version 2.01, software (SPSS, Chicago, IL). The coating efficiency of all the proteins was checked using a BCA protein assay kit (Pierce).

RESULTS

Bioisosteres and Secretion of Wild Type and Mutant MBLs Expressed in Sf9 Cells—The amino acids deleted for each mutant and their localization within the MBL molecule are presented in Fig. 2. Both wild type and mutant recombinant hMBL proteins were expressed in Sf9 cells and secreted as documented by the presence of immunoreactive MBL protein in culture media. The levels of mutant protein secreted into the cell culture media were similar to that of wild type MBL (Table 1), suggesting that the mutations introduced into the collagen-like domains did not seriously affect the biosynthesis and secretion of MBL under our experimental conditions. ELISA assays demonstrated that the recovery of these proteins (Wt, Del, −2, −3, −5, and A) after purification was 37–56% of the starting material (Table 1). Interestingly, mutant A, which does not have the break in the Gly-X-Y sequence that is proposed to mediate the bend or kink in the collagen domain of the wild type protein, was also expressed suggesting that lack of this structural characteristic has no deleterious effect on expression.

Biochemical Assessment of Purified Recombinant MBL—SDS-PAGE of native and recombinant wild type hMBL under reducing conditions revealed that native hMBL migrates as a single band with an apparent molecular mass of 29 kDa, whereas rhMBL appears with a slightly lower apparent molecular mass under the same conditions (Table 1). This difference in molecular mass between native and recombinant wild type protein may be attributable to differences in glycosylation between mammalian cells and insect cells (29) and/or the lack of hydroxylation of proline residues. (An additional protein band with an apparent molecular mass of 24 kDa is reactive with anti-MBL in Western blots; this is presumed to be a proteolytic fragment of MBL.))

SDS-PAGE of rMBL and its various mutants under nonre-
The data in Fig. 5 demonstrate that wild type rMBL produced in insect cells mimics the enhancing effect of C1q on FcR-mediated phagocytosis of suboptimally opsonized targets. The phagocytic activity was increased in a dose-dependent manner above that seen with cells adhered to the control protein HSA when measured both as an increase in the percentage of cells ingesting the target particles (Percent Phagocytosis, Fig. 5A) and as an increase in the number of targets ingested per cell (Phagocytic Index, Fig. 5B). Three of the MBL mutants (A, Del, and −3) were equivalent to wild type MBL in enhancing FcR-mediated phagocytosis. In contrast, mutants −5 and −2 failed to enhance FcR-mediated phagocytosis (Fig. 5). These findings were reproducible in that although the absolute amount of phagocytosis relative to the basal level seen with HSA varied among the experiments, wild type and mutants A, Del, and −3 MBL consistently augmented phagocytosis to the same degree as C1q, whereas the mutant MBL proteins −5 and −2 had no such effect. The difference between the ability of wild type MBL and either the −2 or the −5 mutant to enhance the phagocytosis was significant (p < 0.001) as analyzed by Student’s t test (n = 4). Table II presents 10-fold enhancement of the percent phagocytosis and phagocytic index mediated by the different proteins relative to the HSA control (using a coating solution of 8 µg/ml) from four different experiments.

Although the failure in mutants −2 and −5 to enhance phagocytosis could be because of the deletions in these mutants, an alternative possibility is that it was because of a difference in the coating efficiency of mutants −2 and −5 relative to the other active proteins, resulting in reduced levels of the proteins adhering to the well. To investigate this possibility, the amount of each MBL protein adhered to the wells was determined. The data in Table III show that approximately equal amounts of wild type and mutant proteins were bound per well for all proteins tested (n = 3). Thus, the common amino acid sequence deleted in MBL mutants −2 and −5 (GEKGEP) appears to be critical for enhancing phagocytosis.

**MBL (−2) and MBL (−5) Lack Ability to Enhance CR1-mediated Phagocytosis**—In addition to FcR, there are a number of cell surface molecules present on various phagocytes that bind to and facilitate ingestion of foreign particles. One such receptor is CR1. To determine whether the sequence identified by the −2 and −5 mutants that is important for enhancing FcR-mediated phagocytosis is also critical for enhancing CR1-mediated phagocytosis, we tested whether targets opsonized with IgM and C4b (such that they are bound by the C3b/C4b receptor, CR1) were also ingested to a similar degree upon interaction of the phagocytes with MBL or various mutants of MBL. Thus, monocytes were adhered to the wells coated with HSA, C1q, and different mutants of rMBL, EA IgMcC4b targets were added to the wells, and phagocytosis assessed. Similar to FcR-mediated phagocytosis, rMBL and mutants −3, A, and Del augmented CR1-mediated phagocytosis to roughly the same extent as C1q. In contrast, mutants −5 and −2 failed to enhance either the percentage of cells ingesting the target particles or the number of targets ingested per cell (Fig. 6).

**Monoclonal Antibody R3 Inhibits MBL-mediated Enhancement of Phagocytosis**—Previous experiments from our laboratory had shown that anti-C1qRc monoclonal antibody was able to inhibit both C1q- and MBL-mediated enhancement of phagocytosis (16). To verify the involvement of C1qRc in the enhancement of phagocytosis by rMBL and its various mutants, monocytes were pretreated with buffer, R3 (anti-C1qRc), and control IgM antibody for 15 min prior to their addition to wells coated with HSA, C1q, rMBL, or MBL mutants, and phagocytosis was assessed. R3 antibody inhibited the number of monocytes engaged in phagocytosis (Fig. 7A) and the number of
Fig. 4. Deletion of specific GXY triplets does not alter the collagen-like secondary structure. Circular dichroism spectra of the −2, −5, and Del MBL were compared with the wild type MBL. Circular dichroism spectra are presented as millidegrees of ellipticity. Data were collected at 0.5-nm intervals, and the reported results are the average of 4 scans. The protein concentration used was 1 mg/ml in TBS-T Ca^{2+}.

Fig. 5. MBL (−2) and MBL (−5) fail to enhance FeR-mediated phagocytosis. LabTek chambers were precoated with 200 μl of 2, 4, 8, and 16 μg/ml HSA, C1q, or different recombinantly expressed MBL proteins. Human monocytes were then adhered to the LabTek chambers. After 30 min of adherence, suboptimally opsonized EAαG were added, and phagocytosis was assayed after 30 min. A, the percentage of monocytes ingesting at least one EAαG target. B, the number of targets ingested/100 monocytes (Phagocytic Index). Values presented are the mean ± S.D. of duplicate samples from one of four similar experiments.

The percent of monocytes triggered by C1q, wild type rhMBL, and −5 mutant MBL to phagocytose targets was inhibited by 76.9 ± 2.6, 72.0 ± 3.9, and 73.9 ± 2.7%, respectively, in the presence of anti-C1qR, whereas the average inhibition of phagocytic index by R3 was 89.1 ± 14.8% for C1q, 68.7 ± 13.4% for wild MBL, and 60.9 ± 6.9% for the −3 mutant of MBL. Inhibition of enhancement of phagocytosis was not observed in parallel samples incubated with control IgM, nor was there any effect of the R3 antibody on basal phagocytosis seen with cells adhered to HSA or mutants MBL −2 or −5. These data indicate that the enhancement of phagocytosis induced by C1q and the active rMBL proteins requires C1qR function.

DISCUSSION

The generation of rMBL and its mutants provided the reagents for defining the critical interaction site necessary for C1qR-mediated enhancement of phagocytosis. In this study we have shown that rMBL generated in the baculovirus expression system is functionally active in enhancing FeR- and CR1-mediated phagocytosis. Using the recombinant mutant proteins, we have demonstrated that the removal of the sequence GEGEP (amino acids 37–42) from the collagen-like domain of MBL abolishes the ability of MBL to enhance phagocytosis. These data suggest that these two GXY triplets are critical for the interaction of MBL with cells leading to the enhancement of phagocytosis.

MBL, SP-A, and C1q, all of which have been shown to increase the phagocytic activity of cells of myeloid lineage via C1qR, have similar macromolecular structures with a globular COOH-terminal “recognition” domain that mediates binding to specific sugar or lipid moieties or, in the case of C1q, the Fc domain of specific immunoglobulin molecules and select pathogens (30). The contiguous collagen-like domain has a characteristic interruption in the GXY repeat pattern and hydroxylated amino acids. Previously reported studies have shown that the C1q cell interaction site that mediates enhanced phagocytic capacity is located in the collagen-like portion of the C1q molecule (18, 31). However, because intact C1q has not been successfully recombinantly expressed in any system, further localization of the site in the collagen-like domain of defense collagens that enhances phagocytosis with C1q was not possible. Because functional MBL has been generated in recombinant expression systems, we chose to use MBL as the model C1qR ligand and to construct various mutants using the baculovirus expression system.

Phagocytosis assays performed using these mutants revealed that wild type MBL, mutant A, and mutant Del enhanced both FeR- and CR1-mediated phagocytosis in a manner similar to C1q, whereas the −5 mutant, which had five GXY triplets deleted below the “kink,” did not. Construction of two mutants, one with a deletion of three of those five GXY triplets (mutant −3) and the second with the two other triplets deleted (mutant −2), allowed the localization of the critical interaction region in that the −3 mutant retained the wild type MBL ability to enhance phagocytosis, whereas the −2 mutant showed no ability to enhance phagocytosis. In contrast, a mutant with two different GXY triplets deleted above the kink
These data together suggest that: 1) the deletion of any two
implicated sequence (GEKGEP) also showed no loss of activity.
In addition, deletion of the coding sequence for the two amino
acids responsible for the kink in the collagen-like structure of
MBL adjacent to the kink in the collagen-like structure of MBL
showed that all of the mutants have a similar macromolecular
structure, which was confirmed by similar sucrose density gra-
dient centrifugation profiles. In addition, CD analysis demon-
strated that mutants have similar secondary structure, and
dient centrifugation profiles. In addition, CD analysis demon-
strated that mutants have similar secondary structure, and
thus loss of phagocytic activity of Del); 2) deletion of triplets
below the kink does not necessarily nonspecifically destabilize
the collagen-like structure necessary for functional cell interac-
tion (mutant –3); and 3) changes at or near the kink region are
not particularly more likely to induce loss of function (mutant
A). Thus, the sequence GEKGEP in the MBL molecule appears to
be critical for the enhancement of phagocytosis.

SDS-PAGE analysis of rMBL and its various mutants
showed that all of the mutants have a similar macromolecular
structure, which was confirmed by similar sucrose density gra-
dient centrifugation profiles. In addition, CD analysis demons-
trated that mutants have similar secondary structure, and
thus loss of phagocytic activity of –2 and –5 mutants is not due
to the loss of secondary structure of the molecule. Furthermore,
no statistical difference among the coating efficiencies of native
and mutant MBL proteins was detected, suggesting that the
failure of mutants –2 and –5 to enhance phagocytosis is not due
to a decreased density of protein on the plates.

As reported previously, a monoclonal anti-C1qR P inhibited

Fig. 6. MBL mutants –2 and –5 fail to enhance CR1-mediated
phagocytosis. Human monocytes were added to LabTek chambers
that had been precoated with 8 or 16 µg/ml HSA, C1q, or various
mutants of rMBL. After 30 min of adherence, EA IgG C4b were added,
and phagocytosis was assayed as described under “Experimental-Pro-
duces.” A, the percentage of monocytes ingesting at least one
EA IgM C4b target. B, the number of targets ingested/100 mono-
cytes. Error bars indicate the S.D. of duplicate samples.

Fig. 7. Monoclonal anti-C1qRP, antibody, R3, inhibits C1q/
MBL-mediated enhancement of phagocytosis. Purified monocytes
were preincubated for 15 min at room temperature with buffer, R3
(anti-C1qRP), or control IgM prior to being added to chambers precoated
with HSA, C1q, or various rMBL (8 µg/ml). After 30 min of adherence,
suboptimally opsonized EA IgM targets were added, and phagocytosis
was assayed after 30 min. A, the percentage of monocytes ingesting at
least one EA IgM target. B, the number of targets ingested/100 mono-
cytes. Error bars indicate the S.D. of duplicate wells.

TABLE II

| Protein bound/well (µg) | 8 µg/ml | Mean ± S.D. | Range |
|------------------------|---------|-------------|-------|
| HSA                    |         | 0.6 ± 0.23  | 0.45–0.86 |
| C1q                    |         | 1 ± 0.05    | 0.97–1.1 |
| MBL (wild)             |         | 0.97 ± 0.17 | 0.78–1.1 |
| MBL (A)                |         | 0.8 ± 0.07  | 0.76–0.89 |
| MBL (Del)              |         | 0.88 ± 0.31 | 0.52–1.1 |
| MBL (–3)               |         | 0.85 ± 0.58 | 0.41–1.5 |
| MBL (–2)               |         | 0.77 ± 0.08 | 0.68–0.83 |
| MBL (–5)               |         | 0.67 ± 0.17 | 0.61–0.79 |

TABLE III

| Protein bound/well (µg) | 8 µg/ml | Mean ± S.D. | Range |
|------------------------|---------|-------------|-------|
| HSA                    |         | 0.6 ± 0.23  | 0.45–0.86 |
| C1q                    |         | 1 ± 0.05    | 0.97–1.1 |
| MBL (wild)             |         | 0.97 ± 0.17 | 0.78–1.1 |
| MBL (A)                |         | 0.8 ± 0.07  | 0.76–0.89 |
| MBL (Del)              |         | 0.88 ± 0.31 | 0.52–1.1 |
| MBL (–3)               |         | 0.85 ± 0.58 | 0.41–1.5 |
| MBL (–2)               |         | 0.77 ± 0.08 | 0.68–0.83 |
| MBL (–5)               |         | 0.67 ± 0.17 | 0.61–0.79 |

"-Fold enhancement of percent phagocytosis and phagocytic index were calculated relative to human serum albumin (i.e. HSA = 1; n = 4).
the stimulation of phagocytic activity by the active MBL mutants (Fig. 7), indicating that the assessed activity is mediated through C1qRp. The antibody had no effect on the base-line phagocytosis when cells were assayed in the presence of human albumin or when mutants −2 and −5 were the proteins coated on the well. Examination of the amino acid sequence of other defense collagens that have been shown previously to enhance phagocytosis via C1qRp revealed that human SP-A and rat and mouse MBL had the identical sequence, GEKGEP (Fig. 8), although the sequence in SP-A was found at a considerable distance above the kink rather than below it. In contrast, the A chain of human and mouse C1q contains a sequence that is similar but not identical to the MBL sequence in that the lysine chain of human and mouse C1q contains a sequence that is far from the kink rather than below it.

Interestingly, five of the six MBL molecules studied, including the inactive −2 and −5 mutants, have the sequence GQKGDP within their collagen-like domains. Thus, four of the amino acids (GXGXGP) in this motif are not sufficient for activity, suggesting that the two negatively charged amino acids (GEKGEP) are critical for the functional interaction. In addition, the presence of this motif with the conservative substitutions of Gln and Asp for the glutamic acid residues suggests that the two acidic residues in the middle of the triplets in this identified active motif are critical for induction of this function, although location within the protein structure has not been eliminated as a contributing factor. (It should also be noted that there is no evidence that this similar 6-amino acid sequence contributes to activity, because mutant Del lacks the GQK and yet retains full activity.) As shown in Fig. 8, the murine and human C1q molecules have the conservative substitutions Gln and Arg for the Lys in position 3 of the motif, and thus the lysine residue has some flexibility. Taken together, the data implicate the negatively charged residues (Glu) at positions 2 and 5 of the identified motif as critical in some way for the functional interaction of MBL with the cell. Although it is established that these six amino acids are required for activity, it remains to be established whether only one or both glutamic acid residues are required, whether the requirement is only for an acidic amino acid in both positions, and whether any of the remaining amino acids contribute to the enhancement of phagocytosis. A model of this region of MBL based on the known crystal structure of a synthetic collagen peptide (32) presented in Fig. 9 demonstrates a possible orientation of the residues in this motif.

It should be noted that this sequence and location differ from the sequence identified as critical for the interaction of C1q with neutrophils that leads to the induction of superoxide production in vitro (33, 34). This is consistent with other observations supporting the hypothesis that the cell surface structures involved in the interaction of C1q that leads to enhancement of phagocytic activity is somehow critically different from that which leads to the induction of neutrophil NADPH oxidase activity and thus superoxide generation (35, 36).

C1qRp was first detected on professional phagocytic cells such as peripheral blood monocytes, neutrophils, umbilical cord endothelial cells, microglia, and myeloid cell lines (35, 37, 38). However, recent reports investigating the tissue expression of this receptor suggest that other cell types such as endothelial cells (39, 40), early hematopoietic cells in the fetal mouse (41), and NK cells in rat (42) also express C1qRp, indicating that C1qRp may be important in hemopoietic and vascular development and other cell-cell or cell adhesion events apart from regulating phagocytosis. As the role of C1qRp in these other cell types is defined, it will be important to determine whether this consensus motif for enhancement of phagocytosis is also required for the functional responses of these distinct cell types.

In summary, our results demonstrate that a conserved amino acid motif of the collagen-like domains of the defense collagens, GE/KQ/RGEP, is necessary for C1qRp-induced enhancement of FcR- and CR1-mediated phagocytosis in human monocytes. Future studies involving both site-specific mutagenesis and the synthesis of specific peptides that form a collagen-like triple helix would allow further resolution of the molecular interaction mechanism, as well as the determination of whether this peptide motif is sufficient to induce enhancement of phagocytosis. The ability to modulate phagocytosis, a powerful effector mechanism of the innate immune system, could be particularly beneficial in the early stage of infection.

**Fig. 8.** Comparison of Sequences of MBL, SP-A, and C1q in different species. The amino acid (AA) position number is shown as a subscript at the far left of each sequence.

**Fig. 9.** Molecular model of residues 37–42 of MBL. The MBL residues were superimposed on the coordinates of the three-dimensional structure of a collagen triple helix peptide consisting of proline-hydroxyproline-glycine repeats (32) using the Biopolymer module in the Biosym Insight molecular modeling package. The amino acid side chains were rotated to match the well established favored rotamers for side chains.
This capacity might limit disease from infection, while allowing the adaptive response to be induced for future rapid and specific protective immunity.

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REFERENCES

1. Holmskov, U., Malhotra, R., Sim, R. B., and Jensenius, J. C. (1994) Immunol. Today 15, 67–74
2. Kawasaki, N., Kawasaki, T., and Yamashina, I. (1983) J. Biochem. 94, 937–947
3. Drickamer, K., Dordal, M. S., and Reynolds, L. (1986) J. Biol. Chem. 261, 6878–6887
4. Takada, F., Takayama, Y., Hata, S., and Kawakami, M. (1993) Biochem. Biophys. Res. Commun. 196, 1093–1098
5. Sato, T., Endo, Y., Matsushita, M., and Fujita, T. (1994) Int. Immunol. 6, 665–669
6. Thiel, S., Vorup-Jensen, T., Stover, C. M., Schwabe, W., Laursen, S. B., Poulsen, K., Willis, A. C., Eggleton, P., Hansen, S., Holmskov, U., Reid, K. B. M., and Jensenius, J. C. (1997) Nature 386, 506–510
7. Ji, Y. H., Matsushita, M., Okada, H., Fujita, T., and Kawakami, M. (1998) J. Immunol. 141 No. 12, 4271–4275
8. Ji, Y. H., Fujita, T., Hata, S., Takahashi, A., Matsushita, M., and Kawakami, M. (1993) J. Immunol. 150, 571–578
9. Krieger, M., Arton, S., Ashkenas, J., Pearson, A., Penman, M., and Resnick, D. (1995) J. Biol. Chem. 268, 4569–4572
10. Medzhitov, R., and Janeway, C. A., Jr. (1997) Curr. Opin. Immunol. 9, 4–9
11. Matsushita, M., Endo, Y., Taira, S., Sato, Y., Fujita, T., Ichikawa, N., Nakata, M., and Mizuochi, T. (1996) J. Biol. Chem. 271, 2448–2454
12. Wright, J. R. (1997) Physiol. Rev. 77, 931–962
13. Tenner, A. J. (1999) Curr. Opin. Immunol. 11, 34–42
14. Kohlman, M., Joiner, K., and Ezekowitz, R. A. B. (1989) J. Exp. Med. 169, 1733–1745
15. Tenner, A. J., Robinson, S. L., Borchelt, J., and Wright, J. R. (1989) J. Biol. Chem. 264, 13923–13928
16. Tenner, A. J., Robinson, S. L., and Ezekowitz, R. A. B. (1995) Immunity 3, 485–493
17. Nepomuceno, R. R., Ruiz, S., Park, M., and Tenner, A. J. (1999) J. Immunol. 162, 3583–3589
18. Bobak, D. A., Gault, T. G., Frank, M. M., and Tenner, A. J. (1987) J. Immunol. 138, 1150–1156
19. Tenner, A. J., Lesavre, P. H., and Cooper, N. R. (1981) J. Immunol. 127, 648–653
20. Young, K. B., Ambrus, J. L., Jr., Malbran, A., Fauci, A. S., and Tenner, A. J. (1991) J. Immunol. 146, 3356–3364
21. Zhao, H., and Stahl, G. L. (2000) FASEB J. 15, A685–A685
22. Guan, K., Robinson, S. L., Goodman, E. B., and Tenner, A. J. (1994) J. Immunol. 152, 4005–4016
23. Lionetti, F. J., Hunt, S. M., and Valeri, C. R. (1980) Methods of Cell Separation (Catsimpoolas, N., ed) pp. 141–156, Plenum Publishing Corp., New York
24. Bobak, D. A., Frank, M. M., and Tenner, A. J. (1986) J. Immunol. 136, 4604–4610
25. Ma, Y., Shiida, H., and Kawasaki, T. (1997) J. Biochem. (Tokyo) 122, 810–818
26. Matsushita, M., and Fujita, T. (1992) J. Exp. Med. 176, 1497–1502
27. Bohnsack, J. F., Kleinman, H. K., Takahashi, T., O’Shea, J. J., and Brown, E. J. (1985) J. Exp. Med. 161, 912–923
28. Bobak, D. A., Frank, M. M., and Tenner, A. J. (1988) Eur. J. Immunol. 18, 2901–2907
29. Burdick, M. D., Harris, A., Reid, C. J., Iwamura, T., and Hollingsworth, M. A. (1997) J. Biol. Chem. 272, 24198–24202
30. Cooper, N. R. (1985) Advances in Immunology 37, 151–216
31. Tenner, A. J., and Cooper, N. R. (1980) J. Immunol. 125, 1658–1664
32. Bella, J., Eaton, M., Brodsky, B., and Berman, H. M. (1994) Science 266, 75–81
33. Ruiz, S., Henschen-Edman, A. H., Nagase, H., and Tenner, A. J. (1999) J. Leukocyte Biol. 66, 416–422
34. Ruiz, S., Henschen-Edman, A. H., and Tenner, A. J. (1995) J. Biol. Chem. 270, 30627–30634
35. Tenner, A. J. (1998) Immunobiology 199, 250–264
36. Tenner, A. J. (1989) Behring Inst. Mect. 84, 220–235
37. Webster, S. D., Park, M., Fonseca, M. I., and Tenner, A. J. (2000) J. Leukocyte Biol. 67, 109–116
38. Nepomuceno, R. R., and Tenner, A. J. (1998) J. Immunol. 160, 1929–1935
39. Fonseca, M. I., Carpenter, P. M., Park, M., Palmari, G., Nelson, E. L., and Tenner, A. J. (2001) J. Leukocyte Biol., in press
40. Dean, Y. D., McGreal, E. P., Akatsu, H., and Gasque, P. (2000) J. Biol. Chem. 275, 34382–34392
41. Petrenko, O., Beavis, A., Khaine, M., Kittappa, R., Godin, I., and Lemischka, I. R. (1999) Immunity 10, 691–700
42. Lovik, G., Vaage, J. T., Dissen, E., Szpirer, C., Ryan, J. C., and Bolstad, B. (2000) Eur. J. Immunol. 30, 3355–3362