THE HUMAN MANNOSE-BINDING PROTEIN
FUNCTIONS AS AN OPSONIN

BY MARCELLA KUHLMAN,* KEITH JOINER,‡ AND R. ALAN B. EZEKOWITZ*

*From the *Division of Hematology/Oncology, Harvard Department of Pediatrics,
Children's Hospital and Dana Farber Cancer Institute, Boston, Massachusetts 02115;
and the ‡Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Disease,
National Institutes of Health, Bethesda, Maryland 20892*

Phagocytes play a key role in host defense against bacterial invasion. Bacterial clearance is mediated in large part by phagocytosis via distinct receptors for different isotypes of Ig (FcR) and fragments of the third component of complement (CR, CR3) (reviewed in references 1, 2). Although these receptors recognize antibody- and complement-coated organisms, respectively, they may also directly engage certain microorganisms in the absence of added opsonins (3, 4). Lectin-like molecules on the surface of bacteria can mediate adhesion to phagocyte cell surface glycoproteins (5-8). Conversely, opsonin-independent phagocytosis can also occur via phagocyte lectin-like receptors that recognize specific configurations of carbohydrate on target organisms. The phagocyte lectin-like receptors include the β glucan receptor on monocytes (9) and a 170-kD macrophage-specific mannose receptor (MR)[1] expressed on the surface of tissue macrophages but not on circulating phagocytes (10, 11). Engagement of mannose-rich particles like zymosan by the MR (12) results in engulfment of the particles and the release of biologically active secretory products such as reactive oxygen intermediates (13), arachidonate metabolites (14), and neutral proteinases (15). Although the MR may recognize certain configurations of high mannose glycans expressed on the surface of some bacteria (16-18), yeasts, and fungi (19), experiments performed with *Leishmania donovani* provide the clearest evidence for involvement of the MR in cell binding and uptake of a pathogenic microorganism.

Although circulating monocytes do not express the MR (10), there exists in serum a mannose-binding protein which shares similar binding characteristics and cross reactive epitopes with the MR. The mannose binding protein (MBP) was first isolated from the serum of rabbits (20) and subsequently found in the serum and liver of humans and rodents (21-26). Analysis of the human MBP, like its two rat homologues (27), reveals that the protein is divided into three domains: a cysteine-rich NH_2-terminal domain that stabilizes the α-helix of the second collagen-like domain, and a third COOH-terminal carbohydrate binding region (28). The function of MBP is unknown, although a role in host defense is suggested by its ability to bind yeast mannans (20) and our finding (29) that MBP prevents infection of H9 lymphoblasts

[1] *Abbreviations used in this paper:* MBP, mannose binding protein; MR, mannose receptor.

by HIV by binding to the high mannose glycans expressed on the envelope glyco-protein of HIV (17, 30). Further, MBP synthesis by liver is induced as part of the acute-phase response (28) and its in vitro synthesis also appears to be upregulated by cytokines (Ezekowitz, R. A. B., unpublished data). We hypothesized that MBP would serve an opsonic role in serum and thereby enhance clearance of mannose-rich pathogens by phagocytes.

We investigated this idea using strains of salmonella that vary in the amount of mannose within their cell wall LPS. Enteric Gram-negative bacteria bear LPS con-taining one copy per molecule of lipid A and core oligosaccharide regions, and a variable number of O-polysaccharide repeating units extending from the core-oligosaccharide. In *Salmonella montevideo*, each repeating subunit contains four man-nose residues with one galactose and one *N*-acetylglucosamine (31). Enteric bacteria that lack O-polysaccharide (rough phenotype) are generally avirulent. These organisms are readily cleared from the circulation of experimental animals, are avidly ingested by phagocytes, perhaps due to direct binding to adhesion molecules on the phago-cyte surface, and are directly killed by complement. A critical density of O-poly-saccharide (smooth phenotype) masks cognate epitopes on the outer membrane for cell surface phagocyte adhesion molecules (4), outer membrane–specific bacterio-phages (32), and core-specific antibodies (33). Perhaps more importantly, the O-poly-saccharide confers resistance to complement-mediated killing (reviewed in reference 34). Enteric bacteria bearing O-polysaccharide within LPS are relatively resistant to host defences and therefore provide a physiologically relevant target to test our proposed role for MBP in host defence.

In this report we demonstrate that purified native and recombinant MBP bind to wild-type *S. montevideo* expressing the mannose-rich O-polysaccharide. Interac-tion of MBP with these organisms results in attachment, uptake and killing of the opsonised bacteria by phagocytes. MBP does not bind to a Salmonella mutant that is incapable of synthesizing complete LPS in the absence of added galactose and mannose. However, when this mutant is growth with exogenous D+ galactose and D+ mannose, complete LPS-containing mannose-rich O-polysacchride is synthe-sized, and the organism is readily opsonized by MBP.

Materials and Methods

Reagents. D-mannose, ferricytochrome *c*, D-galactose, yeast mannan, PMA were obtained from Sigma Chemical Co., St. Louis, MO. ^{3}[H]-D-glucose and ^{125}I sodium iodide were ob-tained from Amersham Corp. (Arlington Heights, IL).

Purification of Human MBP. MBP was isolated from serum as described (26, 29). Briefly, plasma from ill patients undergoing plasmapheresis was recalcified to 20 mmol $CaCl_2$ and incubated with mannan-Sepharose beads overnight at 4°C. After extensive washing, the column was eluted with 10 mmol Tris, 2 mmol EDTA and the peak was identified. After recalcification, the pooled samples were passed down a smaller mannan-Sepharose column and eluted with Tris-buffered saline plus 10 mmol calcium chloride (TBS-$CaCl_2$) + 50 mmol D-mannose. The purity of the eluted protein was assessed on an 11% SDS-polyacrylamide Laemmli gel and biological activity assayed by using ^{125}I-labeled mannose-BSA glycoconjugate as de-scribed (35).

Preparation of Cells. Polymorphonuclear leukocytes (PMNs) were isolated from peripheral blood of healthy volunteers and neutrophils were separated on a Ficoll hypaque gradient and were further purified from the pellet by hypotonic lysis of erythrocytes as described (10).

Monocytes were purified on a Ficoll hypaque gradient and further purified from other

mononuclear cells by agglutination at 4°C and passage through a 3-ml column of FCS. Analysis of the population revealed <10% CD3⁺ cells and >90% of cells expressed mature macrophage marker LeuM3. In some experiments, monocytes were further purified by adherence on glass Labtek slides as described elsewhere (10).

Bacterial Strains. Wild-type *Salmonella montevideo* (strain SH5770, tm-mv hybrid rfc 0-6, 7) (provided by Helena Mäkelä, National Public Health Institute, Helsinki, Finland) was cultivated overnight at 37°C in 5 ml of LB broth. The strain produces a mannose-rich *O*-polysaccharide within its LPS (31). The bacterial culture was diluted 1:10 in fresh LB broth and grown in the presence of 50uc/ml [^{3}H]glucose. At midlog phase (OD$_{660}$ = 0.5, 4–5 × 10^8 CFU/ml) the cells were washed three times in HBSS plus 0.1% gelatin containing 10 mmol CaCl$_2$ (GHBSS). The bacteria were then resuspended in 2 ml of GHBSS to a specific activity of 5 × 10^5 cpm/5 × 10^8 bacteria. A mutant of *S. montevideo* (SL5222 mv-P14, lysogen Hbc pmc462, gal E715) (36) was used in some experiments. This strain is deficient in UDP galactose epimerase and phosphomannose-isomerase. When grown in the absence of exogenous galactose or D+mannose, these mutations prevent, respectively, the synthesis of complete core oligosaccharide and *O*-polysaccharide, resulting in production of a rough Rc chemotype isolate. This rough strain is nonpathogenic and susceptible to complement lysis (34). When SL5222 is in the presence of 5 mmol mannose and 5 mmol galactose, complete core oligosaccharide is produced. The *O*-polysaccharide is identical to the wild-type strain SH5770. For experiments reported here, we compared SL5222 grown in the absence or the presence of galactose and mannose. To standardize assays and to prevent piliation, only bacteria in the log phase of growth were used. Electron micrographs of representative samples of bacteria revealed the absence of pili (not shown), thereby avoiding the possible complication of lectin phagocytosis (37).

Binding of MBP to Bacteria. 200 μl of GHBSS containing 10^7 bacteria was prepared. The bacteria were then incubated at 4°C with 20 μg/ml of purified native human MBP or 1 μg/ml of recombinant human MBP (described below). Binding assays were done in the presence or absence of 10 mg/ml of yeast mannan that binds MBP with high affinity and serves as a competitive inhibitor. After incubation for 30 min, the bacteria were washed five times in GHBSS + 10 mmol CaCl$_2$ at room temperature in a microfuge at 1,300 g for 3 min. Thereafter, a 1:1,000 dilution of rabbit anti–human MBP antiserum was incubated with the bacteria for 1 h in PBS + 5% FCS and 10 mmol CaCl$_2$ and then the bacteria were washed three times in GHBSS + 10 mmol CaCl$_2$ plus 0.05% Tween 20. Binding of anti–human MBP to bacteria was detected by a 1:1,000 dilution of goat anti–rabbit IgG conjugated to rhodamine (Fisher Pharmaceuticals, Orangeburg, NY). After incubation, the bacteria were washed five times in GHBSS + 10 mmol CaCl$_2$ plus 0.05% Tween 20, smeared onto glass slides and allowed to air dry. Thereafter, bacteria were fixed with 100% ice cold methanol for 5 min. A rabbit antiserum raised against SH5770 served as a positive control for binding of primary step antibody. Negative controls included the absence of MBP, no first step antibody, and preincubation of MBP with mannan before addition to the bacteria. Numerous fields of triplicate assays were examined by fluorescence microscopy.

Association of Radiolabeled Bacteria with Cells. 10^7 radiolabeled wild-type and mutant strains of bacteria were resuspended in 200 μl of GHBSS + 10 mmol CaCl$_2$ and were incubated with varying concentrations of native MBP ranging from 2 μg/ml to 40 μg/ml at 4°C for 30 min. In all assays some aliquots of MBP were preincubated with 10 mg/ml yeast mannan before addition to the bacteria. The bacteria were then washed three times in GHBSS + 10 mmol CaCl$_2$ for 3 min at room temperature in a microfuge at 1,300 g, resuspended in 50 μl of GHBSS and added to a 1-ml suspension containing either 10^6 PMNs or monocytes (GHBSS). The bacteria and phagocytes were gently rocked at 37°C for 20 min and thereafter the unattached bacteria were removed by five washes using differential centrifugation at 82 g in a microfuge. Before the last wash, an aliquot from each sample was plated on a Labtek slide and cells were adhered for 10 min, fixed in methanol, stained with Geimsa, and scored by light microscopy. To score the cells plated on the Labtek slides, at least 400 cells were counted. The phagocytic index represented the number of attached or ingested particles per 100 PMNs. The pellet from above containing cells and radiolabeled bacteria was then lysed in 100 μl PBS + 0.5% Triton X-100 and the radioactivity was measured in a scintillation

spectometer (Packard Instrument Co., Inc., Downer's Grove, IL). Nonspecific radioactivity of the buffer alone was subtracted. All assays were performed in triplicate.

Bacterial Killing. To assess whether opsonization of *S. montevideo* SH5770 by MBP enhanced the killing of bacteria by PMNs and monocytes, we performed a bacterial killing assay that is a slight modification on a method described by C. Farber and C. Nathan (manuscript in preparation). Bacteria were grown up to midlog phase (OD$_{620}$ = 2.0 × 10^9 CFU/ml) and 3.0 × 10^8 CFU were incubated at 4°C for 30 min with either MBP (10 μg/μl) or MBP (10 μg/μl) + mannan (10 mg/μl). Bacteria, bacteria + MBP, or bacteria + MBP + mannan were washed twice and resuspended in 20 μl of GHBSS and added to 2 × 10^6 PMNs or monocytes that had been plated in a 96-well plate to make up a final volume of 50 μl. Three wells were prepared for each time point and the plate was incubated on a shaking platform at 37°C for either 1 or 2 h. For time 0, control bacteria alone were incubated in triplicate. At the appropriate time, 200 μl of ice cold water containing 0.1% Triton X-100 was added per well. The contents of each well were agitated by repeated pipetting and then 22 μl was removed from each row using a multiloader pipetteman and added to a second microliter plate containing 200 μl of M63 medium (7 g potassium phosphate [dibasic], 3 g potassium phosphate [monobasic], 2 g ammonium sulphate, 0.5 ml of ferrous sulphate solution [1.83 mg/ml]). By repeating this step a fivefold dilution was prepared. Then 20 μl of each serial dilution was plated in duplicate on LB agar plates and incubated overnight at 37°C. Colonies were quantitated in those dilutions that had grown 30 to 300 CFU. The log of CFU/ml was calculated and subtracted from the control which was bacteria alone.

Superoxide Assays. The ability of *S. montevideo* SH5770 opsonized by MBP to trigger superoxide release by 10^6 PMNs or monocytes was measured by a discontinuous assay of superoxide dismutase-inhibitable reduction of ferricytochrome c, as previously described (10), or histochemically by reduction of nitroblue tetrazolium (10).

Preparation of Recombinant Human MBP. A truncated cDNA representing the coding region of the human MBP was inserted into the Eco RI site of the polycistronic expression vector, pMT-ADA (38) (a gift from Dr. R. Kaufman, Genetics Institute, Cambridge, MA 02140). Chinese Hamster Ovary cells were transfected with the vector containing the MBP coding region by protoplast fusion using polyethylene glycol 1000 (J. T. Baker Chemical Co., Phillipsburg, NJ). Transfectants were selected and amplified as described (38). Four clones synthesized recombinant MBP as assessed by RNA analysis and immunoprecipitation of radiolabeled supernatants with antibody to MBP (not shown). Cells from one clone were expanded and grown to confluence in ten 100-mm tissue culture dishes. Thereafter the fetal calf containing medium was removed and the cells were incubated overnight with DME plus 1% BSA. Recombinant MBP was purified from 100 ml of pooled medium collected from the 10 dishes by elution from a mannan-Sepharose affinity column with TBS-CaCl$_2$ and 50 mmol mannose. The purity of the material was assessed by PAGE and immunoblot as described (29).

Results

Human MBP Binds Selectively to Salmonella montevideo SH5770 Bearing Mannose-rich O-antigen Polysaccharide. We first sought to assess MBP binding to wild-type and mutant strains of *S. montevideo* varying in mannose content within LPS. The results shown in Fig. 1 *A* demonstrate that MBP selectively recognizes SH5770, which has a mannose-rich polysaccharide. The binding of MBP is abrogated by preincubation of MBP with mannose-rich yeast mannan (Fig. 1 *C*). Interestingly, if mannan and MBP are added simultaneously to the bacteria, only partial inhibition of MBP binding is observed (not shown). This suggests that the configurations of high mannose on yeast mannan and bacteria are recognized with similar affinity by MBP. The SL5222 mutant of *S. montevideo*, when grown in the absence of galactose and mannose and hence lacking complete core oligosaccharide and all *O*-polysaccharide is not recognized by MBP (Fig. 1 *E*). When grown in the presence of galactose and mannose,
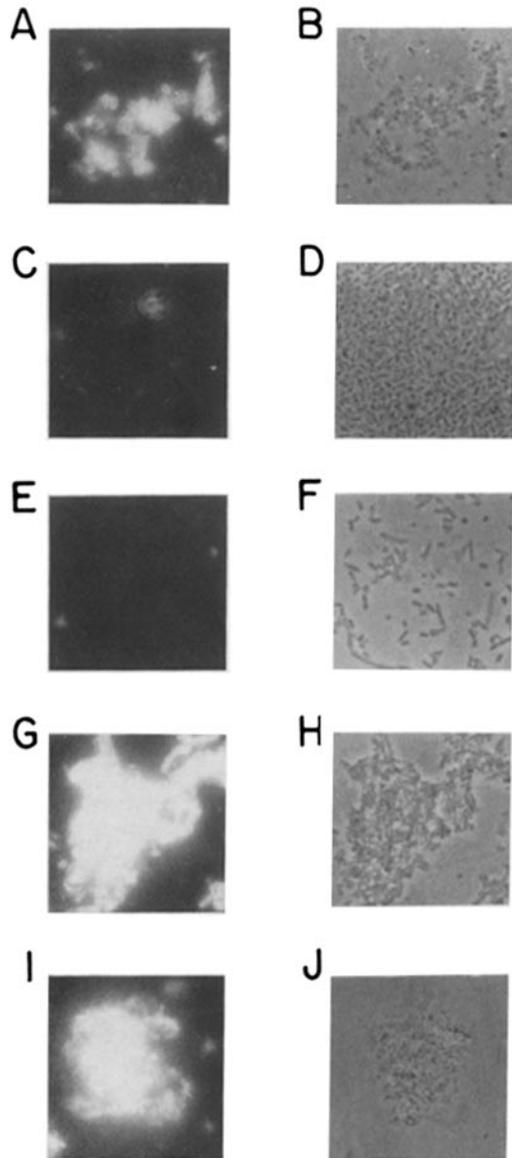
FIGURE 1. Binding of MBP to *Salmonella montevideo*. Binding of MBP was detected by anti-MBP serum and a rhodamine goat anti–rabbit IgG. (*A*) Binding to the wild-type *O*-polysaccharide bearing SH5770 strain. (*C*) Inhibition of MBP binding to SH5770 by 10 mg/ml yeast mannan. (*E*) Lack of binding of MBP to the mutant strain SH5222 grown under conditions (no galactose or mannose) precluding *O*-polysaccharide synthesis. (*G*) MBP binding to SL5222 grown in galactose and mannose, resulting in synthesis of mannose-rich *O*-polysaccharide. (*B*, *D*, *F*, *H*, and *I*) Light micrographs of the same fields. No fluorescence was observed in the absence of MBP or anti-MBP antiserum (not shown). These results are representative of five independent experiments.

however, these bacteria synthesize complete core oligosaccharide and an *O*-polysaccharide identical to wild type bacteria and are recognized by MBP (Fig. 1 *G*). SL5222 grown in galactose alone permitting complete core oligosaccharide synthesis showed dim fluorescence. SH5770 alone in the absence of MBP displayed no specific fluorescence (not shown).

MBP-dependent Binding and Uptake of S. montevideo SH5770 by PMNs. We next sought to determine if MBP mediated binding of mannose-rich *S. montevideo* to neutrophils.
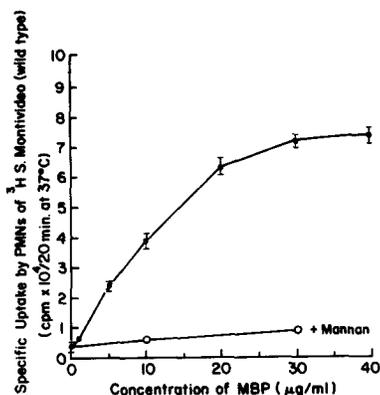
FIGURE 2.   Specific MBP-dependent uptake of radiolabeled *S. montevideo* by neutrophils. (□) Bacteria opsonized with varying concentrations of MBP; (O) inhibition of MBP-mediated opsonization of bacteria by 10 mg/ml of yeast mannan. Results show the mean of triplicate samples for each time point from one experiment and are representative of nine independent experiments.

Approximately 10^7 radiolabeled SH5770 (mannose-rich O-polysaccharide) were preincubated with increasing amounts of MBP and added to 10^6 PMNs. After 20 min at 37°C the non–cell-associated bacteria were removed by differential centrifugation and cell-associated radioactivity was measured. MBP enhanced the binding and uptake of bacteria in a dose-dependent manner that was maximal at 30 μg/ml of MBP (Fig. 2). These results were highly reproducible and are representative of nine independent assays using multiple batches of MBP. Preincubation of MBP with 10 mg/ml yeast mannan competitively inhibited the ability of MBP to bind the bacteria (Fig. 1) and abrogated the opsonic role of MBP. Association of bacteria with cells was also assessed visually on preparations stained with Wrights/Giemsa. Bacteria opsonised by MBP had a phagocytic index (number of cell associated bacteria/100 PMN) of 740 ± 50 compared with 55 ± 15 for bacteria alone. Although the majority of cell associated bacteria appeared to be intracellular, we did not attempt to rigorously distinguish between attached and ingested organisms.

MBP Mediates Binding and Uptake of SL5222 Mutants Only if They Are Grown in the Presence of Exogenous D (+) Mannose and D + Galactose.   The requirement for mannose within the O-polysaccharide for binding of MBP-coated Salmonella to PMN was tested next. SL5222 is a mutant (described in Materials and Methods) which when grown in the absence of exogenous galactose and mannose has a "rough" phenotype and is not recognized by MBP (Fig. 1 *E*). We, like others (4), found that these bacteria bind directly to PMNs but are poorly phagocytosed in the absence of serum (Fig. 3 *A*). In addition, we show that this binding is not enhanced by MBP and is unaffected by mannan (Fig. 3 *A*). However, if SL5222 is grown in the presence of galactose and mannose, the core oligosaccharide is covered by the mannose-rich O-polysaccharide (smooth LPS phenotype) and direct recognition by PMNs is greatly reduced (Fig. 3 *B*). Addition of MBP, which binds directly to SL5222 grown under these conditions (Fig. 1 *G*) enhances bacterial binding and uptake fivefold by PMNs (Fig. 3 *B*). Total uptake in the presence of MBP was similar to that described above for the wild-type SH5770 strain of *S. montevideo*-bearing MBP. The MBP-dependent binding and uptake of bacteria is in large part inhibited by preincubation of MBP with 10 mg/ml of mannan.

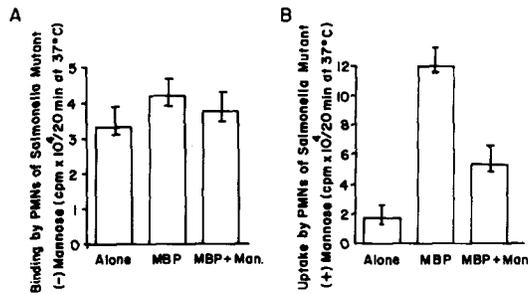*MBP Enhances Uptake and Binding of SH5770 by Monocytes.*   We next assessed the

FIGURE 3. (*A*) MBP does not affect attachment of Salmonella SL5222 grown without mannose and galactose (rough LPS phenotype). (*B*) Salmonella SL5222 when grown in the presence of galactose and mannose, has a mannose-rich *O*-polysaccharide that reduces direct recognition by PMNs. However, 20 µg/ml of MBP enhances binding and uptake of these bacteria. The MBP dependent opsonization is inhibited 50% by 10 mg/ml of yeast mannan. The results shown are the mean determinants of triplicate samples for each treatment from one experiment and are representative of six independent experiments.

MBP-dependent binding and uptake of wild-type *S. montevideo* SH5770 by human monocytes. Fig. 4 shows that 20 µg/ml of MBP significantly enhanced the uptake of SH5770 by monocytes in suspension and this activity was almost entirely abrogated by preincubation of MBP with 10 mg/ml of mannan. In some experiments the monocytes were adhered for 1 h on glass Labtek slides. Thereafter the nonadherent cells were removed and bacteria were added after preincubation with or without MBP in the presence or absence of mannan. Quantitative analysis using radiolabeled bacteria gave similar results (data not shown) to suspension assays, as shown in Fig. 4. There were at least five MBP opsonized bacteria associated with the vast majority (90%) of monocytes, as detected by specific immunofluorescence (Fig. 4, *inset*). No phagocytosis was observed if MBP was omitted.

Recombinant Human MBP Is an Opsonin. The ability of recombinant MBP to serve as an opsonin was tested next. The MBP mRNA in stably transfected CHO cells was amplified several hundredfold when compared with the constitutive levels observed in human hepatoma cells HepG2 (not shown). MBP expressing CHO cells were metabolically labeled with [35S]cysteine and [35S]methionine for 4 h and chased
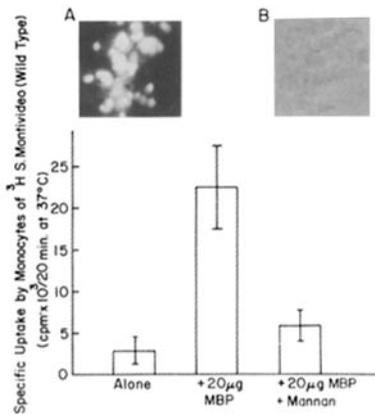

FIGURE 4. Specific uptake by monocytes of radiolabeled *S. montevideo* opsonized by 20 µg/ml of MBP. (*Inset A*) Fluorescence staining of MBP-coated bacteria within monocytes as detected by a rabbit anti-MBP antiserum and a rhodamine-coupled goat anti-rabbit IgG. (Inset *B*) Phase contrast microscopy of the same field. Results shown are the mean of triplicate samples for each treatment from one experiment and are representative of three independent experiments.
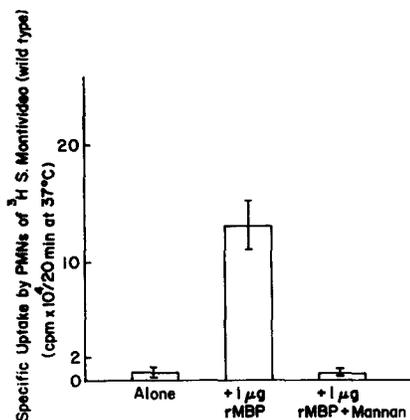
FIGURE 5.   Specific rMBP mediated uptake of *S. montevideo* by monocytes. Bacteria were radiolabeled with [³H]glucose and incubated with rMBP with or without mannan as described in Materials and Methods. The results represent the mean from triplicate samples of one experiment and are representative of three independent experiments.

overnight with medium containing excess unlabeled cysteine and methionine and immunoprecipitation with anti-MBP antibody revealed a 32-kD under protein reducing conditions. Supernatant containing recombinant MBP was collected under serum-free conditions from CHO cells that stably expressed MBP and MBP was purified on a mannan sepharose column. The eluted material co-migrated with native protein purified from serum at an apparent molecular weight of 32×10^3 (not shown).

Like native MBP, recombinant MBP bound specifically to SH5770 (Fig. 1 *I*) and binding was inhibited by 10 mg/µl of yeast mannan (not shown). Radiolabeled bacteria were then preincubated with rMBP with and without mannan and added to PMNs. rMBP, like the native MBP, enhanced the uptake of *S. montevideo* into monocytes (Fig. 5) and activity was totally abrogated in the presence of 10 mg/ml of yeast mannan. The above results indicate that rMBP is functionally equivalent to native MBP in binding to and opsonizing *S. montevideo* for ingestion by PMNs.
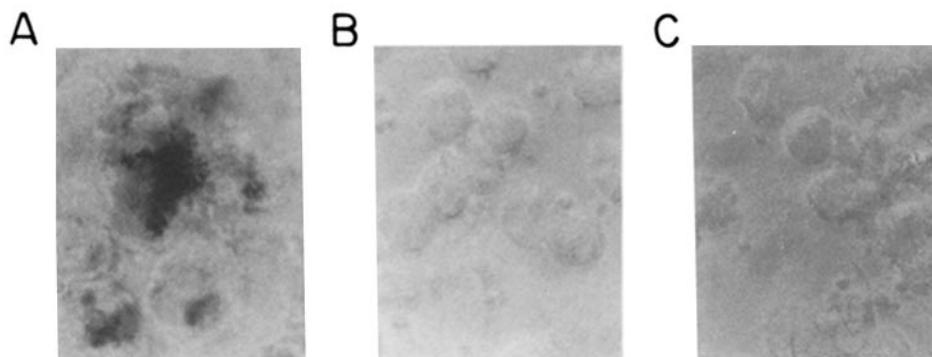

FIGURE 6.   *S. montevideo* opsonized with native MBP mediate reduction of nitroblue tetrazolium by PMNs. (*A*) *S. montevideo* opsonized with MBP triggered NBT reduction by PMNs. Quantitative assays reveal superoxide dismutase inhibitable O_2^- release was 12 ± 3 nmol $O_2^-/10^6$ PMNs for 5 min. (*C*) *S. montevideo* alone failed to trigger PMN reduction of NBT. (*B*) MBP alone failed to trigger PMN reduction of NBT.
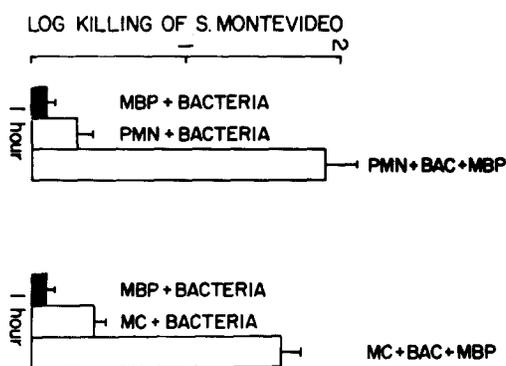
LOG KILLING OF S. MONTEVIDEO

FIGURE 7. Log killing by PMNs and monocytes of bacteria opsonized with native MBP. The ordinate represents the log of CFU/ml on input bacteria (3 × 10^8 CFU/ml) minus the log of CFU/ml of bacteria cultivated for 1 h at 37°C after indicated treatments. MBP + bacteria = 2.5 × 10^8 CFU/ml; PMN + bacteria = 1.5 × 10^8 CFU/ml; PMN + bacteria + MBP = 4.5 × 10^6 CFU/ml; monocytes (mc) + bacteria = 1.7 × 10^8 CFU/ml; MC + bacteria + MBP = 7.5 × 10^6 CFU/ml. The standard deviations are the mean of values of three wells per time point plus or minus the standard deviation. The experiments shown are representative of three independent experiments.

S. montevideo Opsonized with MBP Trigger the Respiratory Burst. The capacity of MBP to elicit an oxidative burst from PMNs was tested. Respiratory burst activity was determined histochemically in individual cells by means of nitroblue tetrazolium reduction assays. *S. montevideo* opsonized with MBP scored positive (Fig. 6 *A*), but no activity was observed when cells were incubated with bacteria alone (Fig. 6 *C*) or MBP alone (Fig. 6 *B*). Quantitative assays of superoxide production by pooled cells revealed that *S. montevideo* opsonized with MBP resulted in the release of 12 ± 3 nmol $O_2/10^6$ PMNs for 5 min, whereas PMNs incubated with either bacteria or MBP alone did not release any detectable superoxide. The maximum release triggered by PMA was 29 ± 2 nmol $O_2/10^6$ PMNs for 5 min.

MBP Enhances Killing of S. montevideo by PMNs. We next determined whether phagocyte killing of MBP opsonized bacteria was enhanced. Opsonization of *S. montevideo* by MBP resulted in a 1.8 ± 0.2 and 1.6 ± 0.1 log killing by PMNs and monocytes respectively after 1 h incubation at 37°C (Fig. 7). In the absence of phagocytes, MBP agglutination of bacteria did occur to some extent. This may account for the small but reproducible reduction in CFU/ml from 3.0 × 10^8 at time 0 to 2.5 × 10^8 CFU/ml at 1 h. There was measurable but low killing of bacteria in the presence of phagocytes and in the absence of MBP. No enhanced killing was observed at the 2-h time point and mannan abrogated the MBP effect (not shown).

Discussion

In this study we demonstrate that purified human MBP and recombinant human MBP bind selectively to wild-type *S. montevideo* that expresses a mannose-rich O-polysaccharide. In contrast, MBP does not bind to nonpathogenic mutants of *S. Montevideo* that lack the mannose-rich O-polysaccharide within LPS. MBP enhances attachment, ingestion, and killing of the bacteria by phagocytes, suggesting that MBP has a role in host defense against pathogens bearing surface-exposed mannose residues.

The selective binding of MBP to the mannose-rich O-polysaccharide of LPS is evident from the experiments with mutant strains of *S. montevideo* that lack the enzymes galactose epimerase and phosphomannose-isomerase. When grown in the absence of exogenous D+ galactose or D+ mannose, these bacteria are unable to assemble polysaccharide side chains (36, 39) on their outer surface and do not bind

to MBP (Fig. 1 *E*). However, if exogenous substrate is added, they assemble a mannose-rich *O*-polysaccharide that is similar to the wild type *S. montevideo* and are recognized by MBP (Fig. 1 *G*).

The *O*-polysaccharide confers resistance to the bacteria against several host defense mechanisms. It masks the determinants on the LPS that mediate direct recognition by phagocytes (4). *O*-polysaccharide decreases access to core-specific antibodies and phages (33, 40) and confers resistance to complement-mediated lysis (39). However, presence of the *O*-polysaccharide is an absolute requirement for substantial MBP opsonization and illustrates the physiological role for MBP in host defense. Killing of the organisms can be explained in large part by the release of reactive oxygen metabolites to which the bacteria are known to be sensitive.

MBP appears to selectively recognize high mannose glycans found on the surface of bacteria, yeasts, and the envelope glycoprotein of certain viruses, such as HIV (41). The engagement of ligand may result in a conformational change of the MBP molecule, like C1q (42), to which it has an analogous structure (27, 28), thereby enabling recognition by cell surface receptor(s). In the absence of ligand, MBP does not bind to the surface of cells implying that the configurations of carbohydrates normally exposed on cell surface glycoproteins are not recognized by MBP. This selectivity contrasts with nonvertebrate mannose lectins, like the plant lectin Con A (43) and mannose-sensitive bacterial adhesins found on the pili of some stationary phase bacteria (37). It is not surprising that the binding specificities of Con A and MBP should differ as these two proteins bear no structural homology (28, 44) to one another and illustrate that the vertebrate host defense molecule has a more defined specificity than its more primitive counterparts, Con A and bacterial adhesins.

As yet we have not identified the cellular receptor or receptors that mediate uptake of MBP opsonized bacteria. The structural analogy of MBP with the first complement component C1q suggests that the C1q receptor (45, 46) may be a likely candidate as are members of the integrin family of molecules to which CR3 belongs (47). Experiments are in progress to assess the relative contributions of these and or other receptor(s) in MBP-mediated phagocytosis.

Although the experiments with MBP reported in this manuscript were performed in the absence of serum, MBP most likely functions in conjunction with other serum molecules in host defense. In particular, MBP is able to enhance complement deposition on mannan-treated erythrocytes (48), suggesting a cooperative role for MBP and complement. Furthermore, MBP enhances complement deposition on mannose bearing isolates of Salmonella (Schweinle, J., A. Ezekowitz, M. Kuhlman, and K. Joiner, manuscript submitted for publication). These results emphasize that in the bloodstream and at inflammatory sites MBP and complement molecules most likely cooperate in opsonization and clearance of particles by phagocytic cells.

This study demonstrates that vertebrate lectin-like proteins that recognize certain configurations of mannose oligosaccharides have a key role in host defense against some microorganisms. MR$^+$ macrophages form a lattice beneath epithelial surfaces guarding the portals of maximum antigen entry. In these and other tissue sites, the MR$^+$ macrophages may bind and ingest pathogens directly. Since circulating phagocytes do not express the MR, the MBP serves an analogous function in the serum. This acute-phase reactant directly mediates recognition and killing of mannose-rich pathogens by neutrophils and monocytes.

Summary

The human mannose-binding protein (MBP) is a multimeric serum protein that is divided into three domains: a cysteine-rich NH2-terminal domain that stabilizes the α-helix of the second collagen-like domain, and a third COOH-terminal carbohydrate binding region. The function of MBP is unknown, although a role in host defense is suggested by its ability to bind yeast mannans. In this report we show that native and recombinant human MBP can serve in an opsonic role in serum and thereby enhance clearance of mannose rich pathogens by phagocytes. MBP binds to wild-type virulent *Salmonella montevideo* that express a mannose-rich O-polysaccharide. Interaction of MBP with these organisms results in attachment, uptake, and killing of the opsonized bacteria by phagocytes. These results demonstrate that MBP plays a role in first line host defense against certain pathogenic organisms.

References

1. Russell, D., and S. D. Wright. 1988. Complement type 3 receptor (CR3) binds to an RGD containing region of the major surface glycoprotein, gp63, of Leishmania promastigotes. *J. Exp. Med.* 168:279.
2. Brown, E. J. 1985. Interaction of grampositive microorganisms with complement. *Curr. Top. Microbiol. Immunol.* 121:159.
3. Salmon, J. E., S. Kapur, and R. P. Kimberly. 1987. Opsonin-independent ligation of Fc Gamma receptors. The 3G8-bearing receptors on neutrophils mediate the phagocytosis of Concanavalin-A treated erythrocytes and non-opsonised Escherichia coli. *J. Exp. Med.* 166:1798.
4. Wright, S. D., and M. T. C. Jong. 1986. Adhesion promoting receptors on human macrophages recognize *Escherichia coli* by binding to lipopolysaccharide. *J. Exp. Med.* 164:1876.
5. Bar-Shavit, Z., I. Olek, R. Goldman, D. Mirelman, and N. Sharon. 1977. Mannose residues on phagocytes as receptors for attachment of *Escherichia coli* and *Salmonella typhi*. *Biochem. Biophys. Res. Commun.* 78:455.
6. Silverblatt, F. J., J. S. Dreyer, and S. Schauer. 1979. Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. *Infect. Immun.* 24:218.
7. Bar-Shavit, Z., R. Goldman, I. Ofek, N. Sharon, and D. Mirelman. 1980. Mannose-binding activity of *Escherichia coli*: a determinant of attachment and ingestion of the bacteria by macrophages. *Infect. Immun.* 29:417.
8. Ofek, I., and E. H. Beachey. 1980. Bacterial adherence. *Adv. Intern. Med.* 25:503.
9. Czop, J. K., and K. F. Austen. 1985. A glucan-inhibitable receptor on human monocytes: its identity with the phagocytic receptor for particulate activators of the alternative complement pathway. *J. Immunol.* 134:2588.
10. Ezekowitz, R. A. B., R. Sim, M. Hill, and S. Gordon. 1984. Local opsonization by secreted macrophage components. Role of receptors for complement in uptake of zymosan. *J. Exp. Med.* 159:244.

11. Ezekowitz, R. A. B., and P. S. Stahl. 1988. The structure-function of vertebrate mannose lectin-like proteins. *J. Cell Sci. Suppl.* 9:121.

12. Sung, S. J., R. S. Nelson, and S. C. Silverstein. 1983. Yeast mannan inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. *J. Cell Biol.* 96:160.

13. Berton, G., and S. Gordon. 1983. Modulation of macrophage mannosyl-specific receptors by cultivation on immobilised zymosan. Effects on superoxide-anion release and phagocytosis. *Immunology.* 49:705.

14. Rouzer, C. A., W. A. Scott, A. L. Hamill, and Z. A. Cohn. 1982. Synthesis of leukotriene C and other arachidonic acid metabolites by mouse pulmonary macrophages. *J. Exp. Med.* 155:720.

15. Palva, E. T., and P. H. Makela. 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Eur. J. Biochem.* 107:116.

16. Sutherland, I. W. 1985. Biosynthesis and composition of gram-negative bacterial extracellular cell wall polysaccharides. *Annu. Rev. Microbiol.* 39:243.

17. Hunter, S. W., H. Gaylord, and P. Brennan. 1986. Structure and antigeneity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. *J. Biol. Chem.* 261:12345.

18. Tonn, S. J., and J. E. Gander. 1979. Biosynthesis of polysaccharides by prokaryotes. *Annu. Rev. Microbiol.* 33:169.

19. Pistole, T. G. 1981. Interaction of bacteria and fungi with lectins and lectin-like substances. *Annu. Rev. Microbiol.* 35:85.

20. Kawasaki, T., R. Eton, and I. Yamashura. 1978. Isolation and characterization of a mannose-binding protein from rabbit liver. *Biochem. Biophys. Res. Commun.* 81:1018.

21. Townsend, R., and P. Stahl. 1981. Isolation and characterization of a mannose/N-acetylglucosamine/fucose binding protein from rat liver. *Biochem. J.* 194:209.

22. Kawasaki, N., T. Kawasaki, and I. Yamashura. 1983. Isolation and characterization of a mannose-binding protein from human serum. *J. Biochem.* 94:937.

23. Wild, J., D. Robinson, and B. Winchester. 1983. Isolation of mannose-binding proteins from human liver. *Biochem. J.* 210:167.

24. Summerfield, J. A., and M. E. Taylor. 1986. Mannose-binding proteins human serum: identification of mannose-specific immunoglobulins and a calcium dependent lectin, of broader carbohydrate specificity secreted by hepatocytes. *Biochem. Biophys. Acta.* 883:197.

25. Mizuno, Y., Y. Kozutsumi, T. Kawasaki, and I. Yamashina. 1981. Isolation and characterization of a mannose-binding protein from rat liver. *J. Biol. Chem.* 256:4247.

26. Maynard, Y., and J. U. Baenziger. 1982. Characterization of a mannose and N-acetylglucosamine-specific lectin present in rat hepatocytes. *J. Biol. Chem.* 257:3708.

27. Drickamer, K., M. S. Dordal, and L. Reynolds. 1986. Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to collagenous tail. *J. Biol. Chem.* 261:6578.

28. Ezekowitz, R. A. B., L. Day, and G. Herman. 1988. A human mannose-binding protein is an acute phase reactant that shares sequence homology with other vertebrate lectins. *J. Exp. Med.* 167:1034.

29. Ezekowitz, R. A. B., M. Kuhlman, J. Groopman, and R. Byrn. 1989. A human serum mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. *J. Exp. Med.* 169:185.

30. Montagnier, L., F. Clavel, B. Krust, S. Chamaret, R. Rey, F. Barre-Sinoussi, and J. C. Chamon. 1978. Identification and antigeneity of the major glycoprotein of lymphoadenopathy associated virus. *Virology.* 144:283.

31. Grossman, M., and L. Lieve. 1984. Complement activation via the alternative pathway

by purified Salmonella lipopolysaccharide is affected by its structure but not its O-antigen length. *J. Immunol.* 132:376.

32. Szmeleman, S., and M. Hofrung. 1975. Maltose transport in *Escherichia coli* K-12: involvement of bacteriophage Lambda receptor. *J. Bacteriol.* 124:112.

33. van der Lay, P., P. de Graeff, and J. Tommassen. 1986. Shielding of *Escherichia coli* outer membrane proteins as receptors for bacteriophages and colicins by O-antigenic chains of lipopolysaccharide. *J. Bacteriol.* 168:449.

34. Joiner, K. A. 1988. Complement evasion by bacteria and parasites. *Annu. Rev. Microbiol.* 42:201.

35. Ezekowitz, R. A. B., J. Austyn, P. D. Stahl, and S. Gordon. 1981. Surface properties of Bacillus Calmette-Guerin activated mouse macrophages: reduced expression of mannose-specific endocytosis, Fc receptors and antigen F4/80 accompanies induction of Ia. *J. Exp. Med.* 154:60.

36. Elbein, A. D., and E. C. Heath. 1965. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. I. The biochemical properties of a uridine diphosphate galactose-4-empimeraseless mutant. *J. Biol. Chem.* 240:1919.

37. Ofek, I., and N. Sharon. 1988. Lectinophagocytosis: a molecular mechanism of recognition between cell surface sugars and lectins in the phagocytosis of bacteria. *Infect. Immun.* 56:539.

38. Kaufman, R. J., P. Murtha, and M. V. Davies. 1987. Translational efficiency of polycistronic mRNAs and their utilization to express heterologous genes in mammalian cells. *Embo* 6:187.

39. Grossman, N., M. A. Schmetz, J. Foulds, E. N. Klima, V. Jiminez, L. L. Leive, and K. A. Joiner. 1987. Lipopolysaccharide size and distribution determine serum resistance in salmonella montevideo. *J. Bacteriol.* 169:856.

40. van der Lay, P., O. Kuipers, J. Tommassen, and B. Lugtenberg. 1986. O-antigenic chains of lipopolysaccharide prevent binding of antibody molecules to an outer membrane pore protein in Enterobacteriaceae. *Microb. Pathog.* 1:43.

41. Mizuochi, T., M. W. Spellman, M. Larkin, J. Solomon, L. J. Basu, and T. Feizi. 1988. Carbohydrate structures of the human immunodeficiency virus (HIV) and recombinant envelope glycoprotein gp120 produced in Chinese Hamster Ovary cells. *Biochem. J.* 254:599.

42. Reid, K. B. M. 1983. Proteins involved in the activation and control of the two pathways of human complement. *Biochem. Soc. Trans.* 11:1.

43. Weinbaum, D. L., J. A. Sullivan, and G. L. Mandell. 1980. Receptors for concanavalin A cluster at the front of polarized neutrophils. *Nature (Lond.).* 286:725.

44. Carrington, D. M., A. Auffret, and D. E. Hank. 1985. Polypeptide ligation occurs during post-translational modification of concanavalin A. *Nature (Lond.).* 313:64.

45. Tenner, A. J., and N. R. Cooper. 1981. Identification of types of cells in human peripheral blood that bind C1q. *J. Immunol.* 126:1174.

46. Ghebrehiwet, B., L. Silvestri, and C. McDevitt. 1984. Identification of the Raji cell membrane-derived C1q inhibitor as a receptor for human C1q. *J. Exp. Med.* 160:1375.

47. Hynes, R. O. 1988. Integrins: a family of cell surface receptors. *Cell.* 48:549.

48. 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.