

Interaction of Mannose-Binding Lectin with HIV Type 1 Is Sufficient for Virus Opsonization But Not Neutralization

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ABSTRACT

Mannose-binding lectin (MBL), a microbe-recognition protein in serum, binds to high mannose glycans on HIV-1 gp120 and has been reported to neutralize the cell line-adapted strain HIV_{IIIB}. Because HIV primary isolates (PI) are generally more resistant to neutralization by antibodies and considering that PI are produced in primary cells that could alter the number of high mannose glycans on HIV relative to cell lines, we assessed the ability of MBL to neutralize HIV PI. MBL at concentrations up to 50 $\mu\text{g/ml}$ mediated relatively little neutralization (<20%) of HIV PI infection of peripheral blood mononuclear cells (PBMCs). MBL-neutralizing activity was slightly higher for cell line-adapted HIV infection of the H9 T cell line (up to 64% at 50 $\mu\text{g/ml}$). However, this effect was specific for H9 cells since MBL did not neutralize cell line-adapted virus infection of PBMCs, HIV PI infection of the GHOST cell line, or VSV pseudotyped with HIV gp160 from cell line-derived virus or PI. In contrast to its low activity in neutralization assays, MBL efficiently bound infectious HIV PI and opsonized HIV PI for uptake by monocytic cells. These results show that both PI and cell line-adapted HIV, despite binding of MBL, are relatively resistant to neutralization by levels of MBL normally present in serum. However, binding and opsonization of HIV by MBL may alter virus trafficking and viral-antigen presentation during HIV infection.

INTRODUCTION

MANNOSE-BINDING LECTIN (MBL) is a member of the collectin family of proteins that contains both collagen-like and C-type lectin domains. MBL is present in serum, binds to carbohydrates on microorganisms, and mediates several innate immune functions including opsonization of microbes through interaction with collagen receptors on phagocytic cells, initiation of the lectin complement pathway, and direct neutralization of some viruses.^{1–3} A number of studies show that MBL deficiency is a risk factor for bacterial infection in situations where immunity is compromised such as in cancer patients and transplant recipients or in infants before full development of antibody responses.^{2–4} There are also reports that AIDS progression is affected by MBL deficiency⁵ and that progression of HIV disease affects the serum levels of MBL.⁶

The gp120 envelope protein of HIV-1 contains a relatively high level of carbohydrate with about half of the molecular mass consisting of N-linked glycans. Approximately half of the N-

linked sites on gp120 are of the high mannose or hybrid type.^{7–9} MBL has been reported to bind to HIV via N-linked carbohydrates on gp120^{10–14} and a recent study shows that MBL binding to recombinant gp120 or intact virus is dependent on the high mannose type N-linked glycans.¹⁴ The MBL/gp120 high mannose glycan interaction is consistent with the known MBL carbohydrate-binding specificity of repetitive terminal mannose residues.¹⁵ MBL was also reported to neutralize the cell line-adapted strain HIV_{IIIB} at levels of the lectin found in serum of most donors (50% neutralization at 1 $\mu\text{g/ml}$).¹¹

Two other soluble proteins, the cyanobacterium-derived lectin, cyanovirin, and human monoclonal antibody 2G12, have also been shown to bind to the high mannose type glycans on HIV gp120.^{16–18} Both of these proteins neutralize a broad array of HIV strains including HIV primary isolates (PI). Additionally, the cell surface lectins DC-SIGN and DC-SIGNR bind to high mannose type glycans on HIV gp120 of both cell line-adapted and HIV PI strains.¹⁹ In contrast to 2G12 and cyanovirin, there are no reports of neutralization of HIV PI by

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MBL. Several findings suggest the possibility that HIV PI may not be neutralized by MBL. First, PI are generally more resistant to neutralization by anti-HIV antibodies and soluble CD4 than cell line-adapted virus.^{20–22} Also, the type of cell that produces virus is known to affect glycosylation of viral proteins²³ and thus may affect binding of MBL to HIV PI. In fact, a recent study showed that differences between virus-producing cells affect the interaction of DC-SIGN with gp120 by influencing the relative numbers of high mannose type glycans on gp120.²⁴ These studies show that it is important to compare the interaction of MBL with virus produced by T cell lines with the virus produced by peripheral blood mononuclear cells (PBMCs). Therefore, the goal of this study was to determine if MBL can interact with HIV PI and compare this interaction with HIV produced in cell lines. HIV binding by MBL as well as MBL-mediated neutralization and opsonization were assessed.

MATERIALS AND METHODS

Mannose-binding lectin

Recombinant MBL (rMBL) was obtained by infecting the HLF liver cell line with a recombinant vaccinia virus expressing the cDNA sequence for human MBL (provided by Dr. Toshisuke Kawasaki, Kyoto University, Japan) as previously described.²⁵ Supernatants from infected cells were collected and MBL was purified by passage over a mannan-sepharose 4B column and elution with 10 mM ethylenediaminetetraacetic acid (EDTA). The purified MBL was estimated to be >95% pure as determined by silver staining of reducing gels. The majority of the purified material was estimated by Western blot of nonreducing gels to contain mainly MBL multimers between approximately 90,000 and 300,000 Da with some material of higher molecular weight. Serum MBL (sMBL) was similarly purified by passage over a mannan-sepharose 4B column.²⁶

MBL neutralization of HIV primary isolates produced by PBMCs

To produce virus stocks, phytohemagglutinin- (PHA; Sigma Chemical Co., St. Louis, MO) stimulated PBMCs were infected with HIV PI HIV_{GP} (X4), HIV_{TH} (R5), HIV_{Ada} (R5), or HIV_{BAL} (R5) in the presence of 30 U/ml recombinant interleukin-2 (IL-2) (obtained through the AIDS Research Reference and Reagent Program, ARRRP, Rockville, MD, from Dr. Maurice Gately, Hoffmann LaRoche, Inc.). HIV_{GP} and HIV_{TH} were isolated directly from patients by our laboratory and have been minimally passaged in primary lymphocytes.²⁷ Ten days after infection, culture supernatants were harvested and tested for virus production by p24 enzyme-linked immunosorbent assay (ELISA) (AIDS Vaccine Program, Frederick, MD).²⁷ The infectious titer (tissue culture infectious dose, TCID₅₀) of virus preparations was determined by limiting dilution; PHA-stimulated PBMCs (2×10^5 cells) were infected for 24 hr at 37°C with 0.1 ml of serial 10-fold dilutions (1:10 to 1:100,000; three wells per dilution in 96-well plates). Cells were washed and resuspended in 200 μ l culture medium containing IL-2 (20 U/ml). After 7 days, culture wells were scored as positive or negative for virus growth by p24 ELISA. To assess HIV neutralization mediated

by MBL, 100 TCID₅₀ of virus was treated with MBL or monoclonal antibodies for 2 hr at 37°C. Treated virus was then cultured with PHA-stimulated PBMC for 24 hr. Cells were washed and resuspended in 200 μ l culture media containing IL-2, and on Day 7, supernatants were assayed for virus production by p24 ELISA.

Neutralization by MBL was also assessed by titration of virus on GHOST cells.²⁸ HIV PI were incubated either in the presence or absence of MBL as above and then incubated with GHOST cells expressing either CXCR4 or CCR5 (obtained from ARRRP) overnight in the presence of 8 μ g/ml DEAE-dextran. Fresh medium was added and the cells incubated an additional 30 hr. The percent of cells expressing green fluorescent protein (GFP) was determined by flow cytometry.

MBL neutralization of cell line-derived HIV

HIV_{IIB} or HIV_{MN} (100 TCID₅₀) was incubated with MBL or antibodies for 1 hr at 37°C. Treated virus was added to H9 T cells (10^5 cells/well/200 μ l) in 96-well microtiter plates. Medium was exchanged after 24 and 96 hr of culture and p24 was measured by ELISA 8–10 days after infection.

Binding of HIV to MBL

Ninety-six-well, flat-bottom polystyrene tissue culture plates (Costar, Cambridge, MA) were coated with 100 μ l of either rMBL or bovine serum albumin (BSA) at 10 μ g/ml in serum-free RPMI-1640 culture medium. After overnight incubation at room temperature, wells were blocked with 1% bovine serum albumin (BSA) for 1 h, washed with serum-free medium, and incubated for 2 hr with 100 μ l of HIV_{TH} containing 136 pg p24. Wells were washed and PHA-stimulated PBMCs (2×10^5) in culture medium supplemented with IL-2 (20 U/ml) were added to each well. Virus replication was measured by p24 ELISA 7 days after infection. To measure the amount of HIV initially bound to wells, replicate wells were treated with 0.5% Triton X-100 and p24 measured by ELISA.

Opsonization of HIV by MBL

HIV_{GP} (0.1 ml at 20,000 pg/ml) was preincubated with either rMBL (6–25 μ g/ml) or medium for 1 hour at 37°C. Treated virus was added to THP monocytic cells (1.5×10^6) (American Type Culture Collection, Manassas, VA) and incubated either at 37°C or on ice for 3 hr. After incubation, the cells were washed in serum-free RPMI-1640 two times. Cell pellets were obtained by centrifugation, cells were lysed by incubation with 0.5% Triton X-100, and bound virus levels determined by p24 ELISA.

Neutralization assay using HIV-gp160-pseudotyped VSV

VSV Δ G-gp160G-GFP pseudoviruses were provided by John Rose (Yale University School of Medicine, New Haven, CT).²⁹ In these viruses, the gene encoding the VSV G protein was deleted and replaced by an HIV gp160–VSV G hybrid gene encoding the extracellular and transmembrane domain of HIV gp160 fused to the cytoplasmic domain of VSV-G protein. Two different strains of GFP-expressing viruses were used in the neutralization assay: VSV Δ G–gp160G(JRFL)–GFP expresses

the gp160 of HIV-1_{JRFL} (R5) and VSVΔG-gp160G(IIIB)-GFP expresses the gp160 of HIV-1_{IIIB} (X4). The propagation and titration of VSVΔG-gp160G(IIIB)-GFP pseudoviruses were performed as described by Boritz *et al.*²⁹ using BHK-G cells, cells that were derived from BHK cells and stably transfected with VSV-G (also provided by John Rose).²⁹ CEM.NKR-CCR5 cells, obtained from the AIDS Research and Reference Reagent Program (ARRRP),³⁰ were used as infection targets. MBL, monoclonal antibodies, heat-inactivated patient sera, or cyanovirin (kindly provided by Michael R. Boyd, Frederick, MD) were serially diluted in serum-free RPMI-1640 and incubated with pseudotyped viruses (MOI = 0.3 ~ 0.5) at 37°C for 1 hr. Aliquots (100 μ l) of the virus-antibody mixtures were transferred to 96-well flat-bottom microplates containing 2×10^5 CEM.NKR-CCR5 cells in 100 μ l of culture medium. Immediately after addition of virus, spinoculation was used to enhance infection by centrifuging plates at $1000 \times g$ for 2 hr at 25°C.³¹ After incubation of plates at 37°C overnight, the cells were washed with phosphate-buffered saline (PBS), resuspended in PBS, and the percent GFP⁺ cells determined by flow cytometry (CYTORON ABSOLUTE, Johnson & Johnson Company).

RESULTS

MBL does not efficiently neutralize HIV primary isolates

The ability of MBL to prevent infection of PHA-stimulated PBMCs by HIV PI was assessed. In multiple experiments, preincubation of either R5 or X4 HIV PI with either recombinant MBL (rMBL) or serum-derived MBL at concentrations of

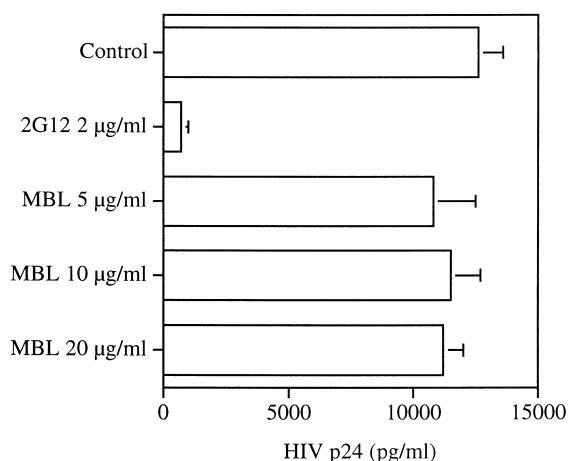


FIG. 1. Effect of MBL on HIV primary isolate infection of PBMCs. Virus (R5 primary isolate HIV_{TH}) was incubated with either rMBL, 2G12 monoclonal antibody, or medium (control) for 2 hr and added to PHA-stimulated PBMCs. Cells were cultured for 7 days and virus replication evaluated by measuring HIV p24. Values shown are the mean \pm SD of triplicate cultures for a representative experiment. This experiment was repeated five times with similar results (see Table 1).

TABLE 1. EFFECT OF MBL ON HIV PRIMARY ISOLATE INFECTION OF PBMCs^a

Virus (type)	MBL (μ g/ml)	Neutralization (% \pm SD)
HIV _{TH} (R5)	5	8 \pm 10
	10	12 \pm 7
	20	6 \pm 6
HIV _{GP} (X4)	20	4 \pm 4
HIV _{B-AL} (R5)	5	2 \pm 2
	20	2 \pm 2
HIV _{Ada} (R5)	20	0 \pm 1

^aShown are the average neutralization values for five experiments for HIV_{TH}, three for HIV_{GP}, two for HIV_{B-AL}, and one for HIV_{Ada}. None of the neutralization values was significantly different than 0 ($p > 0.05$, t test).

5–20 μ g/ml resulted in low levels of neutralization (<15%, Fig. 1 and Table 1). In contrast, in controls for each experiment, anti-gp120 monoclonal antibodies 2G12 or IgG1b12 at 2–5 μ g/ml neutralized all viruses at >80% (Fig. 1 and not shown).

To determine if the type of cell used for infection targets could influence the ability of MBL to neutralize HIV, neutralization of PI by MBL was also studied using GHOST cells expressing CXCR4 or CCR5. Treatment of PI with 20 μ g/ml MBL did not result in detectable neutralization (not shown). In contrast, in the same experiment, anti-gp120 monoclonals 2G12 and IgG1b12 at 2 μ g/ml neutralized both R5 and X4 PI by more than 70% (not shown).

MBL mediates partial neutralization of cell line-adapted HIV

The original study that reported that HIV is neutralized by MBL used the cell line-adapted strain HIV_{IIIB} with the H9 T cell line as targets.¹¹ Therefore, this combination of targets cells and virus was also tested for neutralization by MBL. Modest but significant neutralization of up to 64% was observed when HIV_{IIIB} was incubated with 10–50 μ g/ml MBL (Table 2). Infection of the H9 cell line by another cell line-adapted strain, HIV_{MN}, was neutralized at a similar level (up to 54%) by MBL. In contrast, MBL did not mediate neutralization of HIV_{MN} above 10% when PHA-stimulated PBMCs or GHOST cells were used as infection targets (not shown).

TABLE 2. EFFECT OF MBL ON CELL LINE-DERIVED HIV INFECTION OF H9 CELLS^a

Virus	MBL (μ g/ml)	Neutralization (% \pm SD)
HIV _{MN}	10	18 \pm 11
	50	50 \pm 6 ^b
HIV _{IIIB}	10	31 \pm 16 ^b
	50	51 \pm 18 ^b

^aValues shown are the average of three experiments.

^bSignificant neutralization ($p < 0.05$, t test).

MBL binds to infectious virus

Although the above studies indicated that MBL mediated relatively low levels of neutralization of HIV, previous studies that assessed virus capture by immobilized MBL showed that MBL bound HIV PI with relatively high efficiency. Thus, while neutralizing monoclonal antibodies such as 2G12, 2F5, and IgG1b12 bind much less than 0.5% of input virus when immobilized in microtiter wells,^{13,32–34} MBL can bind as much as 5% of the input virus.¹³ However, since several studies showed that less than 1% of HIV particles in a virus preparations are actually infectious, one possible explanation for the low neutralization by MBL could be that MBL binds only to the non-infectious fraction of virions. To determine if MBL bound to infectious virus, the HIV_{TH} PI was captured on MBL-coated microtiter plates and PHA-stimulated PBMCs were then added to wells. The amount of virus replication observed in MBL-coated wells after 7 days of culture was about six times higher than in BSA-coated wells (Fig. 2B) indicating that MBL captured infectious virus. The amount of virus replication was roughly proportional to the amount of virus captured in the wells immediately before addition of indicator cells (Fig. 2A).

F2

Opsonization of HIV by MBL

Previous studies showed that MBL opsonizes certain microbes for uptake by phagocytic cells. To determine if MBL can bind to HIV particles when in solution as opposed to immobilized on microtiter plates, we assessed the ability of MBL to opsonize an HIV PI. HIV was incubated with MBL and then added to THP monocytic cells, which do not express CD4. While the THP cells bound relatively low levels of HIV in the absence of MBL either at 4°C or 37°C (Fig. 3), preincubation of HIV with 6 µg/ml MBL increased HIV binding by 6-fold at 37°C and 2-fold at 4°C. The peak increases in binding at both temperatures occurred with MBL at 12 µg/ml while higher MBL slightly decreased the amount of HIV associated with cells.

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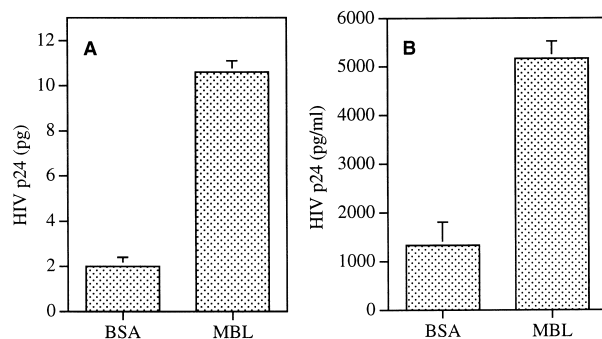


FIG. 2. MBL binds to infectious virus. The HIV_{TH} PI was incubated in MBL-coated or BSA-coated microtiter wells for 2 hr. The amount of virus bound to wells was determined by p24 ELISA after detergent lysis (A). The presence of infectious virus in wells was assessed by adding PHA-stimulated PBMC indicator cells into replicate wells (B). The level of virus replication was determined 7 days after infection. This experiment is representative of three independent experiments. The mean \pm SD of triplicate wells is shown.

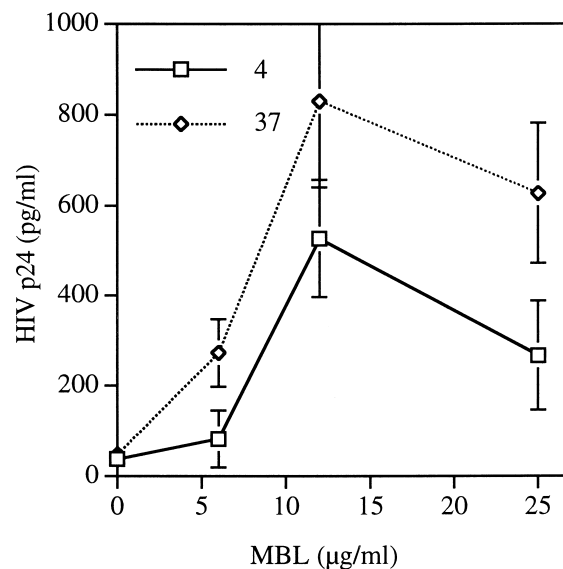


FIG. 3. Opsonization of HIV by MBL. HIV was incubated with MBL (0, 6, 12, or 25 µg/ml) and then added to THP monocytic cells for 3 hr at either 4°C or 37°C. Cells were washed and lysed and the amount of p24 associated with cells determined by ELISA. This experiment is representative of two independent experiments. The mean \pm SD of triplicates is shown.

MBL does not neutralize VSV pseudotyped with HIV-gp160

The above studies suggested that virus strains and/or infection targets could influence the ability of MBL to mediate neutralization of HIV. Therefore, in further studies to determine the level of neutralization of HIV by MBL we tested an additional target, the CEM.NKR-CCR5 T cell line that can be infected by both X4 and R5 HIV isolates.³⁰ The cells were infected with recombinant viruses consisting of VSV lacking the G glycoprotein (VSVΔG) but expressing the HIV gp160 from either the PI JRFL (R5) or cell line-adapted IIIB (X4) strains of HIV. The viruses were produced in BHK cells and previous studies show that production in BHK cells results in normal glycosylation of gp160.^{35–37} These viruses were modified to direct infected cells to express GFP and therefore infection was measured by flow cytometric detection of green fluorescence 24 hr after infection. Uninfected cells were <1% GFP⁺ and cells infected with the VSVΔG-IIIB virus were 40% GFP⁺ (Fig. 4A, panels A and B, respectively). Treatment of this virus with the broadly neutralizing monoclonal antibodies 2F5 or 2G12 substantially neutralized VSVΔG-IIIB (Fig. 4, panels E and F). Pretreatment of CEM.NKR-CCR5 with anti-CD4 or anti-CXCR4 monoclonal antibodies also prevented infection (not shown). Treatment of virus with either serum-derived MBL or recombinant MBL at 10 µg/ml did not detectably neutralize VSVΔG-IIIB (Fig. 4A, panels C and D). Testing of a range of concentrations of MBL, 2F5, and 2G12 showed that VSVΔG-IIIB was approximately 50% neutralized by 2 µg 2F5/ml and was about 90% neutralized by the same amount of 2G12 (Fig. 4B). No significant neutralization (<20%) was observed when virus was treated with 0.4–10 µg/ml of serum-derived MBL or

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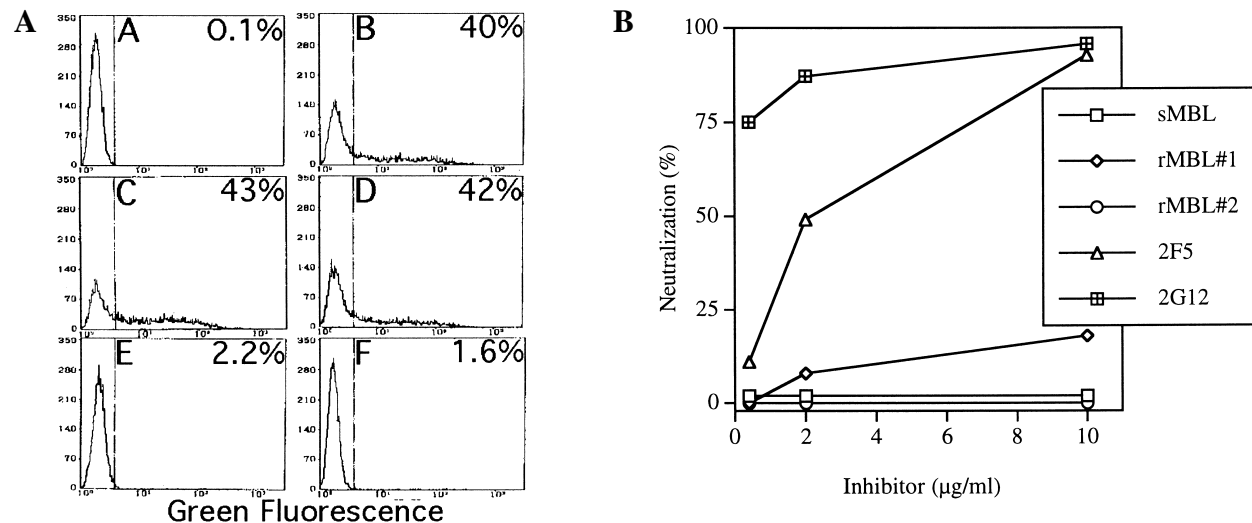


FIG. 4. The effect of MBL on infection by HIV gp160-pseudotyped VSV. VSV pseudotyped with HIV gp160 was preincubated with MBL or antibodies and CEM.NKR-CCR5 cells were infected with treated virus. After 20 hr of culture, the number of GFP⁺ cells was determined by flow cytometry. (A) Histograms of uninfected or VSV Δ G-IIIB-infected cells with percent positive shown for each condition: (A) no virus; (B) control-treated virus; (C) 10 μ g/ml serum-derived MBL; (D) 10 μ g/ml rMBL; (E) 10 μ g/ml 2F5; (F) 2 μ g/ml 2G12. (B) Neutralization of VSV Δ G-IIIB by different concentrations of either serum-derived MBL (sMBL), two different preparations of rMBL, 2F5, or 2G12. Neutralization percent was calculated using the formula $100 - (100 \times \text{experimental GFP}^+ \text{ cells} - \text{uninfected GFP}^+ \text{ cells} / \text{control-treated virus GFP}^+ \text{ cells} - \text{uninfected GFP}^+ \text{ cells})$. Results shown are the mean of duplicate cultures and are representative of at least two experiments.

F5

two different preparations of rMBL ($p > 0.05$, t test). In parallel experiments (not shown and Fig. 5C), MBL also did not neutralize the VSV Δ G-JRFL virus, which expresses the gp160 cloned from a PI.

Since both MBL and antiviral antibodies have been shown to bind to HIV gp120 and since MBL has been reported to interact with carbohydrates on antibodies,³⁸ we next determined if MBL and antibodies could additively or synergistically mediate neutralization. Two sera from HIV-infected persons that neutralized the VSV Δ G-IIIB virus were utilized for this experiment; one neutralized at a relatively high titer and one at a low titer (sera 1 and 2, respectively). Addition of rMBL at 10 μ g/ml to dilutions of either sera did not increase neutralization over the sera alone for VSV Δ G-IIIB (Fig. 5A). Similar experiments were performed with VSV Δ G-JRFL but with an additional serum sample. Although the three sera neutralized VSV Δ G-JRFL at varying titers, MBL at 10 μ g/ml did not augment neutralization (Fig. 5B). Serum from an HIV-seronegative person with or without MBL did not neutralize either virus (data not shown).

The capacity of MBL to mediate neutralization in an additive or synergistic manner with anti-HIV monoclonal antibodies was also assessed. The VSV Δ G-JRFL virus was >85% neutralized by as little as 0.8 μ g/ml of IgG1b12 while 20 μ g/ml of 2G12 was required for about 65% neutralization (Fig. 5C). Although the relatively high neutralization mediated by IgG1b12 alone made it difficult to assess synergy with MBL, addition of rMBL at 10 μ g/ml to the monoclonals did not increase neutralization.

In the above experiments, MBL did not appreciably neutralize either the VSV Δ G-IIIB or the VSV Δ G-JRFL virus.

Since previous studies showed that HIV could be neutralized by cyanovirin,^{16,39} a lectin-like mannose-binding protein isolated from cyanobacteria, we next determined if cyanovirin could neutralize either VSV Δ G-IIIB or VSV Δ G-JRFL. As little as 0.8 μ g/ml cyanovirin neutralized both of the viruses by approximately 80% while 10 μ g/ml resulted in 100% neutralization (not shown). These experiments show that while VSV pseudotyped with HIV gp160 are not neutralized by MBL, they are susceptible to neutralization by another mannose-binding lectin-like protein as well as the 2G12 anti-gp120 antibody that is believed to interact with carbohydrates on gp120.^{17,18}

DISCUSSION

These studies show that while MBL binds to HIV, it does not cause appreciable neutralization. Thus, MBL at ≥ 20 μ g/ml generally caused <20% neutralization of HIV PI and <65% neutralization of cell line-adapted virus. Median serum levels of MBL in both HIV-seropositive and -seronegative persons is reported to be 1–3 μ g/ml with levels in a few individuals reaching as high as 5–10 μ g/ml.^{5,40,41} Approximately 5–10% of individuals have substantially lower levels of MBL (<1 μ g/ml) due to heterozygosity or homozygosity of alleles that code for unstable MBL protein.⁴² The levels of MBL in serum increase during the acute phase⁴³ but rarely exceed 10 μ g/ml. Thus, MBL serum levels are generally much lower than the highest levels tested in this study and we therefore conclude that *in vivo* MBL does not reach a concentration that has significant neutralizing activity for HIV. The one previous study that reported that MBL mediated neutralization of HIV tested only cell line-

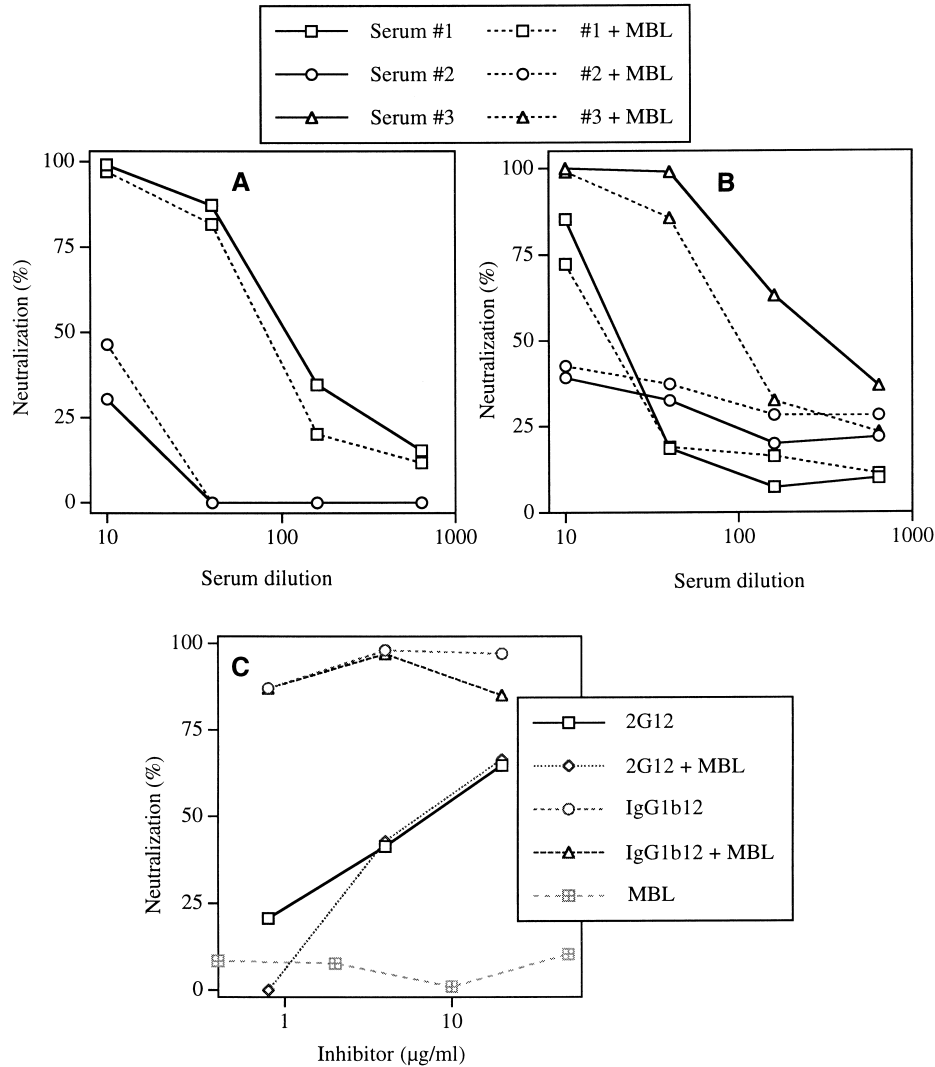


FIG. 5. MBL does not increase antibody-mediated neutralization of HIV gp160-pseudotyped VSV. VSVΔG-IIIB (A) or VSVΔG-JRFL (B) was treated with dilutions of heat-inactivated sera from HIV-seropositive individuals either in the absence or presence of 10 $\mu\text{g/ml}$ rMBL (preparation 1 from Fig. 3B). In (C) VSVΔG-JRFL was treated with 2G12, IgG1b12, rMBL, or monoclonal antibodies plus 10 $\mu\text{g/ml}$ rMBL. Neutralization (%) was calculated as described in the legend to Figure 3. Results shown are the mean of duplicate cultures and are representative of at least two experiments.

derived HIV_{IIIB} using the H9 T cell line as target cells.¹¹ That study showed that 1 $\mu\text{g/ml}$ MBL caused 50% neutralization. In our studies HIV_{IIIB}/H9 was the virus/cell combination that was most sensitive to neutralization although 50% neutralization was achieved only with approximately 50 $\mu\text{g/ml}$ MBL. The difference in efficacy of MBL neutralization between the previous study and this study could be due to variations in the H9 cell line, virus strain, or MBL preparation. However, in the current study, two different serum-derived MBL preparations and two different recombinant MBL preparations were tested for neutralization and all preparations showed little or no neutralization of HIV. When other virus/cell systems were assessed, including PI virus tested on PBMCs, PI virus tested on GHOST cells, and gp160-pseudotyped VSV tested on CEM cells, virus was substantially less sensitive to MBL-mediated neutralization

than cell line-derived virus tested on H9 cells. In contrast, broadly neutralizing human monoclonal antibodies potentially neutralized HIV in all these cell/virus combinations. Additionally, sera from HIV-seropositive patients and cyanovirin neutralized the gp160-pseudotyped VSV. Previous studies showed that cyanovirin also broadly neutralizes PI.¹⁶

Recent studies showed that MBL binds to high mannose N-linked glycans on gp120.¹⁴ Thus, treatment of gp120 with endoglycosidase H or endoglycosidase F1 eliminated binding of MBL as assessed by lectin blot. Enzyme treatment of intact virus also substantially reduced binding to MBL. Blomstedt *et al.*³⁹ showed that cyanovirin also binds to high mannose N-linked glycans on gp120. Thus, endoglycosidase H or PnGase treatment of gp120 released glycans that bound to cyanovirin. In contrast, treatment of a protein known to contain only com-

plex glycans, the envelope protein gC from herpes simplex virus, with either enzyme did not release MBL-binding glycans.

The 2G12 human monoclonal antibody, capable of neutralizing a broad range of PI and cell line-adapted strains of HIV, appears to also bind to high mannose glycans on gp120.^{17,18} Variation or mutation of the gp120 peptide backbone has little effect on 2G12 binding unless the amino acid change affects glycosylation. Mannosidase or endoglycosidase H treatment of gp120 eliminates 2G12 binding and mannose also inhibits binding. Interestingly, preincubation of gp120 with cyanovirin prevented binding of 2G12 while preincubation of gp120 with 2G12 did not prevent binding of cyanovirin.¹⁶

The studies of binding sites on HIV gp120 for MBL, cyanovirin, and 2G12 point to an apparent discrepancy: all three proteins bind to high mannose glycans on gp120 but MBL does not neutralize HIV while cyanovirin and 2G12 do neutralize HIV. One difference between 2G12, cyanovirin, and MBL is the affinity and avidity of interaction with HIV-binding sites. An MBL molecule has on average about 9–12 carbohydrate recognition domains (CRD), but each CRD has weak affinity for monovalent sugars. MBL therefore recognizes pathogens by binding avidly to repetitive sugars.^{5,15,44} Multiple CRD on an MBL molecule cannot bind to the same mammalian high mannose N-linked glycan due to steric constraints⁴⁴ and therefore it is likely that an MBL molecule binds to HIV by binding to multiple high mannose glycans on either one gp120, the gp120 trimer, or multiple gp120 trimers.

In contrast to MBL, 2G12 and cyanovirin each contains only two ligand-binding sites and each binds to gp120 with relatively high affinity.^{17,45,46} While the gp120 protein forms trimers on the surface of virus, recent studies show that HIV PI may contain as many as 14 gp120 trimers on the surface of one virion.⁴⁷ One possibility that could explain MBL binding to HIV but a lack of neutralization is that MBL binds to multiple sites on multiple gp120 molecules or even several gp120 trimers with high enough avidity to remain associated with virus during opsonization or virus capture. However, when virus comes into contact with a target cell, the binding of MBL is of too low affinity to each gp120 molecule to effectively block CD4 interaction with the CD4 binding site on that gp120. The affinity of MBL for gp120 has not yet been determined. Alternatively, MBL may bind at a site on gp120 that does not obscure CD4 binding or interfere with gp120 conformational changes that must occur for gp120 to interact with the chemokine receptor.

A recent study by Poignard *et al.*⁴⁸ suggests another possible explanation for the binding and opsonization but inefficient neutralization due to MBL. That study presented data that suggest that on the surface of HIV there are two forms of gp120. One form is relatively accessible to antibodies but is nonfunctional while the other form is less accessible but functions during infection. Thus it is possible that MBL binds mostly to a nonfunctional form of gp120 on virions.

Although these studies suggest that MBL does not efficiently neutralize HIV *in vivo*, MBL may mediate other important antiviral functions *in vivo* since it binds to HIV and opsonizes it. For example, since MBL enhances opsonization, MBL binding to HIV may alter the interaction of HIV with antigen-processing cells and consequently affect antigen presentation to T cells. MBL could also affect clearance of HIV from blood by neu-

trophils or cells in liver or lung that express MBL receptors.⁴⁹ Since MBL binds to HIV gp120, it could be exploited to selectively target antiviral compounds to virions or HIV-infected cells.

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