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Mannose-binding lectin binds to Ebola and Marburg envelope glycoproteins, resulting in blocking of virus interaction with DC-SIGN and complement-mediated virus neutralization

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Mannose-binding lectin (MBL), a serum lectin that mediates innate immune functions including activation of the lectin complement pathway, binds to carbohydrates expressed on some viral glycoproteins. In this study, the ability of MBL to bind to virus particles pseudotyped with Ebola and Marburg envelope glycoproteins was evaluated. Virus particles bearing either Ebola (Zaire strain) or Marburg (Musoke strain) envelope glycoproteins bound at significantly higher levels to immobilized MBL compared with virus particles pseudotyped with vesicular stomatitis virus glycoprotein or with no virus glycoprotein. As observed in previous studies, Ebola-pseudotyped virus bound to cells expressing the lectin DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin). However, pre-incubation of virus with MBL blocked DC-SIGN-mediated binding to cells, suggesting that the two lectins bind at the same or overlapping sites on the Ebola glycoprotein. Neutralization experiments showed that virus pseudotyped with Ebola or Marburg (Musoke) glycoprotein was neutralized by complement, while the Marburg (Ravn strain) glycoprotein-pseudotyped virus was less sensitive to neutralization. Neutralization was partially mediated through the lectin complement pathway, since a complement source deficient in MBL was significantly less effective at neutralizing viruses pseudotyped with filovirus glycoproteins and addition of purified MBL to the MBL-deficient complement increased neutralization. These experiments demonstrated that MBL binds to filovirus envelope glycoproteins resulting in important biological effects and suggest that MBL can interact with filoviruses during infection in humans.

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INTRODUCTION

The filoviruses Ebola virus and Marburg virus can cause severe haemorrhagic fevers that are frequently fatal (Mahanty & Bray, 2004). Marburg and Ebola envelope glycoproteins consist of a GP1 protein and membrane-bound GP2 protein that are covalently linked by a disulfide bond (Sanchez *et al.*, 1996). Although the causes of filovirus virulence are not well defined, there is evidence that glycans on the viral glycoproteins play distinct roles in pathogenesis (Takada & Kawaoka, 2001). For example, expression of Ebola glycoprotein in cells causes a reduction in host cell-surface protein expression that is associated with cell rounding and detachment (Simmons *et al.*, 2002; Sullivan *et al.*, 2005). Removal of *O*-linked carbohydrates from the glycoprotein reduces the cell rounding and detachment. Additionally, the Ebola envelope glycoprotein interacts with the cell-surface lectins DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin) and DC-SIGNR (DC-SIGN-related molecule), which are expressed on several potential target cells including dendritic cells, alveolar macrophages and endothelial cells in the liver (Alvarez *et al.*, 2002; Baribaud *et al.*, 2002; Lin *et al.*, 2003; Simmons *et al.*, 2003). The Ebola glycoprotein carbohydrate that interacts with DC-SIGN is likely to contain mannose residues, since DC-SIGN and DC-SIGNR have a high affinity for high-mannose sites on *N*-linked glycans and because the interaction is inhibited by the high-mannose carbohydrate mannan (Alvarez *et al.*, 2002; Feinberg *et al.*, 2001). Further evidence that mannose residues are displayed prominently on Ebola glycoprotein is that cyanovirin-N inhibits Ebola virus infection of cells (Barrientos *et al.*, 2003, 2004b). Cyanovirin-N is an antiviral lectin derived from cyanobacteria, which binds to mannose residues of *N*-linked glycans (Botos & Wlodawer, 2003).

Mannose-binding lectin (MBL) is a C-type lectin found in serum with specificity for mannose, *N*-acetylglucosamine and fucose (Hansen & Holmskov, 1998). MBL has several important innate immune functions including initiation of the lectin complement pathway, opsonization of microbes for uptake by phagocytic cells and direct neutralization of some viruses (Jack *et al.*, 2001; Matsushita & Fujita, 2001; Petersen *et al.*, 2001). MBL binds strongly to human immunodeficiency virus type 1 (HIV-1), and high-mannose *N*-linked glycans on the HIV-1 glycoprotein gp120 are critical for this interaction (Ezekowitz *et al.*, 1989; Hart *et al.*, 2002; Haurum *et al.*, 1993; Ohtani *et al.*, 1999; Saifuddin *et al.*, 2000). HIV-1 gp120 also binds to DC-SIGN, DC-SIGNR and cyanovirin-N through high-mannose *N*-linked glycans (Bashirova *et al.*, 2001; Bolmstedt *et al.*, 2001; Esser *et al.*, 1999; Geijtenbeek *et al.*, 2000). Since Ebola virus interacts with DC-SIGN, DC-SIGNR and cyanovirin-N, we hypothesized that the Ebola glycoprotein would also bind to MBL. This study assessed the ability of Ebola and Marburg virus glycoprotein-pseudotyped viruses to bind to immobilized MBL and to activate the lectin complement pathway. We also evaluated the ability of MBL to inhibit interaction between the Ebola virus glycoprotein and DC-SIGN. Virus pseudotyped with the envelope glycoprotein of vesicular stomatitis virus (VSV-G) was used as a control in these experiments, since both VSV and HIV particles pseudotyped with VSV-G are neutralized by the classical pathway of human complement (Beebe & Cooper, 1981; DePolo *et al.*, 2000).

METHODS

Cell lines and reagents. Human embryonic kidney 293 cells were obtained from the AIDS Research and Reference Reagent Program (ARRRP), National Institutes of Health (NIH, Rockville, MD, USA), and cultured in Dulbecco's modified Eagle's medium containing 10 % heat-inactivated fetal bovine serum, 1 % L-glutamine and 50 μg gentamicin (BioWhittaker) ml^{-1}. The B-THP-1 cell line (Wu *et al.*, 2004) expressing DC-SIGN and control DC-SIGN-negative cells were kindly provided by Dr Dan Littman (New York University School of Medicine, NY, USA) and grown in RPMI 1640 (BioWhittaker) containing 10 % heat-inactivated fetal bovine serum, L-glutamine and gentamicin. Sera from two donors (one male aged 49 years, one female aged 30 years) with normal levels of MBL (3·9 and 4·8 μg ml^{-1}, respectively, as determined by ELISA; Statens Serum Institut, Copenhagen, Denmark) were pooled as a complement source (normal human pooled sera, NHPS). Sera from three MBL-deficient donors (0·4–0·8 μg ml^{-1}) were pooled as a source of MBL-deficient complement (MBL-deficient pooled sera, MDPS). The three donors were all healthy females, aged 22–38 years. All donors were recruited from healthy laboratory personnel. To inactivate complement, pooled sera were heat inactivated at 56 °C for 1 h.

As described previously (Hart *et al.*, 2003), supernatants from a human liver fibroblast cell line infected with recombinant vaccinia virus expressing human recombinant MBL (rMBL) were collected and rMBL was purified on a mannan–Sepharose 4B column. MBL was also purified from freshly frozen plasma as previously described (Suankratay *et al.*, 1998). Briefly, MBL was affinity isolated using mannan-coupled Sepharose, passed over protein A–Sepharose to remove residual immunoglobulins and concentrated. The concentration of MBL was determined by ELISA.

Preparation of pseudotyped viruses. HIV particles lacking gp120/gp41 and pseudotyped with Ebola and Marburg envelope proteins were used in all experiments since filoviruses present a significant biohazard (Chan *et al.*, 2000). Pseudotyped viruses were prepared by co-transfecting 293 cells using Lipofectamine (Gibco-BRL) with env-negative HIV plasmid pNL 4-3 (E^-) (ARRRP) expressing luciferase together with plasmids expressing either VSV-G (DePolo *et al.*, 2000), Marburg (Ravn strain), Marburg (Musoke strain) or Ebola (Zaire strain) envelope glycoproteins. The filovirus glycoprotein plasmids were kindly provided by Dr Connie Schmaljohn (US Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, USA). Supernatants containing virus were collected after 2 days of incubation. Virus concentration was determined by ELISA for the HIV p24 core protein (AIDS Vaccine program, Frederik, MD, USA).

Binding of pseudotyped viruses to MBL. Flat-bottomed, 96-well polystyrene tissue culture plates (Costar) were coated overnight with 100 μl of either BSA or rMBL (10 μg ml^{-1}) diluted in veronal-buffered saline (VBS-Ca: 5 mM veronal pH 7·5, 0·15 M NaCl and 10 mM $CaCl_2$). Wells were blocked with 1 % BSA, washed with VBS-Ca and incubated for 4 h with 100 μl pseudotyped virus (8 ng p24 ml^{-1}) diluted in VBS-Ca. Wells were washed with VBS-Ca, bound viruses were lysed with 0·25 % Triton X-100 and p24 was detected by ELISA. The percentage of p24 bound for each virus was calculated as: [(p24 bound in MBL-coated wells−background binding to BSA-coated wells)/(input p24−background binding)] $\times 100$.

Binding of pseudotyped viruses to DC-SIGN. Virus pseudotyped with the Ebola envelope glycoprotein was pre-incubated with rMBL (0–10 μg p24 ml^{-1}) for 1 h at 37 °C before incubation with 1×10^6 $DC\text{-}SIGN^+$ or $DC\text{-}SIGN^-$ THP-1 cells for 3 h at 37 °C. Cells were washed, lysed with detergent (0·2 % Tween 20) and cell-bound virus was measured by p24 ELISA.

Measurement of lectin complement pathway activity. Lectin pathway-dependent C4 deposition was assayed as previously described (Valdimarsson *et al.*, 1998). Microtitre plate wells (Maxisorp Nunc) were coated overnight with 10 μg mannan ml^{-1} and wells were then blocked with 1 % BSA. Sera diluted in buffer containing 20 mM Tris/HCl pH 7·4, 10 mM $CaCl_2$, 1 M NaCl, 0·05 % Triton X-100 and 1 % BSA (100 μl per well) were incubated in wells overnight at 4 °C. Purified C4 (12 μg per well; Calbiochem) was added to each well and incubated for 1·5 h at 37 °C. Wells were washed, 8 ng biotinylated goat anti-human C4 antibody (Calbiochem) was added per well and wells were incubated at room temperature for 1 h. Wells were washed and streptavidin–HRP (Biosource) was added to the wells for 45 min at 37 °C. After further washing, substrate was added and the absorbance at 405 nm was determined.

Complement neutralization of pseudotyped viruses. Pseudotyped viruses were incubated with dilutions of NHPS, MDPS or heat-inactivated sera at 37 °C for 50 min. Treated virus (100 μl) was transferred to 48-well flat-bottomed culture plates containing $0{\cdot}7 \times 10^5$ 293 cells per well. Plates were centrifuged at 1000 ***g*** for 2 h at 25 °C to facilitate infection (Ying *et al.*, 2004) and then incubated for 4 h at 37 °C. The virus–complement mixture was replaced with fresh medium and cells were harvested after 40 h of culture. Luciferase activity in cells was measured and neutralization calculated by the formula: neutralization (%) = 100−(complement-treated luciferase units/heat-inactivated complement luciferase units) × 100.

RESULTS

Binding of pseudotyped viruses to MBL

Previous studies have shown that MBL binds HIV via high-mannose carbohydrate residues on gp120/gp41 (Hart *et al.*, 2002), while HIV virions lacking gp120/gp41 bind at relatively low levels to MBL (Saifuddin *et al.*, 2000). To determine whether filovirus glycoproteins interact with MBL, viruses pseudotyped with Ebola, Marburg, HIV or VSV envelope glycoproteins were incubated in rMBL-coated microtitre wells. HIV particles lacking a viral envelope glycoprotein ($gp120^-$) bound at relatively low levels to MBL (0·2 % of input; Fig. 1), while HIV particles containing HIV envelope glycoprotein ($gp120^+$) bound at significantly higher levels to MBL (3·1 % of input, $P<0·05$, *t*-test). As an additional control, we assessed binding to MBL of virus pseudotyped with VSV-G since these virus particles activate the classical, but not the lectin, complement pathway (DePolo *et al.*, 2000). HIV particles pseudotyped with VSV-G bound to MBL at significantly lower levels than HIV (0·6 % of input, $P<0·05$), although binding was consistently higher than binding of particles with no viral glycoproteins. Particles pseudotyped with Ebola (Zaire) and Marburg (Musoke) envelope proteins bound at significantly higher levels (4·1 and 3·0 %, respectively) than $gp120^-$- or VSV-G-pseudotyped particles ($P<0·05$ vs $gp120^-$). In contrast, particles pseudotyped with Marburg (Ravn) bound at a level (1·3 %) intermediate between $gp120^-$- and Ebola-pseudotyped particles. These data showed that incorporation of Ebola and Marburg virus envelope glycoproteins into HIV particles significantly increased the ability of virus particles to interact with MBL.

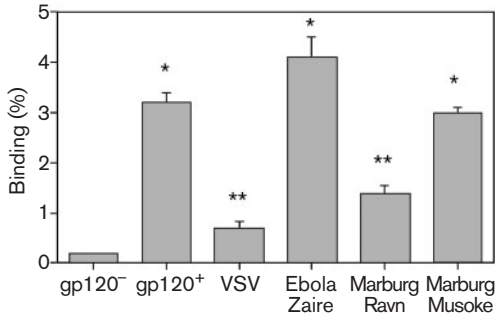

Fig. 1. Binding of pseudotyped viruses to MBL. Microtitre plate wells were coated with MBL overnight. Wells were blocked with BSA, washed and incubated for 4 h with HIV particles pseudotyped with no envelope protein ($gp120^-$), HIV gp120 ($gp120^+$), or envelope glycoprotein from VSV-G, Ebola (Zaire), Marburg (Ravn) or Marburg (Musoke). Bound virus was detected by ELISA for HIV p24. The mean percentage ± SD of triplicate wells of p24 bound for each virus was calculated as: [(p24 bound in MBL-coated wells−background binding to BSA-coated wells)/(input p24−background binding)] × 100. One representative experiment of three independent experiments is shown. *, $P<0·05$ (*t*-test) compared with $gp120^-$ virus; **, $P<0·05$ compared with both $gp120^-$ and $gp120^+$ viruses.

MBL blocks Ebola virus glycoprotein interaction with DC-SIGN

Previous studies have shown that Ebola virus envelope glycoprotein binds to the dendritic cell lectin DC-SIGN (Alvarez *et al.*, 2002; Baribaud *et al.*, 2002; Lin *et al.*, 2003; Simmons *et al.*, 2003). We evaluated the ability of MBL to block binding of Ebola-pseudotyped particles to cells expressing DC-SIGN. Ebola-pseudotyped virus bound at significantly higher levels to DC-SIGN-expressing THP-1 cells than to control $DC\text{-}SIGN^-$ THP-1 cells (Fig. 2, $P<0·05$, *t*-test). Pre-incubation of virus with as little as 0·1 μg MBL ml^{-1} reduced virus binding by 60 % ($P<0·05$). Addition of higher amounts of MBL inhibited binding by up to 98 %. These data demonstrated that MBL binds to virus pseudotyped with the Ebola glycoprotein and blocks Ebola glycoprotein-bearing virus interaction with DC-SIGN.

Complement-dependent neutralization of pseudotyped viruses

Since the above experiments indicated that MBL binds to filovirus glycoproteins on the surface of virus particles, we wanted to determine whether MBL could directly neutralize virus pseudotyped with the Ebola virus glycoprotein. No neutralization of infection of 293 cells was observed when virus was pre-incubated with 10 or 30 μg MBL ml^{-1} for 1 h at 37 °C (not shown). We next determined whether the lectin complement pathway was activated, as assessed by neutralization. Two pools of sera were used as sources of

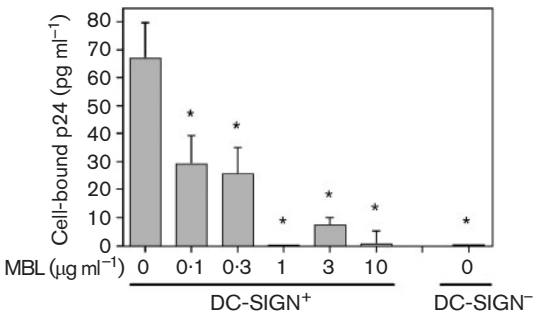

Fig. 2. MBL blocks Ebola envelope glycoprotein-mediated virus binding to DC-SIGN. Virus pseudotyped with the Ebola envelope glycoprotein was pre-incubated with 0–10 μg MBL ml^{-1} before incubation with THP-1 cells expressing DC-SIGN ($DC\text{-}SIGN^+$) or not expressing DC-SIGN ($DC\text{-}SIGN^-$). Cells were washed and lysed with detergent, and cell-bound virus was detected by p24 ELISA. The mean ± SD of triplicates from one representative experiment of three independent experiments is shown. *, $P<0·05$ (*t*-test) compared with virus bound to $DC\text{-}SIGN^+$ cells in the absence of MBL.

complement in neutralization assays: NHPS, a pool of equal volumes of sera from two individuals with normal levels of serum MBL, and MDPS, a pool of sera from three individuals with low levels of serum MBL. Previous studies have shown that when MBL in serum binds to immobilized mannan it activates MBL-associated serine proteases leading to activation of C4 (Valdimarsson *et al.*, 1998). Therefore, in order to compare the lectin pathway activity of the two serum pools, the complement sources were diluted and added to mannan-coated microtitre wells. Addition of NHPS to mannan-coated wells resulted in detectable C4 deposition with dilutions from 1 : 3 to 1 : 30 (Fig. 3), while, in contrast, MDPS induced lower C4 deposition at these dilutions. For example, a 1 : 3 dilution of MDPS and a 1 : 30 dilution of NHPS resulted in similar C4 activation. Thus, lectin complement pathway activation in MDPS was lower by approximately five- to tenfold compared with NHPS.

Neutralization of VSV-G-pseudotyped particles by sera was next evaluated, since previous studies have shown that these virus particles interact with natural IgM antibodies in sera, resulting in neutralization by the classical complement pathway (Beebe & Cooper, 1981; DePolo *et al.*, 2000). NHPS at low dilutions (1 : 2 and 1 : 4) significantly neutralized VSV-G-pseudotyped particles compared with heat-inactivated NHPS (Fig. 4a). When expressed as the percentage of neutralization, VSV-G-pseudotyped virus was neutralized by 98, 97 and 59 % by 1 : 2, 1 : 4 and 1 : 8 dilutions of NHPS, respectively (Fig. 4b). MDPS neutralized VSV-G-pseudotyped virus at levels similar to NHPS (Fig. 4b).

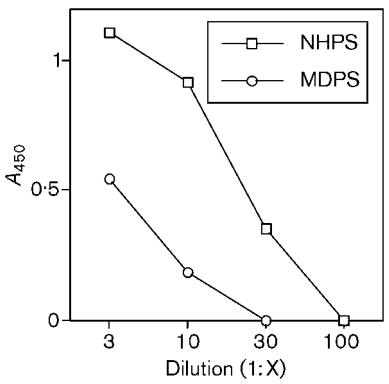

Fig. 3. C4 activation by MBL-deficient and normal sera. Microtitre wells were coated with mannan. Dilutions of normal human pooled sera (NHPS) or MBL-deficient pooled sera (MDPS) were incubated in mannan-coated wells overnight at 4 °C to allow binding of MBL. After washing, purified C4 was added to each well and incubated for 1·5 h at 37 °C. Wells were subsequently incubated with biotinylated anti-C4, streptavidin–HRP and substrate. One experiment representative of two independent experiments is shown.

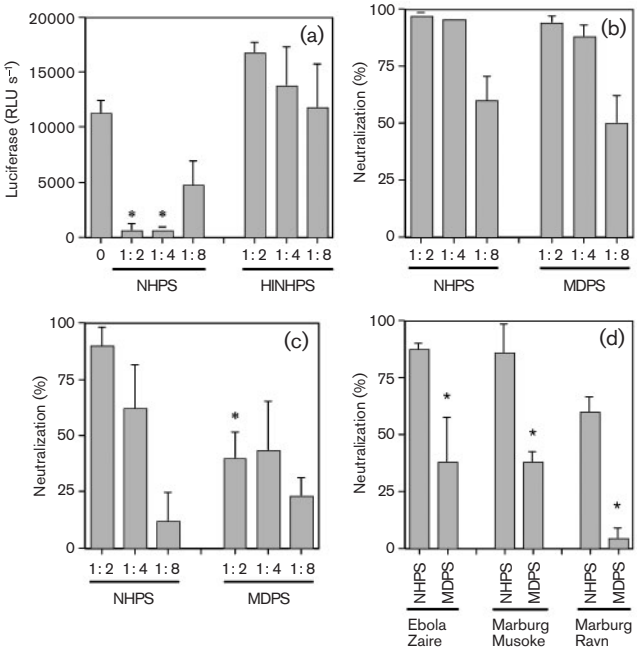

Fig. 4. Complement-mediated neutralization of pseudotyped viruses. Pseudotyped viruses were incubated with dilutions of pooled sera from either normal donors (NHPS), MBL-deficient donors (MDPS) or heat-inactivated pooled sera from these two sources (HINHPS and HIMDPS) at 37 °C for 50 min. Treated virus was then incubated with 293 cells. After 40 h, luciferase activity was measured and neutralization was calculated using the formula: neutralization (%) = 100−(complement-treated luciferase units/heat-inactivated complement-treated luciferase unit) × 100. Each graph shows one representative experiment of three experiments that were performed. (a) Luciferase activity of VSV-G-pseudotyped virus was measured as relative light units (RLU) s^{-1}. *, $P<0{\cdot}05$ (*t*-test) compared with the same dilution of heat-inactivated complement. (b) Neutralization of VSV-G-pseudotyped virus. The data shown in (a) was recalculated as percentage neutralization. Neutralization by NHPS and MDPS was not significantly different for each dilution. (c) Comparison of percentage neutralization of Ebola-pseudotyped virus by NHPS and MDPS. *, $P<0{\cdot}05$ (*t*-test) when comparing the same dilutions of NHPS and MDPS. (d) Neutralization of Ebola- and Marburg-pseudotyped viruses by either NHPS or MDPS at a serum dilution of 1 : 2. *, $P<0{\cdot}05$ comparing NHPS and MDPS for each virus.

Ebola-pseudotyped virus was also neutralized by NHPS, although neutralization was less than with VSV-G-pseudotyped virus with 85, 60 and 10 % neutralization with 1 : 2, 1 : 4 and 1 : 8 dilutions of NHPS, respectively (Fig. 4c). MDPS was significantly less effective in neutralizing Ebola-pseudotyped virus at a 1 : 2 dilution (50 % neutralization, Fig. 4c), but at 1 : 4 and 1 : 8 dilutions NHPS and MDPS neutralized Ebola-pseudotyped virus at similar levels. Thus, complement efficiently neutralized Ebola-pseudotyped virus, and MBL-deficient complement was less

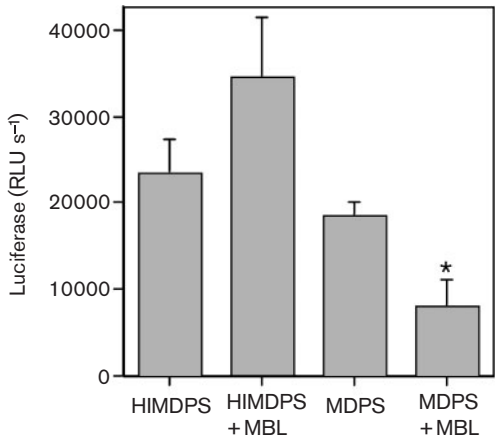

Fig. 5. MBL enhances complement neutralization of Ebola envelope glycoprotein-mediated infection. Ebola-pseudotyped virus was incubated with heat-inactivated MDPS (HIMDPS) or MDPS at a serum dilution of 1 : 2 with or without 10 μg purified MBL ml^{-1}. Neutralization of infection of 293 cells was measured as described in the legend to Fig. 4. *, $P<0{\cdot}05$ (*t*-test) comparing MDPSS with MDPS plus MBL. One representative experiment of two experiments that were performed is shown.

effective at neutralization, indicating that neutralization was partially mediated through the lectin complement pathway.

Viruses pseudotyped with either Ebola, Marburg (Musoke) or Marburg (Ravn) glycoproteins were compared next using the highest concentration of complement (1 : 2 dilution of sera). The Ebola- and Marburg (Musoke)-pseudotyped viruses were 87 and 85 % neutralized, respectively, while Marburg (Ravn)-pseudotyped virus was 65 % neutralized by treatment with NHPS (Fig. 4d). In contrast, MDPS neutralized each pseudotyped virus at significantly lower levels than NHPS (Fig. 4d).

To confirm that MBL plays a role in neutralizing the Ebola glycoprotein-pseudotyped virus, we assessed the effect on neutralization of reconstituting the lectin complement pathway in MDPS by adding MBL. Addition of 10 μg purified MBL ml^{-1} to heat-inactivated MDPS did not increase neutralization of the Ebola glycoprotein-pseudotyped virus when compared with heat-inactivated MDPS alone (Fig. 5). In contrast, addition of MBL to MDPS significantly neutralized the pseudotyped virus when compared with MDPS alone (Fig. 5).

DISCUSSION

The current study is the first description of MBL binding to Ebola and Marburg glycoproteins. MBL binding effectively blocked Ebola glycoprotein interactions with DC-SIGN. Furthermore, when other complement components were present, the lectin pathway initiated by MBL neutralized virus pseudotyped with the filovirus glycoproteins.

Filovirus infections are associated with both the inability to mount effective adaptive immune responses and down-regulation of innate immune responses (Bray, 2001; Geisbert *et al.*, 2003; Mahanty & Bray, 2004; Reed *et al.*, 2004; Sanchez *et al.*, 2004). While vaccination has been investigated as a way of boosting adaptive immune responses to filoviruses and increase survival during infection, few studies have evaluated innate immunity. Innate immune responses are an important early response by the host, since they can inhibit viral growth while simultaneously initiating and modulating adaptive immune responses (Baron *et al.*, 2000; Biron, 1998; Biron *et al.*, 1999; Hackett, 2003; Singh & Baron, 2000). In the mouse model of Ebola, the innate immune response, and specifically type I interferon production, was shown to be important for protection (Bray, 2001; Mahanty *et al.*, 2003), while the viral protein VP35 functions as an interferon antagonist (Basler *et al.*, 2000, 2003). More recently, Warfield *et al.* (2004) demonstrated a role for NK cells in protection when mice were vaccinated with Ebola virus-like particles. While these observations describe antiviral effects of the innate immune response, only a few studies have evaluated the role of the complement system. One study compared complement levels with Ebola virus-induced disease outcome (Zabavichene & Chepurnov, 2004) and an association between complement activation and lethal outcome in guinea pigs was observed. Specifically, early activation followed by a steady decline in complement activity was associated with a lethal outcome. Takada and co-workers (Takada *et al.*, 2003; Takada & Kawaoka, 2003) showed that complement component C1q mediated antibody-dependent enhancement of Ebola infection *in vitro*. In contrast, a potential beneficial role for complement in adaptive responses was demonstrated by Wilson *et al.* (2000), since complement boosted neutralization of Ebola by monoclonal antibodies. Similarly, *in vitro* neutralization of Marburg virus by specific monoclonal antibodies was dependent on complement (Hevey *et al.*, 2003). Interactions between filoviruses and the lectin or alternative complement pathways have not been investigated.

MBL recognizes a variety of clinically relevant pathogens by binding to repeating sugar structures found on exposed proteins (Ezekowitz, 2003). For example, MBL binds to influenza virus, herpes simplex virus type 2 and HIV through *N*-linked high-mannose carbohydrate structures on their respective glycoproteins (Anders *et al.*, 1994; Gadjeva *et al.*, 2004; Hartshorn *et al.*, 1993; Reading *et al.*, 1995; Saifuddin *et al.*, 2000). The GP1 and GP2 glycoproteins of filoviruses are heavily glycosylated and predicted to have both *N*-linked and *O*-linked carbohydrate structures (Feldmann *et al.*, 1994; Geyer *et al.*, 1992), although *O*-linked carbohydrates are rarely terminated with mannose residues. DC-SIGN and cyanovirin-N, which bind to *N*-linked high-mannose oligosaccharides, have been shown to

bind to Ebola and Marburg glycoproteins (Alvarez *et al.*, 2002; Barrientos *et al.*, 2003, 2004a, b; Marzi *et al.*, 2004; Simmons *et al.*, 2003). A role for macrophage cell-surface C-type lectin in binding the virus has also been demonstrated (Takada *et al.*, 2004). In the current study, the existence of high-mannose *N*-linked carbohydrate structures on a filovirus envelope protein was supported by the demonstration that MBL binds to both Ebola and Marburg virus glycoproteins. Overall, binding of the Ebola and Marburg (Musoke) glycoprotein-pseudotyped viruses to MBL was similar to binding of HIV. There was less binding by Marburg (Ravn)-pseudotyped virus to MBL. These differences in binding may be due to different glycosylation patterns in the envelope glycoproteins. An analysis of GenBank sequences shows that the Ravn and Musoke strains are only 70 % homologous in amino acid sequence. Furthermore, while Ebola and Marburg viruses share a number of predicted *N*-linked glycosylation sites, there are also sites that differ between the two. Similarly, Marburg strains appear to share some *N*-linked sites but are divergent at other sites. As expected, the VSV-pseudotyped virus did not bind efficiently to MBL, presumably because VSV-G lacks high-mannose carbohydrate structures. VSV-G contains only two to six potential glycosylation sites (Coll, 1995).

In this study, neutralization of filoviruses was dependent on complement activity since heat inactivation reduced neutralization. However, MBL has been shown to alter virus infectivity directly by mechanisms that are independent of complement activation. Thus, MBL neutralizes influenza A virus by blocking glycoprotein interactions with receptors and viral aggregation (Hartshorn *et al.*, 1997; Saifuddin *et al.*, 2000). MBL could neutralize filoviruses by similar mechanisms. While several potential receptors have been proposed for Ebola, DC-SIGN and C-type lectins specific for galactose and *N*-acetylgalactosamine have been implicated as an Ebola-binding protein (Alvarez *et al.*, 2002; Takada *et al.*, 2004). In the absence of complement, MBL treatment of virus blocked the Ebola glycoprotein-mediated attachment to cells expressing DC-SIGN. During infection, MBL binding to circulating virus could effectively reduce viral infection of lectin-expressing cells, including dendritic cells that modulate later adaptive responses.

There is disagreement on the role of MBL in disease progression due to viral agents. For example, there are conflicting reports on the association between MBL variants and HIV infections (Ji *et al.*, 2005). A large percentage of the population are MBL deficient and low MBL levels could have a significant impact on viral infection. Despite the ability of MBL to bind and inhibit filovirus glycoprotein-pseudotyped virus infection *in vitro*, natural disease in humans is characterized by explosive virus replication with high mortality. No study has evaluated MBL expression in the populations that have been afflicted with filovirus disease. The current study suggests that MBL levels in patients should be studied. Alternatively, filoviruses could avoid complement activity by a variety of ways (Spear *et al.*, 2001). Binding of MBL to virus and subsequent deposition of complement components on the viral membrane could lead to enhanced virus infection of cells that express complement receptors. Furthermore, as observed with other enveloped viruses, filoviruses may incorporate host complement control proteins that would limit the ability of complement to eliminate the cell-free virus.

In summary, we have demonstrated the ability of MBL, a recognition and effector molecule of the innate immune system, to bind to filovirus glycoproteins, resulting in antiviral activity. These findings suggest that human MBL may play important roles in innate immunity during filovirus infections such as direct inhibition of infection and inhibition of viral spread, as well as mediating opsonization and complement activation. Further studies are needed to evaluate the role of MBL and complement during filovirus infections.

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REFERENCES

Alvarez, C. P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A. L. & Delgado, R. (2002). C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in *cis* and in *trans*. *J Virol* **76**, 6841–6844.

Anders, E. M., Hartley, C. A., Reading, P. C. & Ezekowitz, R. A. (1994). Complement-dependent neutralization of influenza virus by a serum mannose-binding lectin. *J Gen Virol* **75**, 615–622.

Baribaud, F., Pohlmann, S., Leslie, G., Mortari, F. & Doms, R. W. (2002). Quantitative expression and virus transmission analysis of DC-SIGN on monocyte-derived dendritic cells. *J Virol* **76**, 9135–9142.

Baron, S., Singh, I., Chopra, A., Coppenhaver, D. & Pan, J. (2000). Innate antiviral defenses in body fluids and tissues. *Antiviral Res* **48**, 71–89.

Barrientos, L. G., O'Keefe, B. R., Bray, M., Sanchez, A., Gronenborn, A. M. & Boyd, M. R. (2003). Cyanovirin-N binds to the viral surface glycoprotein, $GP_{1,2}$ and inhibits infectivity of Ebola virus. *Antiviral Res* **58**, 47–56.

Barrientos, L. G., Lasala, F., Delgado, R., Sanchez, A. & Gronenborn, A. M. (2004a). Flipping the switch from monomeric to dimeric CV-N has little effect on antiviral activity. *Structure* **12**, 1799–1807.

Barrientos, L. G., Lasala, F., Otero, J. R., Sanchez, A. & Delgado, R. (2004b). In vitro evaluation of cyanovirin-N antiviral activity, by use of lentiviral vectors pseudotyped with filovirus envelope glycoproteins. *J Infect Dis* **189**, 1440–1443.

Bashirova, A. A., Geijtenbeek, T. B., van Duijnhoven, G. C. & 10 other authors (2001). A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J Exp Med* **193**, 671–678.

Basler, C. F., Wang, X., Muhlberger, E., Volchkov, V., Paragas, J., Klenk, H. D., Garcia-Sastre, A. & Palese, P. (2000). The Ebola virus VP35 protein functions as a type I IFN antagonist. *Proc Natl Acad Sci U S A* **97**, 12289–12294.

Basler, C. F., Mikulasova, A., Martinez-Sobrido, L., Paragas, J., Muhlberger, E., Bray, M., Klenk, H. D., Palese, P. & Garcia-Sastre, A. (2003). The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. *J Virol* **77**, 7945–7956.

Beebe, D. P. & Cooper, N. R. (1981). Neutralization of vesicular stomatitis virus (VSV) by human complement requires a natural IgM antibody present in human serum. *J Immunol* **126**, 1562–1568.

Biron, C. A. (1998). Role of early cytokines, including alpha and beta interferons (IFN-α/β), in innate and adaptive immune responses to viral infections. *Semin Immunol* **10**, 383–390.

Biron, C. A., Nguyen, K. B., Pien, G. C., Cousens, L. P. & Salazar-Mather, T. P. (1999). Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* **17**, 189–220.

Bolmstedt, A. J., O'Keefe, B. R., Shenoy, S. R., McMahon, J. B. & Boyd, M. R. (2001). Cyanovirin-N defines a new class of antiviral agent targeting *N*-linked, high-mannose glycans in an oligosaccharide-specific manner. *Mol Pharmacol* **59**, 949–954.

Botos, I. & Wlodawer, A. (2003). Cyanovirin-N: a sugar-binding antiviral protein with a new twist. *Cell Mol Life Sci* **60**, 277–287.

Bray, M. (2001). The role of the Type I interferon response in the resistance of mice to filovirus infection. *J Gen Virol* **82**, 1365–1373.

Chan, S. Y., Speck, R. F., Ma, M. C. & Goldsmith, M. A. (2000). Distinct mechanisms of entry by envelope glycoproteins of Marburg and Ebola (Zaire) viruses. *J Virol* **74**, 4933–4937.

Coll, J. M. (1995). The glycoprotein G of rhabdoviruses. *Arch Virol* **140**, 827–851.

DePolo, N. J., Reed, J. D., Sheridan, P. L., Townsend, K., Sauter, S. L., Jolly, D. J. & Dubensky, T. W., Jr (2000). VSV-G pseudotyped lentiviral vector particles produced in human cells are inactivated by human serum. *Mol Ther* **2**, 218–222.

Esser, M. T., Mori, T., Mondor, I., Sattentau, Q. J., Dey, B., Berger, E. A., Boyd, M. R. & Lifson, J. D. (1999). Cyanovirin-N binds to gp120 to interfere with CD4-dependent human immunodeficiency virus type 1 virion binding, fusion, and infectivity but does not affect the CD4 binding site on gp120 or soluble CD4-induced conformational changes in gp120. *J Virol* **73**, 4360–4371.

Ezekowitz, R. A. (2003). Role of the mannose-binding lectin in innate immunity. *J Infect Dis* **187**, S335–S339.

Ezekowitz, R. A., Kuhlman, M., Groopman, J. E. & Byrn, R. A. (1989). A human serum mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. *J Exp Med* **169**, 185–196.

Feinberg, H., Mitchell, D. A., Drickamer, K. & Weis, W. I. (2001). Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* **294**, 2163–2166.

Feldmann, H., Nichol, S. T., Klenk, H. D., Peters, C. J. & Sanchez, A. (1994). Characterization of filoviruses based on differences in structure and antigenicity of the virion glycoprotein. *Virology* **199**, 469–473.

Gadjeva, M., Paludan, S. R., Thiel, S. & 8 other authors (2004). Mannan-binding lectin modulates the response to HSV-2 infection. *Clin Exp Immunol* **138**, 304–311.

Geijtenbeek, T. B., Kwon, D. S., Torensma, R. & 9 other authors (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells [see comments]. *Cell* **100**, 587–597.

Geisbert, T. W., Hensley, L. E., Larsen, T. & 7 other authors (2003). Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *Am J Pathol* **163**, 2347–2370.

Geyer, H., Will, C., Feldmann, H., Klenk, H. D. & Geyer, R. (1992). Carbohydrate structure of Marburg virus glycoprotein. *Glycobiology* **2**, 299–312.

Hackett, C. J. (2003). Innate immune activation as a broad-spectrum biodefense strategy: prospects and research challenges. *J Allergy Clin Immunol* **112**, 686–694.

Hansen, S. & Holmskov, U. (1998). Structural aspects of collectins and receptors for collectins. *Immunobiology* **199**, 165–189.

Hart, M. L., Saifuddin, M., Uemura, K., Bremer, E. G., Hooker, B., Kawasaki, T. & Spear, G. T. (2002). High mannose glycans and sialic acid on gp120 regulate binding of mannose-binding lectin (MBL) to HIV type 1. *AIDS Res Hum Retroviruses* **18**, 1311–1317.

Hart, M. L., Saifuddin, M. & Spear, G. T. (2003). Glycosylation inhibitors and neuraminidase enhance human immunodeficiency virus type 1 binding and neutralization by mannose-binding lectin. *J Gen Virol* **84**, 353–360.

Hartshorn, K. L., Sastry, K., White, M. R., Anders, E. M., Super, M., Ezekowitz, R. A. & Tauber, A. I. (1993). Human mannose-binding protein functions as an opsonin for influenza A viruses. *J Clin Invest* **91**, 1414–1420.

Hartshorn, K. L., White, M. R., Shepherd, V., Reid, K., Jensenius, J. C. & Crouch, E. C. (1997). Mechanisms of anti-influenza activity of surfactant proteins A and D: comparison with serum collectins. *Am J Physiol* **273**, L1156–L1166.

Haurum, J. S., Thiel, S., Jones, I. M., Fischer, P. B., Laursen, S. B. & Jensenius, J. C. (1993). Complement activation upon binding of mannan-binding protein to HIV envelope glycoproteins. *AIDS* **7**, 1307–1313.

Hevey, M., Negley, D. & Schmaljohn, A. (2003). Characterization of monoclonal antibodies to Marburg virus (strain Musoke) glycoprotein and identification of two protective epitopes. *Virology* **314**, 350–357.

Jack, D. L., Klein, N. J. & Turner, M. W. (2001). Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. *Immunol Rev* **180**, 86–99.

Ji, X., Gewurz, H. & Spear, G. T. (2005). Mannose binding lectin (MBL) and HIV. *Mol Immunol* **42**, 145–152.

Lin, G., Simmons, G., Pohlmann, S. & 8 other authors (2003). Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. *J Virol* **77**, 1337–1346.

Mahanty, S. & Bray, M. (2004). Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect Dis* **4**, 487–498.

Mahanty, S., Gupta, M., Paragas, J., Bray, M., Ahmed, R. & Rollin, P. E. (2003). Protection from lethal infection is determined by innate immune responses in a mouse model of Ebola virus infection. *Virology* **312**, 415–424.

Marzi, A., Gramberg, T., Simmons, G. & 12 other authors (2004). DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. *J Virol* **78**, 12090–12095.

Matsushita, M. & Fujita, T. (2001). Ficolins and the lectin complement pathway. *Immunol Rev* **180**, 78–85.

Ohtani, K., Suzuki, Y., Eda, S. & 7 other authors (1999). High-level and effective production of human mannan-binding lectin (MBL) in Chinese hamster ovary (CHO) cells. *J Immunol Methods* **222**, 135–144.

Petersen, S. V., Thiel, S. & Jensenius, J. C. (2001). The mannan-binding lectin pathway of complement activation: biology and disease association. *Mol Immunol* **38**, 133–149.

Reading, P. C., Hartley, C. A., Ezekowitz, R. A. & Anders, E. M. (1995). A serum mannose-binding lectin mediates complement-dependent lysis of influenza virus-infected cells. *Biochem Biophys Res Commun* **217**, 1128–1136.

Reed, D. S., Hensley, L. E., Geisbert, J. B., Jahrling, P. B. & Geisbert, T. W. (2004). Depletion of peripheral blood T lymphocytes and NK cells during the course of Ebola hemorrhagic fever in cynomolgus macaques. *Viral Immunol* **17**, 390–400.

Saifuddin, M., Hart, M. L., Gewurz, H., Zhang, Y. & Spear, G. T. (2000). Interaction of mannose-binding lectin with primary isolates of human immunodeficiency virus type 1. *J Gen Virol* **81**, 949–955.

Sanchez, A., Trappier, S. G., Mahy, B. W., Peters, C. J. & Nichol, S. T. (1996). The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc Natl Acad Sci U S A* **93**, 3602–3607.

Sanchez, A., Lukwiya, M., Bausch, D., Mahanty, S., Sanchez, A. J., Wagoner, K. D. & Rollin, P. E. (2004). Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: cellular responses, virus load, and nitric oxide levels. *J Virol* **78**, 10370–10377.

Simmons, G., Wool-Lewis, R. J., Baribaud, F., Netter, R. C. & Bates, P. (2002). Ebola virus glycoproteins induce global surface protein down-modulation and loss of cell adherence. *J Virol* **76**, 2518–2528.

Simmons, G., Reeves, J. D., Grogan, C. C. & 10 other authors (2003). DC-SIGN and DC-SIGNR bind Ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology* **305**, 115–123.

Singh, I. P. & Baron, S. (2000). Innate defences against viraemia. *Rev Med Virol* **10**, 395–403.

Spear, G. T., Hart, M., Olinger, G. G., Hashemi, F. B. & Saifuddin, M. (2001). The role of the complement system in virus infections. *Curr Top Microbiol Immunol* **260**, 229–245.

Suankratay, C., Zhang, X. H., Zhang, Y., Lint, T. F. & Gewurz, H. (1998). Requirement for the alternative pathway as well as C4 and C2 in complement-dependent hemolysis via the lectin pathway. *J Immunol* **160**, 3006–3013.

Sullivan, N. J., Peterson, M., Yang, Z. Y., Kong, W. P., Duckers, H., Nabel, E. & Nabel, G. J. (2005). Ebola virus glycoprotein toxicity is mediated by a dynamin-dependent protein-trafficking pathway. *J Virol* **79**, 547–553.

Takada, A. & Kawaoka, Y. (2001). The pathogenesis of Ebola hemorrhagic fever. *Trends Microbiol* **9**, 506–511.

Takada, A. & Kawaoka, Y. (2003). Antibody-dependent enhancement of viral infection: molecular mechanisms and *in vivo* implications. *Rev Med Virol* **13**, 387–398.

Takada, A., Feldmann, H., Ksiazek, T. G. & Kawaoka, Y. (2003). Antibody-dependent enhancement of Ebola virus infection. *J Virol* **77**, 7539–7544.

Takada, A., Fujioka, K., Tsuiji, M. & 7 other authors (2004). Human macrophage C-type lectin specific for galactose and *N*-acetylgalactosamine promotes filovirus entry. *J Virol* **78**, 2943–2947.

Valdimarsson, H., Stefansson, M., Vikingsdottir, T., Arason, G. J., Koch, C., Thiel, S. & Jensenius, J. C. (1998). Reconstitution of opsonizing activity by infusion of mannan-binding lectin (MBL) to MBL-deficient humans. *Scand J Immunol* **48**, 116–123.

Warfield, K. L., Perkins, J. G., Swenson, D. L., Deal, E. M., Bosio, C. M., Aman, M. J., Yokoyama, W. M., Young, H. A. & Bavari, S. (2004). Role of natural killer cells in innate protection against lethal Ebola virus infection. *J Exp Med* **200**, 169–179.

Wilson, J. A., Hevey, M., Bakken, R., Guest, S., Bray, M., Schmaljohn, A. L. & Hart, M. K. (2000). Epitopes involved in antibody-mediated protection from Ebola virus. *Science* **287**, 1664–1666.

Wu, L., Martin, T. D., Carrington, M. & KewalRamani, V. N. (2004). Raji B cells, misidentified as THP-1 cells, stimulate DC-SIGN-mediated HIV transmission. *Virology* **318**, 17–23.

Ying, H., Ji, X., Hart, M. L., Gupta, K., Saifuddin, M., Zariffard, M. R. & Spear, G. T. (2004). Interaction of mannose-binding lectin with HIV type 1 is sufficient for virus opsonization but not neutralization. *AIDS Res Hum Retroviruses* **20**, 327–335.

Zabavichene, N. M. & Chepurnov, A. A. (2004). Dynamics of complement hemolytic activity in experimental Ebola infection. *Vopr Virusol* **49**, 21–25 (in Russian).