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The lectin pathway of complement activation contributes to protection from West Nile virus infection

Anja Fuchs [a,c], Amelia K. Pinto [a], Wilhelm J. Schwaeble [e], Michael S. Diamond [a,b,c,d,*]

[a] Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
[b] Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA
[c] Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
[d] Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research, Washington University School of Medicine, St. Louis, MO 63110, USA
[e] Department of Infection, Immunity, and Inflammation, University of Leicester, Leicester LE1 9HN, UK

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ABSTRACT

The function of the lectin pathway of complement activation in vivo against West Nile virus (WNV) or many other pathogenic viruses has not been defined. Mice deficient in lectin pathway recognition molecules (mannose binding lectin-A (MBL-A) and mannose binding lectin-C (MBL-C)) or the effector enzyme mannan-binding lectin-associated serine protease-2 (MASP-2), were more vulnerable to WNV infection than wild type mice. Compared with studies of mice deficient in factors of the classical or alternative pathway, *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ or *MASP-2*$^{-/-}$ mice showed a less severe course of WNV infection. Indeed, a deficiency in lectin pathway activation did not significantly affect the kinetics of viral spread to the central nervous system (CNS) nor did it profoundly alter generation of adaptive B and T cell immune responses. We conclude that MBL-mediated recognition and lectin pathway activation have important yet subordinate functions in protecting against WNV infection and disease.

Introduction

Mannose-binding lectin (MBL) is a pattern recognition component of the complement system that binds carbohydrate groups on the surface of microbial pathogens and triggers the lectin activation pathway of complement (Takahashi et al., 2006). In humans, one MBL gene is expressed, whereas mice produce two closely related proteins, MBL-A and MBL-C. MBL proteins are expressed primarily in the liver and circulate in the blood, where they complex with mannan-binding lectin-associated serine proteases (MASP) (Takahashi et al., 2007). In addition to MBL, there is a family of structurally related plasma proteins, called ficolins, which may serve as carbohydrate recognition molecules and drive lectin pathway mediated complement activation upon binding to a pathogen surface. MBL and ficolin engagement activates the associated MASP-2, which cleaves the downstream complement components C4 and C2 to form the C3 convertase complex C4b2a. The cleavage of C3, results in C3a release and C3b deposition (Ip et al., 2009). C3b marks pathogens for clearance by phagocytosis through one of several complement receptors. Additionally, complement deposition targets pathogens for destruction by forming a C5 convertase, C4b2a(C3b) that cleaves C5 to release the complement anaphylatoxin C5a and the larger cleavage fragment C5b, which promotes formation of the membrane-attack complex (MAC) by the terminal (C5–C9) complement components.

MBL and ficolins recognize many pathogens, including viruses, bacteria and fungi, and are believed to function both in direct innate immune restriction and induction of adaptive immunity. In humans, inherited MBL deficiencies are common, and are caused by frequently occurring polymorphisms in the MBL gene. MBL deficiencies are associated with more severe disease after *Neisseria meningitides* infection, and greater susceptibility to infection with HIV, hepatitis B virus, hepatitis C virus, and herpes simplex virus (HSV) (Bathum et al., 2006; Chong et al., 2005; Sasaki et al., 2000; Seppanen et al., 2009; Tan et al., 2009). Studies in *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice have established a role for MBL in limiting infections with *Staphylococcus aureus*, *Pseudomonas aeruginosa, Candida albicans,* and herpes simplex virus (Gadjeva et al., 2004; Held et al., 2008; Moller-Kristensen et al., 2006; Shi et al., 2004).

West Nile virus (WNV) is an enveloped, single-stranded positive polarity RNA virus in the *Flaviviridae* family. The virus cycles between mosquitoes and birds, but also infects humans, horses and other vertebrate animals (Mackenzie et al., 2004). In humans, WNV infection is often asymptomatic, or manifests with a mild fever;

Abbreviations: WNV, West Nile virus; DENV, dengue virus; CNS, central nervous system; MBL, mannose binding lectin; MASP, mannan-associated serine protease; PFU, plaque forming units.

* Corresponding author. Departments of Medicine, Molecular Microbiology, and Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Ave. Box 8051, Saint Louis, MO 63110, USA. Fax: +1 314 362 9230.
E-mail address: diamond@borcim.wustl.edu (M.S. Diamond).

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however, in some cases, especially in elderly or immunosuppressed individuals, it can cause life-threatening encephalitis (Diamond, 2009; Petersen et al., 2003). The immunological basis of protection against WNV has been analyzed extensively in inbred mice, which are susceptible to WNV encephalitis. From these studies, it is evident that both innate and adaptive immune mechanisms are required for protection against neuroinvasive disease. Mice deficient in type I or II interferon, or T cell subsets such as γδ, regulatory, $CD4^+$ or $CD8^+$ T cells succumb to WNV disease with enhanced kinetics and frequency (reviewed in Diamond et al., 2009; Samuel and Diamond, 2006).

Protection against WNV encephalitis also requires an intact complement system as mice lacking the central complement component C3 uniformly succumbed to infection (Mehlhop et al., 2005). Both the classical and alternative activation pathways are required, as mice deficient in C1q (classical pathway) or fB (alternative pathway) also showed greater susceptibility (Mehlhop and Diamond, 2006). As $C4^{-/-}$ mice showed a more severe phenotype after WNV infection than $C1q^{-/-}$ mice, we hypothesized that the lectin pathway independently protects against WNV infection. Consistent with this, mouse serum lacking MBL showed a decreased ability to directly neutralize insect cell-derived WNV in vitro (Fuchs et al., 2010).

Here, using mice that were genetically deficient in MBL-mediated pathway recognition and activation, we directly evaluated the contribution of MBL to immunity against WNV infection. *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice had relatively mild defects in WNV clearance, resulting in only moderately enhanced susceptibility. Contrary to our expectations, the in vivo studies revealed similar viral tissue burdens in several peripheral organs between wild type and *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice. Furthermore, a deficiency in MBL genes did not significantly impair induction of adaptive antiviral immune responses. Thus, while the lectin pathway contributes to protection against WNV, its effect is subordinate compared to the antiviral and priming functions of the classical and alternative pathways of complement activation.

Results

A deficiency of MBL or MASP-2 results in increased susceptibility to WNV infection

We recently observed that MBL in serum can bind to insect cell-derived WNV, leading to complement activation and neutralization of viral infectivity in vitro (Fuchs et al., 2010). To investigate whether MBL contributes to immune protection in vivo, we infected *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice on a C57BL/6 background with 10^2 PFU of WNV via footpad injection (Fig. 1A). As seen previously (Samuel and Diamond, 2006), ~30% of wild type C57BL/6 mice succumbed to WNV infection. Congenic *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice reproducibly showed a small yet statistically significant increased mortality rate (~55%, $P<0.005$). However, *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice showed similar kinetics of survival with a mean time to death that was not substantially different compared to wild type mice (mean time to death of 11.6 versus 11.9 days, $P>0.6$). For both wild type and *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice, animals that succumbed to WNV infection showed similar clinical signs of encephalitis and limb paralysis. As our previous studies suggested that MBL preferentially bound to insect cell-derived WNV (Fuchs et al., 2010), we assessed whether infection with mammalian cell-derived WNV altered the susceptibility in *MBL-A*$^{-/-}$ × *MBL*−*C*$^{-/-}$ mice. However, similar to insect-cell derived virus, WNV propagated in Vero African green monkey cells also showed greater virulence in *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice with increased mortality observed (66% versus 24%, $P=0.006$) (data not shown).

MBL proteins associate with MASP molecules to trigger downstream complement activation, with MASP-2 believed to be the functionally dominant serine protease (Gal et al., 2009). Infection of congenic *MASP-2*$^{-/-}$ mice with WNV showed a similar phenotype of modestly increased susceptibility to lethal infection (Fig. 1B, $P<0.0005$). Overall,

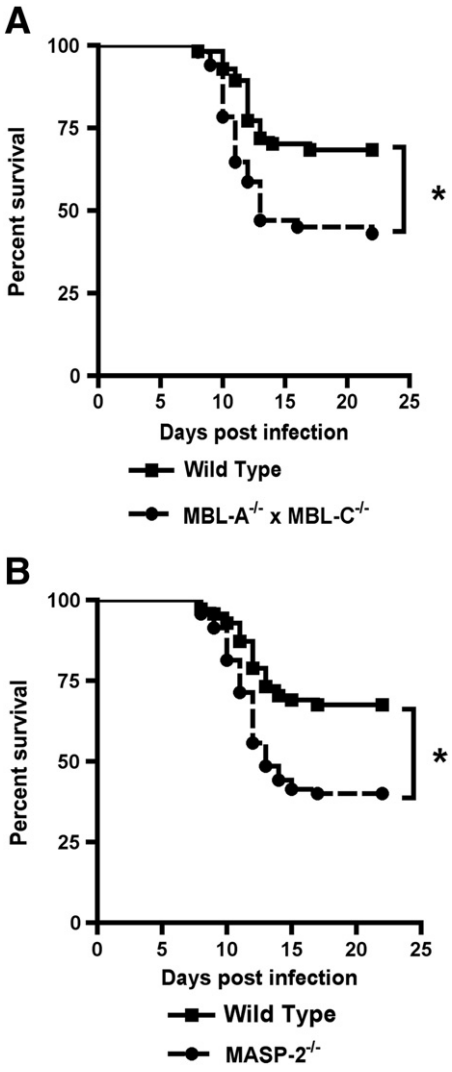

Fig. 1. Mice deficient in components of the lectin pathway are more susceptible to lethal WNV infection. Wild type (A, n = 78; B, n = 71), *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ (n = 65) (A), or *MASP-2*$^{-/-}$ (n = 70) (B) C57BL/6 mice were infected subcutaneously with 10^2 PFU of WNV. Survival was monitored over the course of 22 days. Asterisks denote differences that were statistically significant ($P<0.05$) as judged by the log rank test.

an absence of key components of the lectin activation pathway resulted in increased susceptibility to WNV infection, although the difference was less than that observed with deficiencies in either the classical or alternative pathways (Mehlhop and Diamond, 2006).

Viral burden in wild type and MBL-A$^{-/-}$ × *MBL-C*$^{-/-}$ *mice after subcutaneous infection*

To begin to determine the cause for the higher mortality, we measured viral burden in tissues from WNV-infected wild type and *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice at several time points after infection. Because of its ability to neutralize insect cell-derived WNV (Fuchs et al., 2010), we hypothesized that MBL would limit viral spread at early time points in lymphoid tissues or the intravascular compartment. Surprisingly, no statistical difference in magnitude or kinetics of WNV infection was observed in the draining inguinal lymph node, serum, or spleen of wild type mice and *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice (Figs. 2A, B, and C, $P>0.1$). At day one after infection, we found slightly lower levels of WNV RNA that did not achieve statistical significance in the draining lymph node of *MBL-A*$^{-/-}$ × *MBL-C*$^{-/-}$ mice, as half of the samples had undetectable levels of viral RNA. However, by day 2, no difference in WNV RNA was observed, confirming that viral