Role of Complement in Dengue Virus Infection: Protection or Pathogenesis?

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ABSTRACT Dengue viruses (DENV) cause a spectrum of disease in humans, ranging from dengue fever (DF) to a severe, life-threatening syndrome called dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Despite the global morbidity and mortality associated with DENV infection, mechanisms of immune control and viral pathogenesis are poorly understood. In a recent article, Avirutnan et al. [mBio 2(6):e00276-11, 2011] demonstrated that DENV can be directly neutralized via the mannose binding lectin (MBL) pathway of the complement system and that deficiency in MBL level or activity due to host polymorphisms in the *MBL2* gene correlates with reduced levels of DENV neutralization. These findings implicate a role for the MBL pathway in controlling DENV infections and modulating DHF/DSS manifestations.

The four serotypes of dengue viruses (DENV) cause 100 to 500 million infections each year in over 100 countries, resulting in 40 million clinically apparent cases and 20,000 deaths (1). The clinically apparent cases represent a spectrum of illnesses ranging from an acute febrile disease called dengue fever (DF) to the severe and life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Severe disease is preferentially associated with secondary infection with a different serotype and with primary infection of infants born to DENV-immune mothers. To explain these epidemiological observations, a process called antibody-dependent enhancement of infection (ADE) has been proposed to explain DENV pathogenesis (2). Two other dominant hypotheses in the field involve serotype-cross-reactive memory T cells (3) and viral virulence (4). These three, non-mutually-exclusive processes are postulated to increase viral load and trigger "cytokine storm" and activation of the complement system, resulting in DHF/DSS. The precise mechanism of DHF/DSS pathogenesis and the relationships between viral load, cytokine storm, and complement activation are uncertain. Moreover, host genetic factors may influence disease pathogenesis, as polymorphisms present in several host immune response genes, including those encoding tumor necrosis factor (TNF), DC-SIGN, and FcγRIIa (5), have been correlated with altered susceptibility to DHF/DSS. However, the virus-host interactions that lead to protective immunity as opposed to disease pathogenesis have yet to be elucidated. In fact, little is known about the mechanisms of anti-DENV immunity, as most studies to date have focused on examining the role of the immune system in the context of pathogenesis. Understanding the mechanisms that regulate the balance between immune-mediated pathology and protection is critical for developing safe and effective treatments and vaccines against DENV.

The recent study by Avirutnan et al. (6) begins to shed light on the dual role of the complement system in protection against and pathogenesis of DENV infection. The complement system, composed of over 30 different soluble and cell surface proteins, is an important component of innate immune responses against various pathogens. It is activated via the classical, lectin, and alternative pathways, and it controls viral infections through multiple mechanisms, including lysis of virions or infected cells, production of anaphylatoxins, and priming of T and B cell responses. The classical pathway is activated by C1q binding to antigen-antibody complexes; the lectin pathway involves recognition of carbohydrate structures on pathogens by mannose binding lectin (MBL); and the alternative pathway is constitutively active at low levels through spontaneous hydrolysis of C3 (reviewed in reference 7). A majority of studies examining complement-DENV interactions have focused on the role of complement in the context of DHF/DSS pathogenesis. In particular, a retrospective clinical study has shown that excessive consumption of complement proteins correlated with DHF/DSS (8), and an *in vitro* study has demonstrated that complement could enhance DENV infection of myeloid cells by promoting viral entry through CR3 (9). Based on these earlier studies suggesting that complement activation is pathogenic, later studies have examined potential mechanisms of complement activation in DHF/DSS cases. In an *in vitro* study, anti-DENV antibodies could activate complement on the surface of infected endothelial cells (10). In a prospective study, the DENV nonstructural protein 1 (NS1) could activate complement, and levels of NS1 and several complement proteins correlated with disease severity (11). In another prospective study, the levels of complement factors D and H (i.e., regulatory proteins of the alternative pathway) and the MBL protein were found to be higher in DHF patients than in DF individuals (12). These later studies thus supported the earlier studies by postulating a relationship between NS1 activity and complement activation (11) or an altered regulation of complement activation (12) in dengue pathogenesis. However, recent findings have begun to suggest a role for the complement system in the context of protective immunity. In particular, *in vitro* ADE studies have demonstrated that complement proteins reduce DENV infection (13, 14), suggesting that complement may play a role in limiting ADE-mediated disease. More recently, *in vitro* studies with DENV NS1 have shown that this viral protein binds C4 and C1s (15) or C4BP (16) to antagonize complement activation, implicating NS1 as an immune evasion molecule *in vivo*. These most recent studies showing how DENV may employ multiple mechanisms to subvert complement

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activation suggest that the complement system is an important player in the host defense against DENV. Avirutnan et al. (6) examined the role of the complement system in protection against DENV infection, and the results provide support for an important role of the complement system in controlling DENV infection and potentially influencing the severity of dengue disease in humans.

Specifically, Avirutnan et al. performed initial experiments using naive mouse sera to determine which complement pathways contribute to neutralization of DENV *in vitro* (6). Experiments with loss-of-function models for various mouse complement proteins demonstrated that the MBL pathway was critical for neutralization of both insect and mammalian cell-derived DENV serotype 2 (DENV2), although insect cell-derived DENV2 was neutralized more efficiently than mammalian cell-derived virus. Based on these results, the authors performed additional experiments using purified human MBL and showed that MBL could directly neutralize insect but not mammalian cell-derived DENV2 in the absence of other complement proteins and that this direct MBL-mediated neutralization was more efficient at higher temperatures (37°C and 40°C) than at room temperature. Further experiments using purified human MBL and serum from MBL-deficient mice showed that MBL-mediated neutralization of insect cell-derived DENV2 was enhanced by other complement proteins. Similar to results obtained with mouse sera, human sera from some individuals could neutralize both insect and mammalian cell-derived DENV2 in an MBL-dependent manner. In this case, neutralization of insect cell-derived DENV2 was more efficient than that of mammalian cell-derived virus. Sera from other individuals neutralized only insect cell-derived DENV2, not mammalian cell-derived DENV2. A positive correlation was observed between the MBL concentration in human serum and the level of DENV2 neutralizing activity. Moreover, experiments with sera obtained from individuals with different levels of MBL2 due to known polymorphisms in the *MBL2* gene corroborated the positive correlation between human MBL2 levels and neutralization of insect cell-derived DENV2. Finally, experiments were performed with both mouse and human serum samples to show that the MBL pathway could neutralize the remaining three serotypes other than DENV2. Collectively, these results tie together initial observations made using mouse models and human donors to subsequent findings related to humans with particular polymorphisms in the *MBL2* gene and suggest that the MBL pathway contributes to protection against DENV infection in humans.

Based on the findings of this study, a depressed level of MBL protein or activity may be an independent risk factor for morbidity and mortality associated with DENV infection. Deficiencies in MBL are relatively common in humans. MBL deficiency has been associated with increased susceptibility to many infectious diseases, including viral infections (17), and *MBL2* polymorphisms have been associated with disease pathogenesis (18). Examination of a limited number of Vietnamese individuals with low serum MBL concentrations due to a variant MBL allele suggested that MBL deficiency does not impact the risk of DHF/DSS (5). However, studies with DENV-infected patients in Brazil suggested that low levels of MBL may be associated with protection against thrombocytopenia (19), whereas high MBL levels appear to be correlated with severe disease (12). Accumulating evidence indicates that DF and DHF/DSS are complex diseases that are likely affected by multiple viral and host immune and genetic factors. Future studies with statistically significant numbers of individuals with the same MBL genotypes analyzed in the published studies and with individuals with other known *MBL2* gene polymorphisms that are associated with decreased MBL levels should clarify the precise role of the MBL pathway in determining the outcome of DENV infection.

The present study has thus provided an impetus for investigating the role of the MBL pathway and other elements of the complement system in anti-DENV protection versus disease pathogenesis using animal models and patient samples. This line of research may help answer two central questions in the DENV field: why are the vast majority of individuals with either primary or secondary DENV infections asymptomatic, and why do secondary infections with DENV result in more severe illness than primary infections?

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