General Introduction
CHAPTER 1

GLOBAL BURDEN OF DENGUE VIRUS AND WEST NILE VIRUS

Dengue virus (DENV) and West Nile virus (WNV) are emerging mosquito-borne pathogens belonging to the Flavivirus genus in the family Flaviviridae. The past decades have witnessed a global resurgence of DENV, now causing an estimated 50 million infections annually (WHO, 2009). Some 2.5 billion people worldwide are at risk of being infected with DENV. Although most dengue cases are asymptomatic, infection may result in dengue fever, a debilitating febrile illness, or progress into more severe manifestations known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler, 1998; Halstead, 2007). There are four dengue serotypes (DENV 1-4) all of which may cause similar clinical manifestations. Importantly, the development of severe disease is associated with secondary infections with a heterotypic DENV serotype (Halstead, 1970; Thein et al., 1997; Guzman et al., 2002). Co-circulation of multiple serotypes due to the global spread of DENV has been responsible for an increased number of epidemics (Gubler, 2002; Mackenzie et al., 2004). WNV is common in regions throughout the Old World and Oceania where it causes incidental outbreaks associated with only minor illness (Mackenzie et al., 2004). However, with the emergence of a more virulent strain of WNV lineage 1, outbreaks in the past decades have involved high numbers of neuroinvasive disease. In recent epidemics, approximately 1% of infections resulted in meningitis, encephalitis or flaccid paralysis, with fatality rates of ~10 % among hospitalized patients (Petersen et al., 2003; Gubler, 2007). Many survivors experience long-term cognitive and neurological impairment (Petersen et al., 2003; Mackenzie et al., 2004). Moreover, WNV lineage 1 has recently emerged in North America and subsequently spread to South America and the Caribbean (Gubler, 2007). Currently, no vaccines or antiviral drugs are available to prevent or treat disease caused by DENV or WNV.

SCOPE OF THE THESIS

Antibodies (Abs) play a central role in controlling flavivirus infections. Individuals that have undergone flavivirus infection are protected upon reinfection due to the presence of protective Abs (Klockmann et al., 1991; Gubler, 1998). At the same time, the presence of Abs may predispose individuals to more severe disease upon infection with DENV (Halstead, 1970; Kliks et al., 1988; Thein et al., 1997; Guzman et al., 2002). A more fundamental insight into the molecular basis of protection against flavivirus infection is crucial for the development of antiviral strategies. Therefore, the studies described in this thesis focus on the role of the membrane fusion process in the cellular entry of flaviviruses, with the ultimate aim to gain a better understanding of the mechanisms of Ab-mediated neutralization and enhancement of flavivirus infection.

Chapter 2 reviews the steps involved in the receptor-mediated endocytosis of flavivirus particles with particular emphasis on the molecular mechanisms that govern the process of membrane fusion. Furthermore, the influence of particle maturation on the infectious properties of flaviviruses is discussed. Finally, the mechanisms by which Abs may neutralize or enhance and thus critically influence the outcome of flavivirus infections are addressed.
Chapter 3 presents a detailed characterization of the basic requirements for WNV membrane fusion with liposomal target membranes. This study provides a kinetic analysis of WNV fusion by monitoring the redistribution of fluorescent probes during membrane merger in an on-line fashion. It is shown that WNV fuses at mildly acidic pH and does not share the stringent lipid requirements of alphaviruses. Furthermore, it is demonstrated that fully immature particles can be rendered fusogenic upon in vitro maturation.

In Chapter 4, the inhibitory mechanism of the potently neutralizing anti-WNV monoclonal Ab (MAb) E16 is investigated. Using confocal microscopy, it is established that E16-opsonized WNV particles readily enter cells but are then targeted for lysosomal degradation. Subsequently, it is demonstrated that E16 strongly inhibits WNV fusion in a concentration-dependent manner. Moreover, fusion with liposomal target membranes could be completely blocked. These results suggest that MAb E16 inhibits infection by preventing fusion with the endosomal membrane.

In Chapter 5, the neutralizing properties of two potent anti-WNV MAbs raised during natural WNV infection are investigated. Both MAbs were mapped to epitopes that likely span the E dimer-interface. Binding affinity was shown to be pH-dependent, suggesting the involvement of a conformational epitope. In fusion measurements with liposomes, opsonization with these MAbs strongly inhibited the fusogenicity of WNV. This study shows that naturally occurring MAbs that bind epitopes outside of DIII of the E-protein can potently inhibit infection through blockade of membrane fusion.

Chapter 6 establishes a biological role for immature WNV in the presence of anti-prM MAbs. Otherwise non-infectious immature WNV particles were demonstrated to readily infect Fc-receptor bearing murine macrophages when opsonized with anti-prM MAbs. Infectivity was dependent on the activity of the endoprotease furin, as no infectious titer was observed upon addition of a furin-inhibitor to cells prior to infection with opsonized immature WNV. Moreover, WNV could be detected in the blood serum and brain of mice that received anti-prM opsonized immature WNV, while no virus was found in sera or brain from mice infected with immature WNV alone. The observation that antibody-opsonized immature WNV can cause mortality due to encephalitis in these mice suggests that immature virus should be regarded as an important component of flavivirus infection.

Chapter 7 provides evidence that MAbs directed against the E-protein can also enhance the infectious properties of immature flaviviruses. We found that opsonization with the anti-E MAb E53 rendered both immature DENV and WNV infectious in a furin-dependent manner. Interestingly however, while E53 readily promoted infectivity of immature DENV on murine P388D1 macrophages, the increase in infectivity was not observed on human leukemia K562 cells. It is shown that the ability of furin to cleave immature DENV is altered by the presence of MAb E53, possibly explaining the differences in enhancement observed in the two cell lines. The relevance of anti-E MAbs that preferentially recognize immature particles and their role in flavivirus disease pathogenesis is discussed.

Chapter 8 provides a summary and discussion of the findings described in this thesis.
REFERENCES
