Chapter 1

General Introduction and Scope of the Thesis
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Global burden of Dengue virus and Chikungunya virus

Dengue virus (DENV) and Chikungunya virus (CHIKV) are rapidly emerging single-stranded positive-sense RNA viruses that are prevalent in the tropical and subtropical regions of the world (figure 1). Both viruses are transmitted to humans by mosquitoes of the *Aedes* (*Ae.*) species, with the yellow fever mosquito (*Ae. aegypti*) and the tiger mosquito (*Ae. albopictus*) being the main vectors [1-3].

The incidence of DENV increased more than 30-fold over the last 60 years due to large scale unplanned urbanization, international trade and travel, and population growth [1,4]. Accordingly, DENV is the most rapidly spreading and also most common mosquito-borne viral infection worldwide. Yearly, an estimated 390 million individuals are infected with DENV and about 100 million develop symptomatic disease [5]. Four serotypes of DENV have been described (DENV-1 to 4) and each of them can cause symptomatic disease. Disease is classified in three categories: “dengue without warning signs”, “dengue with warning signs”, and “severe dengue”. In most cases, dengue is mild with symptoms including fever, rash, muscle and joint pain, and nausea. Patients that have additional symptoms like vomiting, lethargy, bleedings, abdominal pain and/or show liver enlargement and/or increased haematocrit combined with decreased platelet levels are classified as dengue with warning signs. These patients are considered at risk for severe disease development. Severe dengue is characterized by severe plasma leakage, bleedings, and organ impairment. If not

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**Figure 1 Global distribution of Dengue Virus (yellow) and Chikungunya Virus (blue).**
Areas in which both viruses co-circulate are marked in green. The red line represents the 10°C isotherm line. Within these zones, the vectors *Ae. aegypti* and *Ae. albopictus* can survive. The latter can also adapt to lower temperatures and is sometimes present in more temperate climates (adapted from [12,29]).
properly treated patients may develop Dengue shock syndrome [4]. About 500,000 dengue-infected patients are hospitalized per year, and in approximately 25,000 cases dengue is fatal [6]. Individuals are life-long protected from disease upon re-infection with the same DENV serotype. Remarkably, severe disease is most often seen in individuals experiencing a secondary infection with a heterologous DENV serotype or in infants born to dengue-immune mothers [7-9]. These observations indicate that pre-existing antibodies play an important role in severe disease development, which will be discussed in detail in subsequent chapters. Third or tertiary infections on the other hand are often mild and usually do not lead to disease manifestations [10].

The global distribution of CHIKV rapidly expanded since the end of 2004. Before, only small focal epidemics within Africa and Asia were reported. In only ten years, the virus has spread worldwide causing millions of infections in the (sub-)tropical regions of the world (figure 1)[11,12]. Due to the recent emergence and hence naïve population, attack rates of CHIKV are high [13,14]. The first large-scale CHIKV outbreak was caused by adaptation of the virus to *Ae. albopictus* without compromising viral fitness in the original vector *Ae. aegypti*. However, not only strains that are primarily transmitted by *Ae. albopictus* are circulating. For example, the ongoing outbreak within the Americas is caused by a CHIKV strain that relies on transmission by *Ae. aegypti* [15]. Importantly, the flexibility in vector usage increases the epidemic reach of the virus. In contrast to DENV infection, CHIKV infection is in about 75%-95% of the cases symptomatic [16,17]. Affected individuals develop Chikungunya fever, which is characterized by high fever, rash, headache, myalgia, and arthralgia. Although these symptoms normally resolve within 7-10 days, a significant number (12%-49%) of patients experience debilitating joint pain that can persist from month to years [18,19]. Infection with CHIKV usually leads to a protective adaptive immune response with neutralizing antibodies and CD4+ T cells being the main antiviral effectors [20,21]. The time intervals between historical epidemics have led to the assumption that infection confers long-term, if not life-long, immunity against re-infection with CHIKV [22,23].

Taken together, both DENV and CHIKV have an enormous health-economic impact in the affected areas. In spite of continuous effort, no specific antiviral treatment or vaccine has been found for either of the viruses and treatment is currently focused on relieving the symptoms of disease. For the rational design of vaccines and antiviral drugs it is imperative to better understand the dynamic interactions that occur between the virus and the host during virus reproduction.
Scope of the Thesis

The research described in this thesis focuses on the early events in DENV and CHIKV infection. Both viruses are known to enter the cell via receptor-mediated endocytosis and fuse from within acidic endosomes. In this thesis we aimed to further investigate the molecular mechanisms that are involved in viral cell entry. Moreover, the role of pre-existing immunity on infectivity was assessed using patient serum and monoclonal antibodies.

Chapter 2 provides an overview of the structure, life cycle and pathogenesis of DENV. Infected cells produce DENV particles that vary in maturation state, and we and others have demonstrated before that immature virions are essentially non-infectious in various cell types [24-26]. The general implementation of this finding was however questioned when the receptor molecule DC-SIGN – which is also expressed on natural target cells of DENV – had been reported to promote the infectivity of both mature and immature West Nile virus, a virus closely related to DENV [27,28]. These results prompted us to investigate in chapter 3 whether immature DENV is infectious in cells expressing DC-SIGN. To this end, we studied the infectious properties of DENV particles of different serotypes and maturation states on target cells with and without DC-SIGN. In addition, using the same cell types, we examined the role of pre-existing immunity on DENV infectivity in relation to DC-SIGN expression.

In recent years, major efforts have been made to provide insights into the route of CHIKV cell entry and the molecular mechanism of membrane fusion. Chapter 4 gives a detailed overview on the early events in CHIKV infection. This chapter summarizes the current knowledge on viral structure, target cells, entry and membrane fusion. In addition, we discuss infection prevention by use of entry inhibitors.

In chapter 5, we unraveled the cell entry pathway of CHIKV by live-cell microscopy. By using fluorescently labeled virions and cellular marker proteins, we were able to dissect the route of CHIKV cell entry and unraveled the dynamics involved. In addition, we investigated the role of the amino acid at E1-226 in lipid dependency of viral entry and membrane fusion.

In chapter 6, the membrane fusion characteristics of CHIKV are further characterized using liposome-based bulk studies and high-resolution single-molecule microscopy. Besides studying basic characteristics like pH- and lipid-dependency of fusion, also a detailed kinetic picture of the fusion process is drawn.

Administration of neutralizing antibodies represents an attractive approach to treat or prevent CHIKV infections. Hence, knowledge on the mechanism of neutralization can promote the development of a vaccine or viral inhibitor. In
Chapter 7, the generation and in vitro characterization of a panel of CHIKV-specific monoclonal antibodies (MAbs) is described. Furthermore, the in vivo activity of several highly neutralizing MAbs is assessed in a mouse model.

In chapter 8, a series of neutralizing MAbs from the previous chapter are tested for their fusion-inhibiting capacities, and the mechanism of neutralization is investigated for a selection of fusion-inhibitory antibodies.

Chapter 9 summarizes and discusses the results obtained in the studies in this thesis.

References

12. Centers for Disease Control and Prevention. Countries and territories where chikungunya cases have been reported. 2015. Available: http://www.cdc.gov/chikungunya/geo/


