Molecular mechanisms of dengue virus infection
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Chapter 1

Brief introduction and scope of thesis
Dengue virus and dengue disease

Dengue virus (DENV) is a positive-sense, single-stranded RNA virus belonging to the genus *Flavivirus* of the family *Flaviviridae*. To date, four antigenically distinct DENV serotypes have been identified. DENV is transmitted by mosquitoes of the *Aedes* family and it is the most common arthropod-borne viral infection in humans, with an estimated 390 million infections in 2010. Most infections occur in the (sub-)tropical areas of the world. Indeed, dengue is hyper-endemic within Asia, and both Central and South America. Furthermore, the awareness of this virus and its potency to settle within Europe is increasing. In part, this is due to the 2011-2012 epidemic on the island of Madeira, resulting in 2,168 reported dengue cases. Moreover, around the same time, another report was published highlighting the alarming invasion of ‘dengue mosquitoes’ (i.e. *Aedes aegypti* & *Ae. albopictus*) within Europe.

Yet, dengue epidemics within Europe and North America are not a recent phenomenon. One of the earliest reports of a dengue-like, break-bone fever epidemic includes a report by Benjamin Rush on “bilious remitting fever” in Philadelphia, USA. During the summer and autumn of 1780, Dr. Rush describes how the epidemic evolved in the city while noting that “the muschetoes were uncommonly numerous during the autumn”. Those falling ill with the disease were noted to suffer amongst others from fever and “… pains … exquisitely severe in the head, back and limbs. The pains in the head were sometimes in the back parts of it, and at other times they occupied only the eyeballs.” Moreover, some of the patients had haemorrhagic signs, e.g.: “… the discharge of a few spoons-full of blood from the nose…. In others, a profuse haemorrhage from the nose, mouth, and bowels…”

Dengue was also present in Europe. In 1801, Queen Maria Luisa of Spain reported of falling ill with a disease commonly called dengue, describing symptoms as “… distorts my whole visage, making me yellow as saffron, … the hips and womb hurt..., … my body is very painful and especially (?) the head.”

In the late 1920s, Greece experienced one of the most serious dengue epidemics in Europe with more than 90% of the Athens population falling ill with dengue resulting more than 1000 deaths. Thereafter, dengue had a low profile within Europe till the Madeira epidemic.

Importantly, the historical epidemics are based on symptomatic cases, while ~75% of infections actually is asymptomatic. Hence, Bhatt and colleagues estimated that there had been a total of 390 million infections in 2010. This estimate makes dengue one of the most common arthropod-borne infections worldwide with...
major implications for health care systems in epidemic countries. Symptomatic dengue can display a wide range of signs from e.g. relatively mild flu-like disease with head pains to severe bleedings (i.e. dengue haemorrhagic fever), or shock syndrome and potentially death.

The role of immunity in dengue disease severity

The question why some patients develop relatively mild dengue fever and others experience life-threatening haemorrhagic fever or shock syndrome is continuously under investigation. Strikingly, reports of, amongst others, Scott Halstead and colleagues noted a relationship between the immune status and disease severity. Indeed, dengue virus infectivity was enhanced in vivo with sub-neutralizing properties, a phenomenon called antibody-dependent enhancement of dengue virus infection.

Severe disease was most often observed in patients experiencing a secondary DENV infection with a heterologous serotype. Moreover, the observation that infants born to dengue-immune mothers had higher risk of severe disease, even though it was their first infection, particularly points to the role of antibodies in severe disease. Indeed, dengue virus infectivity was enhanced in vivo and in vitro by waning concentrations of homotypic antibodies, or heterotypic antibodies with sub-neutralizing properties, a phenomenon called antibody-dependent enhancement of dengue virus infection.

The observation that antibodies aggravate dengue disease, has hampered the development of a dengue vaccine as incomplete immunization may sensitize individuals for enhanced disease. Yet, meanwhile, larger epidemics with a higher incidence of severe disease are seen due to globalization, urbanization, and hyper-endemicity. Vaccine development is thus essential yet the major challenge is to provoke neutralizing immunity to all serotypes. Recent clinical trials of a tetravalent DENV vaccine showed that seroconversion alone is not sufficient for protection. It is therefore of utmost importance to gain a better understanding of the mechanisms behind neutralizing versus enhancing antibodies in dengue infection.

Scope of thesis

The research presented in this thesis centres around the interactions of dengue virus with its human host cells. In the past, the phenomenon of antibody-dependent enhancement was mostly studied in cell lines. Within this thesis, we wished to take it further using primary human cells. Despite the intrinsic variety between donors, using primary cells will bring important insights as it better reflects the in vivo interactions between dengue virus and its host cells.

Given the complexity of the theory of antibody-dependent enhancement of dengue virus infection, we first reviewed the state of knowledge at the beginning of the project. This review, chapter 2, presents an overview of the known host cell factors and molecular mechanisms contributing to dengue virus infection of human cells in the absence as well as in the presence of dengue antibodies. Moreover, within this review, several challenges were identified related to the mechanisms of enhanced infection. Some of these challenges were subsequently investigated and will be discussed in the chapters below.

First, in chapter 3, we investigated how the cell tropism of dengue virus serotype 2 affects the infectious properties of progeny virions. This was investigated to gain more insight in the interaction between dengue virus and its human host cells, and the sequential rounds of cellular infection. We performed side-by-side comparisons of the infectious properties of DENV in macrophages, immature dendritic cells and mature dendritic cells, derived from the same donors. Moreover, we differentiated between infection in the absence of dengue antibodies as well as infection in the presence of dengue antibodies.

In chapter 4, we identified the molecular mechanisms underlying the phenomenon of antibody-dependent enhancement of dengue infection in primary macrophages. Completion of the viral life cycle can be considered as the product of a successfully orchestrated symphony of virus – host interactions. Hence, we quantified the effects of enhancing antibodies on each of the distinct stages in the viral life cycle.

Furthermore, we employed microarray technology to gain insight in the response of macrophages towards dengue virus. Notably, dengue-infected macrophages were found to respond to infection by secreting cytokines. Several of these cytokines were previously identified as being pro-viral or antiviral for dengue virus. Hence, within chapter 5, we further investigated if and how interleukin 6, tumour necrosis factor alpha and interferon alpha influenced dengue virus infection of primary human macrophages.

In the experimental chapters described above, we elucidated the molecular mechanisms involved in antibody-dependent enhancement of dengue infection in primary human macrophages.

Recently, several publications were published on the host antibody repertoire in dengue vaccinated or infected humans. In chapter 6, we reviewed these publications to present a ‘systems vaccines’-approach towards: (i) the human antibody response in the context of the recent CYD-TDV vaccine trials of Sanofi-Pasteur, and (ii) the role of these antibody epitopes in neutralization of disease.

Lastly, the results presented in this thesis are summarized and discussed in chapter 7.
References


