CHAPTER 1

INTRODUCTION

1.1 Background

Zika virus (ZIKV) is an enveloped positive-sense single-stranded RNA flavivirus which is classified to the family of Flaviviridae, together with other clinically significant viruses, for example, dengue virus (DENV), Japanese encephalitis virus (JEV), and Yellow Fever virus (YFV) (Lauriti et al., 2018). It has been widely recorded that 75% to 80% exposure to ZIKV produces asymptomatic infection. In symptomatic cases, the infection produces broad clinical presentations, from acute febrile illness to severe neurological complications (Calvet et al., 2015; Muñoz, Barreras, & Pardo et al., 2016; Song, Yun, & Lee, 2017). Zika disease has gained the attention of the healthcare community after its re-emergence on French Polynesia in 2013 and Brazil in 2015 as it is associated with congenital Zika Syndrome (CZS), that includes congenital microcephaly and myelitis, as the consequence of maternal infection (Song, Yun, & Lee, 2017).

ZIKV has infected hundred thousands of people worldwide and caused devastating outcomes in both healthcare and socioeconomic settings across the endemic regions (Mayer, Tesh, & Vasilakis, 2017). In 2017, about 560 000 Zika cases, with as much as 3000 confirmed congenital Zika syndromes, have been documented in the Americas (Lim, Lim, & Yoon, 2017). In 2019, the World Health Organization (WHO) corroborated the presence of autochthonous transmission of ZIKV within 87 countries and territories. The reason for the extensive distribution of ZIKV is not well understood; however, increasing international mobility, climatic and other environmental factors, population density, as well as suitable vector habitat may increase the vulnerability to ZIKV emergence and widespread distribution (Rees et al., 2018).

More recently, co-circulation of ZIKV and other flaviviruses has been shown in some tropical countries. As a result, the likelihood of co-infections and subsequent infections by

multiple flaviviruses has increased (Dupont-Rouzeyrol et al., 2015; Pessôa et al., 2016; Rico-Mendoza et al., 2019). The relatively high degree of structural and antigenic similarity between DENV and ZIKV increases the propensity of pre-existing DENV immune sera and T cells to cross-react with ZIKV (Stettler et al., 2016). Cross-reactivity has been widely evaluated for its relation to the disease-enhancing effects mediated by the interaction of pre-existing antibodies with heterologous virus present during a subsequent infection, which is known as antibody dependent enhancement (ADE) of infection. This phenomenon is commonly assumed to occur in cases of DENV, where immune responses produced after primary infection confers protection against secondary infections by viruses under the same serotype, while enhancing sequential viral infection with different serotypes (Stettler et al., 2016). DENV-specific antibodies were also demonstrated to cross-react with ZIKV and intensify ZIKV infection *in vitro* and *in vivo* (Dejnirattisai et al., 2016; Langerak et al., 2019; Priyamvada et al., 2016; Rathore et al., 2019).

On the contrary, ADE was not identified in DENV-immunized mice after receiving infection with ZIKV (Watanabe et al., 2018). The potential therapeutic role of DENV specific-antibodies against lethal ZIKV infection has been illustrated (Stettler et al., 2016). While antibodies observed to contribute in disease enhancement, pre-existing DENV specific T-cell provide functional immune outcomes in secondary infection with ZIKV (Elong Ngono et al., 2017; Pardy & Richer, 2019). Overall, the evidence of ADE phenomenon between DENV-immunity and ZIKV infection *in vivo*, as well as in human, is insufficient. Therefore, further investigations for contribution antibody cross-reactivity to ADE involving a better animal model remains necessary.

Nonhuman primates (NHPs) are a prominent animal model as they mimic various aspects of human flavivirus infection, such as susceptibility, viral replication, pathogenesis, and immunological responses despite its practical issue of cost and ethics (Osuna & Whitney, 2017). Although NHPs have provided clinical relevance of ADE induced by heterogenous re-infection by different DENV serotypes in dengue-immune and zika-immune macaques, the contribution of

DENV immunity and ZIKV cross-reactivity to immune protection or disease enhancement remains debatable (George et al., 2017; Pantoja et al., 2017; Valiant et al., 2018).

In this study, the antibody responses following DENV infection was observed for its cross reactivity to ZIKV, which is known to contribute disease enhancement outcome, in NHPs. Pregnant DENV2-immune Cynomolgus macaques were infected with ZIKV. Antibody responses were monitored for 91 days post infection. The antibody cross-reactivity of DENV-specific antibodies against ZIKV was measured and ADE was demonstrated *ex vivo.*

1.2 **Objective(s)**

This study aims to characterized Zika cross-reactive immune responses in dengue immune primates.

1.3 **Hypothesis**

H_0: Anti-DENV IgG produced upon primary challenge of DENV2 does not cross-react with ZIKV

H_1: Anti-DENV IgG produced upon primary challenge of DENV2 cross-react with ZIKV