Journal of Immunological Methods 410 (2014) 34–38

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Mouse models for dengue vaccines and antivirals

Emily M. Plummer, Sujan Shresta *

La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 3 January 2014
Accepted 6 January 2014
Available online 15 January 2014

Keywords:
Dengue virus
Mouse model
Non-human primate

ABSTRACT

Dengue virus (DENV) has substantial global impact, with an estimated 390 million people infected each year. In spite of this, there is currently no approved DENV-specific vaccine or antiviral. One reason for this is the difficulty involved with development of an adequate animal model. While non-human primates support viral replication, they do not exhibit signs of clinical disease. A mouse model is an ideal alternative; however, wild-type mice are resistant to DENV-induced disease. Infection of interferon receptor-deficient mice results in disease that recapitulates key features of severe dengue disease in humans. For the development of vaccines, interferon receptor-deficient mice provide a stringent model for testing vaccine-induced immune components from vaccinated wild-type mice.

1. Introduction

An estimated 390 million people are infected with one of the four serotypes of Dengue virus (DENV1-4) every year (Bhatt et al., 2013). Of these, 96 million infected individuals present with clinical or subclinical severity (Bhatt et al., 2013), and 20,000 annual cases result in death (Gubler, 2012). Half the world's population, generally in tropical and subtropical climates, is at risk for infection with DENV (Tapia-Conyer et al., 2012). Southeast Asia, the Western Pacific, Central and South America, Africa, and the Caribbean all have widespread risk for dengue. The virus alternately infects mosquitoes (*Aedes aegypti* and *Aedes albopictus*) and humans, in which symptoms can vary from asymptomatic, to acute febrile dengue fever (DF), to severe, potentially lethal dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) (Gibbons and Vaughn, 2002).

1.1. Need for an animal model

The existence and concomitant circulation of four related but distinct serotypes is unique to DENV (Balmaseda et al., 2006). Also unlike other pathogens, a second infection with a different DENV serotype is associated with more severe disease than primary infections. DENV has emerged as an important pathogen (World Health Organization, 2009) due to increasing disease prevalence, which is likely associated with expanding mosquito habitats and widespread concomitant circulation of multiple DENV serotypes. All of these factors make DENV a complex and relatively unexplored pathogen. Treatments that are currently in use to control DENV infection are largely supportive, including rehydration and in-patient monitoring. Efforts towards a better understanding of DENV and an effective targeted treatment, including vaccines and antivirals, have been hindered in large part by the lack of an appropriate animal model. Infection of some non-human primate species results in viremia, but infected animals show limited signs of clinical disease. Lack of replication in immunocompetent mice (Yauch and Shresta, 2008) has led to the use of immunodeficient mouse models, and DENV infection of mice lacking both type I and II interferon (IFN) receptors or type I IFN receptor alone recapitulates many hallmarks of human disease. Herein, we will review the animal models that have been used in studies of DENV. In addition, we will highlight the potential for using the IFN receptor-deficient mice in combination with adoptive transfer of immune components from immunized wild-type mice as a valid model for testing DENV-specific vaccines.

* Corresponding author at: 9420 Athena Circle La Jolla, CA 92037. Tel.: +858 752 6944.
E-mail address: sujan@lji.org (S. Shresta).

0022-1759/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jim.2014.01.001

1.2. Human infection with DENV

Clinical DENV disease manifestations can be separated into two forms. The milder form, DF, is self-limiting and is characterized by fever, headache, rash, nausea, vomiting, and myalgia. The severe form, DHF/DSS, typically occurs during a secondary infection with a different serotype, or in infants born to DENV-immune mothers (Nimmannitya, 1997). Signs associated with DHF/DSS include elevated hematocrit, pleural effusion, hemorrhagic manifestations, thrombocytopenia, and shock (World Health Organization, 2009). Hemorrhagic manifestations are associated with distortion, but rarely severe damage, of blood vessels (Sahaphong et al., 1980). A key hallmark of severe disease is vascular leakage, which is thought to be caused, at least in part, by elevation of pro- and anti-inflammatory cytokines, often referred to as cytokine storm (Rathakrishnan et al., 2012). The risk factor responsible for the largest number of cases of DHF is secondary dengue infection. Primary dengue infections of infants with passively acquired dengue antibodies result in higher rates of severe disease than do secondary dengue infections. In addition to the immune status of the individual, genetic factors and viral virulence could also play a role in determining severity of disease, and the response to infection likely depends on a combination of host and viral factors (Halstead, 2007; Rico-Hesse, 2007).

2. Models of DENV infection

2.1. Nonhuman primate models

Natural hosts for DENV infection are humans and mosquitoes. Serology studies of primates in Africa and Southeast Asia suggest a sylvatic cycle in nonhuman primates (Wang et al., 2000; Diallo et al., 2003). Chimpanzees, as well as rhesus and other monkey species, develop viremia and neutralizing antibody in response to subcutaneous infection with DENV (Scherer et al., 1978; Halstead et al., 1973a,1973b,1973c; Halstead and Palumbo, 1973). However, evidence of clinical disease as seen in humans is scarce in these species, with the exception of limited clinical manifestations seen in rhesus macaques. Following subcutaneous infection of macaques, DENV spreads quickly from the site to draining lymph nodes, and viremia begins 2–6 days later. Virus can be detected in the blood for 3–6 days and during this time can be isolated from skin, non-draining lymph nodes, and infrequently from bone marrow, liver, lung, and thymus (Marchette et al., 1973). In one study of macaque infection, leukopenia or thrombocytopenia was seen following primary or secondary heterologous (respectively) infections with DENV (Halstead et al., 1973a,1973b,1973c; Halstead and Palumbo, 1973). Another study found evidence for petechiae and subcutaneous bleeding 3–5 days after intravenous infection with DENV2 (Onlamoon et al., 2010). Although hemorrhage is a feature of severe disease in humans, the hallmarks, vascular leakage, and fever were not present, and viremia levels were appreciably lower than those in human DHF, limiting this model to studies of early response to infection.

Nonhuman primate models have been widely used for testing of DENV vaccines. Safety and efficacy can be measured by changes in viremia, peak viral levels, and magnitude of antibody response. A tetravalent chimeric dengue vaccine (DENVax) induced virus-neutralizing antibodies in cynomolgus macaques against all 4 serotypes and reduced viremia compared with controls (Osorio et al., 2011). In another study, a chimeric DENV vaccine also reduced viremia in monkeys (Li et al., 2013). The onset and duration of viremia in NHPs is similar to humans, and aspects of the immune response seen in humans are recapitulated in some species. However, the use of NHP models is limited to studies of viremia, which is reduced compared to humans. These differences, along with a lack of manifestations of hallmarks of DHF/DSS, make NHP an inadequate model for vaccine and antiviral testing. It is difficult to even find hallmarks of DF in NHP models. Recording of temperature in DENV-infected primates is logistically difficult, and body temperature varies greatly, making accurate readings difficult (Baker et al., 1976). A live, attenuated vaccine prevented viremia, however, did not block anamnestic antibody response in monkeys and ultimately did not show protection in human phase 2b clinical trials (Sabchareon et al., 2012; Guirakhoo et al., 2004; Halstead, 2013). Based on this result, the use of NHP models should be used in conjunction with other models in order to investigate the efficacy of the vaccine in the context of severe dengue disease manifestations. In particular, a tractable and genetically manipulable small animal model, such as the IFN receptor-deficient mouse model described below, is less expensive than NHPs and can be used to both evaluate vaccine safety and efficacy and immunological mechanisms of vaccine-mediated protection.

2.2. Mouse models

2.2.1. Intracerebral infection with mouse-brain-adapted DENV

Wild-type mice infected by the peripheral route do not support replication of DENV and show no signs of disease manifestation. However, a DENV with increased neurovirulence in mice has been made by serial intracerebral passaging in suckling mice (Cole and Wisseman, 1969; Sabin and Schlesinger, 1945). The resulting virus causes paralysis in a fraction of mice after a long incubation period. The partial, delayed response does not recapitulate severe disease seen in humans, limiting the scope of the model. Additionally, the route of infection is not relevant to humans, and nervous system involvement in human DENV infections is controversial and assumed to be rare by some and frequent by others (Patey et al., 1993; Lum et al., 1996). According to WHO guidelines, this model is not appropriate for testing DENV vaccine safety and efficacy (WHO, 2013).

2.2.2. Humanized mice

Models of mouse–human chimeras have been shown to develop fever, and thrombocytopenia following infection with DENV. By measuring viral titers in severe combined immunodeficiency (SCID) mice transplanted with human liver cells (SCID-HuH-7), the level of attenuation of live DENV vaccines can be evaluated (Blaney et al., 2005). The NOD/SCID/IL-2gamma receptor-null mice reconstituted with human CD34+ cells have been used in DENV studies and demonstrate fever and thrombocytopenia (Mota and Rico-Hesse, 2011).

The advantage of the humanized mice is that one can investigate human cell response to DENV infection better than in the mouse model with intracerebral challenge and paralysis. However, it is laborious to prepare humanized mice, which contributes at least in part to the considerable mouse-to-mouse variation that is often seen (Akkina et al.,