Virology 487 (2016) 41–49

Contents lists available at ScienceDirect

Virology

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

Antibodies are not required to a protective immune response against dengue virus elicited in a mouse encephalitis model

Jaime Henrique Amorim [a,*], Rúbens Prince dos Santos Alves [a], Raíza Bizerra [a], Sara Araújo Pereira [a], Lennon Ramos Pereira [a], Denicar Lina Nascimento Fabris [a], Robert Andreata Santos [a], Camila Malta Romano [b], Luís Carlos de Souza Ferreira [a,*]

[a] Laboratório de Desenvolvimento de Vacinas, Departamento de Microbiologia, Universidade de São Paulo, Brasil
[b] Instituto de Medicina Tropical de São Paulo e Faculdade de Medicina, Departamento de Moléstias Infecciosas e Parasitárias (LIMHC), Universidade de São Paulo, Brasil

ARTICLE INFO

Article history:
Received 20 May 2015
Returned to author for revisions
28 September 2015
Accepted 5 October 2015

Keywords:
Dengue
Nonstructural proteins
Immune response
T lymphocytes
Antibodies

ABSTRACT

Generating neutralizing antibodies have been considered a prerequisite to control dengue virus (DENV) infection. However, T lymphocytes have also been shown to be important in a protective immune state. In order to investigate the contribution of both humoral and cellular immune responses in DENV immunity, we used an experimental model in which a non-lethal DENV2 strain (ACS46) is used to intracranially prime Balb/C mice which develop protective immunity against a lethal DENV2 strain (JHA1). Primed mice generated envelope-specific antibodies and $CD8^+$ T cell responses targeting mainly non-structural proteins. Immune sera from protected mice did not confer passive protection to naïve mice challenged with the JHA1 strain. In contrast, depletion of $CD4^+$ and $CD8^+$ T lymphocytes significantly reduced survival of ACS46-primed mice challenged with the JHA1 strain. Collectively, results presented in this study show that a cellular immune response targeting non-structural proteins are a promising way in vaccine development against dengue.

Introduction

Dengue viruses are arboviruses of the *Flavivirus* genus (Guzman et al., 2010) found as four distinct serotypes (DENV1–4). They cause an acute febrile illness called dengue, which may develop in different degrees of manifestations that were classified by WHO as dengue fever, dengue with alarm symptoms and severe dengue (Horstick et al., 2015). It is estimated that over 300 million people are infected by any of the DENV serotypes per year worldwide and, at least, 90 million develop the severe forms of the disease (Bhatt et al., 2013). Despite the high epidemiological relevance of the disease, no effective vaccine formulation is presently approved for use in humans.

Severity of symptoms displayed by a DENV infection is highly associated with viremia titers (Horstick et al., 2015; Murgue et al., 2000; Vaughn et al., 2000). Historically, antibodies capable of preventing virus of infecting susceptible cells have been thought to represent the main and, perhaps, the sole protection correlate (Guzman and Harris, 2015; Guzman et al., 2010; Sabin, 1952; Whitehead et al., 2007). Such antibodies were extensively demonstrated to be capable of preventing infection of the host cells *in vitro* (Blaney et al., 2005; Chiang et al., 2012; Guzman et al., 2010; Roehrig et al., 2008; Whitehead et al., 2007; Zhang et al., 2007), either by blocking the binding step of viral particles or by preventing conformational changes in the protein required for membranes fusion in endosome (Teoh et al., 2012), or by inducing a structural disruption of the viral envelope (Cockburn et al., 2012; Lok et al., 2008; Pierson and Kuhn, 2012). This rational was reinforced by reports of *in vivo* protection mediated by neutralizing antibodies in non-human primate model (Guirakhoo et al., 2004, 2001, 2000; Guy et al., 2010), and was adopted in the development of presently tested anti-dengue vaccine formulations which are based on chimeric or live-attenuated viruses which induce neutralizing antibodies to the four serotypes of DENV (Whitehead et al., 2007). In particular, chimeric live attenuated viruses between DENV and yellow fever virus (YFV) were constructed with the aim of inducing high titers of neutralizing antibodies against DENV envelope proteins (Guirakhoo et al., 2001). In clinical trials considerable titers of neutralizing

* Correspondence to: Laboratório de Desenvolvimento de Vacinas, Departamento de Microbiologia, ICB II, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1374, Cidade Universitária, São Paulo, SP, 05508-000, Brazil. Fax: +55 11 30917354.
E-mail addresses: jhamorim@usp.br (J.H. Amorim), lcsf@usp.br (L.C. de Souza Ferreira).

http://dx.doi.org/10.1016/j.virol.2015.10.006
0042-6822/

antibodies were induced in vaccinees volunteers (Sabchareon et al., 2012; Villar et al., 2014). Unfortunately, especially for DENV2, the vaccine formulation based on those chimeric viruses did not achieve the expected protective efficacy in a phase III clinical trials in different parts of the world (Sabchareon et al., 2012; Villar et al., 2014).

The relevance of cellular immune responses has also been extensively studied in the control of DENV infection (Bäck and Lundkvist, 2013; Bashyam et al., 2006; Han et al., 2012; Kurane et al., 1991; Yauch et al., 2010; Yoshida et al., 2013; Zellweger et al., 2013). $CD4^+$ and $CD8^+$ T lymphocytes specific for DENV antigens have been demonstrated to be important in controlling virus spread and intracellular replication (Gil et al., 2009; Rivino et al., 2013; Yoshida et al., 2013). Of particular interest, was the recent demonstration that skin-homing DENV-specific T cells occurs during natural infection (Rivino et al., 2015), which may indicate such cells are important to prevent infection at the viral entry site. In addition, $CD8^+$ T lymphocytes with multifunctional cytokine secretion patterns were found in volunteers immunized with a live attenuated tetravalent dengue vaccine (Weiskopf et al., 2015a). Moreover, such lymphocytes target highly conserved epitopes located on non-structural proteins and this immunological pattern fits with those found after natural sequential infections (Weiskopf et al., 2015a). Such reports clearly show that cellular immune responses are also involved in the generation of a protective immunological status, particularly in the context of vaccines to DENV.

In this study we used a mouse encephalitis model to further evaluate the contribution of antibodies and T cells on the generation of a protective immunological status for DENV infection. Based on two different DENV2 strains: the ACS46, unable to cause any harm to mice, and the neurovirulent JHA1 strain, capable to cause encephalitis and kill immunocompetent adult mice; we observed that Balb/C mice intracranially inoculated with ACS46 developed a protective immunity to a subsequent, otherwise lethal, challenge with the JHA1 strain. We found that antibodies generated in mice inoculated with the ACS46 strain, although capable of neutralizing the virus *in vitro*, were not capable to confer passive protection in the encephalitis model. In contrast, depletion of $CD4^+$ and $CD8^+$ T lymphocytes drastically reduced the protection of ACS46-inoculated mice challenged with the neurovirulent DENV2 strain. Collectively, our results clearly show that cellular immune responses, particularly those targeting non-structural proteins, are specifically involved in the control of DENV infection in the mouse encephalitis model.

Results

Infection of Balb/C mice with the DENV2 non-virulent ACS46 strain and the neurovirulent JHA1 strain

Balb/C mice were i.c. inoculated with 10, 1000 or 10,000 PFU of the ACS46 strain or with 100 PFU of the JHA1 strain. The ACS46 strain proved to be non-lethal to Balb/C mice, even when inoculated in a dose 100-fold higher with regard to JHA1, and the survival curves were significantly different ($p < 0.0001$) (Fig. 1A). The ACS46 strain was detected in mice brains in significantly lower numbers than the initial inoculum, which indicates a non-productive infection (Fig. 1B). In addition, in contrast with the JHA1 strain, the ACS46 strain was not capable to induce tissue damage (Fig. 1C), weight loss (Fig. 1D) or hematological disturbances (Table 1) in infected animals even when i.c. administered in high doses. Collectively these results indicate that the ACS46 strain infects Balb/C mice brains in a nonproductive manner without any pathological consequences to the host.

Evaluation of immune responses elicited in mice following i.c. administration of DENV2 ACS46 strain

Animals i.c. inoculated with 100, 1000 or 10,000 PFU of the ACS46 strain were bled fifteen days after inoculation for measurement of virus-specific antibodies and serum IFN-γ

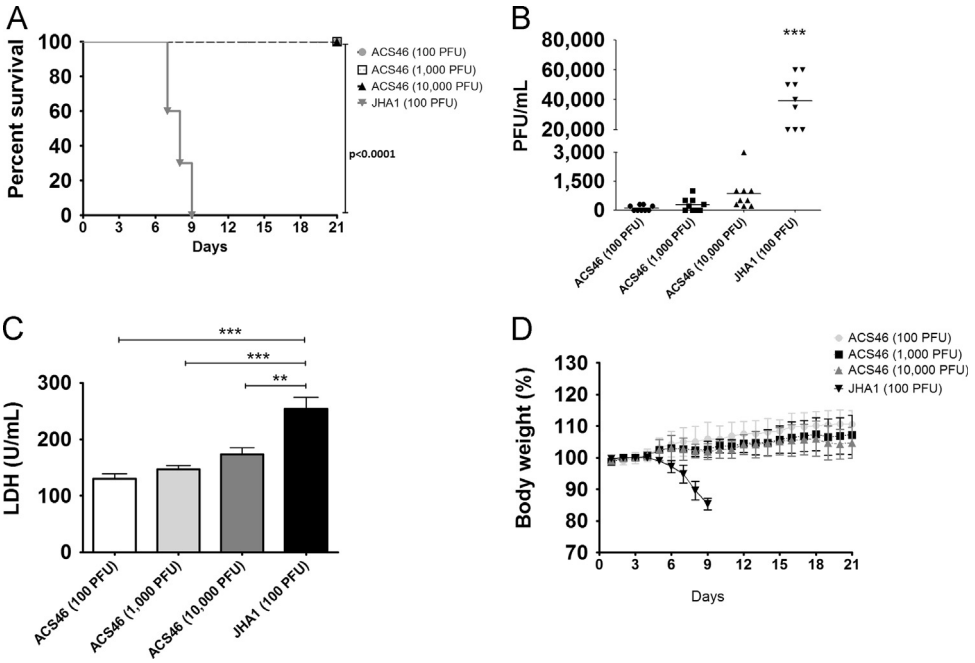

Fig. 1. The ACS46 DENV2 strain is not neurovirulent when i.c. administered to Balb/C mice. (A) ACS46 was not lethal to Balb/C mice even at a dose 100-fold higher than the LD_{100} of the neurovilent JHA1 strain. (B) ACS46 was detected in brain tissue at titers significantly lower than the initial inoculum, in contrast to JHA1, which indicates a non-productive infection. ACS46 was not capable to induce tissue damage (C) or weight loss in infected animals (D). Statistically significant differences are indicated with asterisks: **, $p < 0.01$; and ***, $p < 0.001$ ($n = 10$, for each inoculation group). Data are representative of three independent experiments.