Virology 456-457 (2014) 70–76

Contents lists available at ScienceDirect

Virology

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

Brief Communication

Capsid protein: Evidences about the partial protective role of neutralizing antibody-independent immunity against dengue in monkeys

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ARTICLE INFO

Article history:
Received 15 January 2014
Returned to author for revisions
4 February 2014
Accepted 11 March 2014

Keywords:
Capsid protein
Nonhuman primates
Protection
Dengue-2 virus
Nucleocapsid-like particles

ABSTRACT

The role of cellular immune response in dengue virus infection is not yet fully understood. Only few studies in murine models propose that $CD8^+$ T-cells are associated with protection from infection and disease. At the light of recent reports about the protective role of $CD8^+$ T-cells in humans and the no correlation between neutralizing antibodies and protection observed in several studies, a vaccine based on cell-mediated immunity constitute an attractive approach. Our group has developed a capsid-based vaccine as nucleocpasid-like particles from dengue-2 virus, which induced a protective $CD4^+$ and $CD8^+$ cell-mediated immunity in mice, without the contribution of neutralizing antibodies. Herein we evaluated the immunogenicity and protective efficacy of this molecule in monkeys. Neither IgG antibodies against the whole virus nor neutralizing antibodies were elicited after the antigen inoculation. However, animals developed a cell-mediated immunity, measured by gamma interferon secretion and cytotoxic capacity. Although only one out of three vaccinated animals was fully protected against viral challenge, a viral load reduction was observed in this group compared with the placebo one, suggesting that capsid could be the base on an attractive vaccine against dengue.

Introduction

Dengue virus (DENV) infection is a major emerging disease of tropical and subtropical countries, transmitted by the bite of an infected mosquito, usually *Stegomya aegypti*. Many infections are asymptomatic while the clinical manifestations can range from a self-limiting febrile illness (dengue fever) to a life-threatening disease, characterized by increased vascular permeability, thrombocytopenia, hemorrhagic manifestations and shock (dengue severe) (Kyle and Harris, 2008). It is estimated that nearly half of the world population is at risk of infection and up to 50 million people are infected each year. DENV are positive-stranded RNA viruses belonging to the *Flaviviridae* family. There are four distinct serotypes (DENV-1 to -4), which show 67–75% sequence homology (Fu et al., 1992).

For several years, researchers have associated the generation of neutralizing antibodies as a premise to reach protection against DENV. However, dengue is a non-cytopathic virus that up-regulates the surface expression of MHC-I molecules in the infected cells (Lobigs et al., 2004), thus the cellular immune response should constitute other important mediator of the adaptive immune system against this pathogen. Little is known about the protective role of cell-mediated immunity (CMI) against this pathogen. To our knowledge, only five reports provide evidences about this issue in the mouse model. The first report describes the contribution of $CD8^+$ cells in protecting mice immunized with the Yellow fever-dengue chimeric virus (van der Most et al., 2000). In a second report, our group demonstrated the role of the cellular immune response against DENV-2 after infection with the homologous virus in mice (Gil et al., 2009). The third report has also shown that the immunization of mice with four $CD8^+$ T cell epitopes from DENV-2, which are immunodominant in this animal model, enhances viral clearance (Yauch et al., 2009). Finally our group published new evidence, using recombinant nucleocapsid-like

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http://dx.doi.org/10.1016/j.virol.2014.03.011
0042-6822/

particles from DENV-2 (NLPs-2). This antigen induced IFN-γ-secreting and cytolytic $CD4^+$ and $CD8^+$ cells with protective capacity, without the contribution of neutralizing antibodies (Gil et al., 2012). Further studies on CMI to better understand the immunopathology of dengue and the immunogenicity of vaccine candidates are required.

In the present work, the immunogenicity of the recombinant NLPs-2 was evaluated in Vervet monkeys [*Chlorocebus* (formerly *Cercopithecus*) *aethiops sabaeus*] to have another evidence of the protective role of the neutralizing antibodies independent immunity against DENV.

Results

Recombinant NLPs-2 do not induce antiviral and neutralizing antibodies in monkeys

To evaluate the immunogenicity and protective efficacy of NLPs-2 in monkeys, animals were divided in two groups. One group received the NLPs-2 formulation and the second group acted as a negative control. Placebo group was immunized with the same quantity of ODN 39M contained in the formulation of NLPs-2 and alum. All animals received four doses at days 0, 60, 120 and 180.

The kinetics of anti-capsid antibody response was determined. As shown in Fig. 1A, immunized animals developed anti-capsid antibodies after the first dose. Antibody titers increased after the second and third doses to a geometric mean titer (GMT) of >8000. Administration of the fourth dose increased the GMT to 20,000 for NLPs-2-immune animals.

The antibody response against the whole DENV-2 was also measured by an indirect ELISA system. As expected, monkeys receiving the NLPs-2 or placebo did not exhibit anti-virion antibodies at any of the time points analyzed before challenge (Fig. 1B). Also, the kinetics of neutralizing antibodies was measured by PRNT, using the strain SB8553 of DENV-2 and the Vero cell line. None of the animals receiving the NLPs-2 developed detectable neutralizing antibodies at any of the time points tested before challenge (Fig. 1C). However, all animals developed neutralizing antibodies after viral challenge.

Recombinant NLPs-2 induce IFN-γ-secreting and cytotoxic cells against DENV-2 in monkeys

Peripheral blood mononuclear cells (PBMCs) from the immunized monkeys, isolated in four distinct time points, were stimulated with infective DENV-2 SB8553 to measure IFN-γ secretion.

Fifteen days after the fourth dose, PBMCs from all NLPs-2-immune animals secreted the antiviral cytokine (M2072, 80.3 pg/mL; M2126, 367.4 pg/mL and M2048, 126.5 pg/mL). However, on challenge day only two out three monkeys (M2072 and M2126) immunized with NLPs-2 showed a positive response, with concentration of IFN-γ of 123.9 pg/mL and 178.5 pg/mL, respectively (Fig. 2A). Interestingly, PBMCs collected after challenge from all the animals immunized with NLPs-2 secreted high levels of IFN-γ (M2072, 345.8 pg/mL; M2126, 197.7 pg/mL and M2048, 582.7 pg/mL). At the same time-point, no secretion of IFN-γ was detected in the placebo group.

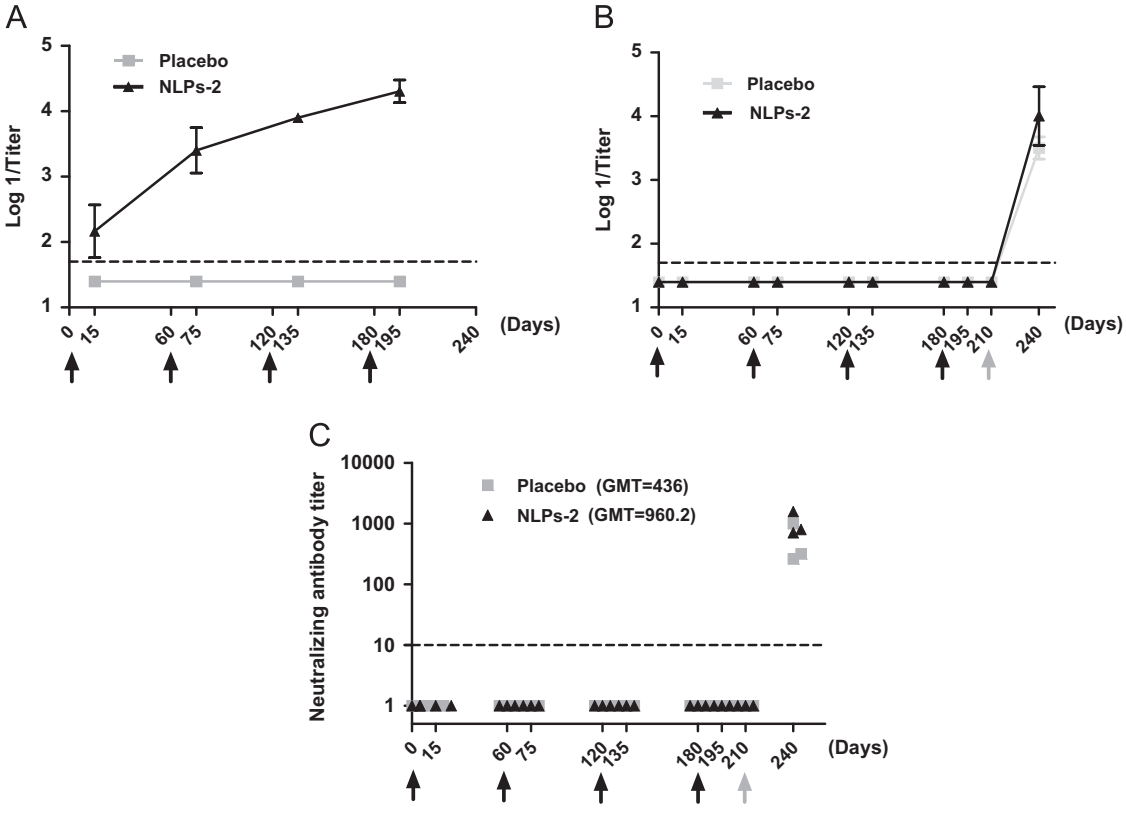

Fig. 1. Kinetics of the humoral immune response induced in monkeys by NLPs-2. (A) IgG antibodies against the recombinant protein, as measured by ELISA. Flat-bottomed 96-well plates were coated with the purified capsid protein (5 μg/mL). Serially diluted samples from sera (starting at 1:50) were assayed and detected with anti-monkey IgG-peroxidase conjugate (B) IgG antibodies against DENV-2, as measured by ELISA. Flat-bottomed 96-well plates were coated with the monoclonal antibody 4G2 (5 μg/mL). Serially diluted samples from sera (starting at 1:50) were assayed and detected with anti-monkey IgG-peroxidase conjugate. In both cases, data represent the mean ± SD of two independent experiments. Animals were considered positive when IgG titers were $>1/50$ (C) Titers of neutralizing antibodies, measured by plaque reduction neutralization test (PRNT), in Vero cells against DENV-2 SB8553 strain. Neutralizing antibody titers are the highest serum dilution which resulted in a 50% reduction in the number of plaques produced by DENV-2. Responders were considered when titers $>1/10$. Data represent the means of two independent experiments. Black arrows indicate days of immunization with the different formulations; the gray arrow indicates day of challenge. The dashed line indicates the cutoff value.