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Investigating the efficacy of monovalent and tetravalent dengue vaccine formulations against DENV-4 challenge in AG129 mice

Jeremy Fuchs[a], Haiyan Chu[a], Peter O'Day[a], Richard Pyles[b], Nigel Bourne[b], Subash C. Das[a], Gregg N. Milligan[b], Alan D.T. Barrett[b], Charalambos D. Partidos[a,*], Jorge E. Osorio[a]

[a] Takeda Vaccines, Inc., Madison, WI, USA
[b] Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555-0436, USA

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ABSTRACT

Dengue (DEN) is the most important mosquito-borne viral disease, with a major impact on global health and economics, caused by four serologically and distinct viruses termed DENV-1 to DENV-4. Currently, there is no licensed vaccine to prevent DEN. We have developed a live attenuated tetravalent DENV vaccine candidate (TDV) (formally known as DENVax) that has shown promise in preclinical and clinical studies and elicits neutralizing antibody responses to all four DENVs. As these responses are lowest to DENV-4 we have used the AG129 mouse model to investigate the immunogenicity of monovalent TDV-4 or tetravalent TDV vaccines, and their efficacy against lethal DENV-4 challenge. Since the common backbone of TDV is based on an attenuated DENV-2 strain (TDV-2) we also tested the efficacy of TDV-2 against DENV-4 challenge. Single doses of the tetravalent or monovalent vaccines elicited neutralizing antibodies, anti-NS1 antibodies, and cellular responses to both envelope and nonstructural proteins. All vaccinated animals were protected against challenge at 60 days post-immunization, whereas all control animals died. Investigation of DENV-4 viremias post-challenge showed that only the control animals had high viremias on day 3 post-challenge, whereas vaccinated mice had no detectable viremia. Overall, these data highlight the excellent immunogenicity and efficacy profile of our candidate dengue vaccine in AG129 mice.

1. Introduction

Dengue disease (DEN) is the most important mosquito-borne viral disease, and a major worldwide public health problem [1]. It is caused by four serologically and distinct viruses or serotypes, termed DENV-1 to DENV-4. The four DENVs are endemic throughout the world's subtropical and tropical regions, especially in Asia and Latin America. In humans, DENV may cause acute febrile illness dengue fever (DF) that is not life threatening. However, DF can progress to a potentially life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) [2,3]. It is estimated that these viruses cause approximately 390 million clinical DEN infections annually [1].

The humoral immune response to DENV primarily targets the premembrane (prM) and envelope (E) structural proteins, and is predominately composed of serotype-cross-reactive antibodies [4–6]. In addition, the secreted nonstructural protein 1 (NS1) is highly immunogenic eliciting antibodies that exhibit complement-fixing activity that can trigger the lysis of virus-infected cells, and induces protective immunity in mice against DENV challenge [7–11]. In contrast, cellular immune responses to DENV, predominantly target epitopes located in the NS proteins [12]. Primary infection with any of the four serotypes is believed to confer lifelong protection to the homologous serotype; however, secondary infection with a different DENV serotype is the major risk factor for severe disease. This may be due to "antibody-dependent enhancement" (ADE) of disease where cross-reactive anti-DENV antibodies facilitate entry of DENV into Fcγ receptor-bearing cells [13].

Current vaccine development efforts for DEN are based on inducing simultaneous immune responses to all four DENV serotypes using different technologies. Several of these candidate vaccines are at various stages of preclinical or clinical development [14]. Following successful Phase 1 clinical trials, our candidate live-attenuated tetravalent dengue vaccine (TDV) (formally known as DENVax) is currently being tested in Phase II trials at various endemic geographical locations. This vaccine consists of a molecularly characterized attenuated DENV-2 strain (TDV-2) and three

* Corresponding author at: Takeda Vaccines, Inc., 504 S. Rosa Rd., Suite 200, Madison, WI 53719, USA. Tel.: +1 6084217316.
E-mail address: harry.partidos@takeda.com (C.D. Partidos).

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chimeric viruses containing the prM and E protein genes of DENV-1, -3 and -4 expressed in the context of the attenuated TDV-2 genome backbone (TDV-1, TDV-3, and TDV-4, respectively) [15–19]. In pre-clinical animal models, and phase I clinical trials this vaccine was shown to elicit tetravalent neutralizing antibody responses, with responses highest to DENV-2, and lowest to DENV-4 [20,21,32] . Type-I & II interferon receptor-deficient (AG129) mice allow efficient replication of DENVs and represent a well-characterized animal model of DENV infection [22]. We used this model to investigate the protective efficacy of TDV-4 vaccine and compared it with that elicited by TDV against a lethal DENV-4 challenge. Since the common backbone of TDV is based on the attenuated TDV-2 virus we also tested the efficacy of this monovalent vaccine against DENV-4 challenge.

2. Materials and methods

2.1. Viruses

The TDV vaccine strains have been previously reported [19]. The human Thai DENV-4 strain 703 was used for the mouse challenge studies (Bourne et al., in preparation). In addition, a number of DENV-4 isolates were used, referred to as; Colombia 2006 (CI/DB039/2006; Indonesian genotype), Ecuador 1999 (EC/DB041/1999; Indonesian genotype), Micronesia 1995 (MC/DB043/1995; S.E Asian genotype), Mexico 2006 (MX/DB042/2006; Indonesian genotype), Virgin Islands 1994 (SC/DB045/1994; Indonesian genotype, and Thailand 2006 (TH/DB060/2006; S.E. Asian genotype). The viruses were obtained from the Division of Vector-Borne Diseases, Dengue Branch, CDC, San Juan, Puerto Rico.

2.2. Immunizations and virus challenge

On day 0 of the study, groups of 4 week-old AG129 mice ($n = 15$) were immunized subcutaneously (SC) with 0.2 ml of the tetravalent-TDV (2×10^4, 5×10^4, 1×10^5 and 3×10^5 pfu/ml for TDV 1–4 vaccines, respectively), monovalent TDV-4 (3×10^5 pfu/ml) or monovalent TDV-2 (5×10^4 pfu/ml) vaccines. In addition, a group of mice were injected with FTA (virus diluent) as a negative control. Mice were bled on day 56 post-immunization to measure neutralizing antibodies against each DENV serotype. On day 60 all animals were challenged intraperitoneally (IP) with $\sim 1.5 \times 10^7$ pfu of DENV-4 strain 703 in a 0.1 ml volume. Following challenge mice were bled on days 1, 2 and 3 (5 mice from each group/bled day) to measure viremia levels by qRT-PCR. In addition, mice were weighed and monitored for morbidity daily to day 28 post-challenge. Animals exhibiting signs of severe disease or with weight loss greater than 20% of initial body weight were euthanized and counted as being dead on the following day for analysis. At the end of the monitoring period a terminal bleed was collected from all surviving animals to quantify the neutralizing antibody response against DENV-4.

To measure cellular responses, two groups of 4–6 week-old AG129 mice were immunized SC with 0.2 ml TDV ($n = 3$) or TDV-4 vaccines ($n = 2$) using the same vaccine doses as described above. Six and seven weeks post-priming, respectively, mice from each group were euthanized and individual spleens were collected for further analysis.

2.3. Measurement of anti-NS1antibodies by ELISA

Purified NS1 antigen from DENV-2 and DENV-4 (abcam, Cambridge, MA) was resuspended in carbonate coating buffer pH 9.6 and coated at 1 ng/μl (50 μl/well) onto 96-well ELISA plates (Corning Polystyrene). Plates were washed with PBS/0.1% Tween 20 (PBST) and blocked with 10% milk in PBST. Sera were serially diluted and incubated at 37 °C for 1 h. Following washing with PBST, goat anti-mouse HRP (Jackson Immuno, West Grove, PA) at 1:10,000 in 10% milk/PBST was added, and plates were incubated at 37 °C for 1hr. Color reaction was developed by adding 100 μl TMB solution and incubating plates at room temperature in the dark for 6 min. Reaction was stopped by adding 1 N HCl. Absorbance was recorded at 450 nm and 630 nm using a Biotek plate reader. To account for optical interference the A630 was then subtracted from the A450.

2.4. Neutralization test

Vero cells (1.5×10^4 cells/100 μl) were plated into 96-well tissue culture plates in DMEM/10% FBS/1% penicillin/streptomycin and incubated at 37 °C with 5% CO_2 for 48 h. Heat-inactivated sera were two-fold serially diluted in BA-1 medium, mixed with 2× virus in an equal volume and incubated at 4 °C, overnight. Dengue viruses used are the parent strains to the vaccine viruses (DENV-1; 16007, DENV-2; 16681, DENV-3; 16562, DENV-4; 1036). In addition, we tested the breadth of neutralizing antibody responses elicited by TDV or TDV-4 vaccines against several DENV-4 isolates collected from different geographical locations (see Section 2.1). Next, 30 μl of the serum-virus mixture was added to Vero cell monolayers in triplicate and adsorbed at 37 °C for 2 h. Both positive and negative control sera samples were included. At the end of the incubation period, 100 μl/well of 1.2% carboxy-methyl cellulose overlay was added and plates were incubated at 37 °C, 5% CO_2 for a previously determined time period (plus or minus 3 h) to allow for the formation of detectable foci (DENV-1; 53 h, DENV-2; 72 h, DENV-3; 53 h, DENV4; 48 h). Cells were fixed with 85% ice cold acetone at ambient temperature for 20 min and stored at −20 °C. Plates were equilibrated to ambient temperature and washed 3 times with PBS-T (PBS/0.1% Tween 20) to remove residual overlay and then incubated with primary rabbit anti-DENV polyclonal antibody (1:1000 dilution in PBS-T/2.5% milk) at 37 °C for 2 h. Plates were washed as before and then incubated with secondary HRP-conjugated anti-rabbit antibody at 37 °C for 2 h. Finally, plates were incubated with 100 μl/well of the HRP substrate 3-amino-9-ethylcarbozole until foci were visible. Following washing with water plates were air-dried and foci were quantified on an ELISpot reader. Titers were defined as the reciprocal of the highest serum dilution that reduced the average virus input in the negative control serum by at least 50%.

2.5. Virus quantitation by qRT-PCR

RNA was extracted from sera using the Aurum total RNA isolation kit (Bio-Rad, Hercules, CA) as previously described [23]. Reverse transcription was accomplished using an iScript™ synthesis kit (Bio-Rad) using the following protocol: 1) 1.5 min, 25 °C, 2) 42 °C, 30 min, 3) 85 °C, 5 min, 4) infinite hold at 4 °C. Samples were evaluated using a DENV-4 serotype-specific qRT-PCR [24] utilizing a TaqMan probe (Sigma–Aldrich, St. Louis, MO) to quantify the specific amplification in each reaction. Each 25 μl qRT-PCR reaction contained: 12.5 μl iQ supermix™ (Bio-Rad) and 1 μl (5 μM) of forward and reverse primer and 1.5 μl of (5.0 μM) of the TaqMan probe [24] and 3 μl of cDNA template or nuclease-free water (no template controls). The qPCR was completed in a C1000 thermocycler equipped with a CFX™ optical module (Bio-Rad) using the following parameters: Cycle 1), 95 °C, 1.5 min; Cycle 2), Step 1. 95 °C, 10 s, Step 2. 62 °C, 30 s, repeat 40×. Fluorescent signal data was collected at the end of each annealing/extension step. Starting quantity values were extrapolated from standard curves of plasmids harboring the PCR targets generated in parallel for each run.