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Delineating the serotype-specific neutralizing antibody response to a live attenuated tetravalent dengue vaccine

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ABSTRACT

The dengue virus (DENV) vaccines that are licensed or in clinical development consist of DENV serotype 1–4 tetravalent formulations given simultaneously and are not acquired sequentially like natural infections. It is unclear what effect this has on development of protective levels of immunity to all four serotypes. Serotype-specific neutralizing antibody (NAb) is considered the most relevant correlate of protection from dengue disease. Here we assessed levels of serotype-specific and cross-reactive NAb in immune sera from 10 subjects vaccinated with a live attenuated tetravalent DENV vaccine developed at the Walter Reed Army Institute of Research. The majority of subjects NAb responses to DENV-2 and DENV-4 were type-specific, while their NAb responses to DENV-1 and DENV-3 were primarily cross-reactive. Vaccine virus RNAemia has been most frequently detected for DENV-2 and DENV-4 in vaccinated subjects, strongly suggesting that replication is important for eliciting serotype-specific immunity.
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1. Introduction

Dengue virus (DENV) serotypes 1–4 are of significant importance to public health in endemic countries, which are widely distributed in tropical and subtropical areas of the world. It has been estimated that 390 million DENV infections occur annually [1]. Dengue vaccine development has been complicated by co-circulation of all four DENV serotypes in endemic countries and the possibility of sequential infections with different serotypes causing more severe disease, which necessitates a tetravalent vaccine for simultaneous protection against all 4 serotypes. Historical evidence suggests that infection with any one DENV serotype will provide life-long immunity to that serotype and short-term cross-protection against the heterologous DENV serotypes that lasts only a few months [2]. This paradigm has been challenged by more recent studies indicating that homologous re-infections, although rare, can occur in some people [3]. The immune responses to post-primary DENV infections display an anamnestic response that is broadly cross-reactive among DENV serotypes and longer periods of cross-protection are observed [4–7].

The DENV vaccines that are currently licensed or in clinical development are tetravalent formulations given simultaneously and DENV 1–4 are not acquired sequentially like they are in endemic areas. It is unclear what effect this has on development of protective levels of immunity in humans. Furthermore, neutralizing antibody (NAb) measured by the plaque reduction neutralization test (PRNT) is considered the most relevant correlate of protection and is routinely used to evaluate vaccine efficacy. However, only moderate clinical efficacy in phase III trials was observed for recipients of the CYD-TDV vaccine (Sanofi Pasteur) who had balanced tetravalent NAb responses [8–10]. This indicates that our understanding of NAbs as a correlate of protection needs to be refined.

The DENV envelope protein is the major target of neutralizing antibodies [11]. Potently neutralizing human NAbs have been mapped to epitopes on the E protein that link multiple E protein monomers [12–18]. The presence of serotype-specific NAb is evidence of previous exposure to a specific DENV serotype and if it were elicited by natural or experimental infection then it would be expected to correlate with protection against homologous reinfection, as previous human studies have demonstrated [2,19]. Cross-reactive NAb is not necessarily an indicator of prior exposure to any specific serotypes but has been shown to reduce the risk of disease from heterologous DENV infection [20]. Recently, virus-specific serum antibody depletion methods have been developed to determine the contribution made by DENV type-specific and cross-reactive antibodies to virus neutralization in vitro after primary and secondary infections or immunizations [7,12,19,21].

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After a primary DENV infection, serotype-specific antibodies contribute most to neutralizing activity against the infecting serotype, while cross-reactive antibodies contribute less to neutralization and can mediate enhancement of heterologous DENV infection in cell culture and in mice [22]. The proportion of cross-reactive antibodies that contribute to virus neutralization appeared to increase after secondary DENV infection or immunization [7,21]. After immunization with the CYD-TDV vaccine, a combination of all four serotypes delivered simultaneously, subjects had type-specific NAb primarily to DENV-4 and cross-reactive NAb to DENV 1–3 [19]. This bias was hypothesized to be the result of better replication of the DENV-4 vaccine component in most subjects.

The live attenuated CYD-TDV vaccine has been licensed for use in several endemic countries (trademarked as Dengvaxia) including Brazil, El Salvador, Mexico, Paraguay, and the Philippines [23–26]. However, the CYD-TDV vaccine had only moderate efficacy in DENV seronegative individuals, limiting its utility in these populations. Two other live attenuated vaccines are in clinical development. The live attenuated TV003/TV005 vaccine (US National Institutes of Health [NIH]) is in phase III development and has been licensed to Merck, and companies in Brazil (Instituto Butantan), India (Panacea Biotec and Serum Institute of India), and Vietnam (Vabiotech) [27–31]. The TDV vaccine (Takeda) is currently undergoing phase III studies [32–35].

A live attenuated tetravalent dengue vaccine was developed at Walter Reed Army Institute of Research (WRAIR) in collaboration with GlaxoSmithKline (GSK) [36–38]. The DENV 1–4 live attenuated vaccine (TDENV) viruses developed at WRAIR were attenuated by serial passage in primary dog kidney (PDK) cells [38–50]. A safe and immunogenic formulation of TDENV was selected based on a phase II clinical study in flavivirus naïve subjects [46]. This formulation demonstrated good safety and efficacy in infants and children [47,48]. The TDENV vaccine viruses were re-derived and tested in a phase II study in flavivirus naïve adults, demonstrating adequate safety and immunogenicity after two doses [49]. The TDENV vaccine, in combination with a tetravalent DENV purified inactivated vaccine [51] is currently undergoing clinical evaluation using a heterologous prime-boost approach. In the current study, sera from 10 flavivirus naive subjects that received two doses of TDENV were evaluated for levels of serotype-specific and cross-reactive NAb.

2. Material and methods

2.1. Viruses and cells

The DENV strains used for in vitro assays are DENV-1 West Pac 74, DENV-2 S16803, DENV-3 CH53489, and DENV-4 TVP-360. Virus stocks were produced by passage in Aedes albopictus mosquito C6/36 cells as described previously [52].

2.2. Vaccine study sera

The vaccine study design is described in detail in Ref. [49] and was approved by the US Army Human Subjects Research Review Board and the Office of the Surgeon General (www.clinicaltrials.gov identifier: NCT00350337). A total of 10 sera from study day 210 (30 days post dose two) were selected from subjects that were flavivirus naïve, had received two doses of vaccine, and had consented to use of their sera.

2.3. Purification of dengue viruses

The DENV 1–4 were amplified in Vero cells, concentrated by tangential flow ultrafiltration, and purified by sucrose gradient ultracentrifugation as described previously [53].

2.4. Depletion of DENV-specific antibodies from human sera

The antibody depletion method was carried out as described previously [12]. Purified DENV or BSA control protein was adsorbed onto 4 μm Polybead polystyrene microspheres following the manufacturer's instructions (Polysciences, Inc.). The polystyrene microspheres were washed three times with 0.1 M borate buffer (pH 8.5) and were incubated with purified DENV or BSA control protein in borate buffer overnight at room temperature. The control and DENV-adsorbed beads are blocked three times with BSA (10 mg/ml) in borate buffer for 30 min at room temperature and washed six times with PBS. Human immune sera were depleted of DENV-specific antibody by incubation with virus-adsorbed beads for 2 h at 37 °C with gentle mixing. Sequential rounds of antibody depletion were performed and the depleted sera were tested for successful removal of the desired DENV-specific antibodies by ELISA.

2.5. ELISA

Binding of human immune sera to purified DENV 1–4 was measured as described previously [12]. ELISA plates were coated with 50 ng of purified virus. Bound antibody was detected with an alkaline phosphatase conjugated goat anti-human IgG antibody and p-nitrophenyl phosphate substrate and was read at an absorbance of 405 nm on a spectrophotometer. End-point titration of serum samples was initially performed to assess the relative levels of DENV-reactive antibody. To confirm successful removal of antibody by the depletion method the virus antigen-depleted or control-depleted sera were tested for binding at a 1:100 dilution. The DENV antigens used for depletion of a given serum sample matched those used for testing that depleted sample by ELISA.

2.6. Flow cytometry-based 50% neutralization (FlowNT50) assay

Neutralizing antibody titers in heat-inactivated sera were determined using a flow cytometry-based neutralization assay in U937 cells expressing DC-SIGN as previously described [12,54]. Data were analyzed by nonlinear regression to determine 50% neutralization titers in GraphPad Prism 6. Some sera had low NAb titers near the 1:40 limit of quantification of the assay and made it more difficult to fully resolve the type-specific and cross-reactive NAb populations. Also, in some cases, the control depleted sera had marked reductions in NAb titer compared to undepleted sera, which is likely due to non-specific removal of DENV antibody and neutralization assay variability.

2.7. Viremia determination by RT-qPCR (RNAemia)

A DENV serotype-specific RT-qPCR assay (described in Ref. [49]) to assess vaccine virus RNAemia in all study subjects on two separate study visits in a 14 day window after both doses of vaccine. Depending on the subject, the first sampling time-point was day 2, 5, 8 or 12 and the second sampling time-point was day 8, 12, or 14. The limit of detection of the assay was 6500 genome equivalents (GEQ)/ml for DENV-1, 160,000 GEQ/ml for DENV-2, 1250 GEQ/ml for DENV-3, and 33,000 GEQ/ml for DENV-4.

2.8. Statistical analysis

All statistical analyses were performed with GraphPad Prism version 6.02. The levels of seroconversion to DENV 1–4 were compared among and within groups by Fisher's exact test with $p < 0.05$ required for significance. The NAb responses to DENV 1–4 were compared among and within groups using log10 transformed titers

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