

# Analyzing the Human Serum Antibody Responses to a Live Attenuated Tetravalent Dengue Vaccine Candidate

Jessica A. Swanstrom,<sup>1</sup> Sandra Henein,<sup>2</sup> Jessica A. Plante,<sup>1</sup> Boyd L. Yount,<sup>1</sup> Douglas G. Widman,<sup>1</sup> Emily N. Gallichotte,<sup>2</sup> Hansi J. Dean,<sup>3</sup> Jorge E. Osorio,<sup>3</sup> Charalambos D. Partidos,<sup>3</sup> Aravinda M. de Silva,<sup>2</sup> and Ralph S. Baric<sup>1,2</sup>

<sup>1</sup>Department of Epidemiology, School of Public Health, and <sup>2</sup>Department of Microbiology and Immunology, School of Medicine, University of North Carolina, Chapel Hill; and <sup>3</sup>Takeda Vaccines, Cambridge, Massachusetts

**Background.** Dengue virus serotypes 1–4 (DENV-1–4) are the most common vector-borne viral pathogens of humans and the etiological agents of dengue fever and dengue hemorrhagic syndrome. A live-attenuated tetravalent dengue vaccine (TDV) developed by Takeda Vaccines has recently progressed to phase 3 safety and efficacy evaluation.

**Methods.** We analyzed the qualitative features of the neutralizing antibody (nAb) response induced in naive and DENV-immune individuals after TDV administration. Using DENV-specific human monoclonal antibodies (mAbs) and recombinant DENV displaying different serotype-specific Ab epitopes, we mapped the specificity of TDV-induced nAbs against DENV-1–3.

**Results.** Nearly all subjects had high levels of DENV-2-specific nAbs directed to epitopes centered on domain III of the envelope protein. In some individuals, the vaccine induced nAbs that tracked with a DENV-1-specific neutralizing epitope centered on domain I of the envelope protein. The vaccine induced binding Abs directed to a DENV-3 type-specific neutralizing epitope, but findings of mapping of DENV-3 type-specific nAbs were inconclusive.

**Conclusion.** Here we provide qualitative measures of the magnitude and epitope specificity of the nAb responses to TDV. This information will be useful for understanding the performance of TDV in clinical trials and for identifying correlates of protective immunity.

**Keywords.** Flavivirus; dengue; vaccine; antibody; immunity; live-attenuated vaccine.

Dengue viruses (DENVs) are positive-sense RNA viruses transmitted to humans through mosquito vectors. DENV consists of 4 serotypes (DENV-1–4) that cocirculate in tropical and subtropical regions, where an estimated 390 million individuals are infected per year [1]. While most infections are clinically inapparent or mild, about 25% of infections cause acute febrile illness and occasionally progress to severe dengue hemorrhagic syndrome [2, 3].

Neutralizing antibodies (nAbs) are considered a necessary component of protective immunity to DENVs [4, 5]. Primary infection with a DENV serotype induces a nAb response that provides long-term protection against the infecting serotype but limited and transient protection against other serotypes. Secondary infections with a different serotype may stimulate an immune-enhanced severe disease [5, 6]. Given the risk of immune-enhanced disease, leading vaccine candidates are

tetravalent to simultaneously induce a balanced protective response to all 4 serotypes [7–9].

The DENV envelope (E) protein binds to cellular receptors, mediates viral entry and fusion, and is the main target of nAbs and protective Abs [10–12]. The ectodomain of E is composed of 3 domains: I, II, and III (EDI, EDII, and EDIII). Each DENV virion has 180 monomers of E, organized into 90 dimers that cover the entire virus surface. Type-specific nAbs have been isolated from individuals infected with different DENV serotypes [13–16]. Many human DENV nAbs bind to complex quaternary E protein epitopes displayed on intact virions but not on recombinant E protein monomers [17]. Our recent studies have demonstrated that DENV type-specific epitopes defined by human monoclonal Ab (mAbs) are also targeted by polyclonal serum nAbs in people exposed to DENV infections [13, 14].

A live attenuated tetravalent DENV vaccine (TDV) developed by Sanofi Pasteur (Dengvaxia) has been evaluated in 2 large efficacy studies and licensed for use in several countries [18]. In Dengvaxia clinical trials, some people with DENV nAbs experienced breakthrough infections demonstrating that mere presence of nAb was insufficient for protection [18]. Recently, Sanofi announced that Dengvaxia should only be used in people with preexisting immunity to DENV because naive individuals who receive the vaccine may be at increased risk of developing severe disease. These data suggest the need for new diagnostic approaches to determine the quality and molecular

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Correspondence: R.S. Baric, PhD, Department of Epidemiology, CB 7435, University of North Carolina School of Public Health, Chapel Hill, NC 27599 ([rbaric@email.unc.edu](mailto:rbaric@email.unc.edu)).

specificity of nAbs to identify correlates and mechanisms of protective immunity.

Takeda's vaccine candidate consists of an attenuated DENV-2 virus (TDV2) and 3 recombinant chimeric viruses for DENV-1, -3, and -4, generated by reverse genetic replacement of the genes encoding structural surface proteins prM and E of TDV2 with genes from representative members of the other 3 serotypes [7, 19–22]. TDV is immunogenic and efficacious in animal models, and, on the basis of immunogenicity and safety results of phase 1 and 2 trials in humans, pivotal phase 3 trials were initiated in late 2016 in dengue-endemic regions [21, 23–26]. In this study, we used DENV serotype-specific human mAbs and recombinant DENVs that display Ab epitopes of interest [14, 27] to map the specificity of the nAb responses elicited by an early formulation of Takeda's TDV [21].

## MATERIALS AND METHODS

### Ethics Statement

This study used deidentified human samples under University of North Carolina Institutional Review Board exemption approval 15-0015. Samples were collected by Takeda Vaccines in 2 completed clinical trials (studies 104 and 203) in accordance with the Edinburgh revision of the Declaration of Helsinki, International Conference on Harmonization and Good Clinical Practice guidelines, and applicable national and local regulations and requirements. The protocols were approved by the local internal review boards and are registered on ClinicalTrials.gov (NCT01542632 and NCT01511250).

### Source of Serum Samples

Study 104 was a double-blinded, randomized phase 1b trial conducted in the United States among participants aged 18–45 years [24]. For study 104, all subjects were DENV seronegative prior to vaccination. To facilitate epitope mapping, only subjects with a 50% nAb titer ( $NT_{50}$ ) of  $\geq 60$  to particular DENV serotypes were selected for the current study. Study 203 was a double-blinded, randomized, placebo-controlled, phase 2 trial conducted in Puerto Rico, Colombia, Singapore, and Thailand among participants aged 1.5–45 years [25]. As reported previously [25], among participants who were DENV seronegative prior to vaccination, >94% developed nAbs to DENV-1, DENV-2, or DENV-3, and 58.6% developed nAbs to DENV-4 on day 28 after the first dose. The frequency with nAbs to DENV-4 increased to 87.7% following the second dose. Among participants who were seropositive to at least 1 DENV serotype at baseline, the frequency of nAbs to DENV-1–4 were 91.3%–99.1% after 1 dose and 96.5%–100% after 2 doses. Study 203 samples were selected for the current study without prescreening for particular nAb profiles.

### Cells and Viruses

Viruses were propagated in C6/36 *Aedes albopictus* cells, which were grown in minimal essential medium (Gibco, Grand Island, NY) at 32°C. Vero-81 cells were grown in Dulbecco's modified

Eagle's medium (Gibco), while U937+DC-SIGN cells were maintained in Roswell Park Memorial Institute 1640 medium (Gibco) at 37°C. Media were supplemented with 5% fetal bovine serum (HyClone, Logan, UT), 0.1 mM nonessential amino acids (Gibco), 100 U/mL penicillin (Gibco), and 100 mg/mL streptomycin (Gibco). U937+DC-SIGN medium was supplemented with 2 mM GlutaMAX (Gibco), 10mM HEPES (Cellgro, Manassas, VA), and 2-mercaptoethanol (Sigma, St. Louis, MO). Cells were incubated in the presence of 5% CO<sub>2</sub>.

Recombinant chimeric viruses were constructed using a quadripartite complementary DNA clone, the same strategy used to create wild-type DENV [14, 27, 28]. DENV-1 (West Pac '74), DENV-2 (S-16803), DENV-3 (Sri Lanka '89), and DENV-4 (Sri Lanka '92) strains were used in the present study. Epitope-transplanted recombinant DENVs were as follows: DENV-3 with the 1F4 epitope from DENV-1 (DV3/1), DENV-4 with the E domain III from DENV-2 (DV4/2), DENV 4 with the 5J7 epitope from DENV-3 (DV4/3). Full-length cDNA was transcribed into genome-length RNAs using T7 polymerase and recombinant viruses isolated in C6/36 cells as previously described [14, 27–29]. Virus was then passaged twice on C6/36 cells, centrifuged to remove cellular debris, and the 3rd passage was stored at –80°C as a working stock. DENV strains used in blockade of binding assays include DENV-1 West Pac '74, DENV-2 S-16803, DENV-3 CH-53489, and DENV-4 TVP-376.

### In Vitro Neutralization

Human sera or mAbs were serially diluted 3-fold in medium containing 2% fetal bovine serum and mixed with sufficient virus to infect 15% of the U937+DC-SIGN cells. Virus and Ab mixtures were incubated for 45 minutes in 96-well plates at 37°C and mixed with  $5 \times 10^4$  cells for 2 hours at 37°C. Unbound virus was washed with infection media, the volume of medium in each well was increased to 200  $\mu$ L, and the cells were incubated again at 37°C. After 24 hours, the cells were washed with fetal bovine serum, fixed in paraformaldehyde, permeabilized with saponin, blocked with normal mouse serum, and stained with AlexaFluor 488-conjugated (Molecular Probes, Eugene, OR) 2H2 Ab. Unbound Ab was removed, and the cells were resuspended in Hank's buffered salt solution (Gibco, Grand Island, NY) supplemented with 2% fetal bovine serum. Assays were performed twice and in duplicate. Samples were read on a Guava easyCyte 5HT flow cytometer (Millipore) [30].

### Blockade of Binding Assay

Enzyme immunoassay plates were coated with 4G2 mouse Ab to capture DENV-1 or DENV-3. After 1 hour, polyclonal sera were added in 2-fold dilutions and incubated for 1 hour at 37°C. Labeled human mAb (1F4 for DENV-1 and 5J7 for DENV-3) was used to detect quaternary epitopes not blocked by the polyclonal sera. Plates were washed, the color was developed, and the amount of mAb binding that was blocked by polyclonal sera was compared to mAb binding in the absence of sera, with results reported as the percentage of binding blocked.

## Antigenic Cartography

Antigenic cartography was performed using the  $NT_{50}$  values generated from the neutralization assays. Data were normalized as described by Cai et al [31]. Euclidean distances between sera were calculated, and metric multidimensional scaling was used to render data in 3 dimensions [32]. Calculations and images were generated in RStudio, version 0.99.467 (RStudio, Boston, MA).

## Statistical Analysis

$NT_{50}$  values were calculated using the sigmoidal dose response (variable slope) equation in Prism 7 (GraphPad, La Jolla, CA). Log-transformed data from the  $NT_{50}$  values were used to calculate geometric mean titers (GMTs) and 95% confidence intervals (CIs). Variation between groups was measured by the Wilcoxon signed rank test for repeated measurements without normal distribution. *P* values of  $<.05$  were considered statistically significant.

## RESULTS

### Baseline Characterization of Serum Samples From TDV Recipients

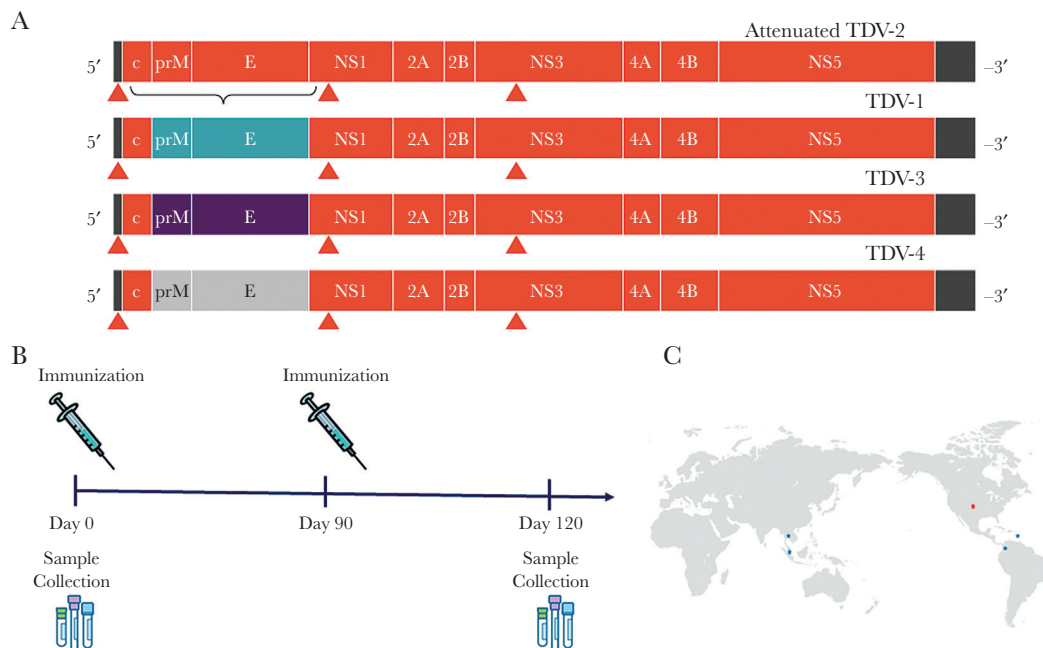
Volunteers from dengue-endemic regions who were enrolled in study 203 received 1 dose of TDV on day 0 and a second dose on day 90 (Figure 1). To map the epitopes targeted by vaccine-induced nAbs, we selected serum samples from 24 study subjects, collected on day 0 (before vaccination) and day 120 (1 month after the second dose). Of the 24 subjects, 11 had DENV-naive

serum samples and 13 had DENV-preimmune serum samples prior to vaccination.

To evaluate epitope-specific responses following vaccination, we first measured levels of serum nAbs against the parental 4 DENV infectious clone-derived recombinant viruses, using flow cytometry and U937 cells expressing DC-SIGN (a DENV attachment receptor; Figures 2 and 3). At day 0, serum from 0 of 11 naive individuals had DENV nAbs (Figure 3A and Supplementary Table 1). At day 120, serum from 6 naive individuals neutralized DENV-1 West Pac '74 (GMT, 86), serum from 11 neutralized DENV-2 S16803 (GMT, 1233), serum from 7 neutralized DENV-3 Sri Lanka '89 (GMT, 74), and serum from 7 neutralized DENV-4 Sri Lanka '92 (GMT, 58; Figure 3A). Of the 13 preimmune subjects, before vaccination serum from 11 neutralized DENV-1 (GMT, 150), serum from 13 neutralized DENV-2 (GMT, 322), serum from 11 neutralized DENV-3 (GMT, 138), and serum from 7 neutralized DENV-4 (GMT, 62). By day 120, all vaccinated preimmune subjects had boosted responses, with elevated neutralizing titers against DENV-1 (GMT, 1637), DENV-2 (GMT, 898), DENV-3 (GMT, 571), and DENV-4 (GMT, 338; Figure 3B and Supplementary Table 1).

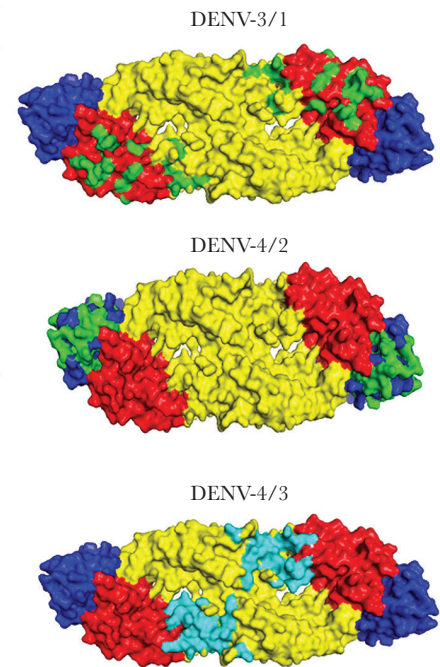
### Mapping the Vaccine-Induced DENV-1 Epitope-Specific Response

A chimeric virus was used to determine whether DENV-1 nAbs induced by TDV targeted a type-specific EDI/EDII hinge region epitope defined by human mAb 1F4 (Figure 2). The DENV-1



**Figure 1.** Study design. *A*, The live attenuated tetravalent dengue vaccine (TDV) vaccine is composed of an attenuated dengue virus serotype 2 (DENV-2) backbone with prM and E from DENV-1 for TDV-1, prM and E from DENV-3 for TDV-3, and prM and E from DENV-4 for TDV-4. *B*, Subjects were immunized with the tetravalent vaccine at days 0 and 90. Serum samples were collected on day 0 before vaccination and on day 120, 30 days after the second dose. *C*, Study sites were located in the United States (Study 104) and Thailand, Colombia, Puerto Rico, and Singapore (Study 203).

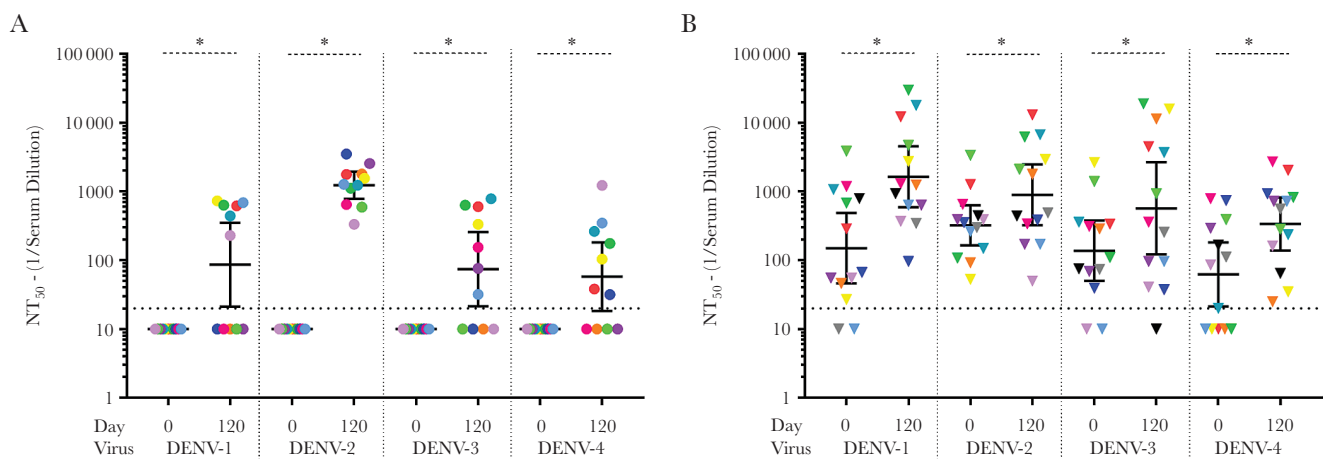
Virus	Strain	Backbone	Epitope	Epitope Donor
DENV-1	West Pac '74			
DENV-2	S16803			
DENV-3	UNC3001 Sri Lanka '89			
DENV-3'	CH-53489			
DENV-4'	D4ic Sri Lanka '92			
DENV-3/1		DENV-3	1F4	DENV-1
DENV-4/2		DENV-4	EDIII	DENV-2
DENV-4/3		DENV-4	5J7	DENV-3



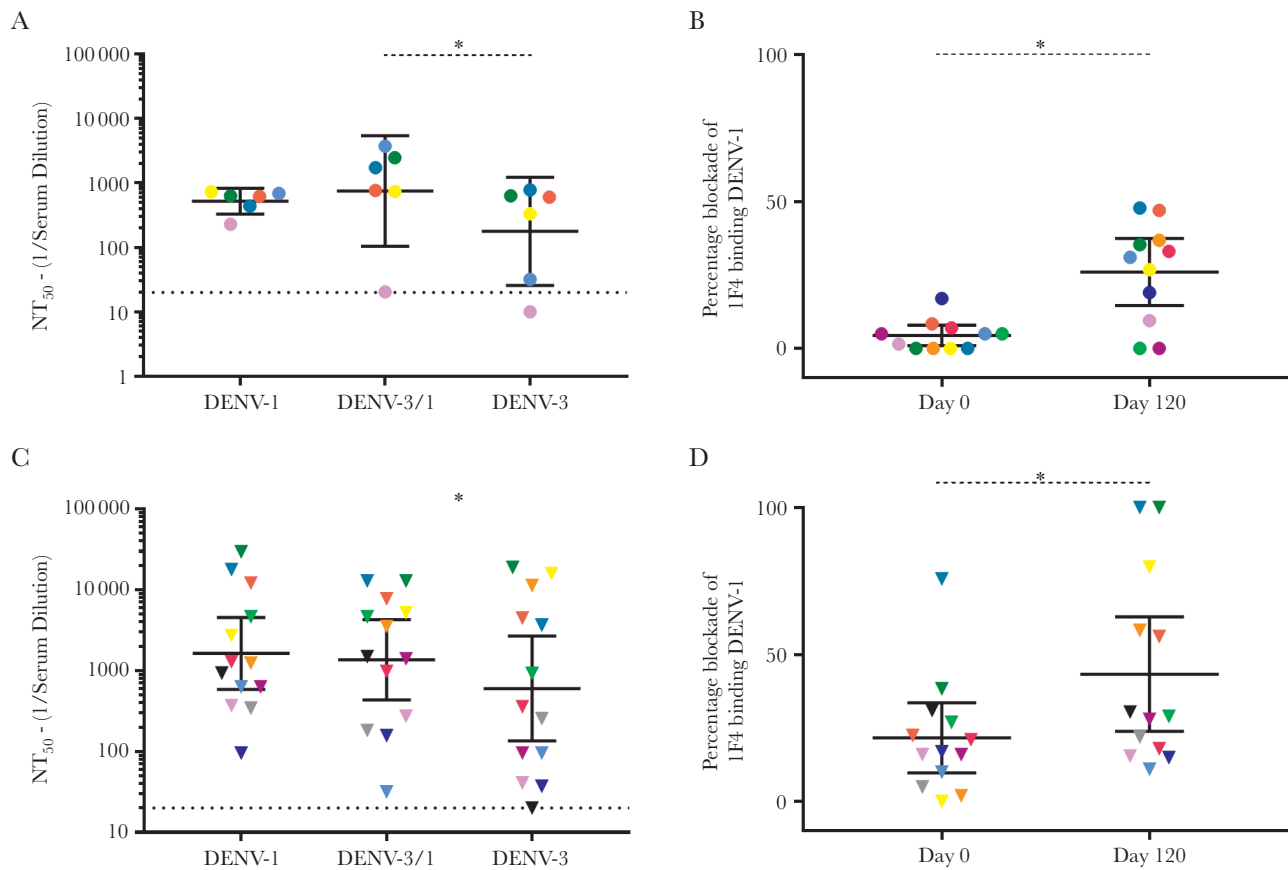
**Figure 2.** Viruses used for characterization. Wild-type dengue virus (DENV) infectious clones of DENV-1 (West Pac '74), DENV-2 (S-16803), DENV-3 (Sri Lanka '89), and DENV-4 (Sri Lanka '92) strains were used in the present study. The following epitope-transplanted recombinant DENVs were used for the U937+DC-SIGN flow-based neutralization assay: DENV-3 with the 1F4 epitope from DENV-1 (DV3/1), DENV-4 with the E domain III from DENV-2 (DV4/2), and DENV-4 with the 5J7 epitope from DENV-3 (DV4/3). Natural isolates of the World Health Organization reference strains DENV-1 West Pac '74 and DENV-3 CH-53489 were used for the blockade of binding assay.

type-specific 1F4 epitope is sensitive to the angle of the EDI/II hinge and is present in DENV-1 virions but not recombinant E protein secreted from cells as a monomer [13]. From the TDV-receiving naive cohort, 6 subjects with DENV-1 nAb titers >1:20 at day 120 were tested for neutralization of DENV-1, DENV-3, and the DENV-3/1 chimera (Figure 4A). Among the 6 naive subjects with a postvaccination DENV-1 response, nAb titers

against rDENV-3/1 were significantly higher (GMT, 755) than the nAb titers against the parental DENV-3 (GMT, 178;  $P < .05$ ; Figure 4A). Blockade of 1F4 binding assays also demonstrated a significant increase in mean blockade of 1F4 binding to DENV-1 at a serum dilution of 1:20 between day 0 (2%) and day 120 (33%;  $P < .05$ ; Figure 4B). In study 104 subjects (all of whom were naive prior to vaccination) [24], neutralization



**Figure 3.** Preimmunization and postimmunization neutralizing antibody (nAb) titers against wild-type dengue viruses (DENVs). Neutralization assays were performed and the geometric mean titer (GMT) calculated against DENV-1, DENV-2, DENV-3, and DENV-4 on day 0 and day 120 in subjects who were naive at day 0 (A) or preimmune at day 0 (B). Bars represent the GMT and whiskers the 95% confidence interval. Dashed line indicates the limit of detection for the assay. Nonresponders were assigned a value of half of the limit of detection, for visualization. Within each graph, each individual is assigned the same color, to enable visualization of each person's nAb titers to the different viruses. Dashed lines indicate the limit of the detection for the assay. \* $P < .05$ , by the Wilcoxon test, for comparison of day 0 to day 120. NT<sub>50</sub>, 50% nAb titer.



**Figure 4.** Mapping the dengue virus serotype 1 (DENV-1) epitope-specific neutralizing antibody (nAb) response. Immune sera from naive subjects (*A* and *B*) and preimmune subjects (*C* and *D*) who received the vaccine and developed DENV-1 nAbs (50% nAb titer [ $NT_{50}$ ]  $>1:20$ ) were selected for mapping DENV-1 responses. Neutralization assays were performed using a recombinant chimeric DENV-3/1 and the wild-type (WT) DENV-1 and DENV-3 parental strains of the chimera. Geometric mean  $NT_{50}$  values (GMTs) were calculated for the naive (*A*) and preimmune (*C*) groups after vaccination. Bars represent the GMT and whiskers the 95% confidence interval. Dashed line indicates the limit of detection for the assay. DENV-3 and DENV-3/1 nonresponders were assigned a value of half the limit of detection, for visualization. Prevacination and postvaccination immune sera from naive (*B*) and preimmune (*D*) subjects were also tested for ability to block the binding of monoclonal Ab 1F4 to DENV-1. Within each graph, each individual is assigned the same color, to enable visualization of each person's nAb titers to the different viruses. \* $P < .05$ , by the Wilcoxon test, for comparison of the parental backbone DENV to the rDENV.

findings for DENV-1 and DENV-3 was not statistically different (Supplementary Figure 1A), yet an increase in blockade of 1F4 binding was observed between day 0 (0%) and day 120 (26.9%;  $P < .05$ ; Supplementary Figure 1C). Preimmune individuals with DENV-1 nAb titers after vaccination (Figure 4C) did not have significantly different levels of nAb to rDENV-3/1 (GMT, 1368) and DENV-3 (GMT, 602). However, in the blockade of binding assay, blockade at day 120 (43%) was significantly greater than blockade at day 0 (22%;  $P < .05$ ; Figure 4D). These results demonstrate that TDV can elicit type-specific DENV-1 1F4-like nAbs in some individuals.

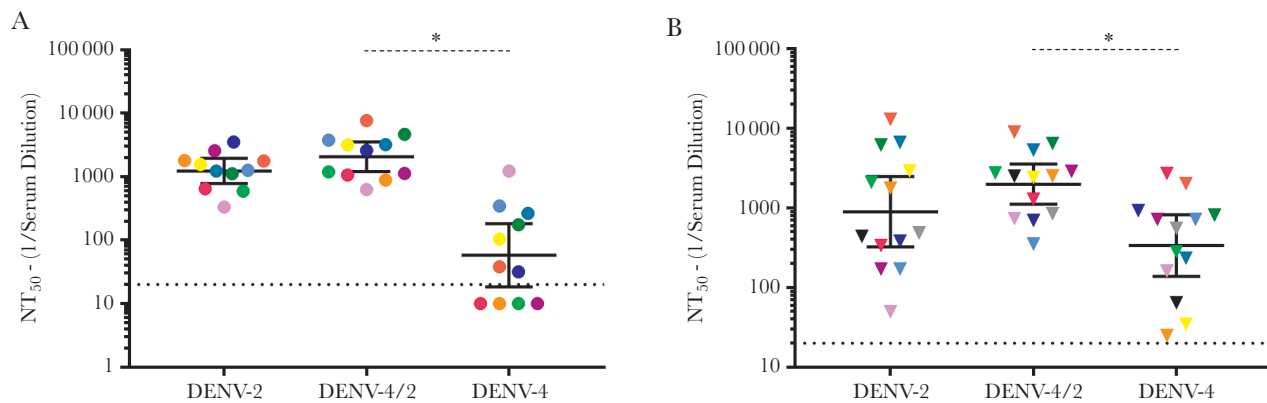
#### Mapping the Vaccine-Induced DENV-2 Response

We previously described a recombinant DENV-4/2 chimeric virus containing an E protein with EDI and II from DENV-4 and EDIII from DENV-2 [14, 33]. This chimera displays a DENV-2 quaternary structure epitope centered on EDIII recognized by strongly neutralizing human mAb 2D22 [14]. After

vaccination, all naive subjects had high DENV-2 nAbs titers (GMT, 1233; Figure 5A) and modest DENV-4 nAbs titers (GMT, 58; Figure 5A). DENV-4/2 neutralization (GMT, 2058) was significantly higher than observed for the parental DENV-4 strain ( $P < .05$ ; Figure 5A). Study 104 subjects (all of whom were naive prior to vaccination) had higher titers of nAbs to DENV-4/2 as compared to DENV-4, as well (Supplementary Figure 1B;  $P < .05$ ). DENV-preimmune subjects who received TDV developed higher titers against rDENV-4/2 (GMT, 1993) as compared to DENV-4 (GMT, 338;  $P < .05$ ; Figure 5B). These results demonstrate that, in both naive and DENV-preimmune subjects, TDV induces robust DENV-2 nAbs that target type-specific epitopes centered on EDIII of DENV-2.

#### Mapping the Vaccine-Induced DENV-3 Response

We used a recombinant DENV-4/3 M14 chimeric virus to determine whether DENV-3 nAbs induced by TDV targeted a quaternary epitope, centered on the EDI/II hinge that spans 2 adjacent



**Figure 5.** Mapping the dengue virus serotype 2 (DENV-2) epitope-specific neutralizing antibody (nAb) response. Immune sera from naive (A) and preimmune (B) subjects who received the vaccine and developed DENV-2 nAbs (50% nAb titer [ $NT_{50}$ ] >1:20) were selected for mapping DENV-2 responses. Neutralization assays were performed using a recombinant chimeric DENV-4/2 and the wild-type DENV-2 and DENV-4 parental strains of the chimera, and the geometric mean  $NT_{50}$  values (GMTs) were calculated for the naive (A) and preimmune (B) groups after vaccination. Bars represent the GMT and whiskers the 95% confidence interval. Dashed line indicates the limit of detection for the assay. Nonresponders were assigned a value of half the limit of detection, for visualization. Within each graph, each individual is assigned the same color, to enable visualization of each person's nAb titers to the different viruses. \* $P < .05$ , by the Wilcoxon test, for comparison of the parental backbone DENV to the rDENV.

monomers, defined by human mAb 5J7 (Figure 2) [34]. We transplanted the 5J7 epitope core from DENV-3 into DENV-4; this recombinant chimeric virus was efficiently neutralized by mAb 5J7 [29]. The 7 naive subjects with DENV-3 nAb titers of >1:20 after vaccination were tested for neutralization of DENV-3, DENV-4, and DENV-4/3 (Figure 6). In these individuals, the nAb titers were not significantly different between DENV-4/3 (GMT, 243) and the parental DENV-4 (GMT, 81;  $P < .1841$ ; Figure 6A). From the naive group, immune sera collected after vaccination blocked the binding of mAb 5J7 to DENV-3, demonstrating the presence of serum Abs that bind at or near the epitope (Figure 6B). However, no significant neutralization or blockade was observed with Study 104 subjects (all naive prior to vaccination) after vaccination (Supplementary Figures 1B and 1D). After vaccination, DENV-3 nAbs in the preimmune group did not significantly track with the 5J7 epitope displayed on the DENV-4/3 chimera (Figure 6C). However, the sera blocked binding of 5J7, suggesting the presence of Abs that bind at or near the epitope (Figure 6D).

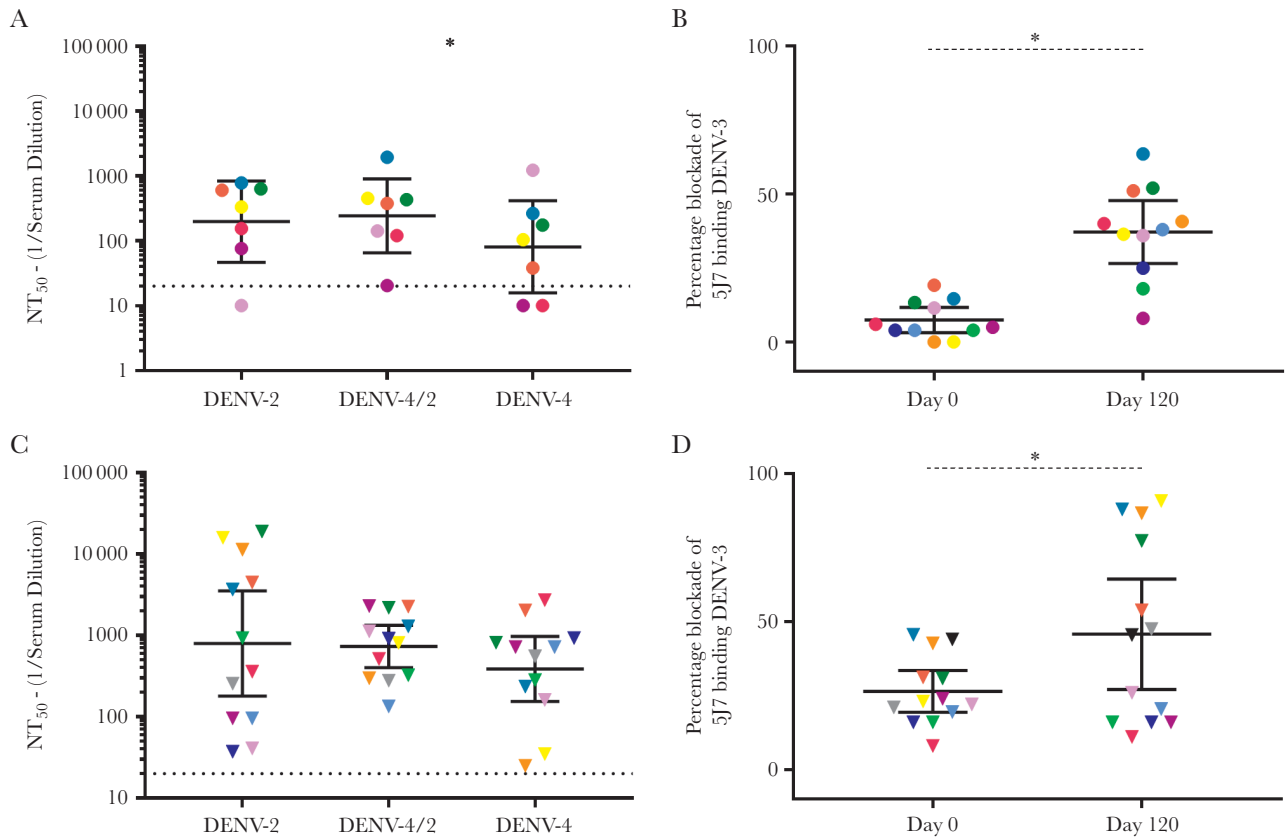
#### Antigenic Cartography

As an alternate strategy to detect the tracking of TDV-induced Ab with transplanted epitopes, we performed antigenic cartography of the wild-type and recombinant DENVs, using the neutralization data generated from TDV immune sera (Figure 7 and Supplementary Figure 2). In the antigenic map created by this analysis, the antigenic position of the DENV-4/2 recombinant virus moved away from the parental backbone of DENV-4 toward that of DENV-2, indicating that TDV induces responses directed to the DENV-2 domain displayed on DENV-4 (Figure 7). Cartography findings indicate that TDV induces a robust type-specific response to DENV-2 and variable responses to DENV-1 and 3 epitopes.

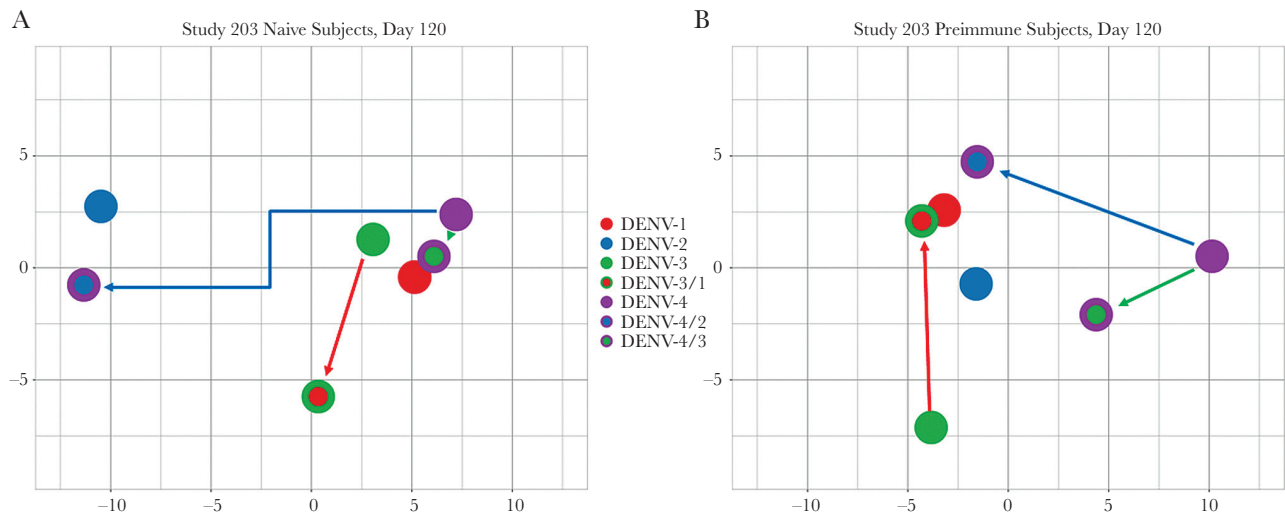
#### DISCUSSION

We have evaluated the epitope specificity of DENV nAbs induced by TDV in naive and dengue-preimmune individuals. Epitope-specific measurements are important because people exposed to primary DENV infection develop a strong nAb response that targets serotype-specific epitopes that protect against homologous-serotype infection. It is unclear whether individuals who receive tetravalent dengue vaccines develop primary serotype-specific responses or broadly cross-reactive Ab responses that target each vaccine component. Also unclear is the role of these responses in durable protection against the 4 serotypes. A recent study demonstrated that dengue-naive individuals who received a tetravalent chimeric yellow fever-dengue (CYD) vaccine mainly had type-specific nAbs to DENV-4 and cross-reactive nAbs to the other serotypes [9]. The DENV-2 response, in particular, was dominated by nAbs directed to cross-reactive epitopes, and overall CYD vaccine efficacy was lowest against this serotype in clinical trials [9].

Natural DENV-2 infection elicits human mAb 2D22-like type-specific nAbs that bind to a quaternary epitope spanning the EDIII and EDII of a single E protein homodimer [14, 33]. The recombinant chimeric DENV-4/2 displays the 2D22 quaternary epitope, allowing us to track linear EDIII and quaternary “2D22-like” responses in human immune sera after infection and vaccination [14, 33]. The Takeda TDV induced robust DENV-2-specific nAbs that tracked with EDIII on DENV-4/2. These studies could not differentiate between nAbs directed to simple epitopes contained with EDIII and more-complex “2D22-like” responses that require both EDIII and residues on adjacent E molecules. Blockade of binding assays with TDV immune sera and human mAb 2D22 could not be performed, as the 2D22 epitope encompasses the highly conserved fusion



**Figure 6.** Mapping the dengue virus serotype 3 (DENV-3) epitope-specific neutralizing antibody (nAb) response. Immune sera from naive (*A* and *B*) and preimmune (*C* and *D*) subjects who received the vaccine and developed DENV-3 nAb (50% nAb titer [NT<sub>50</sub>] >1:20) were selected for mapping DENV-3 responses. Neutralization assays were performed using a recombinant chimeric DENV-4/3 and the wild-type (WT) DENV-3 and DENV-4 parental strains of the chimera, and the geometric mean NT<sub>50</sub> values (GMTs) were calculated for the naive (*A*) and preimmune (*C*) groups after vaccination. Bars represent the GMT and whiskers the 95% confidence interval. Dashed line indicates the limit of detection for the assay. Nonresponders were assigned a value of half the limit of detection, for visualization. Prevacination and postvaccination immune sera from naive (*B*) and preimmune (*D*) subjects were also tested for ability to block the binding of monoclonal Ab 5J7 to DENV-3. Within each graph, each individual is assigned the same color, to enable visualization of each person's nAb titers to the different viruses. \**P* < .05, by the Wilcoxon test, for comparison of the parental backbone DENV to the rDENV.



**Figure 7.** Study 203 cartography. *A*, In Study 203 naive subjects, antigenic mapping shows that putting the 1F4 epitope from dengue virus serotype 1 (DENV-1) into the DENV-3 backbone shifts the neutralization away from DENV-3 and DENV-1 (red arrow). Putting the EDIII domain from DENV-2 into DENV-4 moves the neutralization from DENV-4 to DENV-2 (blue arrow). Putting the 5J7 epitope from DENV-3 into the DENV-4 backbone moves the response away from the DENV-4 and closer to the DENV-3 response (green arrow). *B*, In Study 203 preimmune subjects, antigenic mapping shows that putting the 1F4 epitope from DENV-1 into the DENV-3 backbone shifts the neutralization away from DENV-3 and toward the DENV-1 response (red arrow). Putting the EDIII domain from DENV-2 into DENV-4 moves the neutralization from DENV-4 to DENV-2 (blue arrow). Putting the 5J7 epitope from DENV-3 into the DENV-4 backbone moves the response away from the DENV-4 and closer to the DENV-3 response (green arrow).

loop domain of the E glycoprotein, which also elicits binding Abs that interfere with the specificity of the 2D22 blockade assay [33].

We also evaluated subjects with DENV-1 nAbs responses after vaccination. Among naive subjects who developed DENV-1 nAbs after vaccination, Abs that neutralized rDENV-3/1 displaying the 1F4 epitope were significantly greater than the nAb titer against the parental DENV-3 strain. Consistent with these findings, the blockade of binding assay demonstrated the presence of Abs in dengue-naive TDV recipients that prevented human mAb 1F4 from binding to DENV-1. Together, these results demonstrate that TDV can elicit nAbs directed to the complex serotype-specific epitope region defined by mAb 1F4 in dengue-naive subjects. The use of the rDENV-3/1 proved more challenging in the preimmune subjects, owing to the high titers of nAbs to both the parental DENV-1 and DENV-3 strains. However, sera from vaccinated dengue-preimmune subjects also blocked 1F4 binding to its epitope on DENV-1, indicating that vaccination elicited Abs that bind to or near the 1F4 epitope. While we recognize that Abs binding close to the 1F4 epitope may sterically hinder 1F4 binding, the combined approaches provide support for the hypothesis that the TDV-1 vaccine component elicited binding and functionally neutralizing Abs directed to the 1F4 epitope in some individuals.

The majority of subjects evaluated in this study from both the dengue-naive and preimmune groups had Abs that neutralized DENV-3. While we were unable to conclusively detect nAbs that tracked with the DENV-3 5J7 epitope, these results should be interpreted with caution. Many individuals selected for this study had high levels of nAbs to both DENV-3 and DENV-4. In rDENV-4/3, a gain of DENV-3 nAbs could be masked by a loss of an epitope targeted by DENV-4 nAbs [35]. Mapping the DENV-3 response is also complicated by the genotype differences between the TDV and UNC DENV strains. As shown by our group previously, DENV-3 genotypes have up to 10-fold significant differences in polyclonal neutralization phenotypes [28]. There are 5 amino acid differences within the mAb 5J7 epitope, 3 of which are contact residues, between the TDV3 and UNC3001 strains used for the study. A single contact residue change can dramatically reduce neutralization, complicating interpretation of DENV-3 epitope-specific responses. While 5J7 can bind and neutralize the both the UNC3001 and TDV3 strains, new recombinant viruses may be required to specifically map the 5J7 epitope-specific responses in vaccinees receiving TDV. Recently, several DENV-4 type-specific nAbs have been identified from natural human infections and experimental non-human primate studies [35]. We are currently developing reagents to map DENV-4 type-specific nAb responses and anticipate using these reagents to map TDV responses in the near future.

Conventionally, antigenic cartography has been widely used to compare antigenic relatedness between different strains of viruses [32, 36]. Here we modified the approach to determine

whether the Takeda TDV induced nAbs that tracked with epitopes displayed on recombinant DENVs. In the cartography map based on nAbs induced by TDV, the DENV-4/2 chimera mapped closer to DENV-2 than to DENV-4, suggesting that most if not all TDV2 Ab responses target the DENV-2 EDIII displayed on DENV-4. Both rDENV-3/1 and rDENV-4/3 demonstrated shifts toward the donor strain, as well, although the shift was not as pronounced as in the case of rDENV-4/2 strain. In summary our data demonstrate that, in both naive and preimmune individuals who received TDV, the TDV-2 vaccine component elicited robust type-specific nAb responses to epitopes on EDIII of DENV-2. TDV also induced nAb responses that tracked with DENV-1 type-specific 1F4 epitope in some subjects. While our data demonstrate the presence TDV-induced Abs that bind at or near the DENV-3 5J7 epitope, we were unable to detect functionally nAbs directed to this epitope.

As TDV progresses through its phase 2 and 3 clinical trials, the data and methods reported in this study can be used to understand how Abs elicited by vaccination protect people from infection and disease. Our study has some limitations that need to be addressed before large sample sets from large clinical studies can be tested. Many individuals selected for the current study had high levels of nAbs to both the transplant and recipient serotypes. In these individuals, a gain of neutralization due to the transplanted epitope could be masked by a loss of a critical neutralizing site in the recipient strain. This is issue is particularly relevant to rDENV-3/1 and rDENV-4/3 because the known type-specific epitopes on these serotypes are adjacent to each other. In future studies, we plan to deplete all Abs to the recipient strain so that we can unambiguously track the properties of the remaining type-specific Abs. Our studies are also confounded by sequence differences between TDV strains and the recombinant chimeric viruses used for mapping. In particular, the region defined by the DENV-3 5J7 epitope are variable between TDV-3 and the UNC DENV-3 infectious clone. We are currently building recombinant viruses that are matched to epitopes on TDV viruses. Finally, some of the vaccine sera used in this study had detectable DENV-1 or DENV-3 nAbs in the Vero cell assay used at Takeda but not in the U937+DC-SIGN neutralization assay used for epitope mapping. Further studies are needed to understand these cell-type-dependent differences and how they relate to vaccine performance. In conclusion, this study provides a foundation for the development of high-throughput assays for use in DENV vaccine trials to understand the contribution of Abs to protective immunity.

#### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.



## Notes

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**Potential conflicts of interest.** A. M. d. S. has consulted on dengue vaccines for Takeda Vaccines, Sanofi Pasteur, GSK, and Merck Pharmaceuticals and is an inventor in patents related to dengue vaccines. H. J. D. is an employee of Takeda Vaccines. C. D. P. was an employee of Takeda Vaccines at the time the study was conducted. J. E. O. was an employee of Takeda Vaccines at the time the study was conducted and is an inventor on patents related to dengue vaccines. R. S. B. has consulted on dengue vaccines for Takeda Vaccines and Sanofi Pasteur and is an inventor in patents related to dengue vaccines. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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